EFFECTS OF WATER-SOLUBLE FRACTION OF GASOLINE ON THE ION REGULATION OF THE FRESHWATER FISH, PROCHILODUS LINEATUS.

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Objectives:

In fish ion regulation occurs mainly by the gills and the chloride cells (CC), present in these organs play an important role in ion uptake in freshwater environment. This study evaluated the effect of water-soluble fraction of gasoline (WSFG), rich in hydrocarbons on ion regulation and chloride cells of a Neotropical fish species, Prochilodus lineatus.

Methods and Results:

The water-soluble fraction of gasoline was prepared by mixing gasoline in water (1:4) followed by exposure to intense sunlight for 6h. The aqueous phase was separated (WFSG) and the organic phase (insoluble) was discarded. The WFSG showed high concentrations of BTEX [benzene (5700 μg.L⁻¹), toluene (1050 μg.L⁻¹), ethylbenzene (10500 μg.L⁻¹), xylene (7550 μg.L⁻¹)] and 12 polycyclic aromatic hydrocarbons from which naphthalene was the most abundant (2138 μg.L⁻¹). The fishes (11.23 ± 4.67 g) was exposed to WSFG diluted to 5% or only to water (control fishes) during 6 and 96 h. The osmolality and the concentration of sodium, potassium and chloride in plasma and, the activity of enzyme Na⁺/K⁺-ATPase (NKA) of gills were determined. The density and the fractional area of the chloride cells were analyzed using light and SEM microscopy. Six hours exposure to WSFG did not affect ion regulation, NKA activity and chloride cell density and morphology. However, after 96 hours exposure, the osmolality and potassium concentration in plasma increased 9 and 70%, respectively, and the activity of NKA increased 36% in relation to controls. The CC density and fractional surface area did not changed. CC surface showed numerous cells exhibiting apical sponge-like organization or microvilli.

Conclusions:

The results suggested that the mono e polyaromatics hydrocarbons present in the diluted WSFG induce ionic and osmotic disturbance in P. lineatus after 96 hours exposure but, did not affected the density and fractional surface area of CC. The increase in plasma potassium may be the result of hemolytic cells.

Keywords: Chloride cell, Fish, Gasoline, Gill, Prochilodus lineatus

Financial Support: FAPESP; CNPq
Objectives:

The humic substances, resulting from the decomposition of the remains of plants and animals, are found in most waters in different concentrations, mainly in the Amazon basin, causing physiological changes in aquatic organisms. This study aimed to evaluate the oxidative parameters in catfish *Rhamdia quelen* exposed to different levels of humic substances.

Methods and Results:

Humic synthetic substance used was (CAT: H1,675-2 Aldrich® - humic acid sodium salt - HA). Juveniles catfishs (143±6.41g, 23.33±1.20cm) were divided in 3 groups (N=7) and acclimated to laboratory conditions for 30 days in fish tanks (maximum capacity of 250 liters) under controlled temperature (22.21±0.28ºC) and dissolved oxygen (6.60±0.09mg/L). After this period, the animals were submitted for 9 days to 2.5 mg/L and 5 mg/L of humic acid, serving the group without HA as control. At the end of experimental period the animals were killed by sectioning the spinal cord, and their livers were subsequently dissected out and frozen for posterior analyses to thiobarbituric acid reactive substances – TBARS, as indicator of lipid peroxidation, according to Wills (1987), thiol groups, nonenzimatic antioxidant, according to Ellman, (1959), and glutathione-S-transferase, enzyme antioxidant detoxified, using the method described in Habig et al. (1974). The statistical analysis was performed with Software GraphPad Instat®, 3.0. used one-way ANOVA complemented with Dunnett’s test (p

Conclusions:

The presence of humic acid decrease GST activity, which is probably linked to prevention of oxidative damage. Further studies are needed to elucidate the mechanism of action of humic acid in fish.

Keywords: humic substances, oxidative stress, silver catfish

Financial Support: CAPES, CNPq

Objetivos:

Metabolismos alterados induzidos por pesticidas em morcegos frutíferos (*Artibeus lituratus*).

Concluções:

A presença de ácido humico diminui a atividade da GST, que provavelmente está associada à prevenção do dano oxidativo.

Palavras-chave: substâncias humáticas, estresse oxidativo, morcego pratinho.

Finanças: CAPES, CNPq.

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**Resumo:**

**METABOLIC ALTERATIONS INDUCED BY PESTICIDES IN FRUIT-EATING BATS (*ARTIBEUS LITURATUS*)**

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Objectives:

Evaluate the metabolic effects induced by acute environmental exposure to two relevant fungicides: tebuconazole (Folicur®) and mancozebe (Mancozeb BR®) in fruit-eating bats *Artibes lituratus*.

Methods and Results:

male bats (N=18) were captured near Viçosa, MG, Brasil. They were maintained in a bat cage at the University Museum of Zoology from UFV. After two days of adaptation, animals were randomly distributed in 3 groups: (1) Control – animals were
offered papaya (200 g) immersed in adhesive spreader solution at 10% for 7 days; (2) MZB – treatment with Mancozeb BR® (2g/L) and adhesive spreader (10%) solution for 7 days (N=6) and (3) TBZ – treatment with Folicur® (1mL/L) and adhesive spreader (10%) solution for 7 days (N=6). Fruits and water ad libitum were offered at 1800 pm and removed at 0800 am. After treatment, animals were killed by decapitation, and blood and tissues (breast and limbs muscles, adipose tissue and liver) were collected for glycogen, protein and fat determination. Liver enzymes ALT and AST activities were also performed. Data were compared by ANOVA or by the non-parametric Kruskal-Wallis test (p

Conclusions:

Our results suggest that the exposure to acute low concentrations to mancozebe and tebuconazole induced little metabolic alterations in fruit-eating bats, and we found no evidences for liver damage. TBZ group presented a greater number of metabolic alterations, but apparently none of tested pesticides interfered on the animals glucose homeostasis or survival. Energy reserves, mobilized during reproductive periods and necessary to adapt to different seasons food availability, were also not significantly affected, suggesting that reproduction or environmental adaptations would not be impaired by the acute low concentration exposition to the pesticides. Financial support: FAPEMIG.

Keywords: bats, glucose, mancozebe, metabolism, tebuconazole

Financial Support: FAPEMIG

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Resumo:01-013

PARTIAL PROTEIN CHARACTERIZATION OF FREE-LIVING NEMATODES IN TWO COASTAL ENVIRONMENTS

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Objectives:

This study aimed to determine the partial protein profile of nematofauna in two coastal environments including digestive enzymes.

Methods and Results:

The collection was performed in sediments from Maracaípe beach and Pina estuary, Pernambuco, Brazil. Nine replicas were taken from random samples to constitute three treatments: (S1) fixed with 4% neutral formalin, (S2) 4% formaldehyde buffered with borax and (S3) without formaldehyde. The biosedimentological samples were elutriated (at least 10 times) and supernatant from this procedure was poured into 0.045 mm geological sieves for nematofauna extraction. For each treatment were used 200 individuals per replica which were sonicated and centrifuged to analyze protein content by BCA method. Total proteolytic activity was colorimetric assayed as well as trypsin, chymotrypsin, dipeptidase, carboxipeptidase and pepsin activities. Molecular weight was estimated in 12.5% polyacrylamide gel electrophoresis (SDS-PAGE). The protein content of extracts obtained from treatments without formaldehyde (S3) were approximately 75% (beach) and 30% (estuary) lower than the extracts of S1 and S2 treatments (approximately 115 µg/mL). Total proteolytic activity of S3 were about 12.3-fold higher than treatments using formaldehyde. Trypsin activity exposed to 8 mM TLCK was 31.7% of the control (45.3 mU/mg protein) while chymotrypsin activity exposed to 8 mM TPCK were 27.6% (SApNA as substrate) and 1.7% (Suc-Phe-p-NAN as substrate) of controls (103.7 mU/mg protein). Chymotrypsin specific activity was shown to be higher than the same parameter for trypsin in these organisms.
Dipeptidase and pepsin showed no detectable activities while carboxipeptidase showed 1,309 mU/mg protein (beach) and 5,733 mU/mg protein (estuary).

Conclusions:

The nematofauna from estuarine environment presented higher protein content than beach environment. Carboxipeptidase and chymotrypsin presented the highest activities in contrast to superior organisms where trypsin and pepsin perform the most relevant functions.

Keywords: Nematofauna, BCA protein assay, Trypsin, Chymotrypsin, Free-living

Financial Support: CNPq and FACEPE

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Resumo:01-014

STUDY ON THE BIOCHEMICAL RESPONSES IN FISH AS BIOMARKERS OF AQUATIC CONTAMINATION IN VACAÇA RIVER, SÃO GABRIEL, RS, BRAZIL.

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Objectives:

It is recognized that exposure of aquatic organisms to contaminants is related to physiological, genetic and biochemical changes such as induction of enzymes that act in the metabolism or detoxification of exogenous compounds. The aim of this study was to evaluate biochemical changes in Danio rerio exposed to water from Vacacaí River, São Gabriel – RS.

Methods and Results:

The animals were exposed for 48h to water collected from two distinct points: S1 (30°22'35.45"S/54°21'24.82"O) and S2 (0°20'27.28"S/54°18'19.96"O). Control animals were kept under recommended laboratory conditions for the species. After exposure, the tissues were isolated (brain and muscle) and processed for determination of enzyme activities. Acetylcholinesterase, catalase and glutathione S-transferase were measured by spectrophotometry and the analysis of phospho-p38MAPK/total-p38MAPK as well as PARP cleavage was performed by immunoblotting using specific primary antibodies. There was a significant decrease (29.4.8±4.2%, p

Conclusions:

In conclusion, the study points to an important degree of water pollution in the Vacacaí River, São Gabriel – RS. Given the important rice farming activity in the region targeted by this study, the inhibition of acetylcholinesterase activity and induction of glutathione S-transferase observed may be an indicative of the presence of pesticides in the exposure sites. The absence of PARP cleavage indicates that exposure of animals during 48 hours was not sufficient to induced cell death by apoptosis. More studies are needed to determine the actual levels of biological damage in the region. Moreover, the present data indicate that the species used has potential for studies of biomarkers of environmental contamination in the region of biome Pampa.

Keywords: biomarkers, Danio rerio, biochemical responses

Financial Support: PBDA-Unipampa, CNPq, Fapergs.
EFFECT OF ANESTHESIA WITH ESSENTIAL OIL *ALOYSIA TRIPHYLLA* IN OXIDATIVE PARAMETERS OF SILVER CATFISH

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Objectives:

Anesthetics have been widely employed in aquaculture. Essential oils obtained from leaves of medicinal compounds have been used as an alternative to anesthetize fish, as *Aloysia triphylla*, popularly known as “cedrón”, member of the Verbenaceae Family. This study aimed at verifying alterations in oxidative parameters in liver of silver catfish *Rhamdia quelen* due to the use of essential oil (EO) of *A. triphylla* as an anesthetic for this species.

Methods and Results:

Juveniles of silver catfish (95.63±2.83g, 22.12±0.2cm) were allocated in four groups (N=7): A - anesthesia; B - anesthesia and recovery; C – control and D – vehicle. The fish were anesthetized in a 2 L aquarium with 150 µL/L of EO. Group A remained in the anesthetic bath until loss of reflex activity and no reaction to external stimuli. After reaching such stage, group B was transferred to an aquarium free of EO to recover. Group C and D were transferred from one aquarium to another, both free of EO. After the experimental period, fish were sacrificed by section of the spinal cord and livers homogenized. Supernatants were used to analyze the levels of lipid peroxide, using thiobarbituric acid reactive substances (TBARs) assay, according to Wills (1987), and of the antioxidants catalase (CAT), according to Boveris and Chance (1973), and glutathione-S-transferase (GST), using the method described in Habig et al. (1974). Homogeneity analysis was performed by Bartlett and the comparison was determined by one-way ANOVA followed by Tukey (p

Conclusions:

The increase in CAT and GST during anesthesia occurred due to a decreased metabolism under the anesthetic state, preparing the organism for a possible reoxygenation. The absence of oxidative damage due to the use of EO of *A. triphylla* contributes for its use as an anesthetic for silver catfish.

Keywords: *Aloysia triphylla*, anesthesia, essential oil, oxidative parameters

Financial Support: CNPq, CAPES. FAPERGS/PRONEX
Objectives:

Determining fish eurihalinity degree and physiological plasticity through muscle’s expression of HSP70, gills and renal activity of carbonic anhydrase (CAA), plasma osmolality and muscle water content, we attempted to answer how the freshwater teleosts *Rhamdia quelen*, *Geophagus brasiliensis*, *Clarias gariepinus*, *Ictalurus punctatus*, *Cyprinus carpio* and *Oreochromis niloticus* responds to increases in salinity to 15‰ and 30‰, and then could possibly use estuaries as scatter bridges to other continental waters.

Methods and Results:

The freshwater teleosts *R. quelen* (n= 6-12), *G. brasiliensis* (n= 6-12), *C. gariepinus* (n= 6-12), *I. punctatus* (n= 6-12), *C. carpio* (n= 6-10) and *O. niloticus* (n= 6-12) were exposed to increases in salinity to 15‰ and 30‰ (diluted seawater), for 6 hours in 50L aquariaums with constant aeration and environmental temperature. After the experiments, they were anesthetized with benzocaine (80 mg/L) and was collected an aliquot of plasma for measured the osmolality. The fish was then sacrificed and muscle tissue, kidney and gills were collected and stored at -80°C. With the tissues was analyze expression of HSP70 (muscle), muscle water content (MWC) and branquial and renal carbonic anhydrase activity (CAA). The salinity of 30‰ (between 1.5 – 3 hours of exposition) was lethal for all species, except *O. niloticus*. The species *R. quelen* and *C. gariepinus* increased their expression of HSP70 following the salinity increase; *I. punctatus*, *O. niloticus* and *C. carpio* presented constant levels of HSP70 and remained constant in *I. punctatus*. The renal CAA was elevated only at 30 ‰ for *R. quelen* and *O. niloticus*, increased at 15‰ and 30‰ for *I. punctatus* and remained constant in *C. gariepinus* and *C. carpio*. Osmolality and MWC showed an inverse pattern of variation to each other, in most species, with an increase in osmolality and a decrease in MWC, except for *G. brasiliensis* and *C. carpio*.

Conclusions:

There was no standard response in HSP70, which shows that the action of this protein seems to be species-specific. The increase in CAA may be due to the maintenance osmoregulation or acid-base balance. The inverse pattern of variation between osmolality and MWC indicates a loss of the regulatory capacity of the intra and extracellular mediums due to increase in salinity, not surviving at 30‰. According to the results, the freshwater species could use estuaries as scatter bridges to other continental waters, since the salinity at these places did not exceed 15‰. Invasive species showed similar physiological resistance to native species, all showing some degree of eurihalinity.

Keywords: Ecophysiology, Estuaries, HSP70, Osmoregulation, Teleost
Objectives:

American cutaneous leishmaniasis is one of the clinical forms of protozoan Leishmania genus, transmitted by females of *Lutzomyia* (Diptera: Psychodidae) phlebotomines. The municipality of Adrianópolis, in the Ribeira Valley region, at Paraná state has high epidemiological importance as a transmission area of the disease. Apart from this, to describe and characterize the phlebotomine fauna of this area, and with the aim to isolate the protozoan from these insects, was made an prevalence, abundance and diversity research of phlebotomines in Adrianópolis municipality.

Methods and Results:

The capture of the sand flies occurred while July, August and September of 2010, using luminous traps (CDC), installed at domiciliary, peridomiciliar and wild areas between 18h00 to 06h00. Part of the female sand flies had the intestine dissected, for trying to isolate the parasite. The remaining insects were classified by sex, and conserved in 70% ethanol solution. For identification, the phlebotomines were submitted to a clarification process being immersed to a KOH 20% solution during 24h, and after identified at optic microscope, using NaCl 0,9% solution, without staining. A total of 1.288 specimens were collected, with 809 males (63%) and 479 females (37%), distributed by this way: 432 sand flies collected at domiciliary, 621 at the peridomiciliar and 235 at the wild area. The specimens found are: *Lutzomyia (Nyssomyia) intermedia* (1.287 specimens) and *Lutzomyia (Pintomyia) fischeri* (01 specimen collected at the peridomiciliar area). The *Lutzomyia* population were distributed in this percentages: 18,26% at the forest (117 females and 118 males), 33,57% at domiciliary area (272 females e 160 males) and 48,17% at peridomiciliar (90 female and 530 males).

Conclusions:

The specie *L. intermedia* is highly prevalent at all the ecotopes of the region. The predominance of females at domiciliary site can be related to the affinity of this specie to human sources of blood nutrition, due to the high capacity of adaptation to the anthropic environment. Furthermore, the total dominance of sand flies at peridomiciliar seem to be associated to the collect sites in this kind of ecotope, specially chicken houses, places with high concentration of organic matter and moisture, which gives ideal conditions to larval phlebotomines growth. None parasites were found during the female dissection. However, the high prevalence of *L. intermedia*, due to the high density of females collected at domicile, names this the main suspect of Leishmania cycle in the region, being the domiciliary ecotope a potential risk factor for human contamination by the protozoan.

Keywords: phlebotomines, leishmaniasis, ecology

Financial Support: CNPq
This study was conducted in August 2010 at Farroupilha Park. This park is located in Porto Alegre downtown and it is surrounded by roads with intense automotive traffic. Five sites were determined using a GPS (Garmin International Inc., USA) in order to monitor PM2.5 and NO2 concentrations. Four of these sites were near the traffic roads and one site was located in the middle of the park. The concentrations of NO2 were obtained by passive sampling technique in the five monitoring sites during 12 consecutive days. At the exact same sites, the concentration of PM2.5 was monitored during 6 consecutive days in times of highly intensive vehicular traffic (8-9 AM and 5-6 PM) using DustTrak™ II Aerosol Monitor, Model 8532 (TSI Inc., USA) equipment. One-way ANOVA followed by Student Newman–Keuls post-hoc test was used to evaluate the statistical difference between the concentrations of NO2 at the different sites evaluated. Mann–Whitney Rank Sum Test was used to compare medians of PM2.5 concentration among the different sites. Statistical significance was set at P ≤ 0.05. All statistical analysis was performed using the software SPSS version 11.0 (SPSS Inc., USA). A statistically increase in NO2 levels was observed in the two sites close to the roads when compared to the other sites within the park (P

Conclusions:

The high concentration of NO2 and PM2.5 indicate an impairment of the air quality at Farroupilha Park, probably because of the heavy vehicular traffic near this region. The results of our study are worrisome due to the bad air quality of this park, since NO2 and PM2.5 are known to cause deleterious effects to human health.

Keywords: Air pollution, Nitrogen dioxide, Particulate matter, Urban park

Financial Support: CNPQ

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Resumo:01-019

BIOCHEMICAL ALTERATIONS IN TELEOST FISH TISSUES IN RESPONSE TO ACUTE EXPOSURES TO CADMIUM

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Objectives:

Cadmium (Cd) is a non-essential trace metal with high potential to impair the functions of biological molecules. Cd compounds are widely distributed in environmental and industrial pollutants, and they may bring danger to some physiological mechanisms of aquatic organisms. Fishes has largely been used as bioindicadores, biomonitor or test organisms in ecotoxicological studies. Thus, the objective of this study was to determine the effects of Cd acute exposure on the activity of acetylcholinesterase (AChE), carbonic anhydrase (CA) and Na+/K+-ATPase (NKA) of juveniles of the neotropical fish Prochilodus lineatus (curimba).

Methods and Results:

Fish were acutely exposed (24 and 96 h) to 10 ug L−1 of waterborne Cd (as CdCl2. 2H2O) and animals were killed for the removal of brain, gills, muscle. All samples were diluted 10x (p/v) and homogenized in a motor-driven homogenizer, homogenates were centrifuged at 12000 rpm for 20 min at 4°C and the supernatant was used for the biochemical assays. AChE was measured in brain and muscle, CA and NKA activities were determined in branchial tissue. For AChE determination we used a colorimetric assay based on hydrolysis of acetylcholine. AChE activity was expressed as nM.(min.mg protein)−1 The catalytic activity of AC was measured by using a pHmeter which monitored the time dependent acidification of a medium saturated with CO2. CA activity was expressed as ΔpH. (mg protein. minute)−1. NKA activity was quantified by the difference in the amount of inorganic phosphorous released in the presence or absence of ouabain, and it was expressed as uM Pi h−1 .mg-1 protein. Muscle AChE decreased after 24 h (n=11, p<0.1) or 96 h (n=14 p>0,3) exposures. CA activity showed a decrease after 24 h (n= 12, p=0,02) and 96 h (n=14, p<001).
Conclusions:

These results show that acute exposures of P. lineatus to 10 ug Cd. L-1 promote toxic effects on branchial CA and NKA and on muscle AChE, but not on brain AChE.

Keywords: Cadmium, ecotoxicology, enzymes, fish, teleost

Financial Support: CNPq

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Resumo:02-115

MYOCARDICAL CONTRACTILITY IS PRESERVED IN FEMALE RATS TREATED FOR 15 DAYS WITH TRIBUTYL Tin

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Objectives:

The tributyltin (TBT) is an important silent threat to aquatic ecosystems, owing to its capability to inhibit the attachment of marine organisms on the hulls of ships. TBT is also used as a pesticide on farming. The toxic effects of this compound can be transferred throughout the food chain reaching the humans. Early studies described the TBT modify the synthesis of estrogen by inhibition the aromatase enzyme in mammalian, affecting the action of female gonadal hormones which, have cardioprotective effect in females on reproductive age. Whereas the TBT reduces the production of estrogen, and the cardioprotective function of this hormone is clarified, our aim is verify if the treatment with TBT for 15 days can interfere in the myocardial contractility.

Methods and Results:

Female Wistar rats (230-250 g) were divided on control group treated with vehicle (CT, n = 8) and the TBT group treated with TBT (100 ng/Kg) by gavage for 15 consecutive days (TBT, n = 8). All the protocols were developed was approved by the UFES Animals Ethical Committee (n° 020/2009; 07/2007). At the end of the treatment all animals were anesthetized with pentobarbital (35 mg/kg, i.p.) and the blood was collected for serum levels of 17¥â-estradiol (E2) and progesterone (P4). The papillary muscles of the left ventricle were removed and fixed in a force transducer, being immersed in Krebs solution gasified by carbogenic mixture, pH 7.4, 26¡ÆEC. The papillary muscles were stimulated electrically on 0.5 Hz. The experimental protocols evaluated the contractility response in maximal length (L-max), post pause potentialization (PPP: 15, 30 and 60 s), curve of frequency (0.1 to 1.0 Hz), response to calcium (0.62-3.75 mM) and to isoprenaline (5x10-4 M). Results are presented as mean ¡¾SEM. The differences were analyzed using unpaired Student t-test. P<0.05). The myocardial contractility and temporal parameters were similar between the groups, during basal condition (CT: 0.52 ¡¾ 0.051 vs. TBT: 0.43 ¡¾ 0.03 g/mg; p>0.05), in the calcium curve, in the presence of isoprenaline (CT: 134.8 ¡¾ 5.93 vs. TBT: 133.3 ¡¾ 4.16% of the basal; p>0.05), in the dF/dt + (CT: 2.64 ¡¾ 0.72 vs. TBT: 2.73 ¡¾ 0.43 g/mg/s; p > 0.05) and in the dF/dt - (CT: -1.37 ¡¾ 0.79 vs. TBT: -1.88 ¡¾ 0.58 g/mg/s; p>0.05).

Conclusions:

The present results demonstrated that the treatment with TBT for 15 days in female rats changed the serum levels of gonadal hormones without change the myocardial contractility.
Characterization of Pulmonary and Systemic Inflammatory Responses Produced by Lung Re-expansion After One-Lung Ventilation in Anesthetized Rats

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3 Departamento de Farmacologia, Faculdade de Ciências Médicas, Unicamp

Objectives:

To characterize the pulmonary and systemic inflammatory responses of rats undergoing time-course one-lung ventilation (OLV) with subsequent lung re-expansion under normal conditions.

Methods and Results:

Methods: The experimental protocols were approved by the Animal Ethical Committee of UNICAMP. Male Wistar rats weighing 325 ± 6.87 g were allocated randomly into 5 groups, each consisting of six rats: (i) a control group with no manipulation or mechanical ventilation (Control), (ii) a 1-h right lung collapse/1-h two lung ventilation (OLV1h/TLV1h), (iii) a 3-h right lung collapse/1-h two-lung ventilation (OLV3h/TLV1h), (iv) 1-h right lung collapse with no lung re-expansion (OLV1h) and (v) 3-h right lung collapse with no lung re-expansion (OLV3h). Under anesthesia, the animals underwent atraumatically right lung mobilization, with clamping of the right main bronchus during 1 or 3-h, keeping bilateral pulmonary perfusion and left lung ventilation. The clamp was then removed and both lungs were ventilated during 1-h. The following parameters have been evaluated: pulmonary protein extravasation, pulmonary myeloperoxidase (MPO) activity, cytokine levels in serum and bronchoalveolar lavage (BAL), counts of total and differential cells in BAL fluid, gasometric data and mean arterial blood pressure (MABP). Results: Bronchial occlusion for 1 or 3-h with no lung re-expansion did not significantly change the protein extravasation in the both lungs as compared with control group. However, lung re-expansion after bronchial occlusion (OLV1h/TLV1h and OLV3h/TLV1h groups) markedly increased the serum protein extravasation in the right lung compared with control. Number of neutrophils was markedly higher in OLV1h/TLV1h and OLV1h/TLV3h groups compared with control. Additionally, a significantly higher MPO activity in OLV3h/TLV1h group compared with control rats was found in the right lung, with no significant changes in the continuously ventilated lung. Levels of IL-6 in BAL fluid were significantly higher in both groups subjected to lung occlusion and re-expansion compared with control group. The IL-1β and TNF-α levels were higher in OLV3h/TLV1h group compared to either OLV1h/TLV1h or control animals and no differences in IL-10 levels between groups were found. Systemically, the levels of IL-1β and TNF-α quantified in serum in all groups were below the limit of detection. The IL-6 levels were significantly higher in the OLV1h/TLV1h and in OLV3h/TLV1h groups compared with control values. Similarly, the IL-10 levels were detected in high levels in the OLV1h/TLV1h and OLV3h/TLV1h groups whereas in control group it remained below the detection limit. The blood gas analysis showed that 1-h and 3-h OLV time-points did not differ between each other and almost all parameters returned to the baseline after TLV period. The MABP values were also similar between groups.

Conclusions:

Lung re-expansion after bronchial occlusion for 1 or 3-h promotes inflammatory responses in the right lung, as characterized by edema formation and neutrophil recruitment, which is accompanied by higher levels of IL-6, IL-1β and/or TNF-α in BAL fluid. Additionally, the local lung injury was accompanied by a degree of systemic inflammation, as detected by the increased serum...
levels of IL-6 and IL-10 levels.

Keywords: one-lung ventilation, thoracic surgery, inflammation

Financial Support: CAPES and FAPESP

**Objective:**
It is possible that renal sympathetic activity and renal baroreflex control are involved in the development of sepsis. Aim: The aim of this study was to evaluate cardiovascular parameters such as blood pressure (BP) and heart rate (HR) associated with renal sympathetic nerve activity (RSNA) and baroreflex control of RSNA in animals submitted to the experimental model of sepsis by cecal ligation and puncture for 24h and 48h compared to sham rats.

**Methods and Results:**
Male Wistar rats were submitted to surgery to arterial and venous catheterization for BP recording and drugs administration respectively and for sepsis induction by ligation and 10 punctures in the cecum with needles G18. Animals were divided in three experimental groups: sham, 24h and 48h after sepsis induction. In the experimental day, the rats had body temperature, BP and HR recorded and then were anesthetized with urethane (1.2–1.4g/Kg, IV) and RSNA and its baroreflex control were evaluated. Changes in blood pressure were elicited by IV infusion of phenylephrine and sodium nitroprusside and reflex RSNA responses evaluated and expressed as PPS/mmHg.

**Results:** No significant alterations in basal BP, HR and RSNA were observed in all experimental groups. However a significant increase in body temperature were observed in both sepsis groups (Sepsis 24h 38.9°C, Sepsis 48h 37.9°C vs Sham 36.7°C, n=10). Furthermore in the group sepsis 48h (-1.4±0.13pps/mmHg, n=9) it was observed a significant improvement in the baroreflex sensitivity compared to sham (0.88±0.12pps/mmHg, n=6) and 24h (0.97±0.09pps/mmHg, n=6) animals in the pressor response. It was also observed a significant statistical difference with an enhance of RSNA baroreflex gain for the depressor response between 48h (-1.10±0.08pps/mmHg), 24h (0.97±0.09pps/mmHg) and sham (0.62±0.11pps/mmHg) groups.

**Conclusions:**
Taken altogether the present study suggest that a differential regulation of RSNA and its baroreflex control maybe involved in the sepsis evolution independently of BP changes.

Keywords: sepsis, renal sympathetic nerve activity , baroreflex

Financial Support: CNPq, FAPESP
GABAERGIC MECHANISMS WITHIN THE NUCLEUS OF THE SOLITARY TRACT CONTRIBUTES TO THE ANTI-HYPERTENSIVE EFFECTS OF MOXONIDINE

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Objectives:
The depressor action of the central antihypertensive drug moxonidine has been attributed to activation of α2-adrenergic/imidazoline receptors in the rostral ventrolateral medulla (RVLM) and, more recently, in the nucleus of the solitary tract (NTS). In the present study, we aimed to determine the role of the γ-aminobutyric acid (GABA) mechanisms in the commissural NTS (cNTS) in mediating the sympathoinhibition and hypotension effect of moxonidine in anesthetized normotensive rats.

Methods and Results:
The experiments were recorded in urethane-anaesthetised and artificially ventilated male Wistar rats (280-320 g). A polyethylene tubing (PE-10 connected to PE-50) was inserted into the femoral artery for measure mean arterial pressure (MAP). The splanchnic nerve was isolated via a retroperitoneal approach, and the segment distal to the suprarenal ganglion was placed on a pair of teflon-coated silver wires that had been bared at the tip MAP and sSNA were continuously recorded during 60 min and were analyzed at every 10 min. Injections of moxonidine (5 nmol/50 nl) into the cNTS resulted in sustained (for at least 40 min) hypotension (Δ = -22 ± 4 mmHg).

Conclusions:
The current data suggest that the GABAergic mechanisms in the cNTS are important for the hypotensive and sympathoinhibition elicited by moxonidine in rats.

Keywords: Moxonidine, Antihypertensive, Nucleus of the solitary tract, α2-adrenergic receptor, Imidazoline receptors

Financial Support: FAPESP; CNPq; CAPES
The aim of this work was to carry out the identification test and the biological potency evaluation of raw material and pharmaceutical formulations of heparins demonstrating the advantages for the quality control.

Methods and Results:

The anti-factor IIa and anti-factor Xa were performed evaluating the anticoagulant activity of heparins by reaction with antithrombin III and subsequent addition of factor IIa or Xa, respectively. The reaction with chromogenic substrate results in development a yellow colour inversely proportional to the concentration of heparin. The sheep plasma assay was based on the evaluation of coagulation degrees of citrated and recalcified plasma in the presence of heparin, during 1 hr at 37°C. Samples of pharmaceutical products containing 5000 IU per vial and of raw material with specific activity (>150 IU/mg), were subjected to the potency evaluation by the sheep plasma inhibition assay, anti-factor IIa and anti-factor Xa assays. The statistical analysis of the results showed mean values of 101.63%, 99.85% and 98.88% respectively. Besides, the samples were identified by capillary electrophoresis showing similar profile to the 5th International Standard Heparin (WHO 97/578).

Conclusions:

The results demonstrated the application of the capillary electrophoresis for the characterization of the heparins. The capabilities of the anti-factor IIa assay based on the coagulation mechanism, was demonstrated, evaluating also the correlation between the results of the assays, justifying its use as a valid alternative to replace the sheep plasma inhibition assay. Thus, contributes to improve the quality control of unfractioned heparins, and to assure the safety and therapeutic efficacy.

Keywords: Heparin, Anti-factor IIa, Anti-factor Xa, Pharmaceutical Products, Anticoagulant and Antithrombotic

Financial Support: CNPq

QuebraPagina

Resumo:02-120

THE ASSOCIATION OF ESTROGENS AND TESTOSTERONE BLUNTED THE VASCULAR PROTECTIVE EFFECTS OF ESTROGENS IN OVARIECTOMIZED SHR.

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Objectives:

Cardiovascular disease is more common in postmenopausal than premenopausal women. Besides that menopause has been linked to psychosomatic, vasomotor, bone alterations and hypoactive sexual desire disorder. Hormone Replacement Therapy (HRT) with estrogens is applied to improve mood, general well-being in postmenopausal women. Currently, much interest is focused on the effects of the association of testosterone plus estrogen to improve sexual hypo activity. We have demonstrated that estrogen has a protective effect on the vasculature restoring the endothelium dysfunction (ED) observed in ovariectomized SHR (OVX). Also is already known that testosterone is involved in development and maintenance of the blood pressure in SHR. However little is known about the association of estrogen and testosterone in the cardiovascular system. So the aim of this study is to evaluate the effects of the association of conjugated equine estrogen (CEE) with testosterone in the vascular reactivity, blood pressure (BP) and body weight of OVX.

Methods and Results:

Female SHR 12 weeks-old were divided into two groups: physiological estrous (PE) and OVX. PE was determined by microscopic evaluation of vaginal smears. OVX was performed at 12 weeks of age. Thirty days after OVX animals were treated
with CEE (0.032mg/Kg/day - CEE) during 15 days by gavage. Another OVX group received CEE plus testosterone cypionate (2.85mg/kg/once in a week, im. – CEE+T). Vascular reactivity studies were performed in isolated aorta from the different groups. Concentration-effect curves (CCE) to Angiotensin II (ANGII 0.1nM – 10μM) were constructed in aortic rings with (E+) and without (E-) endothelium. Reactive oxygen species (ROS) generation in aorta was analyzed by dihydroethidium method. In another series of experiments, CCE to acetylcholine (ACH 0.1nM – 10μM) and sodium nitroprussiate (SNP 0.1nM – 10μM) endothelium-dependent and independent vasodilators respectively, were performed in aortic rings (E+) preconstricted with norepinephrine (NE-0.1μM). The BP was measured in anaesthetized animals by an indirect tail-cuff method. The results of vasoconstriction were expressed as a delta of the basal tension(g) and the vasodilators response as a percentage of the NE-preconstriction. The results were shown as mean ± SEM. Statistical analysis was performed by ANOVA one-way analysis of variance, followed by Tukey test, p < 0.05. The maximal response (RMax) to ANGII in aortic rings (+) was increased in OVX (0.49±0.05g) when compared with the PE ones (0.27±0.04g). This difference was abolished by CEE (0.28±0.04g) but not by CEE+T (0.45±0.02g). The RMax to ANGII in aortic rings (E-) did not differ among groups. The basal ROS generation in OVX aorta (E+) was higher than the PE ones. This alteration was restored by CEE but not by CEE+T. The response to ACH in SHR aortic rings (E+) was biphasic (relaxation followed by contraction). The relaxation to ACH was similar in PE (70.4±3.4%) and OVX (60.5±2.4%) however it was increased in CEE (78.0±16.6%) aortic rings (E+). On the other hand, in CEE+T (53.9±3.7%) the relaxation to ACH was reduced when compared with the other groups. There were no differences in NPS vasodilatation and the BP levels among groups. OVX body weight were bigger than PE ones, it was reduced by CEE but not CEE+T.

Conclusions:

We might suggest that association of testosterone plus estrogen in HRT might be applied with caution in hypertensive subjects

Keywords: Estrogen, Hypertension, Testosterone, Vascular reactivity, Vascular endothelium

Financial Support: Fapesp, Capes

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Resumo:02-121

TRANS-[RU(NH3)4(CY)(NO)]3+ EFFECT OF THE COMPOUND IN THE AORTIC RINGS WITH ENDOTHELIUM ISOLATED FROM RATS.

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Objectives:

We demonstrate the effectiveness of the vasorelaxant effect of ruthenium compounds trans-[Ru(NH3)4L(NO)]n+ [L=pyridine, nicotinamide, 4-picoline, Nimidazol, pyrazine, NH3, SO32-, or P(OEt)3] in aortic rings without endothelium, the response induced by the release of NO molecule by these compounds when activated by reduction (Nitric Oxide, 16(2): 189-96, 2007). Due to the relevance of the topic, this study aimed to assess the vascular response of trans-[Ru(NH3)4(Cy)(NO)]3+ (CyNO) in aortic rings with endothelium isolated from rats.

Methods and Results:

For the tests we used six adult male rats. The rings were pre-contracted with noradrenaline (10-6 M). After stabilizing the voltage, single concentration (10-6 M) of the CyNO compound was added to the bath. The responses were recorded over 120 min. The presence of endothelial cells in the rings was assessed by vascular response to acetylcholine (ACH, 10-6 M) and the
integrity of vascular smooth muscle by sodium nitroprusside (SNP, 10-6 M). Results: Results are expressed as mean ± SEM (n=6) of respective responses in gF. Statistics: Student's t-test and ANOVA followed by Tukey test p

Conclusions:

Under experimental conditions the compound showed significant relaxant effect in 120 minutes in the presence of indomethacin and L-Name and contraction after 15, 30 and 60 min in the presence of ODQ.

Keywords: RUTHENIUM, NITRIC OXIDE, AORTIC RINGS, RATS

Financial Support: Financial Support: FAPESP, CAPES, CNPq

QuebraPagina

Resumo:02-122

MITOCHONDRIAL RESPIRATORY CHAIN DYSFUNCTION IN MICE HEARTS INDUCED BY METHYL-MERCURY

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3 Ecodata Exames Médicos Ltda., Ecodata

Objectives:

Aim: Methyl-mercury (MetHg) is an ubiquitous compound found in mining explored in aquatic environments and a relevant pollutant with implications in public health (1). Neurotoxicity is a well-known consequence of exposure. There are also evidences of relationship between cardiovascular disease (hypertension and coronary heart disease) and MetHg chronic exposure, mainly by fish intake in contaminated environment (2). Also, some descriptions about mitochondrial dysfunction and oxidative stress in tissues as brain and liver were published, but data concerning heart tissue functionality are scarcely published. Thus, in this study it was explored the effects of MetHg on heart tissue and heart mitochondria.

Methods and Results:

Methods and results: BALB/c mice received MetHg by daily gavage of 1,5 mg/Kg (cumulative dose = 15 mg/Kg in ten days). Control group received the same volume of water/Kg. The animals were submitted to electrocardiograms (ECG) and echocardiograms (ECHO) a week before and after the intake of MetHg. The exams were done under anesthesia with ketamin (60 mg/Kg) and xylasin (3 mg/Kg). In ECG parameters (heart rate, P-R and QTc intervals, P and QRS wave duration) and in the evaluation of left ventricle systolic function by ECHO (aorta/left atrium ratio and ejection fraction) there were no differences between both groups. Respiratory chain function was assessed in heart tissue fibers permeabilized with saponin. The rates of mitochondrial respiration were assessed at different mitochondrial respiratory states in a high-resolution respirometry according to Gnaiger and Kuznetsov (3). Mice that received MetHg presented evidence of mitochondrial respiratory chain dysfunction, in relation to control group, characterized by: reduced response to ADP+Pi administration, suggesting ATP synthase commitment (24,6±4,58 vs 134,5±42,99 nmol O2/mg.min); O2 maximum flow reduction after a proton ionophore agent (FCCP) administration (113,7±20,99 vs 238,1±41,51 nmol O2/mg.min) and complex IV evidence of dysfunction: reduction of ∆ inhibition of O2 flow after azide administration (63,76±11,88 vs 158,7±19,44 nmol/mg.min).

Conclusions:

Conclusion: In a model of systemic intake in mice the subacute exposure to MetHg is able to develop evidence of multisite
respiratory chain derangement in heart tissue, despite the absence of heart rhythm disturbances and heart failure signs.

Keywords: methyl-mercury, respiratory chain, heart, echocardiography, electrocardiography

Financial Support: INCT-INPeTAm, FAPERJ, CNPq, INCTEN-excito-neurotoxicidade.

QuebraPagina

Resumo:02-123

INDUCTION OF PHYSIOLOGICAL CARDIAC HYPERTROPHY IN MICE BY EXERCISE AND THE ROLE OF REACTIVE OXYGEN SPECIES

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2 Universidade Federal do Rio Grande do Sul, UFRGS

Objectives:

Aim: To evaluate the role of oxidative stress in the development of physiological cardiac hypertrophy induced by voluntary exercise in mice.

Methods and Results:

Methods: We used adults Balb/c mice subjected to a voluntary exercise protocol (EXE, n=63) and a sedentary group (SED, n=48). Trained animals were placed in cages with training wheels and an odometer for monitoring daily distance. EXE and SED animals were divided into two sets that received 200mg/kg/day of vitamin E (vit E) or vehicle (placebo), both by gavage. After 7 and 35 days of training, functional capacity on treadmill was assessed and the animals were euthanized to obtain left ventricle (LV) for analysis of cardiac hypertrophy (LV weight/body weight) and oxidative stress (OS). To evaluate OS we assessed protein and lipid oxidative damage (carbonyl and thiobarbituric acid reactive species assays, respectively). Superoxide dismutase (SOD) and catalase (CAT) activities were also measured. All biochemical analyses were normalized by protein content (Bradford assay). Comparisons between groups were performed by Student t test. Results: EXE groups receiving vit E or placebo ran similar distances during the protocol (7±3km/day vs 6.8±3km/day, p>0.05). Both EXE groups had similar improvements functional capacity tests after 7 days (placebo: 1550±1108m vs 522±124m, p=0.01; vit E: 1681±885m vs 643±350m, p=0.008) and 35 days (placebo: 1858±789m vs 557±141m, p=0.003; vit E: 2250±410m vs 473±181m, p=0.05 for comparisons between placebo and vit E). The LV weight/body weight increased after 7 days (3.61±0.2 vs 3.32±0.1, p=0.05 for all). Changes in catalase activity occurred with exercise and were vitamin E and time-dependent: after 7 days, there was a decrease in CAT with exercise compared to SED group, only in the group receiving vitamin E (33±16U/mg protein vs 54±25U/mg, p=0.025). In contrast, after 35 days, exercise resulted in increased CAT activity in both placebo (50±20U/mg vs 33±11U/mg, p=0.049) and vitamin E (73±29U/mg vs 47±23U/mg, p=0.033) groups. Furthermore, CAT levels tended to be higher in vit E animals compared to placebo (p=0.053). Finally, levels of damaged proteins and lipids and SOD activity were similar between EXE and SED animals in both placebo and vit E groups (p>0.05 for all).

Conclusions:

Conclusion: Voluntary exercise was able to induce cardiac hypertrophy and increased exercise capacity similarly in placebo and vitamin E-treated animals. Vitamin E administration had differential, time-dependent effects on catalase activity changes with exercise: it resulted in a decrease in CAT values early (after 7 days), while after chronic training and stabilised hypertrophy (35 days), there was an increase in catalase activity which seemed to be higher in vitamin E-treated animals. Analyses related to angiogenesis, metabolic adaptation and molecular pathways will complement the characterization of this physiological cardiac
MECHANISMS UNDERLYING THE EFFECT OF ETHANOL EXTRACT OF THE LEAVES OF ASPIDOSPERMA MACROCARPUM MART. (APOCYNACEAE) ON ARTERIAL BLOOD PRESSURE

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Objectives:
Aspidosperma macrocarpum Mart. is a plant popularly known as “peroba-gigante-do-cerrado”. Several alkaloids have been isolated from the genus Aspidosperma. These alkaloids have several pharmacological properties, such as: adrenergic blocking activity, hypotensive and analgesic (J. Pharm. Biom. Ana. 12:10,1994; J. Pharm. Sci. 62:11,1973). The ethanol extract of the stem of Aspidosperma tomentosum produced hypotensive and vasorelaxante effect. We aimed to investigate the mechanisms underlying the effects induced by ethanol extract of the leaves of Aspidosperma macrocarpum Mart. (EEAM) on arterial blood pressure.

Methods and Results:
For the measurement of arterial pressure and heart rate, male Wistar rats (250–300 g) was anesthetized with thiopental (45 mg/Kg; i.p.) and polyethylene catheters was inserted into the abdominal aorta and lower vena cava for pressure recordings and administration of drugs, respectively. Experiments were performed 24 hours after the surgery. The results are presented as mean±standard error of the mean. The study was approved by the Ethics Committee of the Federal University of Alagoas (010151/2008-82). In normotensive non-anesthetized rats (n = 6), bolus intravenous injection of EEAM (1; 5; 10; 20 and 30 mg/Kg; randomly) produced a transient hypotension (-13±4, -10±2, -12±1, -16±3 and -10±3 mmHg) and bradycardia (-2±1, -7±1.1, -4±1 and -20±5 bpm). The hypotensive response and bradycardia induced by EEAM were not altered after the nitric oxide synthase (NOS) inhibitor NG-nitro-L-arginine methyl ester (L-NAME, 20 mg/Kg, i.v.). Hypotensive response was completely abolished after atropine (2 mg/kg, i.v. n = 6) a non-selective antagonist of muscarinic receptors, while bradycardic response was not altered. In hexamethonium treated rats (20 mg/Kg; i.v.), the hypotensive response and bradycardia were not attenuated.

Conclusions:
The results obtained so far show that EEAM produce hypotension, mainly due to a direct stimulation of the endothelial vascular muscarinic receptor. The hypotension induced by EEAM does not involve the activation of the nitric oxide (NO) pathway.

Keywords: Aspidosperma macrocarpum , Bradicardia , Cardiovascular , Hipotensão , receptor muscarinico

Financial Support: CNPq, FAPEAL, PPSUS - MS

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EFFECTS OF REDUCED GLUTATHIONE MICROINJECTION INTO THE NUCLEUS OF TRACTUS SOLITARIUS IN BLOOD PRESSURE MAINTENANCE

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Objectives:
Several studies suggest hypertension as an imbalance between neural networks of excitation or inhibition of neurons within central nervous system and more recently, possibly related to availability of reactive oxygen species (ROS), and appointed the nucleus of tractus solitarius (NTS) as the main region of termination of baroreceptors afferent fibers on sympathetic discharge. The present study evaluates the effects of reduced glutathione (GSH) microinjection into the NTS of non-anesthetized rats in the maintenance of blood pressure, considering its possible modulating effect of glutamatergic neurotransmission in the NTS region.

Methods and Results:
Male Fischer 344 rats (250 grams body weight) were anesthetized with ketamine (80mg/kg) and xylazine (7mg/kg) mixture for implant of stainless steel cannulas directed to the nucleus of tractus solitarius (NTS) and for femoral artery cannulation procedures. The basal levels of blood pressure were recorded during 20 minutes, followed by successive L-glutamate (L-glu, 1nmol/100nL) microinjections for a functional confirmation of NTS region. After the reestablishment of blood pressure basal levels, reduced glutathione was microinjected at a concentration of 20nmol/100nL, followed by a new L-glu microinjection 40 minutes later. The GSH microinjection produced pressor response (17.7 ± 3.2 mmHg, n = 4, p t-test). After GSH microinjection, the responses to a new microinjection of L-glu were virtually abolished (0.7 ± 5.0 mmHg and -6.7 ± 8.6 bpm, n = 4 p t-test).

Conclusions:
The data suggest that changes in the local availability of glutathione in its reduced form in the region of the NTS can modulate neuronal activity leading to an increase in mean arterial pressure and a blockade of new responses to exogenous L-glu.

Keywords: Blood pressure, Glutathione, Hypertension, Nucleus of tractus solitarius, Reactive oxygen species

Financial Support: FAPEMIG, CNPq and UFOP.

EFFECT OF L-ARGININE ON RENOVASCULAR HYPERTENSION IN RATS WITH RENAL DENERVATION

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Objectives:
Studies suggest that enhanced renal sympathetic nervous activity plays an important role in mediating the renal hemodynamic and electrolyte excretion changes associated with two-kidney, one clip hypertension (2K1C). The purpose of this study was to investigate the influence of renal denervation on the mechanisms of anti-hypertensive treatment with L-arginine in renovascular hypertension model (2K1C).

Methods and Results:

The 2K1C rats were prepared and recovery from bilateral surgical-pharmacological renal denervation. Renal denervation was confirmed in every rat at the end of the study by loss of renal tissue norepinephrine content. The rats were studied separately and subdivide into six groups: normotensive control (SHAM), 2K1C, 2K1C treated with L-arginine (+L-arg), denervated normotensive (DN), denervated 2K1C (2K1C+DN) and denervated 2K1C+ L-arg (2K1C+DN+L-arg). Oral administration of L-arginine by seven days started one week after renal artery clipping. Systolic pressure, water intake, urine volume, and sodium excretion were measured. The 2K1C increased systolic pressure (from 106±3 to 183±5.8 mmHg, p< 0.01). L- arginine reduced blood pressure (143±3.4 mm Hg) and surgical denervation reduced blood pressure to normotensive levels in 2K1C rats with and without chronic L-arginine treatment (126±6.1 and 126±7.3 mmHg, respectively). L-arginine and denervation induced a significant increase in water intake and urine volume (2K1C+L-arg: 39±1 and 13±2 ml/day, DN: 39±1.4 and 13±0.4 ml/day; 2K1C+DN: 42±1.5 and 19±0.6ml/day; 2K1C+DN+L-arg: 44±1.9 and 22±0.8 ml/day) compared to sham rats (28±2.8 and 7±0.2 ml/day). Sodium excretion were significantly higher in group chronic treatment with L- arginine compared to the untreated group and Sham (1.1±0.05, 0.8±0.05 and 0.72±0.02 mEq/day, respectively).

Conclusions:

These data suggest that L-arginine reduced blood pressure and surgical denervation contributes to the hypotensive responses. Chronic L-arginine treatment and denervation induced responses renal by increase water intake and urine volume.

Keywords: Hypertension, L-arginine, Renal denervation

Financial Support: CAPES and CNPq (Brazil)

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Resumo:02-127

SUBCHRONIC EXPOSURE TO LOW CONCENTRATIONS OF CADMIUM INCREASES SYSTOLIC BLOOD PRESSURE AND VASCULAR REACTIVITY IN RATS

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Objectives:

Several studies suggest an association between cadmium exposure and the development of hypertension, atherosclerosis and other cardiovascular diseases (Circulation 109: 3196, 2004; Vet. Res. Commun. 27: 807; 2003). The mechanisms involved in the cardiovascular changes induced by cadmium are not yet fully elucidated. Given the above, the objective of this study is to evaluate the effects of subchronic exposure (30 days) to low concentrations of cadmium chloride via drinking water on blood pressure and vascular reactivity of aortic rings of rats.

Methods and Results:
Male Wistar rats, weighing 180 to 200g were given drinking water containing 0 (Ct group), 50 ppm CdCl2 (Cd50 Group) or 100 ppm of CdCl2 (Cd100 Group) for 30 days. Systolic blood pressure was measured by tail plethysmography on days 0, 7, 14, 21 and 28 of treatment. At the end of the treatment animals were anesthetized, euthanized, blood sample was collected and the thoracic aorta was removed and dissected to obtain 3 to 5 mm length rings, which were adapted to an experimental apparatus. Isometric tension was registered and the rings were subjected to a relaxation curve to acetylcholine (10^-11 to 10^-5 M) and then to a phenylephrine concentration-response curve (10^-10 to 10^-4 M). The collected blood was used for Cd concentration measurement by atomic absorption spectrometry. Results were expressed as mean ± SEM and statistical analysis used were nonparametric one way ANOVA and unpaired Student T test (p

Conclusions:

Results here presented suggest that exposure to Cd concentrations below the level considered safe for ingestion (Cd, 1 ìg/ day/ kg body weight), increases systolic blood pressure and vascular reactivity. These effects may lead to health complications such as cardiovascular disease.

Keywords: CADMIUM, HYPERTENSION, VASCULAR REACTIVITY

Financial Support: FAPES / CAPES / CNPq

QuebraPagina

Resumo:02-128

HYPERGLICEMIA PROTECTS THE HEART AFTER MYOCARDIAL INFARCTION: METABOLIC ASPECTS.

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Objectives:

Circulating free fatty acids (FFA) and glucose are the main metabolic substrates for energy generation at the heart. Alterations of the ratio of glucose and FFA oxidation may be associated to insulin resistance leading to type 2 diabetes mellitus and development cardiac dysfunction. This study was designed to evaluate the role of diabetic hyperglycemia on cardiac metabolism after myocardial infarction in rats.

Methods and Results:

The animals were divided into four groups: control (C), diabetic (D), myocardial infarcted (MI) and diabetic myocardial infarcted (DI). After 30 days of diabetes with 15 days of MI, the size of MI, the left ventricular (LV) function and energetic substrate levels were evaluated. The diabetic animals presented hyperglycemia that was not changed after 15 days of myocardial infarction. The 36% reduction of MI area after 15 days observed in DI group (p≤0,05) was accompanied by the improvement of systolic function as expressed by ejection and shortening fractions in comparison with MI group (FS = MI: 25 ± 2 vs DI: 48 ± 2. FE = MI: 55 ± 3 vs DI: 82 ± 3). The increase of plasma levels of FFA and triglycerides (TG) detected after diabetic hyperglycemia were decreased after myocardial infarction and additionally reduced in DI group (FFA = D: 0,043 ± 0,006 vs DI: 0,012 ± 0,002. TG = D: 268,7 ± 55,4 vs DI: 89,2 ± 14,3), suggesting a possible lipolytic activity. On the other hand, the levels of FFA in the LV were increased after myocardial infarction, with a further increase in DI group (D: 0,41 ± 0,08 vs DI: 1,08 ± 0,41). These results indicate higher fatty acids oxidation in the heart to ATP generation in both myocardial infarcted groups. In addition, the glycogen synthesis in the LV was increased significantly in DI group as compared with MI (MI: 73,05 ± 5,78 vs DI: 138,5 ± 14,3).

Conclusions:
These results suggest that the use of glucose may be the preferential source of energetic substrate under stress condition as the myocardial infarction. Moreover, the understanding of these signaling pathways may open the possibility to develop new therapeutic modalities for cardiac protection after ischemia.

Keywords: HYPERGLICEMIA, METABOLISM, MYOCARDIAL INFARCTION

Financial Support: FAPESP n 2010/12449-4, Zerbini Foundation and CAPES

HYPOTENSIVE AND BRADICARDIC EFFECTS INDUCED BY CITRAL IN RATS.

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Objective:
Citral is the major constituent of essential oil of Cymbopogon citratus, medicinal plant popularly known as “capim-limão” or “capim-santo”, widely used to treat hypertension. This study evaluated the cardiovascular effects induced by the citral in normotensive rats by using in vivo approach.

Methods and Results:
For the measurement of arterial pressure and heart rate, male Wistar rats (200–300 g) were anesthetized with thiopental (45 mg/kg; i.p.) and polyethylene catheters was inserted into the abdominal aorta and lower vena cava for pressure recordings and administration of drugs, respectively. Experiments were performed 24 hours after the surgery. In normotensive non anesthetized rats, citral (1, 5, 10, and 20 mg/kg, i.v.; randomly) induced hypotension (-9 ± 2, -39 ± 4, -52 ± 4, and -56 ± 2%, n = 4, respectively) associated with bradycardia (-4 ± 4, -54 ± 5, -71 ± 6, and -83 ± 2%, n = 4, respectively). Both effects were significantly (p < 0.001) attenuated, but not completely abolished, by the pre-treatment with 2 mg/kg of atropine (hypotension: -8 ± 2, -21 ± 4, -18 ± 4, and -24 ± 4%, n = 4, bradycardia: -1 ± 1, -9 ± 7, -19 ± 10, and -11 ± 6%, n = 4, respectively) or 20 mg/kg of hexamethonium (hypotension: -9 ± 1, -15 ± 3, -30 ± 7, and -39 ± 7%, n = 6, bradycardia: 1 ± 1; -7 ± 6; -13 ± 13 and -28 ± 12%; n = 6, respectively). Electrocardiogram records demonstrated that citral (10 and 20 mg/kg) was also able to induce sinoatrial block, which was reverted by atropine (2 mg/kg).

Conclusions:
These results demonstrate that citral induces hypotension associated to bradycardia in normotensive non-anaesthetized rats, which appears to involve, in part, muscarinic and nicotinic receptors.

Keywords: bradycardia, citral, hypotension, rats

Financial Support: CNPq, FAPITEC-SE, Brazil

VASCULAR REACTIVITY INCREASE IN RAT AORTIC RINGS AFTER ACUTE EXPOSURE TO CADMIUM.
Objectives:
Cadmium (Cd) is widely used in industry, and a constant component of agricultural fertilizers, which has increased environmental contamination by this metal. The aim of this study is to evaluate the effects of exposure in vitro to CdCl₂ (Cd) on vascular reactivity and the possible mechanisms involved in this process.

Methods and Results:
Wistar rats 250 to 300g, were anesthetized and the thoracic aorta was removed for dissection and to obtain rings with 3 to 5 mm in length. The registration of isometric tension of each ring was obtained according to the method proposed by Marin et al (1998). Results were expressed as mean ± SEM and difference of the area under the curve (dAUC%). Statistical analysis: unpaired Student t-test (significance p

Conclusions:
These results suggest that Cd increases the maximal response to PHE affecting the vascular endothelium. Furthermore, the mechanisms responsible for this process appear to involve: increased bioavailability of angiotensin II, reduction of the induced release of NO and increased production of ROS.

Keywords: Vascular Reactivity, Cadmium, ROS


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Resumo:02-131

EFFECTS OF CHRONIC GREEN TEA TREATMENT ON CARDIOVASCULAR FUNCTION IN HYPERTENSION INDUCED BY NITRIC-OXIDE SYNTHESIS INHIBITION IN RATS

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Objectives:
Recent studies suggest that antioxidants properties of green tea (GT) may have beneficial cardiovascular actions. However, the mechanisms underlying these actions are poorly understood. The present study aimed to evaluate GT and purified GT extract chronic treatment effects on cardiovascular function in hypertensive rats.

Methods and Results:
Male Wistar rats were treated orally for 2 consecutive weeks with the nitric oxide synthesis inhibitor (L-Name, 20 mg/Kg/day). By the end of the first week of L-Name treatment, the animals were divided in three experimental groups: L-Name treated; L-
Name + GT (9.6g/L) and L-Name + GT extract (20 mg/Kg/day). Additional untreated L-Name control groups submitted or not to GT and purified GT treatments were also evaluated. By the end of 7 days GT and purified GT treatment the rats were submitted to surgery to arterial and venous catheterization for blood pressure (BP) and heart rate (HR) recording and drugs administration, respectively. In the experimental day, the rats had BP and HR recorded and the baroreflex control of BP was evaluated by changes in blood pressure elicited by IV infusion of phenylephrine or sodium nitroprusside and reflex HR responses was evaluated and expressed as beats/minHg. The water/tea ingestion and urinary volume were evaluated in metabolic cages for 24h. There was no statistically difference among groups in urinary or intake volume. Treated L-Name rats (193.1 ± 16.9 mmHg) had a significant increase in BP compared to control group (102.8 ± 4.9 mmHg) as expected. The rats treated with GT (130±6.02 mmHg) or GT extract (131 ± 0.94 mmHg ) presented a significant reduction in BP compared to L-Name treated rats. No changes were observed in HR among groups. Baroreflex sensitivity that was impaired in hypertensive rats was restored in the GT and GT extract treated groups.

Conclusions:

Taken altogether the present study suggest that GT or GT extract treatment were able to reduce BP independently of a diuretic effect. It is possible that the improvement in the baroreflex control is involved in this response.

Keywords: green tea , hypertension, antioxidants properties

Financial Support: CNPq, FAPESP

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Resumo:02-132

GENDER DIFFERENCES IN THE ANTIHYPERTENSIVE EFFECTS OF GRANULOCYTE-COLONY STIMULATING FACTOR (G-CSF) IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR).

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Objectives:

G-CSF is a cytokine which mobilizes hematopoietic cells, including stem cells from bone marrow. Previous reports from our laboratory have demonstrated that systemic administration of G-CSF during two weeks in female SHR significantly reduces arterial blood pressure. The aim of the present study was to compare the antihypertensive effects of G-CSF in both female and male SHR.

Methods and Results:

Both female and male sixteen weeks-old SHRs, receiving G-CSF or saline (CON), were divided in four groups: Male SHR-CON (n=8), Male SHR-GCSF (n=8), Female SHR-CON (n=11) and Female SHR-GCSF (n=9). During fourteen days, all groups have daily received subcutaneous injections of vehicle (saline) or G-CSF (Granulokine, 0.1 mg/Kg). Systolic arterial pressure (SAP) and heart rate (HR) were daily monitored by means of tail occlusion method from five days before to fourteen days after treatment. At the end of this period, after femoral vessels catheterization under anesthesia (sodium thiopental, 40 mg/kg, i.p.) and 48 hours for surgical recovery, direct arterial pressure recordings were performed during 60 minutes in freely moving animals. Following, blood samples were collected to evaluate the hemogram in order to confirm white cell mobilization. At the end, after sacrifice, hearts were removed and weighed. Indirect measurements of SAP and HR by means of tail occlusion method for all groups five days before and during fourteen days of treatment did not show any differences. After direct measurements, however, systolic, diastolic and mean arterial pressures (expressed as mean±SEM) of Female SHR-GCSF group were significantly lower than Female SHR-CON (161±8mmHg, 116±7mmHg and 138±7mmHg, respectively, versus 190±6mmHg, 137±6mmHg and 160±6mmHg. p
Conclusions:
Our results clearly show an antihypertensive effect of G-CSF in female but not in male SHR. The mechanisms involved in this gender difference deserve further investigation.

Keywords: ANTIHYPERTENSIVE EFFECTS, GENDER DIFFERENCES, G-CSF, IN SPONTANEOUSLY HYPERTENSIVE RATS

Financial Support: CAPES, CNPq, FUNEPU, FAPEMIG.

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Resumo:02-133

EFFECTS OF TEMPERATURE ON ELECTROCARDIOGRAPHIC RESPONSES OF TWO ECOLOGICALLY DISTINCT NEOtropical FISH SPECIES

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Objectives:
The physiology of fish is strongly influenced by water temperature which can suffer acute or seasonal fluctuations. The modifications in metabolism are accompanied by adjustments in ventilatory, cardiovascular, hematological and biochemical parameters. Therefore, this study evaluated the ECG at different temperatures of two ecologically distinct Neotropical fish species: trahira, Hoplias malabaricus, a sedentary fish and matrinxã, Brycon amazonicus, a very active fish.

Methods and Results:
The experimental protocols were approved by the Ethics Committee on Animal Experiments of UFSCar. Specimens of matrinxã (~ 216 g) and trahira (~ 307 g) were obtained at fish farms in the region of São Carlos/SP. To record the ECG, fish were anesthetized in 0.1% benzocaine for the implantation of two electrodes that were placed in a ventral position, the first (positive) between the gills and the heart, and the second (negative) close to the pelvic fins. A reference electrode was located in the water of the experimental chamber. After a recovery period of 12 h, fH was recorded at different temperatures. Each species was divided into three experimental groups (fish exposed to 15 °C, n = 10; control - 25 °C, n = 10; fish exposed to 35 °C, n = 10). Fish of all groups were kept in normoxic conditions and their ECG were monitored for 20 min to obtain the fH, the intervals RR, PR, QRS, QT and the duration of P and T waves. The fH of both species showed lower values at 15 °C (~ 20 bpm) and higher values at 35 °C (matrinxã: 161 bpm, trahira: 72 bpm). The fH of matrinxã was significantly lower than that of trahira in 25 and 35 °C. When compared to control group, matrinxãs exposed to 15 °C showed an increase in the RR interval (from ~0.88 to ~3.09 s), PR (from ~0.08 to ~0.19 s), QRS (from ~0.06 to ~0.09 s), QT (from ~0.39 to ~0.77 s) and in the duration of P wave (from ~0.4 to ~0.9 s) and T wave (from ~0.20 to ~0.25 s). Otherwise, the exposure to 35 °C reduced the RR, PR, QRS and QT intervals and the T wave duration. Trahiras exposed to 15 and 35 °C showed similar results of matrinxã, since significant changes were observed in the duration of RR intervals (15 °C: ~ 2.86 s, 35 °C: 0.87 s), PR (15 °C: ~ 0.32 s, 35 °C: 0.11 s), QT (15 °C: ~ 1.17 s, 35 °C: 0.36 s) and P wave duration (15 °C: ~ 0.11 s, 35 °C: 0.07 s), while the QRS interval did not change (~ 0.07 s). Comparing matrinxã and trahira, the RR interval was significantly lower only at 25 and 35 °C in matrinxã. The PR and QT intervals and the duration of P and T waves were significantly lower at all temperatures studied in matrinxã.

Conclusions:
Changes in fH associated with changes in temperature indicate that this variable can modify the sensitivity and the relative influence of cholinergic and adrenergic modulation of this parameter. Moreover, changes in the wave intervals indicate a strong influence of temperature on contractile performance of heart muscles and also in the frequency of discharge of pacemaker cells. Thus, these results indicate that temperature acts in the global metabolism of fish as well as in myocardial metabolism and
membrane permeability to ions involved in their polarity.

Keywords: Brycon amazonicus, Electrocardiography, Heart rate, Hoplias malabaricus, Temperature

Financial Support: FAPESP (Proc. 08/54401-8)

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Resumo:02-134

NITRIC OXIDE SCAVENGE PROPERTY OF RUTHENIUM COMPOUNDS IN RAT AORTIC RINGS.

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Objectives:
To evaluate the nitric oxide (NO) scavenging activity of a series of newly synthesized ruthenium compounds.

Methods and Results:
The thoracic segment of aorta was obtained of adult, male Wistar rats and cut into rings of 3–4 mm width (four rings from each aorta). The aorta segments were suspended under 2 gf of tension in an organ bath containing 15 mL Krebs-Henseleit solution of the following mM composition: NaCl 115.0, KCl 4.6, CaCl2.2H2O 2.5, KH2PO4 1.2, MgSO4.7H2O 2.5, NaHCO3 25.0, glucose 11.0 and ascorbic acid 0.1. The solution was maintained at 36.5 ± 0.1°C, pH 7.2-7.4, and it was continuously gassed with 95% O2 and 5% CO2. The tissues were attached to isometric tension transducers and the developed tension was recorded by a software system (ADInstruments do Brasil, São Paulo, SP). After a 60 min stabilization period, maximum contraction of the aortic rings was obtained in response to noradrenaline (5 µM). One of the ruthenium compounds trans-[Ru(NH3)4(SO4)(4-picoline)](BF4) (Ru-pic); trans-[Ru(NH3)4(SO4)(nicotinamida)](BF4) (Ru-ina); trans-[Ru(NH3)4(SO4)(isonicotinamide)](BF4) (Ru-isn); [Ru(NH3)5Cl]Cl2 (Ru-Cl) and [Ru(edta)Cl] (Ru-EDTA) was then added to the organ bath (5 µM) in the presence or absence of 300 mM L-NAME, an inhibitor of nitric oxide synthase, followed by acetylcholine (5 µM). A control ring was not exposed to any of the compounds to be tested. The number of experiments was 5 for each compound and condition. The value of tension (Mean ± Std. Error in mN) developed following noradrenaline administration was 22.08 ± 1.81 (Ru-pic), 18.48 ± 1.80 (Ru-ina), 19.35 ± 2.87 (Ru-isn), 17.82 ± 1.04 (Ru-Cl) and 23.06 ± 4.03 (Ru-EDTA) and the effect of acetylcholine corresponded to 28.71 % (Ru-pic), 1.73 % (Ru-ina), 31.52 % (Ru-ina), 0.84 % (Ru-Cl) and 12.32 % (Ru-EDTA) of the maximum contraction induced by noradrenaline in presence of compounds. The compound Ru-Cl prevented the aortic rings relaxation induced by acetylcholine, whereas Ru-EDTA, Ru-pic, Ru-ina and Ru-isn did not. However, these compounds reverted acetylcholine effect and restored the maximum contraction after one hour. None effect of acetylcholine or the compounds was observed when they were tested in the presence of L-NAME.

Conclusions:
All the tested compounds (Ru-pic; Ru-ina; Ru-isn; Ru-Cl and Ru-EDTA) have the NO scavenger property but the time course of the effect depends on the compound.

Keywords: aortic rings, nitric oxide, noradrenaline, ruthenium

Financial Support: FAPES / CNPq
EFFECT OF DIFFERENT DOSES OF ESTROGEN ON CARDIAC OXIDATIVE STRESS AND BLOOD PRESSURE IN RATS.

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Objectives:
Estrogen replacement therapy (ERT) has been used to treat menopausal discomforts. Moreover, there is substantial evidence that the benefic effects of estrogen provide protection against the development of cardiovascular disease. However, several adverse effects including higher risk for breast cancer can outweigh the benefits of hormone treatment for postmenopausal women. This study was designed to help the identification of the lower dose of estrogen that it is able to improve the cardiovascular function without the undesirable side effects.

Methods and Results:
Ovariectomized female Wistar rats (n=9/group) were divided in 4 groups to receive pellets subcutaneously (21-days release): 1- sunflower oil (CO); 2- higher dose of estrogen (E=0,5mg/pellet); 3- Median dose of estrogen (ME=0,2mg/pellet) and 4- lower dose of estrogen (LE=0,05mg/pellet). After mean arterial pressure (MAP=mmHg) measurement, frequency domain analysis of heart rate (HR) and blood pressure (BP) variability was performed with an autoregressive algorithm. The spectral components were expressed in absolute (s(2) or mmHg(2)) and normalized units. Spontaneous baroreflex sensitivity (BRS) was estimated by alpha index, defined as square root ratio between low frequency power in BP variability and HR variability. In the end, rats were killed by decapitation, and body and uterus were weighted. Hearts were collected to evaluated the redox balance GSH/GSSG, and blood to measure plasma estradiol levels. Statistical analysis were performed using a 1-way ANOVA followed by Student Newman Keul's post tests. The p value less than 0,05 were considered significant. Estrogen treatment did not change BP compared to CO. We found a decrease on simpathetic cardiac modulation in LE (1.67±0.61) and HE (1.04±1.03) groups vs CO (3.59±2.13). We also observed a decreased in the simpaticovagal balance in these groups (LE=0.15±0.09; HE=0.13±0.05) vs CO(0.27±0.1). The redox balance was significant increased in the estrogen treated groups (EB= 25,40±4,77; EM=14,03±2,29; E=25,57±5,19), compared to CO (8,83±1,42).

Conclusions:
We demonstrated that, similarly to the farmacological dose, the lower dose of estrogen decrease the simpathetic cardiac modulation, and improved the simpaticovagal and the redox balance. Thus, a low dose of estrogen may represent a good option to minimize side effects, especially decreasing the breast cancer risk. More studies are necessary to demonstrate this hypothesis.

Keywords: Estrogen, Oxidative Stress, Autonomic Nervous System

Financial Support: CNPq
Objectives:

The kinin B1 receptors are weakly detectable under physiological conditions, but highly induced in the course of inflammation. Activation of B1 receptor in the vascular endothelium triggers diverse signaling pathways that results in elevation of intracellular calcium and Nitric Oxide Synthase (NOS) activation, followed by NO production and vasodilation. Although much has been investigated about the B1-induction and functionality during inflammation, the importance of B1 subtype in normal vessels remains unclear. To clarify this question, the present study analyzed endothelial function and endothelial NO generation in B1 receptor knockout (B1−/−) and Wild Type (WT) mice.

Methods and Results:

Mesenteric arteriolar bed was perfused with Krebs solution and vascular responses to Acetilcholine (ACh - 0.1, 1 and 10 nmol), sodium nitroprusside (SNP - 0.1, 1 and 10 nmol) and norepinephrine (NE – 5, 10, 50 and 100 nmol) were evaluated by a data acquisition system (PowerLab 8/S, ADInstruments). Plasmatic NO levels were analyzed by NO derivatives nitrate and nitrite using NO Analyzer (Sievers Instruments) and vascular NO generation was assessed in mesenteric arterioles slices using DAF-2 DA, a fluorescent cell permeable dye for NO. NOS activity was measured by the biochemical conversion of L-[3H] arginine to L-[3H] citrulline in homogenates of mesenteric vessels in the presence of optimal levels of substrate and cofactors. Primary cultured endothelial cells were obtained from lung explants and characterized by Ulex europeaus and von Willebrand Factor staining. Endothelial cells were incubated with DAF-2 DA and observed in a confocal microscope (Carl Zeiss SMT). Cells were stimulated with ACh [1 mmol/L] and images were analyzed by optic densitometry. In another set of experiments, cells were stimulated with ACh in presence of the NOS substrate L-arginine (1 mmol/L) or the co-factor tetrahydrobiopterin [BH4 (0.1 mmol/L)]. Mesenteric arterioles from B1−/− exhibited a severe impairment of ACh-vasodilation for all tested doses, with no changes in the response to SNP and NE. Circulating NO (µmol/L) was markedly decreased in B1−/− (49.6 ± 10.5*; n=6) vs WT (141.9 ± 17.3; n=6), accompanied by reduced basal NO release [fluorescence intensity, arbitrary units (a.u.]) in mesenteric arterioles from B1−/− (0.16 ± 0.03*; n=6) when compared to WT (0.58 ± 0.08; n=4). NOS activity (pmol/mg.min) was elevated in mesenteric homogenates from B1−/− (3.4 ± 0.58*; n=4) in comparison to WT (1.9 ± 0.05; n=5). ACh-induced NO release (a.u.) was markedly reduced in primary cultured endothelial cells from B1−/− (35.8 ± 3.1*; n=4) in comparison to WT cells (66.9 ± 3.2; n=4). NO release in endothelial cells from B1−/− was reversed by incubation with BH4 (54.3 ± 1.7; n=4), but not by L-arginine (26.2 ± 8.5*; n=4), while incubation of endothelial cells from WT with BH4 or L-arginine had no effect.

Conclusions:

The severe impairment in the endothelial-mediated vasodilation accompanied by decreased NO bioavailability, despite the augmented NOS activity, strongly indicates an exacerbation of NO inactivation. Reduced NO availability may be preceded by a deficiency in BH4 content in vascular endothelium, resulting in NOS uncoupling and NOS derived production of superoxide anion. These data open new insights in the field of kinins signalization and give support for further studies regarding its relationship with endothelial dysfunction, oxidative stress and NO availability.

Keywords: Endothelial cell, Kallikrein-kinin system, Kinin B1 receptor , Nitric Oxide

Financial Support: FAPESP (07/59039-2, 08/06676-8) and CAPES
**HYDROGEN PEROXIDE INDUCES INSULIN RESISTANCE IN ISOLATED PERFUSED MOUSE HEART**


**Objectives:**

Reactive oxygen species are essential for normal physiology as well as it plays an active role in pathological processes, including Obesity and Diabetes, in which states insulin signaling is impaired. Although several studies have demonstrated that hydrogen peroxide infusion produces mechanical dysfunction and metabolic changes in myocardium, little is known about its regulatory role in regard to insulin action. The aim of the present study was to examine the possible role of hydrogen peroxide in insulin action regulation of isolated mouse cardiomyocyte.

**Methods and Results:**

Twenty-four male swiss mice aged 120 days and weighting 40-50g were submitted to isolated heart perfusion. They were perfused with kreb-Henseleit perfusion fluid according to Langendorff Technique and simultaneously infused with kreb-Henseleit perfusion fluid (control) (n=6), hydrogen peroxide (36 µM) (n=6), hydrogen peroxide (36 µM) plus insulin (1UI/ml) (n=6) and insulin isolated (1UI/ml) (n=6). After 5 minutes of infusion, hearts were freeze-clamped immediately for posterior analyses. Results are presented as means ± S.D. Wilcoxon paired tests were performed at a level of significance of 0.05. Hydrogen peroxide produced a significant percent decrease (25%) in left ventricle systolic pressure (96.2±20.2mmHg baseline versus 71.3±22.2mmHg after 5 min). As opposed to, insulin when administered by itself lead to a significant increase (17%) in left ventricle systolic pressure (88.5±15.0mmHg baseline versus 104.0±22.6mmHg after 5 min). Differently, when insulin was combined by hydrogen peroxide there was a significant decrease (13%) in left ventricle systolic pressure (82.4±25.6mmHg baseline versus 71.48±34.0mmHg after 5min). The same pattern occurred with left ventricle dP/dtmax and dP/dtmin that are indexes of the initial velocity of myocardial contraction and relaxation respectively and thus, characterize the contractile and relaxing ability of the heart. The infusion of hydrogen peroxide alone (2952.8±684.6mmHg/s baseline versus 2201.8±671.5mmHg/s after 5 min) or combined with insulin (2360.7±490.4mmHg/s baseline versus 2048.3±1237.0mmHg/s after 5 min) produced a decrease in left ventricle dP/dtmax while insulin isolated improved this parameter by 24.9% (2581.7±476.4mmHg/s baseline versus 3225.5±489.9mmHg/s). Similarly, hydrogen peroxide alone or combined with insulin produced a decrease in left ventricle dP/dtmin by 25% and 5% respectively while insulin isolated enhanced this parameter by 24.9%.

**Conclusions:**

Exogenous doses of hydrogen peroxide in myocardium lead to a state of insulin resistance as observed by attenuation in the positive effect of insulin in contractile myocardium function of isolated perfused mouse heart.

**Keywords:** hydrogen peroxide, insulin resistance, cardiomyocyte

**Financial Support:** CNPq CAPES FAPERJ

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**CARDIOMYOCYTE ACETYLCHOLINE SYNTHESIS MACHINERY AND ITS CARDIOPROTECTIVE ROLE IN ISOPROTERENOL-TREATED CARDIAC MYOCYTES**

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Objectives:

It is well established that any disturbance in the balance between sympathetic and parasympathetic tones is crucial for the development of cardiac dysfunction. However, little is known about the role of parasympathetic tone in the development of heart disease. Previous work showed that the VACHT KO HOMO mouse (an animal model of cholinergic dysfunction) develop cardiac failure, characterized by alterations not only in genic expression but also in calcium handling in cardiomyocytes (Mol Cell Biol 30; 1746-1756, 2010). Importantly, recent evidence points out the existence of an intrinsic acetylcholine synthesis machinery in cardiomyocytes (FEBS Journal 276; 5111-5125, 2009) suggesting a role for this system in cardiac protection. In this work, we investigated the effects cholinesterase inhibition in neonatal cardiac myocytes treated with beta-adrenergic agonist isoproterenol.

Methods and Results:

Neonatal Rat Cardiomyocytes (NRCMs) were isolated from three old-day male Wistar rats accordingly to guidelines for the human use of laboratory animals of our institute and approved by local authorities. Neonatal cardiomyocytes were exposed to isoproterenol (10 µM) and/or pyridostigmine (1mM) for 48h, and then the cells were used for immunofluorescence, western blot and qPCR analyses. In addition, vesamicol (5µM) and atropine (10µM) were used as inhibitor and antagonist, respectively, of VACHT and M2 receptor. We also performed assays in Adult Ventricular Cardiomyocytes (AVCMs) kept in culture for 24h and treated with isoproterenol. Calcium transients and hypertrophic markers were evaluated in isoproterenol-treated AVCMs with or not pyridostigmine. In NRCMs pyridostigmine blunts cell hypertrophy by 22%, prevents ERK phosphorylation by 50% and inhibits NFAT nuclear localization (27%) in isoproterenol-treated cells. Moreover, isoproterenol treatment increases ChAT (32%), VACHT (78%) and AChR-M2 (31%) mRNA and/or protein levels, an effect which is prevented by pyridostigmine. Isoproterenol-treated ARCMs have an increase in calcium transients (22%), which is blunted by pyridostigmine. Finally, NFAT translocation to the nucleus is also prevented by pyridostigmine in isoproterenol-treated ARCMs (19%).

Conclusions:

Taken together, our results show that cholinesterase inhibition in cardiomyocytes prevents isoproterenol-induced hypertrophy not only in NRCMs but also in ARCMs. Furthermore, acetylcholine synthesis and release machinery has an important anti-hypertrophic effect in cardiomyocytes. Steps toward the characterization of signaling pathways involved in the cellular protective role of this machinery are the next goal of this work.

Keywords: Acetylcholine Synthesis Machinery, Cardiomyocyte, Hypertrophy

Financial Support: CAPES, CNPq, NIH (Fogarty International Award)
Objectives:

Previous data from our laboratory have demonstrated that syngeneic mononuclear cells extracted from bone marrow of spontaneously hypertensive rats (SHR) decreased arterial pressure (AP) in approximately 25-30 mmHg for two consecutive weeks. In this study, we evaluated the effects of intraperitoneal administration of syngeneic mesenchymal stem cells (MSC) from adult bone marrow on systemic AP in female SHR.

Methods and Results:

Adult female SHR (200 - 230g) were divided into 2 groups, SHR-MSC (n = 8) and SHR-CON (n = 8). The animals in each group received an intraperitoneal injection of 5 million MSC expanded until the 5th passage or vehicle solution, respectively. The systolic arterial pressure (SAP) and heart rate (HR) of animals were monitored by means of tail occlusion method during 5 days before and until 15 days after treatment. Then, for direct measurement of cardiovascular parameters, animals were anesthetized with tri bromoethanol (250 mg/kg, i.p.) and the right femoral artery was catheterized. After 24-48 hours of surgical recovery, arterial pressure (AP) was recorded during 60 minutes. Under new anesthesia (urethane -1.2 g/kg) a cannula was inserted into the left carotid artery of rats, as route to inject acetylcholine (3-25 ng/kg) and sodium nitroprusside (0.5 to 4μg/Kg) in order to test systemic endothelial function. At the end, after sacrifice, hearts were removed and weighed. The indirect measures of SAP and HR levels showed no significant differences between groups SHR-MSC and SHR-CON before or after the treatment. However, direct recordings of AP (expressed as mean ± SEM) showed a lower systolic (172±6mmHg versus 189±5mmHg), mean (142±6mmHg versus 162±4mmHg) and diastolic AP (116±6mmHg versus 137±5mmHg) in SHR-MSC compared to SHR-CON, respectively (p

Conclusions:

Our results indicate an anti-hypertensive effect of intraperitoneal administration of bone marrow mesenchymal stem cells in SHR.

Keywords: Hypertension, Spontaneously hypertensive rats, Intraperitoneal, Mesenchymal Stem Cells, Endothelial function

Financial Support: Financial Support: CAPES, FUNEPU, FAPEMIG

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Resumo:02-140

EFFECT OF GONADECTOMY IN THE CORONARY VASODILATION INDUCED BY 17 ß-ESTRADIOL IN RATS

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Objectives:

The relaxation induced by estrogen in the coronary vascular bed from normotensive and hypertensive rats has been well described. However, almost nothing is known about the effects of gonadectomy on the vasodilation induced by 17 ß estradiol (E2) in coronary arteries. We investigated the effect of castration on E2-induced vasodilation in the coronary bed of rats of both sexes.

Methods and Results:

Wistar rats were castrated and divided into two groups: females (N = 68) and males (N = 57). Rats were anesthetized with chloral hydrate (40 mg/Kg, i.p, then the chest was opened, the heart removed and perfused through the aorta with modified Krebs solution with constant flow (10 mL / min) according to the Langendorff technique. Coronary perfusion pressure (CPP) baseline was determined and the effects of 10 μM E2 were assessed by in bolus administration before and after the following protocols: chemical removal of endothelium (deoxycholic acid, 0.25 μM), inhibition of nitric oxide synthase with L-NAME (100 μM), inhibition of cyclooxygenase with indomethacin (INDO, 2.8 μM), inhibition of cytochrome P-450 with clotrimazole (CLOT.,
0.75 μM), inhibition associated with L-NAME + CLOT or L-NAME + INDO. Results were expressed as mean ± standard error of mean. Comparisons between groups were made by paired Student t test and significance level was set at p ≤ 0.05. CPP was higher in females (91 ± 2 mm Hg, N = 68) than in males (77 ± 2 mmHg, N = 57). The relaxing response induced by E2 was also higher in females than in males (-14 ± 1% vs -10 ± 1%, respectively). The removal endothelial reduced the vasodilation in both groups (-14 ± 1 to -5 ± 1% in females and -10 ± 1 to -1 ± 1% in males). Nevertheless, in the presence of L-NAME the 17 B estradiol response was not significantly reduced in any group. In presence of INDO, CLOT., L-NAME plus INDO or L-NAME plus CLOT the relaxing response of E2 was reduced in both groups. These results suggest an important contribution of prostacyclin (PGI2) and endothelium-derived hyperpolarizing factor (EDHF) in E2-induced vasodilation.

Conclusions:
Therefore, we conclude that the relaxing effect of E2 in coronary arteries from castrated rats is partly dependent on the endothelium, and PGI2 and EDHF may have the most important role as mediators of this response.

Keywords: estrogen, sex differences, endothelium, vasodilation

Financial Support: CAPES

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Resumo: 02-141

PYRROLIDINE DITHIOCARBAMATE DOWN-REGULATES VASCULAR MATRIX METALLOPROTEINASES AND AMELIORATES VASCULAR DYSFUNCTION AND REMODELLING IN RENOVASCULAR HYPERTENSION

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Objectives:
We tested the hypothesis that treatment with pyrrolidine dithiocarbamate (PDTC), which interferes with NF-κB-induced MMPs gene transcription, could exert antihypertensive effects, prevent MMP-2 and MMP-9 up-regulation, and protect against the functional alterations and vascular remodelling of two-Kidney, one-clip (2K1C) hypertension.

Methods and Results:
Sham-operated or hypertensive rats (approximately 180 g) were treated with vehicle or PDTC (100 mg/Kg/day) by gavage for 8 weeks. Tail systolic blood pressure (SBP) was assessed weekly by tail-cuff plethysmography. Aortic rings were isolated to assess endothelium-dependent relaxations. Quantitative morphometry of structural alterations of the aortic wall was carried out in haematoxylin/eosin sections. Dihydroethidium (DHE), a sensitive superoxide probe, was used to evaluate vascular production of ROS. Inducible (i) NOS and phosphorilated-p65 (P-p65) NF-κB subunit expression were measured in the aortas by western blot. MMP-2 and MMP-9 aortic levels and gelatinolytic activity were determined by gelatin and in situ zymography and by immunofluorescence. Treatment with PDTC attenuated the increases in SBP (216.2 ± 3.4 x 165 ± 3.9 mmHg, P<0.05).

Conclusions:
These findings suggest that PDTC down-regulates vascular MMPs and ameliorates vascular dysfunction and remodelling in
renovascular hypertension, thus providing evidence supporting the suggestion that PDTC is probably a good candidate to be used to treat hypertension.

Keywords: pyrrolidine dithiocarbamate, 2K1C hypertension, matrix metalloproteinase, vascular remodeling, NF-kappaB

Financial Support: CAPES, CNPq, FAPESP

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Resumo:02-142

MYORELAXANT EFFECTS OF SERTRALINE IN ISOLATED RINGS OF MESENTERIC AND RENAL SEGMENTAL ARTERIES OF RATS

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Objectives:
In a previous work we reported that sertraline, an antidepressant belonging to the selective serotonin reuptake inhibitors family, has inhibitory effects on isolated perfused rat kidneys, probably by inhibiting vascular responses elicited by a contractile agent such as phenylephrine (XXV FeSBE, 07.019, 2010). In this study, we aimed to investigate the effects of sertraline on the responsiveness of isolated ring preparations of renal segmental and mesenteric arteries.

Methods and Results:
After euthanasia of Wistar rats (200 – 250 g) by stunning and exsanguination, first branches of renal artery (segmental artery) or major branch of mesenteric artery were removed under microscope and immersed in Krebs-Henseleit solution (bubbled with 5% CO2 in O2; pH 7.4; 37°C). The mesenteric artery was cleaned of adhering tissue and cut in rings that were suspended by a pair of stainless-steel pins. They were kept in Krebs-Henseleit (pH 7.4, 37°C, 5% CO2 in O2) under basal tension of 0.5 g. Isometric contractions were recorded by force transducers, connected to a digital data acquisition system (PowerLab®). The rings of renal segmental artery were mounted in a 610M-DMT myograph (DMT, Denmark) under basal tension 5 mN, optimal to contraction recordings under isometric conditions. Endothelium-intact rings of segmental artery were firstly exposed to PHE (10 µM; 8.7 ± 2.4 mN; n = 7) or K+ (60 mM; 5.8 ± 1.5 mN; n = 6) and when the steady state was reached, sertraline (0.3 to 100 µM) was added cumulatively. It produced significant (p < 0.001, ANOVA) relaxation of the contractions induced by PHE or K+ with IC50 values of 4.7 [2.9 – 7.7] µM and 7.7 [3.7 – 16.1] µM, respectively. In rings of mesenteric artery contracted with either PHE (1 µM) or K+ (60 mM), sertraline also produced relaxant effects with IC50 values of 16.9 [10.2 – 27.8] µM (n = 11) and 23.3 [12.7-42.7] µM (n = 8), respectively. Statistical comparison showed that IC50 values were significantly higher in mesenteric than in segmental renal artery to a given contractile agent (p < 0.05, Student’s t test).

Conclusions:
Sertraline has myorelaxant effects in isolated rings of rat vessels, being more potent to produce vasodilator effects in segmental renal than in mesenteric artery.

Keywords: selective serotonin reuptake inhibitors, mesenteric artery, antidepressant, renal segmental artery, vasorelaxation

Financial Support: CAPES, FUNCAP
POTENTIAL ROLE OF THE ENDOCANNABINOID SYSTEM MEDIATING THE VASCULAR DYSFUNCTION IN OBESITY

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Objectives:

Endocannabinoids are lipid mediators produced from membrane phospholipids precursors of almost all cell types, including vascular cells. After production and release, these compounds act on cannabinoid receptors in the same or in adjacent cells. In resistance vessels cannabinoids induce a clear vasodilator response, but in conduit vessels, some studies have demonstrated that they do not promote relaxation but evoke contraction. In obesity, the release of endocannabinoids has been demonstrated to be increased. Therefore, we hypothesized that the production of cannabinoids and the consequent activation of contraction pathways through cannabinoids receptors in aorta contributes to impair the vascular function in obesity.

Methods and Results:

Ten young (6-7 week-old) OZRs and their lean counterparts (LZRs) were used. OZRs displayed higher body weight (LZR=193.0±11.1, OZR=283.3±18.2) and fat mass (LZR=0.5±0.03, OZR=2.0±0.3) compared with age-matched LZRs. However, blood pressure (measured in conscious rats by an indirect tail-cuff method) and glucose levels (analysed using a glucometer) were similar between LZRs and OZRs. Therefore, at the age we used, OZRs represent a normotensive and normoglycemic model of obesity. The vascular reactivity was evaluated in a myograph for isometric tension recording. As expected, in aorta with endothelium (E+) or without endothelium (E-), isolated from LZRs or OZR, anandamide did not evoke relaxation. However, the endothelium-dependent relaxation induced by acetylcholine (RMAX, LZR=89.8±2.5, OZR=75.4±2.4) as well as the endothelium-independent relaxation to sodium nitroprusside (pD2, LZR=7.5±0.1, OZR=6.5±0.04) were impaired in aortas from OZRs. The contractile response induced by phenylephrine (pD2, LZR=7.5±0.1, OZR=8.0±0.1) and endothelin-1 (pD2, LZR=9.1±0.1, OZR=9.5±0.1) were also increased in E+ and E- aortas from OZRs. These results indicate that young OZRs display vascular dysfunction as a consequence of alterations not only on the endothelium but also in the vascular smooth muscle. The vascular dysfunction in OZRs was corrected after incubation of E+ and E- aortas with AM251 (1µM), antagonist of cannabinoid receptor CB1 and capsazepine (1µM), antagonist of vanilloid receptors (TRPV1). These results indicate that activation of CB1 and TRPV1 receptors (probably by locally produced anandamide) is associated with activation of intracellular pathways which lead to decreased relaxation and increased contraction in OZRs. Incubation with AM404 (1µM), a cannabinoid transport inhibitor, corrected the vascular dysfunction in OZRs, confirming that the increased release of cannabinoids (probably as a consequence of increased intracellular production) contributes to the decreased relaxation as well as to the increased contraction in aorta from OZRs. Incubation of aortic rings with the CB2 receptor antagonist (AM630, 1µM) did not affect either the contractile or the relaxant responses, indicating that the intracellular pathways associated with CB2 receptors activation are not involved in the vascular dysfunction in aorta from OZRs.

Conclusions:

We conclude that endocannabinoid system mediates the vascular dysfunction in obesity, contributing to increase the vasoconstrictor response and to decrease the relaxant response. These data provide new evidence for a role of the endocannabinoid system accounting for the overall impairment of the vascular function in obesity.

Keywords: endocannabinoid system, vascular dysfunction, obesity, aorta
EVALUATION OF MECHANISMS INVOLVED IN THE REDUCTION OF VASCULAR CONTRACTION IN DIABETIC FEMALE RATS: ROLE OF INOS AND INSULIN.

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Objectives:
Although hypertension may be a complication of diabetes mellitus, reduced blood pressure have been observed in type 1 diabetes. We demonstrated that contraction in aorta of diabetic female rats is reduced. The mechanisms by which this reduction occurs is unknown. Increased expression of the inducible isoform of nitric oxide synthase (iNOS) may have contribution in this change. The aim of this study is to investigate whether the reduced contractile capacity observed in aorta of diabetic females is due to increased expression of iNOS and the effect of insulin in these changes.

Methods and Results:
Female Wistar rats (180-200 g) received an injection of alloxan or saline and were divided into four groups (n = 5-8/group): Control (CT), diabetic (DB), diabetic treated with NPH insulin (6 IU / day) for 15 days (DB-Ins) and diabetic treated with L-NIL (3 mg/kg/day) for 30 days (DB-L-NIL). Treatments started out 15 (insulin) and 3 (L-NIL) days after induction of diabetes concentration-effect curve (CEC) to noradrenaline (NA) (0.1 nM-30 mM) and potassium chloride (KCl) (1 mM-108 mM) were evaluated in aortic rings with (E +) and without (E -) endothelium. In DT-Ins, the KCl response was evaluated only in E+ rings and in DT-L-NIL, the NA response was evaluated only in the E- rings. Expression of iNOS mRNA was evaluated by real time PCR. The maximum response (Rmax) to NA in E+ rings was not different between groups (CT: 1.79 ± 0.11; DB: 1.85 ± 0.09; DB-Ins: 2.01 ± 0.10). On the other hand, the Rmax to NA was reduced in E- rings from DB rats and the treatment with insulin restored this response (CT: 3.22 ± 0.23; DB: 2.20 ± 0.16; DB-Ins: 3.12 ± 0.16; p< 0.05). The contractile response to KCl in E+ rings of DB rats was also reduced and insulin treatment restored this response (CT: 2.42 ± 0.09; DB: 1.78 ± 0.16; DB-Ins: 2.41 ± 0.15; p<0.05).

Conclusions:
The increased expression of iNOS / NO generation appears to be involved with the reduction of contractile responses via adrenergic receptor, whereas this system does not seem to be involved in the reduction of the response that depends directly on smooth muscle, since the response to KCl, which evaluates the receptor-independent contraction was not corrected by L-NIL, and in addition, treatment with insulin was able to restore the decreased response to NA and KCl without reducing the expression of iNOS.

Keywords: diabetes, fêmeas, contração, insulina , iNOS
FILAMENT

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Objectives:

Troponin is a regulatory protein of vertebrate striated muscle. Its three subunits are TnT, which binds to tropomyosin; TnI, the inhibitory component; and TnC, the Ca++-binding subunit. In cardiac muscle, mutations of the troponin gene can lead to cardiomyopathy phenotypes. Recently, four new mutations that cause hypertrophic cardiomyopathy were discovered in cardiac TnC: A8V, in the N-terminus; C84Y, in the central helix; and E134D and D145E, in the C-terminal. It has been shown elsewhere that three of these mutants increase the N-terminus Ca++ affinity. Their effects on the affinity for the thin filament itself, which depends on the C-terminus, are unknown. OBJECTIVE: Investigate the influence of these mutations on the interaction between TnC and the thin filament in striated and cardiac muscle skinned fibers.

Methods and Results:

TnC dissociation experiments were performed in relaxed fibers (in mM: 0.001 Mg++, 20 imidazole propionate, 10 K2EDTA, 3.3 MgATP, pH 7.0, 15°C) to evaluate TnC-thin filament affinity. Troponin C mutant cDNAs were cloned from human cardiac tissue, expressed in Escherichia coli BL21 and purified at Miller School of Medicine (University of Miami). The native TnC was replaced with a mutant, and during 5-60 min of TnC loss the residual tension was measured periodically at pCa 4.4. The results indicate a lower TnC-thin filament affinity (faster TnC loss) for the C-terminus mutants in striated muscle (t½ (min) 8.7 ± 1.2, control; 6.9 ± 2.0, A8V; 15 ± 1.0, C84Y*; 5.4 ± 0.6, E134D* and 2.8 ± 1.0, D145E*; *p

Conclusions:

Taken together, these results indicate that C-domain mutations can alter TnC-thin filament affinity, but have opposite effects in striated and cardiac thin filaments.

Keywords: Troponin C, Cardiomyopathy, Muscle

Financial Support: PIBIC, CNPq, CAPES, FAPERJ, Pronex.

DEHYDROEPIANDROSTERONE IMPROVES ACETYLCHELONE-INDUCED RELAXATION IN SPONTANEOUSLY HYPERTENSIVE RATS BY REDUCING THE RELEASE OF CYCLOOXYGENASE-DERIVED PRODUCTS

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Objectives:

Endothelial dysfunction plays a role in the pathophysiology of cardiovascular disease. Low dehydroepiandrosterone (DHEA)
levels are inversely correlated with the risk of cardiovascular disease. In spontaneously hypertensive rats (SHR), endothelial dysfunction is characterized by a reduced endothelium-dependent relaxation to acetylcholine (ACh), which is due to an increase of the release of cyclooxygenase (COX)-derived products. This study aimed at evaluating if DHEA treatment improves vascular relaxation in SHR by reducing COX-derived products.

Methods and Results:

SHR (16 weeks-old) were treated with DHEA (10 mg/kg/d/sc) for 8 weeks. ACh-induced relaxation was evaluated in precontracted thoracic aorta (AO) rings (% relaxation). Relaxation to ACh was also evaluated in the presence of the COX-1 inhibitor SC-560 (9 nM), the COX-2 inhibitor NS-398 (100 nM), the thromboxane A2 (TXA2) synthesis inhibitor furegrelate (1uM), and the thromboxane receptor antagonist SQ-29548 (1 uM). ACh-induced release of prostaglandin (PG) I2, TXA2, and PGF2alpha from AO was measured by using an enzyme immunoassay kit (pg/mL). DHEA enhanced the relaxation to ACh of SHR (maximal response; SHR: 45.8±2.1; SHR+DHEA: 60.7±4.6; p < 0.05) and PGF2alpha (SHR: 342.8±20.3; SHR+DHEA: 259.0±9.3; p < 0.05) release by AO of SHR. COX-1 inhibition reduced the release of PGI2 (SHR: 554.6±36.5; SHR+DHEA: 553.4±58.0; p

Conclusions:

Our results indicate that activity of both COX isoforms is increased in SHR and that COX-1-derived products have a major role in the reduced endothelium-dependent relaxation, apparently by activating thromboxane receptors. In SHR+DHEA, only COX-1 activity appears to be increased, since we have not observed an additional effect of COX-2 inhibition in these animals. Therefore, our results suggest that DHEA improved the endothelium-dependent relaxation in aorta of SHR by reducing COX-2-derived products.

Keywords: ciclo-oxigenase, DHEA, reatividade vascular

Financial Support: FAPESP/CNPq/INCT-Obesidade/Diabetes

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Resumo:02-147

REPRODUCIBILITY OF HEART RATE VARIABILITY IN SUPINE AND ORTHOSTATIC POSITIONS.

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Objectives:
Assess the reproducibility of heart rate variability (HRV) in the supine (SUP) and orthostatic positions in young adults.

Methods and Results:
The study included five male adults with 29 ± 5 years. In a first moment (M1) continuous recordings of HR, beat-by-beat, were obtained in the supine position (SUP-1) and the orthostatic position (ORT-1) for ten minutes in each position with a total of 20 minutes of testing. In a second moment separated by six months (M2) the volunteers repeated the same protocol described above for the supine (SUP-2) and orthostatic positions (ORT-2). The HR was recorded in M1 and M2 through of cardiac monitor (Polar RS810, Finland). For spectral analysis, RR time series RR were visually inspected, artifacts removed, interpolated (cubic spline) and decimated in order to obtain equally spaced values in time. Spectral power was obtained by an algorithm in Matlab using a Fast Fourier transform (FFT) with the Welch’s Periodogram Method. Spectral power was calculated by integration for the low (LF: 0.04-0.15 Hz) and high (0.15-0.40 Hz) frequency bands. The entire protocol and analysis was performed by the same researcher. Statistical analysis was used to mean ± SD with a Wilcoxon test with p ≤ 0.05. Present for the mean of normal RR intervals (RR, ms): SUP-1: 958.3 ± 88.9, SUP-2: 903.2 ± 183.4, ORT-1: 709.2 ± 82.4, ORT-2: 722.8 ± 65.8. For the standard
deviation of normal RR intervals (SDNN, ms): SUP-1: 72.8 ± 22.7, SUP-2: 75.8 ± 51.3, ORT-1: 55.1 ± 27.1, ORT-2: 55.5 ± 26.4. For the normalized low frequency component (LFN, nu): SUP-1: 57.4 ± 8.45, SUP-2: 60.8 ± 8.12, ORT-1: 82.9 ± 6.85, ORT-2: 68.9 ± 16.4. For the normalized high frequency component (HFn, nu): SUP-1: 37.9 ± 9.06, SUP-2: 32.7 ± 9.83, ORT-1: 12.3 ± 6.54 ORT-2: 21.7 ± 15.8. For the sympathetic-vagal balance (LF/HF): SUP-1: 1.65 ± 0.72, SUP-2: 2.09 ± 1.03, ORT-1: 7.94 ± 2.92, ORT-2: 5.8 ± 4.67. We didn’t find any significant difference for all variables described above between SUP-1 and SUP-2 and between ORT-1 and ORT-2, p > 0.05.

Conclusions:
HRV did not change being evaluated and analyzed at different times for both the supine position as for the orthostatic position.

Keywords: HEART, RATE, VARIABILITY


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Resumo:02-148

EVALUATION OF VASCULAR REACTIVITY IN THE AORTIC OF RATS SUBMITTED CHRONIC EXPOSURE OF CIGARETTE SMOKE

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Objectives:
Of the many dangers of smoking, we elevate the cardiovascular changes. Studies show that smoking induces vasoconstriction of the coronary arteries and particularly affects the elasticity of the peripheral arteries (J. Am. Coll. Cardiol. 22:642,1993 and J. Hypertension 10(S4):S94,1992). However, existing studies regarding the evaluation of vascular damage that involve smoking are restricted to experiments in humans, which are limited in how experimentation and the use of drugs that may further elucidate the vascular damage caused by chronic exposure to smoke cigarette. The aim this study is to evaluate the chronic effects caused by exposure to cigarette smoke in rat aortic rings.

Methods and Results:
Wistar rats were used in approximately 3 months old, weighing 250-300 g and divided into two groups: CON (control, without exposure, n = 6) and FUMO (exposure to cigarette smoke, a rate of 20 cigarettes/day/during 3 months, n = 6). The animals were placed in a transparent chamber connected to a display of smoking, with a volume approximately 90x80x65, where "puffs" of smoke were taken by the cigarette to the vacuum chamber, where the end of three months the animals received a rate 30 cigarette/10 min. with an interval of 10 minutes, twice a day. On the day of the experiment the animals were sacrificed and the aortic rings were placed in organ baths and subjected to a baseline tension of 1.0 g and endothelium was preserved. Vascular reactivity in preparations of isolated aortic rings was evaluated by using increasing doses of phenylephrine (FEN, 10⁻¹⁰ to 10⁻³M) for assessment of contractile response (CR) and to evaluate the vasorelaxant effect of acetylcholine (ACh, 10⁻¹¹ to 10⁻⁸M). The RC of group SMOKE was significantly reduced compared to CON (63.9 ± 16 vs 165.7 ± 16% **, respectively) and the maximal vasorelaxant response to acetylcholine (40.4 ± 3.7 vs 64.4 ± 2.4% *, respectively). The results were expressed as mean ± SEM. ANOVA (Fisher post-hoc).

Conclusions:
Results suggest that chronic exposure (3 months) to cigarette smoke significantly reduces the vascular reactivity to phenylephrine
and acetylcholine in aortic rings of rats with preserved endothelium.

Keywords: SMOKE, VASCULAR REACTIVITY, AORTIC RING

Financial Support: CAPES

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EFFECTS OF POSITIVE AIRWAY PRESSURE IN DIFFERENT BODY POSITIONS ON THE HEART RATE VARIABILITY

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Objectives:
To evaluate the use of continuous positive airway pressure (CPAP) in different body positions on the heart rate variability (HRV). This findings may help in standardization of some methods for our future projects.

Methods and Results:
For this study, six male subjects (24±3y) without any cardiovascular disease were selected. Heart rate was recorded beat-by-beat by a R-R monitor (Polar RS800CX, Finland) during 15 minutes in supine and sitting positions with and without CPAP adjusted for 20cmH2O and the expiratory valve for 15cmH2O). Respiratory frequency was set at 0.25Hz. For HRV analysis, the R-R times series were interpolated (cubic spline) and decimated to be equally series spaced in time, and then processed by a Fast Fourier Transform. Spectral power was obtained for total power, low-frequency (LF: 0.04-0.15Hz) and high-frequency (HF:0.15-0.40Hz) bands. CPAP effects on HRV were tested for the supine and sitting positions on different days. All volunteers were oriented not to drink caffeinated beverages 24 hours before data collection. For statistical analysis a Wilcoxon test were done. All data were reported in mean ± SD. Statistical significance was considered for α ≤ 0.05) had no statistical difference. And so did normalized HF with CPAP (sitting 18.2±9.4 n.u.; supine 29.2±20.2 n.u.) and without (sitting 27.9±13.0 n.u.; supine 38.5±22.6 n.u.).

Conclusions:
The R-R interval was higher when the subjects were in supine position than sitting. This finding is part of a standardization of some methods for a bigger project in order to avoid bias.

Keywords: body pressure, continuous positive airway pressure, heart rate variability

Financial Support: CNPq e CAPES

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HEART RATE VARIABILITY AND SENSITIVITY BAROREFLEX DYNAMICS DURING POSITIVE AIRWAY PRESSURE IN HEALTHY INDIVIDUALS
Objectives:

Oscillatory changes of systolic volume (SV) and of arterial blood pressure (BP) are sensed by baroreceptors, which provoke parallel R-R interval change that are predominantly due to changes in intrathoracic pressure (ITP), however little is known of effects of augmented positive ITP, which occurs continuous positive airway pressure (CPAP), on heart rate variability (HRV) and baroreflex sensitivity (BRS), therefore, we aim to investigate the dynamics of HRV and BRS during the CPAP in healthy subjects.

Methods and Results:

Twelve (seven men) volunteers (23±2 years), with no symptoms of respiratory or cardiovascular disease participated in the study. Subjects rested breathing with or without the application of CPAP (20cmH2O, expiratory pressure of 15cmH2O). Respiratory frequency was set at 0.25Hz. Times series of systolic blood pressure (SBP) and pulse intervals (PI) were recorded continuously by infra-red photoplethysmography (FINOMETER) for fifteen minutes in rest, CPAP and recovery. HRV was obtained by a Fast Fourier Transformation and spectral power obtained for total power, low-frequency (LF) and high-frequency (HF) bands. Spontaneous BRS was assessed by the α-index, for LF (α-LF) and HF (α-HF). For statistical analysis an ANOVA one way test followed by Tukey post hoc test. All data were reported in mean ± SD and with significance of α ≤ 0.05. Cardiac output (CO) reduced during CPAP and recovery (rest = 6.44 ± 1.27, CPAP = 5.52 ± 0.98 and recovery = 6.05 ± 1.06 L.min, p ≤ 0.01). Mean PI was reduced during CPAP (rest = 792.4 ± 101.4, CPAP = 723.2 ± 92.8 and recovery = 802.6 ± 92.8 ms, p 0.05). BRS showed significant difference during CPAP as compared to rest or recovery, α-LF (rest = 10.6 ± 2.22, CPAP = 7.91 ± 2.22 and recovery = 11.3 ± 2.59 ms.mmHg-1) and α-HF (rest = 12.0 ± 3.05, CPAP = 6.22 ± 2.72 and recovery: 11.8 ± 4.51 ms.mmHg-1, p ≤ 0.01).

Conclusions:

The application of CPAP in normal subjects exerts reductions in spontaneous baroreflex sensitivity, probably due to diminished venous return and cardiac output.

Keywords: baroreflex, CPAP, heart rate

Financial Support: CNPq (481434/2008-9) and Capes

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Resumo:02-151

ENDOTHELIUM-INDEPENDENT RELAXATION TO EQUILIN IN RAT MESENTERIC ARTERIES AND THE ROLE OF CALCIUM INFLUX

Filgueira, F. P. ; Lobato, N. S. ; Ceravolo, G. S. ; Dantas, A. P. V. ; Fortes, Z. B. ; Webb, R. B. ; Tostes, R. C. ; Carvalho, M. H. C .
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Objectives:
Several studies have demonstrated vasorelaxant effects of different estrogenic compounds. Equilin (3-hydroxyestra-1,3,5,7-tetraen-17-one) is an equine estrogen and one of the major compound of Premarin (about 25%), the leading prescribed medicine for hormonal replacement therapy for postmenopausal women in the United States. However, its vascular effects have not been studied to date. Therefore, the present study aimed to investigate the effects of equilin in resistance mesenteric arteries from female spontaneously hypertensive rats (SHR).

Methods and Results:

The experiments were performed using rat mesenteric arteries (RMA) (200-300µm) obtained from SHR female (16-18 weeks old, n=21) and mounted in isometric myographs. Cumulative Concentration Effect Curve (CCEE) to equilin (10nM-100µM) were constructed in isolated RMA precontracted with either thromboxane mimetic (U46619 – 1µM) or potassium chloride (KCl – 120mM). Results: Equilin induced concentration-dependent relaxation in RMA. This effect was similar between vessels precontracted with U46619 and KCl (99.94% and 99.15% respectively), indicating that the vasorelaxation induced by equilin is mediated by effects on both receptor-operated and potential-operated calcium (Ca2+) channels. Furthermore, incubation with equilin (10 and 100µM) for 30 minutes decreased the maximal response of endothelium-denuded RMA to increasing concentrations of Ca2+ in high K+ (60mM) depolarizing medium. Additionally, equilin (10 and 100µM) inhibited the effect of (S)-(−)-Bay K8644 (L-type Ca2+ channel activator) in a concentration-dependent manner. On the other hand, the transient contraction induced by caffeine (20mM), which activates Ca2+ release from intracellular stores, was not inhibited by equilin in endothelium-denuded RMA. Equilin effects were not different in tissues with endothelium compared with those without endothelium. Incubation (30min) with the specific estrogen receptor antagonist (ICI182,780 – 10ìM) failed to inhibit equilin-induced relaxation in U46619-precontracted RMA. To determine the contribution of vasodilator prostanoids and/or nitric oxide (NO) to the equilin response, RMA were incubated with indomethacin synthesis and L-NAME. The incubation with indomethacin(10µM), a prostanoid inhibitor ; L-NAME(100µM), a non-specific NO synthase inhibitor, or indomethacin plus L-NAME during (30min), did not modify the response promoted by equilin so those relaxation is not mediated by either prostanoids or NO. Blockade of voltage-dependent (KV), Ca2+-activated (KCa) and ATP-sensitive (KATP) K+ channels did not change equilin-induced responses, showing that vasorelaxation to equilin seems not to be mediated by increasing K+ efflux through KCa, KATP, and KV channels. To elucidate the signaling pathways that could lead to equilin-induced vasorelaxation experiments were performed with SQ22536 (100µM), ODQ (10µM), KT5720 (1µM), and KT5823 (1µM an adenylyl cyclase, guanylyl cyclase, protein kinase A, and protein kinase G inhibitors respectively. None of those pathways were involved in equilin relaxation.

Conclusions:

Our results demonstrate that acute equilin-induced vasodilation can be explained by Ca2+ antagonistic property. Further studies using electrophysiological recordings of voltage-gated Ca2+ currents in RMA smooth muscle cells are needed to substantiate the Ca2+ antagonist effect of equilin.

Keywords: estrogeno, microvasos, reatividade vascular, calcio.

Financial Support: FAPESP, CNPq, NIH (HL074167,HL071138).

Resumo:02-152

ANGIOTENSIN II-INDUCED VASCULAR HYPERTROPHY: INVOLVEMENT OF B1-KININ RECEPTOR

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Objectives:

Angiotensin II (ANG II) is recognized as a pleiotropic factor involved in the regulation of multiple systems. Of prime importance is the pro-inflammatory effect of ANG II in the vasculature, which leads to vascular remodeling. The inducible kinin B1 receptor (B1R) has been described to be also important in cardiovascular homeostasis and inflammation. Because B1R is expressed only in response to injury, as ANG II infusion, we tested the hypothesis that the B1R contributes to ANG II-induced vascular hypertrophy, through a mechanism involving reactive oxygen species (ROS) generation and extracellular signal-regulated kinase (ERK1/2) activation.

Methods and Results:

Male Wistar rats were infused with ANG II (400ng/kg/min-ANG II rats) or ANG II (400ng/kg/min)+B1 receptor antagonist, des-Arg9-Leu8-bradykinin (350ng/Kg/min-ANGII+DAL rats), via osmotic mini-pumps (14 days). The B1 receptor mRNA expression (real time PCR) was higher in aorta of ANG II (2.08±0.3AU*) and ANG II+DAL (2.29±0.4AU*; P

Conclusions:

Our data from in vivo and in vitro studies suggest that B1R contributes to ANG II-induced aortic hypertrophy. This is associated with activation of redox-regulated ERK1/2 pathway that controls vascular smooth muscle cells growth. Our findings highlight important cross-talk between the B1R and ANG II. Such interactions suggest that targeting AT1 and B1R might provide vascular protection in cardiovascular disease, such as hypertension.

Keywords: Hypertension, Angiotensin II, Kinin B1 receptor, Vascular Hypertrophy, Hypertension

Financial Support: FAPESP
days/week, for 8 weeks. After euthanization the large intestine was removed, opened lengthwise, washed and fixed in formalin. After being divided into proximal, medial and distal regions it was stained with methylene blue 0.1% for 1 min and washed in phosphate buffer. The aberrant crypt foci (ACF) were quantified and categorized using a light microscope (100x). ANOVA followed by the Tukey post-hoc test was used to compare groups. This study was approved by the institutional Ethics Committee (protocol: 50/2010). The results showed that animals from ED had a lower (p

Conclusions:

Chronic swimming training prior to the induction of experimental carcinogenesis reduced the number of ACF in the proximal region of the intestine, but it did not alter the number of ACF in the medial and distal regions.

Keywords: Physical activity, Cancer, Bowel

Financial Support: CAPES

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Resumo:03-098

EFFECT OF LOAD ABSENCE ON BONE MINERAL CONTENT OF DIABETIC RATS


Ciências Básicas, Unesp

Objectives:

Diabetes mellitus (DM) and osteoporosis are common diseases, with high impact for the healthcare. Although there is a higher rate of incidence of fractures in patients with diabetes, few data are available about the causing factors of this higher rate. This is well known that during the course of the disease, diabetic patients may have a higher prevalence of bone complications. Because of these co-morbidities associated with diabetes, patients can present a number of factors that can lead them to situations of prolonged immobilization or loss of mobility. These conditions cause a loss in bone mineral density (BMD), leading to osteopenia and subsequently reaching a considerable loss of bone density, causing osteoporosis. The aim of this study was to evaluate the changes in BMD of diabetic rats (induced by injection of streptozotocin in the neonatal period) due to absence of load in a short period of time.

Methods and Results:

Five-day-old male Wistar rats (14 animals) were divided randomly into two groups: 1) Control Group (CN, n=6), which received intra peritoneal (i.p.) injection of citrate buffer (vehicle), 2) Diabetic Group (STZ, n = 8), which received an injection of streptozotocin (150mg/kg b.w., i.p.). After these animals completed 60 days of life, they were anesthetized with ketamine hydrochloride (80 mg/kg b.w., i.m.) and Xylazine (10 mg/kg b.w., i.m.), and under anesthesia, it was done the first BMD measurement of the left tibia of the 2 groups by dual absorptiometry X-ray emission, DPX-ALPHA. After this evaluation, these animals were suspended by the tail for 3 weeks in order to remove the load on their hindlimbs. At the end of the period of suspension, both groups were anesthetized as described above, and again were performed densitometry analysis of the left tibia. The results showed that, before the suspension, there was no significant difference in BMD between the CN and STZ (glycemia around 300 mg / dL) (CN = 0.125 ± 0.006 g/cm² vs. STZ = 0.116 ± 0.006 g/cm²). However, after the suspension period, it was detected a statistically significant difference in BMD between the two groups (CN = 0.130 ± 0.005 g/cm² vs. STZ = 0.106 ± 0.002 g/cm²). And the intra-group comparison has shown that only the diabetic group individuals showed decreased BMD after the suspension period.

Conclusions:
Based on these results, we can conclude that there was a decrease in BMD of the STZ group in relation to the control group, only after the immobilization period. This demonstrates that diabetic patients are more impacted by the effects of the absence of load on the bone and it also highlights the importance of insulin in bone metabolism, especially in these situations without load. As a consequence, it is advisable for diabetic patients, especially after a prolonged period of immobilization, to do a physical activity or physiotherapy sessions to improve the quality of bone tissue.

Keywords: Diabetes, osteopenia, load absence, bone mineral density

Financial Support: FAPESP (Process 2010/14755-5)

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Resumo:03-099

DESCRIPTIVE DATA ABOUT EXERCISE TRAINING PROTOCOL WITH SHORT-TERM SESSIONS: PERFORMANCE, WEIGHT GAIN AND HSP70 EXPRESSION IN MUSCLE

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Objectives:

The aim of this work was to describe a short-term exercise training protocol with different overloads and its effects on HSP70 expression in muscles, body weight gain and performance.

Methods and Results:

Adult male Wistar rats (4/group) were submitted to exercise training protocol (8 weeks, 5d/wk). Rats were submitted to progressive overload divided in 5 groups (GR, G2, G4, G6 and G8). Rats were weighted every week. Rats swam 20 minutes (water at 30±1°C) without overload in the first week. In the second week, four groups received 2% overload (% of body mass attached to the tail). In the third week, three groups swim with 4% overload while one group remained with 2% of overload (G2). In the next week, two groups swim with 6% overload while the G2 group remained with 2% and another group remained with 4% (G4). In the fifth week, only one group had its overload augmented to 8% (G8) and all groups were formed G2, G4, G6, and G8. These groups remained with respective overloads in the follow weeks (sixth, seventh and eighth week) while GR group remained in rest condition (shallow water, 3cm deep) during the same time of the others groups during the same 8weeks. The rats were submitted to weight-loaded forced swimming test before the sixth week of training (after 2 days without exercise to prevent accumulative fatigue). The swimming time was measured from the beginning of swimming with 8% workload attached to the tail of all rats, independently of theirs groups, until the point at which rats could not return to surface of the water 5s (time1) and 10s (time2) after sinking. Rectal temperature was evaluated in randomized days before and after exercise session. The animals were killed by decapitation without anesthesia 72h after the last exercise session to avoid physiological response from the sessions. Intracellular HSP70 expression was detected by Western Blot in soleus and gastrocnemius muscle homogenates. Results: Body weight data shows that high intensity exercise training promotes lower absolute weight gain during the 8 weeks protocol. This effect was more evident if the data is expressed as relative body weight. (% weight gain in 8weeks: REP=55±2,1; G2=57±2,3; G4=50±1,8; G6=38±1,4; G8=44±1,3, p

Conclusions:

These data suggested that a swimming exercise session at 30°C did not promote heat shock. Higher intensities of exercise training promote lower weight gain. Gastrocnemius appear to express more HSP70 than soleus muscle in this protocol of exercise
Objectives:

To investigate the effect of acute exercise and training conducted at different intensities in immunodetection of HSP70 in peritoneal macrophages subjected or not to heat shock.

Methods and Results:

In acute experiment, 15 adult male Wistar rats were adapted to the swimming environment for 3 consecutive days for 8 min in water heated ±30°C without load. On the day of experiment, the rats exercised for 20 min divided into five groups: rest, G2%, G4%, G6% and G8% (load of 2%, 4%, 6% and 8% of body weight animal, respectively) (n = 3 per group). Past 12 hours of exercise, the rats were killed to obtain peritoneal macrophages. In the chronic experiment (8 weeks, 5x/s/wk), 4 rats were kept at rest (REP) and another 16 were trained at different intensities (G2%, G4%, G6% and G8%, as indicated above). The training began with 20 min of swimming without charge (1st week), in the 2nd week, were subjected to 20 min of swimming with G2%, in the 3rd week swam for 20 min: 4 rats (G2%) and 12 (G4%). In the 4th week, rats swam 20 min: 4 (G2%), 4 (G4%) and 8 (G6%). In the 5th week swam 20 min 4 rats (G2%), 4 (G4%), 4 (G6%) and 4 (G8%). In the 6th, 7th and 8th weeks, the animals remained loads and swim for 20 min. 72h after the last session, the animals were killed to obtain peritoneal macrophages. In both experiments, the cells were collected and plated for 30 min in CO2 incubator (5% v/v in air, 37 ° C) for adhesion and removal of non-adherent cells. In the presence of fresh culture medium RPMI 1640, cells were subjected for 2h of heat shock at 42°C water bath or 37°C (controls). After this period the medium was discarded and the cells received fresh culture medium for further incubation of 6h. After incubation, media were discarded and the cells homogenized in lysis buffer with protease inhibitors for SDS-PAGE and immunodetection of HSP70. In animals that underwent acute exercise values of intracellular HSP70 are similar, with increased when comparing the control and heat shock, being higher in the latter (37°C - rest=0.25±0.03, G2%=0.23±0.01, G4%=0.33±0.12, G6%=0.27±0.16; G8%=0.29±0.19; 42°C - rest=0.75±0.07, G2%=0.69±0.17, G4%=1.00±0, G6%=0.71±0.20, G8%=0.52±0.20). In training there was a progressive increase in immunodetection of HSP70 in relation to the load, with a decrease in animals with a charge of 8%. Macrophages exposed to heat shock had increased compared to controls (37°C - rest=0.21±0.06, G2%=0.44±0.26, G4%=0.43±0.37, G6%=0.57±0.30, G8%=0.35±0.07, 42°C - rest=0.62±0.25, G2%=0.71±0.32, G4%=0.51±0.19, G6% 0.56±0.35, G8%=0.40±0.39).

Conclusions:

The immunodetection of HSP70 was higher in peritoneal macrophages of rats that performed moderate-intensity exercise, with a
reduction in those who performed high-intensity exercise. When subjected to a second stimulus, heat shock, the macrophages increased the amount of intracellular HSP70.

Keywords: exercise, heat shock, HSP70, macrophages

Financial Support: CAPES, CNPq, INCT Hormone and Women's Health, Propesq-UFRGS.

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Resumo:03-101

FURTHER EVIDENCES OF EXERCISE-INDUCED NEUROPROTECTION IN 6-OHDA-HEMIPARKINSONIAN MICE

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Objectives:

Parkinson’s disease is the most prevalent motor neurodegenerative disease. Although the currently antiparkinsonian agents offer effective relief of motor deficits, they have not been found to alleviate the underlying dopaminergic neuron degeneration. Indeed, there is general agreement that new Parkinson’s treatments should tackle unresolved problems, such as moving from symptom-alleviating to disease-modifying therapies. Over the last decade, regular physical exercise has been implicated to an increased plasticity in the adult brain and it might also afford protection against dopaminergic neurons death and motor dysfunction elicited in experimental Parkinsonism models.

Methods and Results:

Male C57BL/6 mice were assigned to two groups: untrained and treadmill runners, using a blood-lactate-controlled exercise during 6 weeks. After it, we infused right midstriatum with 6-OHDA (4 μg) or vehicle through stereotactic surgery (anterior 0.4, lateral 1.8, ventral 3.5). Rota-rod performance, cylinder task and apomorphine-induced rotations (0.6 mg/kg, s.c.) were weekly assessed during 4 weeks following surgery. We sacrificed animals 48 h after ending the exercise program to evaluate mitochondrial muscle function and striatal levels of neurotrophins. Four weeks after 6-OHDA surgery, animals were perfused to immunohistochemistry analysis. 6-OHDA induced an asymmetry forepaw use with different degrees in sedentary (60-80%) and exercised animals (20-40%). Moreover, our data indicate that exercise slowdowns progression of 6-OHDA-induced Parkinsonism. Rota-rod performance demonstrated a per se effect of exercise, while 6-OHDA sedentary mice presented poor performance than vehicle-treated animals. Moreover, exercise was also able to ameliorate the rota-rod performance impairments induced by 6-OHDA treatment. Apomorphine challenge (0.6 mg/kg, s.c.) induced a progressive contralateral rotation levels in sedentary animals, which was not observed in the 6-OHDA trained mice, suggesting a reduced dopamine receptors sensitization in trained mice. We associated these behavioral benefits to neuroprotective effects of physical exercise, since it prevented the 6-OHDA-induced decreased in the striatal immunoreactivity of the enzyme tyrosine hydroxylase and reduced the death of dopaminergic neurons in the substantia nigra pars compacts of 6-OHDA-treated animals.

Conclusions:

Taken together, the present findings reinforce and extend the suggestion that basal ganglia plasticity is sensible to environmental manipulation such as the physical exercise. Our evidences are coherent with this statement due to modifying-disease effects induced by exercise in 6-OHDA-treated mice.
Objectives:

We aimed to study the effect of female gonadal steroids and D1 activity on post-exercise GH secretion.

Methods and Results:

Female Wistar rats (200-250g) were divided into: sham-operated sedentary (ShS), ovariec-tomized sedentary (OvxS), sham-operated submitted to exercise (ShEx) and ovariec-tomized submitted to exercise (OvxEx). ShEx and OvxEx were submitted to 20 minutes treadmill acute exercise at 75% of maximum oxygen consumption velocity. The animals were killed by decapitation 10 days after ovariec-tomy, immediately after the exercise and during the recuperation period (30 and 60 min). Pituitary was dissected out and D1 activity was measured. Total serum GH was determined by radioimmunoassay.

Conclusions:

Acute exercise positively regulates pituitary D1 activity and GH secretion, and both effects depend on intact gonadal function. We thus hypothesize the existence of a physiological relationship between pituitary D1 increase and GH secretion.

Keywords: estrogen, exercise, growth hormone, type 1 deiodinase

Financial Support: PRONEX, CNPq, FAPERJ, CAPES
Objectives:

Evaluate if regular swim exercise promotes neuroprotection in a state-dependence way, by decreasing the oxidative stress induced by glutamate and nitric oxide (NO) release in cortical and hippocampal slices after oxygen and glucose deprivation (OGD) in an in vitro model of ischemia.

Methods and Results:

Male Wistar rats weighting 150 - 200 g were divided into two groups: trained (n=18) and sedentary (n=18) groups. Swim training protocol (5 days/week for 8 weeks, 30 min at 60% of maximal capacity-trained) was chose as a neuroprotective agent. To induce oxidative stress, animals were decapitated and cortices and hippocampus were quickly removed and sliced in a tissue Chopper (thickness 400 µM), and then slices were submitted or not to oxygen and glucose deprivation (tOGD) for 60 min. Thereafter, slices from both groups were submitted to reperfusion for 4h without OGD. At the end tissue and incubation medium were collected and stored at -80ºC. The followings parameters were evaluated: cell viability (Lactate dehydrogenase – LDH; Ethidium-homodimer – EthD-1), glutamate and nitrite release (NO2-), superoxide dismutase (SOD) activity, and lipid peroxidation (Thiobarbituric acid reactive substances - TBARS) content. For statistical analysis were used One-way and Two-way ANOVA, followed by Student Neuwman-Keuls (SNK) post hoc test. The results were expressed as mean ± standard errors mean (S.E.M) with significance of p < 0.05. The swim training protocol was effective, since hepatic (50.2 ± 8.5 vs. 86.3 ± 13.5 mg/g; p = 0.02) and muscle (3.1 ± 0.3 vs. 4.2 ± 0.2 mg/g; p = 0.046) glycogen storage increased, as well as, the associated exercise capacity.

Exercise showed to be a neuroprotective agent, since LDH release was reduced in cortical slices (207.1 ± 35 vs. 92.6 ± 19.2 U/L/mg protein; p < 0.01) and the amount of cell death staining decreased in hippocampal slices (317.4 ± 28.64 vs. 183.4 ± 46.8 % cell death; p < 0.05) after OGD. We also demonstrated that exercise decreased glutamate release (568.6 ± 121.0 vs. 328.5 ± 60.55 nmol/mg protein; p < 0.05) after OGD in both cortical and hippocampal slices, as well as, reduced nitrite release (1.93 ± 0.39 vs. 1.0 ± 0.28 µM/mg protein; p < 0.05) and lipid peroxidation content (~ 30%) only in the OGD cortical slices. Moreover, interestingly the exercise per se increased cortical SOD activity in non-OGD slices (0.39 ± 0.06 vs. 1.0 ± 0.22 U/mg protein; p< 0.05) slices were submitted to OGD.

Conclusions:

For the first time was demonstrated that under a metabolic stress situation exercise training prevents in a different way the oxidative damage caused by glutamatergic excitotoxity and free radical-NO, increases antioxidant status, and induces neuroprotection in cortical and hippocampal slices after ischemia-reperfusion in vitro model.

Keywords: ischeamia, neuroprotection, exercise, oxidative stress, nitric oxide

Financial Support: CNPq, Fapemig, Capes

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Resumo:03-104

EXPRESSION OF HSP70 IN THE HYPOTHALAMUS OF RAT AFTER ACUTE SWIMMING EXERCISE AND TRAINING OF DIFFERENT INTENSITIES

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Objectives:
To verify the influence of chronic and acute swimming exercise at different intensities on the expression of HSP70 protein on the rat hypothalamus.

Methods and Results:

Methods: 40 male Wistar rats (200-250g) were adapted to the swimming environment for 1 week and, then, 3 groups were separated: 1. In the acute exercise (n=5 per group), rats were swam for 20 minutes in different intensities (water temperature 30±1°C) constituting the groups G2%, G4%, G6% and G8%, according to the overweight added to the tail and one that stayed in shallow water (3cm) during the same time named the rest group. The animals were killed right after exercise and samples (hypothalamus) were collected from each animal. 2. In the acute 12 hours protocol (n=2 per group), rats were submitted to the same acute exercise bout (rep; G2%, G4%, G6% and G8%) and were killed 12 hours later. 3. In the chronic protocol (n=4 per group), rats were submitted to a swimming training of 8 weeks, 5 days per week (water temperature 30±1°C) for 20 minutes in different intensities as the acute protocols and were killed 72 hours after the last session of training. The immunodetection of HSP70 (arbitrary units HSP70/actina) was verified by western blot technique with specific monoclonal antibody. Results: There was no difference between the intensities of exercise in the acute immediately group (rep=1.22±0.28; G2%=1.08±0.11; G4%=1.17±0.18; G6%=1.20±0.29; G8%=1.34±0.41, p=0.72) neither the acute killed 12 hours after (rep=1.24±0.07; G2%=1.07±0.09; G4%=1.41±0.46; G6%=1.15±0.16; G8%=1.14±0.20, p=0.68) in the HSP70 expression on the hypothalamus. However, high intensities made chronically increased HSP70 on hypothalamus when compared to the rest group (rep=1.05±0.06; G2%=1.11±0.13; G4%=1.28±0.34; G6%=1.59±0.21; G8%=1.84±0.56, p=0.02).

Conclusions:

Our data suggest that acute exercise did not increase the HSP70 expression on hypothalamus. High intensity exercise training increase HSP70 expression on hypothalamus. This increase in the higher intensities just for the trained group may be caused for an adaptation due to the higher requirement and consumption of glucose by the brain during high intensity exercise, to maintain neuronal activity, since hypothalamus controls the body homeostasis. The results show a possible mechanism to be used as treatment and prevention of metabolic disorders such as obesity and this increase in HSP70 also may be related to cytoprotection against neurodegenerative diseases, preventing inappropriate protein interactions, assisting their folding and following the mutant proteins to be degraded.

Keywords: HSP70, hypothalamus, swimming exercise

Financial Support: CNPq, Propesq-UFRGS and INCT Hormones and Women’s Health

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Resumo:03-105

ELDERLY HAVE LOWER SWEAT CAPACITY THAN YOUNG PEOPLE DURING SELF-PACE EXERCISE UNDER THE SUN.

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1 ESCOLA DE EDUCAÇÃO FÍSICA FISIOTERAPIA E TERAPIA OCUPACIONAL, EEFFTO - UFMG
2 ESCOLA DE EDUCAÇÃO FÍSICA FISIOTERAPIA E TERAPIA OCUPACIONAL, EEFFTO - UFMG

Objectives:

Little is known about the effects of aging on the sweating and thermoregulatory capacities in elderly men and women. Objective: To compare thermoregulatory responses between young and older adults of both sexes.

Methods and Results:
After approval by the Ethics Committee UFMG (ETIC 000-09), 47 volunteers, 24 men (M) and 23 women (W), where 24 were young [12 men (YM) and 12 women (YW) (age: 25.6 ± 0.5 and 24.8 ± 0.5 years, body mass: 76.4 ± 2.0 and 56.5 ± 1.2 kg, BSA.mass-1.100: 2.6 ± 0.0 and 2.8 ± 0.0 m2.kg-1 and aerobic capacity: 46.6 ± 1.4 and 38.2 ± 1.3 ml.kg-1.min-1, respectively)] and 23 were old subjects [12 men (OM) and 11 women (OW) (age 66.4 ± 1.1 and 63.3 ± 1.1 years, body mass: 65.4 ± 2.3 and 64.1 ± 3.3 kg, BSA.mass-1.100: 2.6 ± 0.0 e 2.6 ± 0.0 m2.kg-1 and aerobic capacity: 36.4 ± 2.0 and 30.2 ± 1.7 ml.kg-1.min-1, respectively)], considered fit for physical activity, ran a total of 6 km, in four steps of 1.5 km with intervals of 2 minutes at highest voluntary speed possible (self-paced). The total exercise time (TET, in min) to perform 6 km, average speed (VM in km/h), energy expenditure (EE, kcal/min), % heart rate (MHR, %, average heart rate (FCméd in bpm.min−1), delta rectal temperature (delta Tret, in °C), mean skin temperature during exercise (TmpEX, in °C), total sweat rate (TS, in gm−2.min−1), number of activated sweat glands (GSA.cm−2), sweating by GSA (g.min−1.GSA−1), the physiological strain index (PSI) were measured. One-way ANOVA and post hoc test Student Newman Keuls or Tukey were used to compare the results. To investigate the relationship between variables, we used Pearson correlation and the significance was set at α=5%. Results: The TET was lower in YM in comparison with the groups OM and OW, and the OW group was higher than the other groups (YM: 30.6 ± 1.7 min, OM: 41.8 ± 3.7 min YM: 37.2 ± 1.4 min, OW: 51.6 ± 4.0 min; p<0.05). The EE was higher in the YM (15.5 ± 0.8 kcal.min−1; p<0.05). PSI was not different between groups (YM: 8.2 ± 0.3, OM: 7.3 ± 0.2, YW: 8.0 ± 0.2, OW: 7.4 ± 0.2; p>0.05). Regardless of sex, PSI was higher in young compared with old (young: 8.08 ± 0.19, old: 7.37 ± 0.14; p<0.05). Another explanation for the reduction of TS observed in the elderly female group would be to decrease the number of GSA (OW: 54 ± 6 GSA.cm−2 vs. YW: 72 ± 6 GSA.cm−2; p<0.05). The TmpEX was higher (p

Conclusions:

During the self-paced exercise, the elderly had lower sweat rates compared with young people. The lower aerobic capacity of the elderly was the main factor for decreased sweating. The number of active glands, sweat rate per gland active and mean skin temperature during exercise respond to exercise differently between the sexes.

Keywords: total sweating rate, aerobic capacity, age and sex

Financial Support: CNPq, CAPES, FAPEMIG, SANTANDER

THE ROLE OF ANGIOTENSIN CONVERTING ENZIME GENE ON METABOLIC RESPONSES TO PHYSICAL TRAINING IN DIABETIC MICE

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Objectives:

The purpose of this study was to evaluate the effects of physical training(PT) on body weight (BW), energy metabolism and adipose tissue in diabetes mice with different dosage of angiotensin converting enzyme (ACE) gene.

Methods and Results:

Transgenic adult male mice (C57BL/6) harboring 1 or 3 copies of ACE gene were induced to diabetes by Streptozotocin (STZ, 125mg/kg) and separated into groups sedentary or physical trained with swimming during 8 wk (1 session, 90 min, 5 days/week): 1 copy sedentary (1S, n=14), 1 copy trained (1T, n=15), 3 copies sedentary (3S, n=10) and 3 copies trained (3T, n=11), BW, diary food intake (FI), resting metabolic rate (RMR) by indirect calorimetry, exercise tolerance (ETol) by treadmill test,
intraperitoneal glucose tolerance test (IGTT), periepididymal (PF) and retroperitoneal (RF) fat pads weight, adipocyte diameter (AD), lipolytic activity and fatty acid synthetase (FAS) enzyme activity were measured. Experimental procedures were approved by Ethics Committee from School of Physical Education and Sport, University of Sao Paulo (n. 2008/38). Data are showed as means±SE. BW gain and PF fat pad were not different among groups, but 3T reduced RF fat pad and AD compared to 1S, 1T and 3S (p

Conclusions:

Our results are consistent with the idea that metabolic adaptations promoted by PT in diabetes mice are partially associated with 1 copy of ACE gene.

Keywords: renin angiotensin system, body weight, energy metabolism, physical training, streptozotocin

Financial Support: FAPESP and CAPES

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Resumo:03-107

COMPARING QUALITY OF SLEEP INDEX IN ACTIVE AND SEDENTARY STUDENTS OF PHYSIOTHERAPY

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Objectives:

Sleep deprivation causes substantial losses in physical and cognitive performance. Students in the faculty overwhelmed by the content, timetable and activities directly or indirectly linked to the course, may show changes in sleep quality, a condition that affects their learning, relationships, health and quality of life. Although the efficacy of exercise on sleep has been demonstrated and accepted by the American Sleep Disorders Association as a non-pharmacological intervention to improve sleep, few health professionals have recommended and prescribed exercise for this purpose. This study aims to compare the quality of sleep in active and sedentary students of physiotherapy.

Methods and Results:

It was a cross-sectional study, approved by the Ethics Committee of Faculdade Integrada do Recife (Estacio-FIR). Data were collected at the Clinical School of Physiotherapy at Estacio-FIR through the indexes of the Pittsburgh Sleep Quality (PSQI) and the International Physical Activity Questionnaire (IPAQ). Those instruments were translated and validated to Portuguese. Our sample consisted of 260 students, 42 males (16.15%) and 218 females (83.85%), age between 18 and 30 years and mean age of 22.5 ± 2.1 years. In order to compare the quality of sleep in active and sedentary students we used the Student t test for paired samples and the results were presented as mean ± SD, considering as significant p

Conclusions:

Analyzing PSQI and IPAQ indexes, we observed that students who practice regular physical activity for at least three times a week, have a better quality of sleep when compared to sedentary students. It is suggested that the practice of regular physical activity should be useful in the prevention and treatment of sleep disorders. However, we suggest more studies involving students to identify the ideal type and intensive exercise to improve quality of sleep more efficiently.

Keywords: Active, Physiotherapy, Quality of Sleep, Sedentary, Students
SALIVARY CORTISOL IN MALE SWIMMERS SUBMITTED TO A TRAINING PROGRAM BASED ON VERKHOSHANSKY PERIODIZATION MODEL.

Arruda; Garcia; Spadari-bratfisch; Depto. de Biociências/ Universidade Federal de São Paulo, UNIFESP- BS

Objectives:

to investigate whether a training program based on Verkhoshansky periodization model is effective to improve performance in swimmers and whether it alters the behavior of salivary cortisol, a stress hormone related to catabolic state.

Methods and Results:

Four male swimmers (22 ± 2.5 yr, 82.3 ± 3.2 Kg, 1.79 ± 0.03 m, and with 11.58 ± 1.65% of body fat) participated in this study. They were trained two hours/day, six days/week, during 10 weeks. The training program was based in the model of periodization proposed by Verkhoshansky and consisted of blocks of training aimed to develop strength (A, 4 wk), power or strength resistance (B, 3 wk) and speed (C, 3 wk). During this period, the swimmers also participated in competitions. The performance was evaluated by the test time in the swimmers best event, at the beginning and the end of the training program. The athletes rated the perceived exertion after each training session using the Borg scale. Saliva samples were collect immediately after waking up, and also 30 min, 5, 11 and 14 h after awakening, in a resting day, at four specific moments of the training program (before the beginning and after the blocks A, B, C of training). Data are presented as mean ± epm and were analyzed by ANOVA plus Tukey test. Differences were considered significant when p

Conclusions:

The training program based on the model proposed by Verkhoshansky improved swimmers performance. Although classified as maximum level of intensity didn’t alter the basal salivary cortisol in a resting day, showing that the proposed program was effective without threaten the athlete physical health.

Keywords: cortisol, periodization, swimming, concentrated loads, perceived exertion

Financial Support: REUNI, FAPESP

INFLUENCE OF THE INTENSITY OF PHYSICAL EXERCISE IN HSP70 RELEASE FOR PLASMA BY CIRCULATING MONOCYTES

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1 Departamento de Fisiologia, UFRGS
2 PPGCMH, UFRGS
Objectives:

To investigate the correlation between plasma and circulating monocyte HSP70 contents in rats subjected to an acute bout of exercise at different intensities.

Methods and Results:

20 adult male Wistar rats were adapted to the swimming environment for 3 consecutive days (8 min in water at 30 ± 1 °C). The rats were subjected to swimming for 20 min (30 ± 1 °C), 48 h after prior adjustment, were divided into five groups: G2%, G4%, G6%, G8% (corresponding to 2%, 4%, 6% and 8% body weight added to tail of the animal as overload, respectively) and a control group, in which the rats remained at rest (Rep) under the same conditions. The animals were sacrificed immediately after the exercise session by decapitation for the collection of heparinized blood. Of the total blood collection, 1 mL was centrifuged to obtain plasma and the remainder was layered onto a Ficoll 400 / Hypaque (d = 1.084 g / mL) discontinuous gradient to have mononuclear cells isopycnically separated. The cells were then collected, seeded in microplates containing 2 mL of RPMI1640 medium and incubated in CO2 (5% v/v in air) at 37 °C for 2 h. Afterwards, supernatant fractions containing non-adherent cells (lymphocytes essentially) were discarded and the adherent monocytes were scraped and collected in PBS pH 7.4. Cells were homogenized in lysis buffer with protease inhibitors to be analyzed by SDS-PAGE/Immunoblot for HSP70. Plasmas were equally assayed for HSP70. Results: Immediately after acute exercise session, there was an increase in the amount of plasma HSP70 proportional (p = 0.0002, ANOVA) to exercise load (arbitrary units, mean ± DPM): Rep, 0.39 ± 0.142; 2%, 0.42 ± 0.271, 4%, 0.60 ± 0.006, 6%, 0.85 ± 0.141 and 8%, 0.92 ± 0.141. However, monocytes showed no significant differences between groups (p = 0.7322), the values of western blotting of HSP70 obtained after the session very similar between groups (Rep, 0.40 ± 0.26, 2 %, 0.61 ± 0.34, 4%, 0.64 ± 0.47, 6%, 0.66 ± 0.33, 8%, 0.79 ± 0.18).

Conclusions:

While immediately after the end of physical exercise, there is sharp increase in HSP70 plasma concentration proportional to the workload, there was no change in intracellular content of HSP70 in monocytes, suggesting that these cells do not contribute to HSP70 release into the bloodstream during exercise.

Keywords: HSP70, Physical Exercise, Monocytes

Financial Support: CAPES, CNPq, INCT Hormone and Women's Health, Propesq-UFRGS

Resumo:03-110

THE KINETICS OF INTRACELLULAR HSP70 EXPRESSION IN LYMPHOCYTES FROM RATS SUBMITTED TO DIFFERENT INTENSITIES OF EXERCISE: EVIDENCE FOR AN INTENSITY-DEPENDENT THERMOTOLERANT STATE

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¹ Laboratório de Fisiologia Celular, Depto de Fisiologia, ICBS, UFRGS
² Depto de Biologia e Química, UNIJUÍ
³ Faculdade de Biomedicina, UFCSPA
Objectives:

Introduction: The 70-kDa family of heat shock proteins (HSP70s) are important molecular chaperones with anti-inflammatory potential when intracellularly located. They are induced by several stressful events, such as glucose deprivation, heat shock, fever, oxidative stress and exercise. Acute exercise alters the concentration of HSP70s in many cell types, including immune cells. However, the effect of different intensities of exercise on the expression of HSP70 in lymphocytes (whose function can be modulated by exercise) is mostly unknown. Aims: To verify the kinetics of HSP70 expression induced by different intensities of an acute exercise (swimming) bout in lymphocytes and the ability of exercise in imprinting a thermotolerance state in these cells.

Methods and Results:

Adult male Wistar rats (250-300 g, n=5 per group) were previously adapted to the swimming environment for 3 days during 8 min (water at 31°C). Forty-eight hours later, the animals swam for 20 min with different overloads (as a percentage of the body mass tied to the base of the tail) which represent the following intensities: G2% (low intensity), G4% (moderate), G6% (moderate-to-high intensity) and G8% (high-intensity). Control rats were kept at rest (REST group) for the same time in a small amount of water without charge and did not practice exercise. The rats were killed immediately after the exercise and had their mesenteric lymph nodes lymphocytes isolated. The cells from each animal were distributed into 2 groups: one kept in a 37°C water bath (control) for 2h and another challenged with a 42°C water bath (heat shock, HS). After that, the cells were cultured during 6h in a CO2 incubator at 37°C to allow for the HSP70 peak expression. HSP70 immunocontents were analyzed before (Tzero) and after water bath (HS or controls) every 2h during the 6h time of culture. HSP70 expression in the REST lymphocytes was not affected by 6h culturing at 37°C but conspicuously enhanced after HS, peaking at 4 h (ca. 100%, p

Conclusions:

In terms of HSP70 expression, mesenteric lymph node lymphocyte are sensitive to exercise loads, in spite of they are not circulating cells. Furthermore, the expression of HSP70 after a further challenge (HS) reached a plateau at G8%, where the maximum of HSP70 is attained and no additional increment was observed, thus suggesting an acquired thermotolerance in the cells obtained from heavily-exercised animals. The implication of such findings for lymphocyte function is now under investigation in our laboratory.

Keywords: HSP70, physical exercise, lymphocyte, heat shock, thermotolerance

Financial Support: CNPq, CAPES, Propesq-UFRGS, INCT Hormones and Women’s Health

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**Resumo:03-111**

**INFLUENCE OF INTERMITTENT AND CONTINUOUS AEROBIC TRAINING ON ANTIOXIDANT STATUS AND LIPID PEROXIDATION IN HYPERTENSIVE POSTMENOPAUSAL WOMEN**

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Objectives:

Epidemiological studies show that women have a longer life expectance as compared to men. Furthermore, the incidence of
cardiovascular disease in women increases dramatically after menopause. Thus, preventive actions to avoid high incidence of cardiovascular disease are extremely necessary for postmenopausal women. Oxidative stress has been pointed out as a potential cause of arterial hypertension in women whereas physical exercise has been used as a relevant non-pharmacological approach to minimize the deleterious effects of oxidative stress on the cellular function. No studies exist evaluating the health-promoting effect of aerobic training on the blood pressure and redox state in postmenopausal hypertensive and normotensive women. 

PURPOSE: Therefore, the aim of this work was to evaluate whether chronic aerobic training could promote alterations on the oxidant and antioxidant status in hypertensive postmenopausal (PMH) women and normotensive (PMN) women.

Methods and Results:

METHODS: Twenty-four hypertensive (values was considered hypertension SBP 140-159 and DBP 90 and 99 mmHg is considered mild hypertension or degree of the Brazilian Guidelines on Hypertension) postmenopausal women (54±5 years) and twenty-four normotensive (54±5 years) underwent aerobic interval training (IT) and aerobic continuous training (CT) in cycle ergometer and/or treadmill, 3 days/week for 30 min/session during 3 months. Anthropometric parameters, blood pressure, plasma superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) levels were measured. All data were analyzed at the baseline and after 3-months of ET. Each session of IT consisted of 4 min at 60%, 4 min of 70% and 2 min of 80% the frequency of cardiac work (FCW) in which was calculated by the Karvonen formula, where FCT= basal heart rate + % of work (maximum heart rate - Resting heart rate) repeated for 3 times, giving a total of 30 minutes whereas CT consisted of 30 min at 60% of FCW. The statistical test used to work foiob paired t test with a significance level of p

Conclusions:

CONCLUSION: Our findings show that both aerobic ET (continuous and intermittent) was effective in increasing in SOD, CAT, NOx and GMPc plasma levels as well as decrease lipid peroxidation products either in normotensive and hypertensive postmenopausal women.

Keywords: postmenopausal, hypertensive, aerobic training, antioxidant

Financial Support: FAPESP

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EFFECT OF RESISTANCE TRAINING ON VEGF EXPRESSION IN THE VISCERAL ADIPOSE TISSUE OF OVARIECTOMIZED RATS

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Objectives:

The lack of estrogen is related to the development of chronic diseases and the accumulation of abdominal fat. In rats, ovariectomy increases food intake and body weight. Thus, moderate physical training has been used to reduce fat deposition, and provide other benefits to the metabolic profile. The adipose tissue is highly vascularized and it has the ability for production and secretion of VEGF. VEGF is a potent angiogenic mitogen responsible for increasing vascular permeability and endothelial cell proliferation. Therefore, the aim of this study was to analyze the effects of the resistance training in the gene expression of VEGF in visceral adipose tissue of ovariectomized rats.

Methods and Results:

Female Sprague-Dowley rats were randomly assigned to 4 groups (n=6/group), treated with chow diet and submitted or not to resistance training (RT) and to ovariectomy (OVX). The training consisted in three times a week sessions of climbing for 10 wks.
It consisted of 4-8 ladder climbs while carrying progressively heavier loads attached to the tail. The next training sessions consisted of 4 ladder climbs with 65%, 85%, 95% and 100% of previous maximal carrying capacity of the rats, determined in the previous session. The exercise protocol was adapted from HORNBERGER; FARRAR, 2004. The rats were killed 48h after the last session of training and the visceral adipose tissue removed, mRNA extracted with Trizol and VEGF expression was determined by qPCR. Results were normalized to GAPDH gene expression. The ANOVA two way was applied with Fisher pós hoc tests (p≤0.01).The ovariectomy decreased VEGF expression (Sham 1.004±0.0407 versus OVX 0.610±0.0719) while the resistance training increased its expression (Sham 1.004±0.0407 versus Sham RT 1.641±0.0939). However, when OVX was associated with resistance training, the gene expression of VEGF in visceral adipose tissue remained unchanged (OVX 0.610±0.0719 versus OVX TR 0.535±0.0575).

Conclusions:

The resistance training performed was not efficient to minimize and reverse the effects of the lack of estrogen on VEGF expression in the visceral adipose tissue, suggesting that estrogen may have a key role in the expression of this important modulator of angiogenesis.

Keywords: Adipose tissue, estrogen, ovariectomy, resistance training, VEGF

Financial Support: CAPES and CNPq

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Resumo:03-113

EVALUATION OF MODERATE AEROBIC TRAINING ON THE MECHANICAL PROPERTIES OF ACHILLES TENDON IN RATS INDUCED TO EXPERIMENTAL DIABETES

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Objectives:

To evaluate the effect of moderate aerobic training on a treadmill on the mechanical properties of the achilles tendon in rats induced to experimental diabetes.

Methods and Results:

For this study we used 44 male wistar rats randomly distributed into four groups: Sedentary Control Group – SCG (n=11), Sedentary Diabetic Group – SDG (n=11), Trained Control Group - TCG (n=11) and Trained Diabetic Group – TDG (n=11). At 70 days old diabetic groups were chemically induced to diabetes by a single intraperitoneal injection of streptozotocin solution (60mg/Kg). Trained groups were submitted to a protocol of continuous moderate aerobic training on treadmill where the training sessions were held once a day, five days a week for 8 weeks with an intensity of about 70% of VO2max. Training sessions began with 10 minutes and reached to 60 minutes in the last week. At the end of the training period the Achilles tendons were collected and submitted to a traction test in a conventional testing machine. The tendons were fixed in a conventional mechanical testing machine and pulled to the point of failure of the specimen, at a speed of 0.1 mm/s and the strength was constantly measured by a load cell of 500 N. The dislocation of the specimen was registered automatically by the software TESC (Test Script) for automatic testing. The parameters evaluated were: elastic modulus (MPa), Stress Maximum Strength (MPa), Strain Specific Maximum Force (mm), energy / tendon area (N.mm/mm2) and Cross-sectional area (mm2). To describe the sample characteristics we used descriptive measurements, such as measure of central tendency (mean) and dispersion (standard deviation). To compare the means variables between the various treatments we used the Kolmogorov-Smirnov test. For analysis of independent samples to compare the groups we used One Way ANOVA and Post-hoc Bonferroni. Data were analyzed with
SPSS software (Statistical Package for Social Sciences). We accepted 1% as the significance level. The evaluation of the biomechanical properties of the Achilles tendon of the SDG indicated that the elastic modulus (3.73±1.79 MPa) was decreased when compared to the TDG (15.19±4.99 MPa) and the other groups (p 0.01). The cross-sectional area (3.10±0.52 mm²) of TDG decreased when compared to other groups (p

Conclusions:

The results of this study indicated that moderate-intensity aerobic training restored the normal mechanical properties of tendon in diabetic animals.

Keywords: ACHILLES TENDON, DIABETES, MECHANICAL PROPERTIES, MODERATE AEROBIC TRAINING

Financial Support: CNPq/CAPES

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Resumo:03-114

EXERCISE TRAINING RESTAINS SKELETAL MUSCLE WASTING BY REDUCING OXIDATIVE STRESS AND UBQUITIN PROTEASOME SYSTEM ACTIVITY IN MICE AND HUMAN HEART FAILURE


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Objectives:

Skeletal muscle wasting is an independent predictor of poor prognosis in heart failure (HF). Considering that oxidative stress (OS) contributes to muscle wasting activating proteolytic pathways as ubiquitin proteasome system (UPS), our objective was to evaluate whether increased OS would be associated with upregulation of skeletal muscle UPS components and increased 26S proteasome activity in a genetic model of sympathetic hyperactivity-induced HF (SH-HF) and aerobic exercise training (AET) would reverse these changes.

Methods and Results:

AET consisted of treadmill running, 8 weeks, 5 days/week, 60 minutes/day at 60% maximum speed, achieved during a graded treadmill exercise protocol. SH-HF mice presented severe HF evaluated echocardiographically and muscle wasting paralleled by increased plantaris OS vs. control mice (decreased redox balance, increased lipid peroxidation and carbonylated protein levels) and mRNA levels of E3 ligase Atrogin1 (69%) and E3&alpha (86%). Furthermore, SH-HF mice displayed increased ubiquitinated protein levels (30%) and 26S chymotrypsin-like activity (22%). AET decreased OS and UPS components changes in SH-HF mice. The clinical relevance of our findings was demonstrated by 86% increased 26S chymotrypsin-like activity in biopsied vastus lateralis of HF patients (postinfarction HF and ejection fraction

Conclusions:

Collectively, these results provide evidence for the potential contribution of OS to local UPS hyperactivity in skeletal muscle
atrophy in SH-HF mice and HF patients. AET prevented atrophy by reducing OS and UPS components.

Keywords: oxidative stress, ubiquitin proteasome system, skeletal muscle, heart failure, aerobic exercise training

Financial Support: FAPESP

Objectives:

Physical training (PT) has been recommended to prevention and treatment of glucose intolerance (GI) and type 2 diabetes. We investigated whether PT would prevent GI and the role of AMP-activated protein kinase (AMPK)/acetyl CoA carboxylase (ACC) pathway behind this response.

Methods and Results:

Male C57BL/6J mice were assigned into chow-fed controls (C, n=5), cafeteria diet (CAF, n=5), chow-fed trained (TR, n=8), and cafeteria diet plus trained (CAF-TR, n=8). PT was performed simultaneously with diet and consisted of 8-wk running session of 60 min at 60% of maximal speed, achieved during a graded treadmill exercise protocol, 5 days/wk. Body weight (BW), daily food intake (FI), resting metabolic rate (RMR) by indirect calorimetry, intraperitoneal glucose tolerance test (IGTT), fasting glycemia (FG), insulin level and muscle glycogen content (MGly) were measured. Expression of AMPK, AMPK phosphorylated at alpha-Thr172 (P-AMPK), ACC and ACC phosphorylated at Ser 79 (P-ACC) in skeletal muscle were determined by Western blot. Experimental procedures were approved by Ethics Committee from School of Physical Education and Sport, University of Sao Paulo. Data are showed as means±SE. BW was similar among groups before PT. After PT, BW was significantly lower in TR and CAF-TR compared to CAF group. C, CAF and CAF-TR groups increased BW after PT compared to respective BW before PT. CAF-TR showed lower FI compared to C and CAF (p

Conclusions:

PT prevents GI development induced by cafeteria diet and the activation of AMPK/ACC pathway can contribute to this response.

Keywords: ACC, AMPK, GLUCOSE INTOLERANCE , PHYSICAL TRAINING

Financial Support: FAPESP
Objectives:
Physical activity (PA) is an important adjunctive tool in the management of type 2 diabetes. It has been well established that participation in regular PA improves blood glucose control and positively affects several other measures such as lipids, blood pressure, cardiovascular events, mortality, and quality of life. However, despite these evidences, most patients remain sedentary. The aim of this study was to characterize the pattern of PA and to identify factors associated with different levels of PA among patients with type 2 diabetes.

Methods and Results:
This is a cross-sectional observational study of consecutive type 2 diabetic patients seen at outpatient unit over 6 months. Along with demographic and clinical information, individuals were questioned about comorbidity severity (Charlson Comorbidity Index, CCI), sleep quality (Pittsburgh Sleep Quality Index, PSQI), daytime sleepiness (Epworth sleepiness scale), depressive symptoms (Beck Depression Inventory- BDI II), International Physical Activity Questionnaire (IPAQ) and Health-related quality of life (Short-Form Health Survey, SF-36). The study was approved by the local Ethical Committee (CEP-HUWC 031-04-09).

Patients of both genders (N=140; 59% female) aged from 40 to 60 years (mean age 52.8±5.6) were studied. Mean disease duration was 12.0±6.7; Body Mass Index 29.8±4.9 and Body Adiposity Index 50.7±34.2. Individuals were sedentary (24.2%), irregularly active (38.3%), active (29.2%) or very active (8.3%). Quality of life was significantly associated with reduced PA level (p<0.005).

Conclusions:
Poor quality of life is associated with sedentary life in type 2 diabetic patients. Considering the fact that functional capacity was positively associated with improved PA, the evaluation of different types of physical training must take into consideration the state of functional capacity in these patients. In this population, age, disease duration, comorbidity severity, poor sleep, or depressive symptoms were not associated with a sedentary life style.

Keywords: physical activity in type 2 diabetes, quality of life, daytime sleepiness, depressive symptoms

Financial Support: CAPES - Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
Objectives:

Alterations in myocardium morphology and cardiomyocyte contractile function are known to impair cardiac function in Chagas’ disease, however, the role played by such changes on exercise tolerance remains unknown. Thus, we aimed to investigate the relationship between exercise tolerance, myocardium morphology and cardiomyocyte contractile function in rats infected with Trypanosoma cruzi (T. cruzi).

Methods and Results:

Four-month-old male Wistar rats (350-380 g) were randomly assigned into control (CG=14) or infected (IG=14) group. IG was inoculated with T. cruzi Y strain (300,000 trypomastigotes/50g wt.). After nine weeks, all rats were subjected to a graded treadmill running test until exhaustion to assess exercise tolerance. Forty-eight hours later, rats were killed and right atrium (RA) and left ventricle (LV) were carefully harvested for evaluation of myocardial morphological and cardiomyocyte contractile function. IG displayed exercise intolerance as detected by reduced total distance run (CG=288.44±34.82 vs IG=242.38±32.30), time to fatigue (CG=19.24±1.76 vs IG=16.85±1.62) and maximal workload (CG=16.38±2.28 vs IG=12.76±1.69) when compared with CG. LV hypertrophy, increased inflammation and collagen content were also detected in infected animals. RA and LV cardiomyocyte dysfunction was detected by reduced fractional shortening and increased time to half relaxation, while reduced time to maximal shortening was depicted only in LV cells from IG. Reduced responsiveness to β-adrenergic stimulation was also observed in RA and LV myocytes. Striking correlations were found between exercise tolerance and RA and LV myocyte fractional shortening, independently of β-adrenergic stimulation.

Conclusions:

T. cruzi infection significantly reduced exercise tolerance and negatively altered myocardial morphology and mechanical properties of myocytes isolated from RA and LV. Data from myocyte function under β-adrenergic stimulation support the hypothesis of cardiomyocyte contractile dysfunction as an important mechanism underlying cardiac impairment and exercise intolerance induced by T. cruzi infection.

Keywords: Chagas’ Disease, physical exercise, Trypanosoma Cruzi

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Resumo:03-118

THE LACTATE THRESHOLD IS NOT CHANGED IN THE PHYSICAL TRAINING IN RATS WITH INCREASING LOADS AND ESTROGEN DEPRIVATION

Dpto Fisioterapia/UNIVERSIDADE FEDERAL DA PARAIBA, UFPB

Objectives:

Lactate threshold(LT) has been normally used to estimate the contribution of glycolytic system in physical exercise. It is known that estrogen is an important hormone in regulation of mass and muscle strength. However it is not understood how glycolytic system reacts to anaerobic exercise training with increasing loads. Therefore, the aim of this work was to study the behavior of LT at different times of anaerobic physical training through estrogen deprivation.

Methods and Results:

Samples consisted of 12 Wistar rats, ovariectomized, aged 120 days, female, nulliparous, kept in an animal house at UFPB, with temperature of 22 ± 1°C and controlled light/dark cycles (12h-12h). The reduction of estrogen was confirmed by comparison of uterine mass this group (0.07±0.006g) with the uterine mass of not ovariectomized control group (0.48±0.05g, n=11) which had
no physical training. Thirty days after surgical procedures, animals started the protocol of physical training with jumps in water with an adjustable load each week. This was composed of four sets of ten jumps with 30 second intervals between sets, and a total duration of ten weeks. The first week was of adaptation, the second week with an overload of 50% of body weight and for each two weeks the overload was increased by 10% up to 90%. LT blood was measured by the lactimeter Accutrend® Plus - Roche, in three different occasions: 45, 60 and 75 days of training. Data were processed with the statistical program Graph Prism® 5.0 (San Diego, CA, USA) and analyzed by paired t test. A p-value of < 0.05 was considered statistically significant. Values are presented as mean ± SEM. When comparing the mean LT45 (mean 5.84 ± 0.18 mmol / L), LT60 (mean 6.54 ± 1.37 mmol / L) and LT75 (mean 5.67 ± 0.47 mmol / L) these were not statistically significant (P <0.05).

Conclusions:

There was no significant change LT at the end of each exercise period. This can be explained by the energy system used in this model which is primarily anaerobic glycolytic, the possibility of estrogen deficiency does not interfere with gradual adaptation to anaerobic training. Therefore, it is likely that estrogen deprivation did not significantly alter the performance of animals during anaerobic training.

Keywords: Anaerobic Threshold, Ovariectomy, Resistance Training

Financial Support: Cnpq

INVOLVEMENT OF ATRIAL NATRIURETIC PEPTIDE (ANP) IN THE ARTERIAL PRESSURE REDUCTION BY SWIMMING BUT NOT RUNNING PHYSICAL TRAINING IN SPONTANEOUSLY HYPERTENSIVE RATS

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Objectives:

The aim of this study was to compare, under resting conditions, the influence of chronic training in swimming or running on blood pressure (BP), basal heart rate (HR), plasma concentration and mRNA expression of atrial natriuretic peptide (ANP) in the atria were investigated, as well as in the kidney, mRNA expression subtypes of natriuretic peptide receptor (NPR) were quantified and was investigated the affinity of NPR-C by ANP.

Methods and Results:

Methods: Two-month-old male SHR were divided into three groups: sedentary (SD) and swimming (SW) or running (RN) trained. Running training was performed on a motorised treadmill for 8 weeks, five day/week for 60 min, gradually increasing to 24 m/min (12-24 m/min), as previously described by Pittis W et al. (Comp Biochem Physiol 106: 285-89, 1993). The training sessions consisted of swimming sessions five days/week for 60 min for 8 weeks in an apparatus adapted for rats containing warmed water (30-32°C). Exercise duration and workload were increased gradually until the rats could swim for 60 min wearing caudal dumbbells weighing 5% of their body weight (Comp Biochem Physiol A Mol Integr Physiol 130: 21-27, 2001). Thereafter, the duration and workload were constant. These intensities of both exercise training are according to Maximal Lactate Steady State. Forty-eight hours after the end of the exercise training sessions, body weight, such as BP and heart rate (HR) at rest were measured by direct measurement. Plasma and atrial ANP concentrations were measured by radioimmunoassay. ANP mRNA levels in the right and left atria as well as natriuretic peptide receptors (NPR) A and C mRNA in the kidney were determined by real-time PCR. Autoradiography was used to quantify NPR-A and NPR-C in mesenteric adipose tissue.

Results: Both training modalities, swimming and running, reduced mean BP compared to the SD group (110 ± 11, 130 ± 5.9 vs. 105 ± 10, 125 ± 7.9 in the SW and RN group, respectively). The ANP plasma concentration was significantly lower in the SW group compared to the RN and SD group. In the atria, the expression of ANP mRNA was higher in the SW group compared to the RN and SD group. Moreover, the expression of NPR-A mRNA was higher in the RN group compared to the SW and SD group. In the kidney, the expression of NPR-A and NPR-C mRNA was similar in all groups. There were no significant differences in the expression of NPR-B mRNA.

Resumo:03-119

INVOLVEMENT OF ATRIAL NATRIURETIC PEPTIDE (ANP) IN THE ARTERIAL PRESSURE REDUCTION BY SWIMMING BUT NOT RUNNING PHYSICAL TRAINING IN SPONTANEOUSLY HYPERTENSIVE RATS

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Objectives:

The aim of this study was to compare, under resting conditions, the influence of chronic training in swimming or running on blood pressure (BP), basal heart rate (HR), plasma concentration and mRNA expression of atrial natriuretic peptide (ANP) in the atria were investigated, as well as in the kidney, mRNA expression subtypes of natriuretic peptide receptor (NPR) were quantified and was investigated the affinity of NPR-C by ANP.

Methods and Results:

Methods: Two-month-old male SHR were divided into three groups: sedentary (SD) and swimming (SW) or running (RN) trained. Running training was performed on a motorised treadmill for 8 weeks, five day/week for 60 min, gradually increasing to 24 m/min (12-24 m/min), as previously described by Pittis W et al. (Comp Biochem Physiol 106: 285-89, 1993). The training sessions consisted of swimming sessions five days/week for 60 min for 8 weeks in an apparatus adapted for rats containing warmed water (30-32°C). Exercise duration and workload were increased gradually until the rats could swim for 60 min wearing caudal dumbbells weighing 5% of their body weight (Comp Biochem Physiol A Mol Integr Physiol 130: 21-27, 2001). Thereafter, the duration and workload were constant. These intensities of both exercise training are according to Maximal Lactate Steady State. Forty-eight hours after the end of the exercise training sessions, body weight, such as BP and heart rate (HR) at rest were measured by direct measurement. Plasma and atrial ANP concentrations were measured by radioimmunoassay. ANP mRNA levels in the right and left atria as well as natriuretic peptide receptors (NPR) A and C mRNA in the kidney were determined by real-time PCR. Autoradiography was used to quantify NPR-A and NPR-C in mesenteric adipose tissue.

Results: Both training modalities, swimming and running, reduced mean BP compared to the SD group (110 ± 11, 130 ± 5.9 vs.
160 ± 10 mmHg, respectively). Swimming, but not running training increased plasma levels of ANP compared to the sedentary group (SW: 241.6 ± 77.5; RN: 72.7 ± 53.6; SD: 115.3 ± 57.7 pg/mL). The expression of ANP mRNA in the left atrium was reduced in the RN compared to SD group (RN: 87.3±15.6 vs. SD: 169±24.19 mRNA expression ANP/S26 arbitrary units). The expression of NPR-A and NPR-C in the kidneys of SW group decreased significantly compared to SD group (SW: 0.0722±0.036 vs SD: 1.265±0.539, RN: 0.3182±0.1215 mRNA expression NPR-A/s26 arbitrary units; SD: 0.9338±0.4544 vs SW:0.27±0.0108, RN:0.98±0.043 mRNA expression NPR-C/s26 arbitrary units). Although swimming increased 125I-ANP binding to mesenteric adipose tissue, displacement by c-ANF was reduced, indicating reduction of NPR-C.

Conclusions:

The MAP reduction induced by exercise in SHR differs in its mechanisms between the training modalities, as evidenced by the finding that increased levels of ANP were only observed after the swimming regimen.

Keywords: Physical Exercise, Natriuretic Peptide Receptors, NPR-A, NPR-C, Fat Tissue

Financial Support: CNPq, CAPES, FAPES and FACITEC.

ENDURANCE TRAINING PREVENTS SKELETAL MUSCLE ATROPHY BY ACTIVATING IGF-I/AKT/MTOR PATHWAY IN A GENETIC MODEL OF SYMPATHETIC HYPERACTIVITY INDUCED HEART FAILURE

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Objectives:

Skeletal muscle atrophy is a hallmark of heart failure (HF) muscle myopathy being associated with exercise intolerance and poor prognosis. As mechanical stimulus increases IGF-I expression, which is involved in muscle gain of mass by activating AKT/mTOR pathway, we tested whether endurance training (ET) would prevent skeletal muscle wasting by increasing IGF-I/PI3K/AKT/mTOR signaling in mice lacking both &alpha2A and &alpha2C adrenergic receptor subtypes (KO), which display sympathetic hyperactivity-induced HF associated with ventricular dysfunction and 50% mortality at 7 mo of age.

Methods and Results:

We studied a cohort of KO and control (CO) mice randomly assigned into untrained and trained. ET consisted of 8-wk treadmill running sessions of 60 min, 5d/wk. Our results demonstrated that KO mice displayed exercise intolerance, reduced cross sectional area (CSA) of all fibers types in soleus muscle (ATPase histochemistry), and muscle dysfunction (rotarod test) accompanied by decreased soleus protein expression levels of IGF-I, PI3K and pAKT:AKT total (Western blotting) vs. CO mice. ET improved exercise capacity, reestablished CSA, muscle function and increased protein expression levels of IGF-I, PI3K and pAKT:AKT total in KO mice. In a subset of trained KO mice, treatment with rapamycin (1 mg/kg/d), a mTOR inhibitor, along the last month of training precluded the effects of ET on soleus CSA and muscle function, which suggests that mTOR is a key kinase involved in anti-atrophic effects of ET.

Conclusions:
Collectively, our results suggest that endurance training activates IGF-I/Pi3K/AKT/mTOR signaling paralleled by preserved soleus mass in HF mice, which highlights the potential anti-wasting effects of ET in HF.

Keywords: Heart Failure, Muscle Atrophy, Exercise

Financial Support: CAPES, CNPq and FAPESP

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Resumo:03-121

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Objectives:
The increase in brain temperature that occurs during physical exercise in cool or warm environment contributes to the voluntary interruption of exercise. The thermal clamp can also be used to isolate the responses of many physiological systems induced by exercise from the effects that are secondary to the exercise-induced increase in core temperature. Therefore, this study was aimed at investigating if running exercise without the increase of brain temperature and performed until the voluntary interruption of exercise (VIE) changes physical performance when compared to a same-intensity exercise that induces hyperthermia.

Methods and Results:
All procedures were approved by the Ethics Committee for Animal Experimentation of Federal University of Minas Gerais (protocol 278/10). Male Wistar rats (270-315 g) were used in all experiments. Under anesthesia, the animals were fixed to a stereotaxic apparatus, an incision was made in the skin covering the skull at the midline, and a craniotomy was performed. A sterile stainless steel cannula (20 mm in length, 0.8 mm i.d. and 21 gauge) was implanted unilaterally so that the tip of the cannula was aimed at the VMH. The cannula was anchored firmly to the skull with screws and fixed with acrylic cement. The cannula was used as a guide to place the temperature sensor into the brain. After recovery from the surgery, the rats were gradually introduced to exercise on a treadmill by running them at a constant speed of 18 m/min−1 and 5% inclination during 5 min across 5 consecutive days prior to the experiments. The animals were then subjected to an exercise (20 m/min) until the voluntary interruption of exercise or resting on the treadmill belt (60 min) at two ambient temperatures: 12° C (to produce a thermal clamp) or 25° C (without a thermal clamp). Differences between trials were assessed by a multiple factor analysis of variance followed by the least significant difference test. A paired Student’s t-test was used to compare physical performance. Significance level was set as p

Conclusions:
Brain thermal clamp did not change the exercise performance. However, it is possible that the lack of difference between both experimental trials (i.e., with or without thermal clamp) was due to the small sample size.

Keywords: FATIGUE, HYPERTHERMIA, VOLUNTARY INTERRUPTION OF EXERCISE, THERMOREGULATION

QuebraPagina
SERUM NUCLEOTIDASES ACTIVITIES ARE ALTERED BY DIFFERENT PROTOCOLS OF EXERCISE IN WISTAR RATS

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Objectives:

Introduction: The exercise has been considered cardioprotective, improving lipid profile, and blood pressure. It has been recognized that extracellular nucleotides mediate prothrombotic and vasodilatatory responses, their levels are controlled by nucleotidases enzymes. Recently, our laboratory demonstrated that a neuroprotective exercise protocol, daily 20 min of training in treadmill during two weeks, diminished significantly the ADP hydrolysis and there is a trend to reduce the ATP hydrolysis in both hippocampal synaptosomes and blood serum of rats.Interestingly, three times a week during 12-week treadmill training induced an increase in ATP hydrolysis in serum. Objective: The purpose of this work was to examine the effect of training of different programs in treadmill during 12 weeks on extracellular ATP, ADP, and AMP hydrolysis in serum Wistar rats.

Methods and Results:

Methods: Adult male Wistar rats (2-3 months) were assigned to non-exercised (sedentary) group and exercised during 20 min-sessions on different programs (moderate-intensity exercise), one day or five days a week. The exercise training consisted of running sessions on an adapted motorized rodent treadmill at 60% of their maximal oxygen uptake. The effects of physical activity on hydrolysis of ATP, ADP and AMP were assayed in serum approximately 16 h after the last training session. The sedentary groups were taken as 100%. Data were expressed by mean ± standard deviation and analyzed by ANOVA-one way followed by Tukey post-hoc. Results: Both tested protocols increased of ATP hydrolysis in serum. Treadmill training sessions one time and five times a week for a 12 week induced a significant increase (respectively, almost 80% and 115%) on ATP hydrolysis compared with the sedentary group (n=5, 100.0±6.2; one time per week = 181.1±38.9%, n=6; five times a week = 212.1±70.6% of control, n=5) (P

Conclusions:

Conclusion: Both protocols induced an increase in ATP hydrolysis in serum, what may result in ADP accumulation and consequently platelet aggregation. We can suggest that this finding may be related to an enhanced risk of occlusive thrombus formation under exercising conditions. Additionally, five times a week for a 12 week induced a significant increase on AMP hydrolysis, what may result in adenosine accumulation. Since adenosine is recognized as an inhibitor of platelet aggregation and a vasodilator that can decrease arterial blood pressure, this mechanism can be involved with cardiovascular properties of exercise.

Keywords: exercise, nucleotidases, rats

Financial Support: Sources of research support: CAPES, CNPq, FIPE/HCPA.
Objectives:

The aim of this study was to evaluate the effect of different exercise intensities on the changes in thalamic temperature in untrained rats during treadmill running until voluntary interruption of effort (VIE).

Methods and Results:

Six male Wistar rats weighing 333.6 ± 20.4 g were used in the experiments. Rats were housed in individual cages under a 14/10 light-dark cycle at a room temperature of 24 ± 1.3 °C, with free access to water and rat chow. Under anesthesia, a sterile stainless steel cannula (18.0 mm in length, 0.8 mm o.d., 0.6 mm i.d.) was stereotaxically implanted into the thalamus. The rats were allowed to recover for at least 5 days. After this period, they were subjected to a familiarization procedure by running daily on a motor-driven treadmill at 18 m/min; 5% inclination for 5 min/day across 5 consecutive days prior to the experiments. Exercise was performed on treadmill with a velocity of 21 or 24 m/min until the VIE at a room-controlled temperature of 24.44 ± 0.18 °C. Data are expressed as means ± S.E.M. The analysis of thalamic temperature was performed using a two way repeated measures ANOVA followed by the post-hoc Tukey test. Paired Student’s t-tests were used to compare total exercise time (TET) and brain heating rate (BHR) between experimental trials. The association between BHR and TET was assessed using Pearson’s correlation coefficient. Significance level was set at p < 0.05. Thalamic temperature increased from the 3rd minute and remained elevated until the end of exercise in both trials. No differences were observed between the intensities throughout the exercise period (at the beginning of exercise: 38.34 ± 0.35 °C, 21 m/min vs 37.95 ± 0.28 °C, 24 m/min; p = 0.08; and at the IVE: 40.09 ± 0.53 °C, 21 m/min vs 39.88 ± 0.31 °C, 24 m/min; p = 0.33). There was no difference in TET (70.7 ± 27.1 minutes, 21 m/min vs 39.1 ± 17.0 minutes, 24 m/min; p = 0.35) or BHR between trials (0.07 ± 0.03 °C/min, 21 m/min vs 0.11 ± 0.03 °C/min, 24 m/min; p = 0.37). However, regardless of the intensity of exercise, the animals that had higher rates of rise in thalamic temperature were those who ran less, as demonstrated by the inverse correlation between BHR and TET (r = -0.88; p = 0.0002).

Conclusions:

Increasing exercise intensity from 21 to 24 m/min did not change the exercise-induced increase in brain temperature and physical performance.

Keywords: Brain Temperature, Fatigue, Thermorregulation

Financial Support: CAPES, CNPq and FAPEMIG.

Objectives:

Human walking is one of the most complex neuromuscular events, as several muscular groups are activated at different phases of stride cycle. Organized descending motor control commands (central pattern generator), act like a local neural circuit in order to rapidly synchronize muscle activation. Lower limbs, in bipedal walking, have a complex task during gait dynamics and this
complexity is considered as one of factors involving human cortex evolution. Corticospinal and corticobulbar pathways project their information from upper motor neurons to initiate complex voluntary movements. The movement of a single limb during locomotion can be thought as a cycle consisting of two phases: (a) a stance phase (StP), during which the limb is extended (in the present study divided into two halves –StP and +StP); and (b) a swing phase (SwP), during which the limb is flexed to leave the ground and then brought forward to begin the next StP. The study of the neuromuscular activation, i.e., the amount of muscle contraction at different phases of the stride cycle during walking, can lead to practical use, such as functional electrical stimulation (FES) and exoskeletons development for assisted walking. An artificial neural network-based technique for neural prostheses, for example, is only possible by means of detailed knowledge of neuromuscular behavior. Over the last decade, implantable and non-invasive neuroprostheses, such as FES, neural implants, and robotic limbs, hold the promise to improve functional movement in neurorehabilitation programs. The purpose of present study was to quantify neuromuscular activity at different moments of walking stride cycle, i.e., -StP, +StP and SwP, by means of electromyography (EMG) technique.

Methods and Results: 10 healthy subjects (4 men 29.1±5.9 years, leg length 0.97±0.06 m and body weight 82.9±7.9 kg; 6 women 28.7±2.1 years, leg length 0.87±0.04 m and body weight 57.3±8.2 kg) were instructed to walk at 4.5 km.h⁻¹ on a treadmill during 5 minutes. Muscular activation was measured in 7 different muscles (tibialis anterior, soleus, peroneus brevis, medial gastrocnemius, vastus lateralis, rectus femoris and biceps femoris) considered to be representative of the main muscular groups involved in gait activity. The root mean square percentage of EMG activity (relative to the 100% of step cycle) was calculated for each gait phase (i.e., -StP, +StP and SwP) for each subject. Neuromuscular activation along the stride cycle was, for -StP, +StP and SwP respectively, of 54.25±6.09%, 16.76±5.77% and 28.99±4.78% for tibialis anterior; 28.51±6.48%, 53.33±8.81% and 18.15±5.39% for soleus; 31.88±9.23%, 46.06±10.32% and 22.06±7.46% for peroneus brevis; 30.25±10.91%, 15.32±7.01% and 54.43±13.34% for biceps femoris; 22.95±10.52, 61.52±12.62 and 15.52±8.19 for medial gastrocnemius; 53.68±11.05%, 15.33±6.64% and 31.00±7.20% for vastus lateralis; 51.74±9.09%, 23.71±7.70% and 24.55±6.29% for rectus femoris. Neuromuscular activation patterns can be recognized such as higher activation at -StP for tibialis anterior, vastus lateralis and rectus femoris, at +StP for soleus and medial gastrocnemius, at SwP for biceps femoris and balanced activation during the stride cycle for peroneus brevis.

Conclusions: These findings constitute a first step in understanding neuromuscular activation during walking and its practical application, such as FES.

Keywords: electromyography, neuromuscular, walking

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Resumo:03-125

HEART WEIGHT CAN CONTRIBUTE TO INTRINSIC AEROBIC CAPACITY: PRELIMINARY DATA


Objectives: Determine whether animals with different intrinsic aerobic capacities are different on weight of cardiac and/or muscle tissues.

Methods and Results: 39 male Wistar rats, weighting 267 ± 11 g were used in this study. They were kept in a room with controlled light/dark cycle (14-10 h), ambient temperature at 24°C, with free access to chow and water. After one week of treadmill familiarization, rats were
submitted to three progressive exercises tests until fatigue (initial velocity: 10 m.min\(^{-1}\) and increased by 1 m.min\(^{-1}\) each 3 minutes). Based on the time of exercise (TF), a frequency histogram was developed and animals were divided in three groups: low capacity running (LCR), standard capacity running (SCR) and high capacity running (HCR). To be included in the LCR group, the animal needed to have a TF that was lower than one standard deviation below the average. In contrast, animals with a TF of more than one standard deviation above the average were included at HCR group. One week following, animals were euthanized and heart, soleus and gastrocnemius muscles were removed and weighted. All data are expressed as mean ± SD. TF was assessed using normality test and post hoc Shapiro-Wilk. ANOVA following Newman-Keuls test was used for determining differences on weight of tissues between groups. Significance level was set at p ≤ 0.05. Mean TF was 42.0 ± 15.9 min including all groups. Based on this result, 15.4% of the animals were inserted on LCR (TF: below 26.1 min), 66.7% in SCR (TF between 26.1 and 57.9 min) and 17.9% in HCR (TF above 57.9 min). Heart weight of HCR (n = 3; 4.26 ± 0.55 mg/g) was higher compared to SCR (n=6; 3.53 ± 0.33 mg/g; p=0.016) and LCR (n = 4; 3.46 ± 0.21 mg/g; p = 0.037). No significant differences were found on weight of gastrocnemius and soleus.

Conclusions:
Heart weight may be one of the parameters that contribute to the difference on intrinsic aerobic capacity in rats.

Keywords: INTRINSIC AEROBIC CAPACITY, HEART, EXERCISE

Financial Support: FAPEMIG, CNPq, CAPES

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Resumo:03-126

INCOMPLETE PARASYMPATHETIC RECOVERY AFTER SUCCESSIVE BOUTS OF MAXIMAL EXERCISE IN ROWERS

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Objectives:
High intensity exercise induces heart rate variability (HRV) reductions and changes in autonomic balance towards sympathetic dominance. The effects of one exercise bout on HRV recovery depends on intensity and duration. The aim of the present study was to investigate HRV recovery after two bouts of maximal exercise in highly fit subjects.

Methods and Results:
Competitive rowers (n= 14.11 males, 24±6 years old) performed two 2000m maximal bouts (2k1 and 2k2) of rowing in rowergometer (Concept2 D. USA) separated by four hours of rest. Times series of RR intervals were recorded before (PRE1) and immediately after 2k1 (POS1), at rest four hours after POS1 (PRE2) and after the second bout of exercise (POS2). Autonomic markers of parasympathetic modulation of HR were used in time and frequency domains, respectively the root mean squared of the sum of successive differences (rMSSD) and the spectral power for the high (0.15<HF0.05). The rMSSD showed lower values for PRE2 (48±29ms) compared to PRE1 (68±35 ms, p0.05).

Conclusions:
Autonomic modulation of resting heart rate was still modified four hours after maximal whole body exercise, showing incomplete recovery of vagal control of the heart and long lasting effects of exercise. No changes in vagal reactivation were found,
suggesting that different mechanisms may be related during rest and after maximal exercise.

Keywords: exercise, heart rate variability, parasympathetic, post exercise

Financial Support: CNPq (481434/2008-9)

HANDGRIP STRENGTH, GLUCOSE, LACTATE AND RATE OF PERCEIVED EXERTION IN BRAZILIAN JIU-JITSU COMPETITION

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Objectives:
This study aimed to investigate the physiological response to a regional combat performed by male adult Brazilian Jiu-Jitsu athletes.

Methods and Results:
The study included 12 Brazilian Jiu-Jitsu fighters graded blue belt (22.5 ± 4.4 years old, 74.5 ± 4.8 kg). The combats had a maximum duration of six minutes. Blood samples (25ìl) were obtained at rest and immediately after the combats at the earlobe. Lactate was analyzed on a Yellow Springs Sport 1500®. Blood glucose was determined in Optium Xceed® portable analyzer. Maximal isometric grip strength was performed using a Takei Kiki Kogyo® dynamometer, adjusted according to hand size, before and after the combat. Two attempts were performed for each hand in each moment, and the highest value was considered. Rate of perceived exertion (RPE) was also accessed after the combat using the 6-20 Borg Scale. The athletes were asked to indicate, on an anatomical diagram of the anterior and posterior views of the body, the areas they perceived to experience most exertion during combats. The athletes were asked how much they perceived the exertion and which muscles or muscle groups they felt were involved. Data were processed using the Excel® and SPSS® 15.0. Normality was accessed using Shapiro-Wilk test. Student t test and Wilcoxon test were used to compare moments of measurement. Significance level was set in 5%. As a result of the combats the lactate increased significantly (before: 0.5 ± 0.2 mMol/L, after: 6.2 ± 2.3 mMol/L), such much the glucose (before: 96.0 ± 9.3 mg/dL, after: 147.9 ± 34.5 mg/L). The activity performed during the match resulted in significant reductions in handgrip strength (right hand grip before: 37.7 ± 7.0 kgf, after: 33.5 ± 6.6 kgf) (left hand grip before: 32.9 ± 6.1 kgf, after: 29.8 ± 5.5 kgf). The rate of perceived exertion after combats was 15 ± 3. The athletes showed as fatigue points the forearm (67%), wrist (8%) and thighs (8%).

Conclusions:
The results showed that Brazilian Jiu-Jitsu fights affect glycolytic pathways and resulted in significant reductions in handgrip strength. However, the rate of perceived exertion was not high. This may be due to the rotation of large muscle groups and participation of small muscle groups, which do not significantly affect the subjective perception of effort.

Keywords: combat sport, energy demands, performance
SEROTONERGIC SYSTEM IS INVOLVED IN MODULATION OF FOOD INTAKE AND BODY WEIGHT DEPENDENTLY OF DIET OR PHYSICAL ABILITY.

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Objectives:

Serotonin (5-HT) has been associated with neuromodulation of food intake and its greater cerebral availability is related to increased metabolic rate and reduced body mass. It is known that exercise training alters serotonergic system increasing the sensitivity of their receptors and modifies the energy metabolism and body composition. However, it is unclear the role of serotonergic system on food intake of trained animals fed with high palatability diet, a model to induce obesity. This way, the aim was to investigate the influence of the central serotonergic system on food intake in obese and trained rats.

Methods and Results:

Weanling Wistar rats (60-80 g) were kept in individual cages with free access to standard diet (SD) or high palatability diet (HPD) and water for 17 weeks. At the end of eighth week, rats underwent a progressive exercise test until fatigue (PT) to measure the capacity race. In sequence, animals were divided into groups: non-trained (NT) and trained (T) rats and submitted to physical training in a treadmill running for 8 weeks. The intensity and duration were progressively increased during training, starting at 45% maximum velocity (Vmax) of PT for 30 minutes, reaching 65% Vmax and a 1 hour race. At the end of the fourth and eighth weeks of training, rats were conducted at the second and third PT to adjust the exercise intensity and to verify the improving on physical performance. Throughout the experiment, food intake and body mass of all animals were crops. 48 h after the end of the third PT, animals were euthanized and their brains were removed. It was carried out punch of brain areas (hypothalamus and hippocampus) to measure the concentrations of 5HT and 5HIAA. Body weight and food intake were greater in HPD animals compared to SD. Physical training increased Vmax and workload performed. The variation of body mass after exercise training was lower in T compared to NT group (102.5 ± 7.1 g, SDNT; vs 79.2 ± 5.5 g, SDT; vs 183.5 ± 8.8 g, HPDNT; vs 136.6 ± 7.6 g, HPDT; p < 0.01). Furthermore, there was correlation between the ratio of 5HIAA/5HT into hypothalamus and body weight in rats fed with HPD (r = 0.65; p< 0.002). This way, correlation was different between groups trained or treated with HPD.

Conclusions:

Serotonergic system is involved in modulation of food intake and body weight dependently of diet or physical ability.

Keywords: Physical training, Serotonin, food intake, body weight, obesity

Financial Support: CNPq, FAPEMIG and Ministério dos Esportes

THE ROLE OF RB PROTEIN IN THE TUMORIGENESIS OF COLON AND ESOPHAGUS CANCER

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Objectives:
The mediators and cellular effectors of inflammation are important constituents of the local environment of cancer (Nature 420:860, 2002). Patients with chronic inflammation are predisposed to develop cancers. Inflammatory diseases that affect the intestine, such as ulcerative colitis, release mediators of inflammation such as TNFα, leading to an increased risk of developing cancer of the colon and rectum. Similarities between the epithelium and intestinal metaplasia of the esophagus suggest the involvement of similar molecular processes in both diseases (Gut. 44:775, 1999). Among the signaling pathways involved in the pathogenesis of colon cancer and esophagus, the retinoblastoma (Rb), as a tumor suppressor protein, is an important target of study. During the cell cycle progression, Rb protein is inactivated by phosphorylation, and during cell death, Rb is cleaved by caspases at its C-terminal domain. The intestinal tissues from mice carrying Rb mutated at the caspase site were protected from inflammation-induced apoptosis. Therefore, Rb has role in decreasing sensitivity to cell death induced by inflammation. The aim of our study is to evaluate the role of anti-apoptotic phosphorylated Rb in colon cancer, chronic inflammation and cancer associated with inflammation.

Methods and Results:
The human colon cancer cell line HCT 116 was treated with inhibitor of Cycling-Dependent Kinase (roscovitine), which phosphorylate Rb. The labeling of phosphorylated Rb (ppRb) was performed by immunofluorescence. Biopsies from patients suffering from Barrett's esophagus and esophageal adenocarcinoma were subjected to immunohistochemistry for ppRb. The roscovitine pretreatment increased TNF-induced cell death, suggesting that presence of Rb phosphorylated has an anti-apoptotic role. Analysis of biopsies of Barrett's esophagus (n = 14) and esophageal adenocarcinoma (n = 7) revealed that there is a progressive increase in the levels phosphorylated Rb respectively in inflamed tissue and tumor samples. The increased levels of Rb were not detected in adenocarcinomas not related to inflammation (n = 3). We intend to analyze esophageal tumor cell lines TE-7 and TE-13 using the same treatment used in HCT116 with roscovitine. To associate the results of colon cells will work with biopsies of colon cancer with a history of ulcerative colitis.

Conclusions:
Our preliminary results suggest that ppRb has an anti-apoptotic role in both colon and esophageal tissues.

Keywords: Retinoblastoma, Cancer, Esophagus, colon

Financial Support: CNPq, FAF/ONCO III
for its application in national production. Among these animals is silver mojarra (*Diapterus rhombeus*), a widely distributed fish in the estuaries of the Brazilian Northeast region. Because of its good acceptance in the local market and the undeniable reduction in the natural stocks, the production of this species in captivity is promising. However, the lack of information about its digestive physiology, especially of their digestive enzymes, is an obstacle to the viability of commercial cultivation of these organisms. Therefore, aimed to investigate, characterize and purify a trypsin from digestive tract of the *D. rhombeus*.

Methods and Results:

Specimens of *D. rhombeus*, with average weight and length 350 ± 20 g and 28 ± 2 cm, respectively, were obtained from a fishing community in Itapissuma, Pernambuco, Brazil. Fish intestines and pyloric caeca were mixed together and homogenized at a concentration of 40 mg/mL (w/v) of tissue in a solution of 0.01 M Tris-HCl, pH 8.0 with 0.9% NaCl and centrifuged to obtain the enzyme extract. The purification was performed with heat treatment (45 °C for 30 min), ammonium sulphate precipitation and size exclusion chromatography (Sephadex G-75). The molecular mass was estimated in gel electrophoresis (SDS-PAGE). It was used 8 mM BApNA as specific substrate in the enzyme activities, which were performed at 25 °C and pH 8.0. Assays were carried out with specific inhibitors of trypsin (TLCK and Benzanidine) and serine proteases (PMSF). The enzyme activity obtained in the absence of inhibitors was taken as being 100% (control). The influence of pH on the purified enzyme (optimum pH and stability) was determinate using different buffer solutions with pH from 4.0 to 11.0. The optimum temperature and thermal stability were evaluated at temperatures ranging from 25 to 80 °C. The purified enzyme had an estimated molecular mass of 26.5 kDa and its specific activity (100%) was 155.65 ± 0.3 mU.mg⁻¹. It lost 33%, 75% and 100% of its activity on the presence of PMSF, Bezanidine and TLCK, respectively. Higher enzyme activity was observed at pH 8.5 and 55 °C, using BApNA as substrate. The enzyme was completely inactivated after 30 min at 55 °C and it was significantly more stable at alkaline pH.

Conclusions:

The results of the present study suggest that the peptidase purified from *D. rhombeus* is a trypsin. It was observed, by in vitro tests, that it dependent of the basic conditions such as pH and temperature in the hydrolysis of the substrate. Thus, it was possible to better understand the potential of this digestive enzyme, which provides information for the development of an adequate diet for the intensive cultivation of this fish.

Keywords: *Diapterus rhombeus*, Peptidase, Trypsin

Financial Support: CNPq, SEAP/PR, FINEP/RECARCINE, FACEPE PETROBRAS AMBIENTAL e Embrapa

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**THE POTENTIAL SPASMOLYTIC ACTIVITY OF CARVONE IN GASTROINTESTINAL TRACT OF MICE.**

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Objectives:

The essential oil of *Carum carvi* possesses pharmacological activities, although the effects of these activities on the gastrointestinal tract are unknown. Carvone, the principal constituent of this oil, is widely used in aromatherapy and alternative medicine (Food chem. 95:413, 2006).
Methods and Results:

In our work, we evaluated the influence of carvone on gastric retention (GR), intestinal transit (IT) and changes in intragastric pressure (IP) of a liquid test meal in awake mice. Male Swiss mice (30 - 40g, N=115) were randomly treated to oral doses of vehicle (Cremophor 2%, control group) or Carvone with doses 10, 50, 100 or 300 mg/Kg (experimental groups). Thirty min later, they were fed with a liquid test meal (0.3 mL phenol-red 0.5 mg/ml in glucose 5%). After 10, 20 or 30 min post-prandial period, the animals were sacrificed by a cervical dislocation, so as to study GR or measure the IT (J Physiol Sci. 60:75, 2010). In addition, we evaluated changes in IP (Neurogastroenterol Motil. 21:430, 2009) after vehicle or Carvone 100 mg/kg treatments. The GR at 10 - min post-prandial period in the control group was (40.7 ± 4.0%), a value that is significantly (p>0.05) between the control and the carvone 100 mg/kg group at 20 - min post-prandial period. Yet in relation the basal values, the Carvone treatment induced a decrease (p>0.05) in the amplitude of gastric tonus (24.2 ± 3.6 vs. 10.7 ± 2.8 mmHg), which however remained in stable before and after Cremophor treatment in control mice (29.6 ± 2.9 and 24.6 ± 3.0 mmHg, respectively). Besides, Carvone treatment also delayed IT of the liquid test meal (54.5 ± 3.6 %) in comparison to that of the respective control group (72.9 ± 3.1%).

Conclusions:

Carvone inhibits gastrointestinal motility, expressing probable antispasmodic properties; thus could be used in problems involving gut dismotility.

Keywords: Alternative Medicine, Carvone, Gastrointestinal tract, Mice

Financial Support: CNPq and UNIVASF

QuebraPagina

Resumo:04-040

EXPRESSION OF THE RECEPTOR TIE2 BY RT-QPCR IN LIVERS FROM CHILDREN WITH BILIARY ATRESIA.

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2 Universidade Federal do Rio Grande do Sul, UFRGS
3 Hospital de Clínicas de Porto Alegre, HCPA

Objectives:

To study the expression of the receptor Tie2 in the livers from children with biliary atresia (BA), comparing with those from patients with intrahepatic cholestasis (IHC). Tie2 is a tyrosine kinase receptor of the angiopoietins 1 e 2, angiogenic factors involved in formation, remodeling and maturation of blood vessels in physiological and pathological conditions such as chronic cholestasis and hypoxia, in this case leading to fibrogenesis.

Methods and Results:

This study was approved by the ethical and research commit of the Hospital de Clínicas de Porto Alegre. Expression of Tie2 was studied by RT-qPCR. Liver samples obtained from patients during exploratory laparotomy were frozen at -80°C. mRNA was extracted using the BRAZOL kit (LGC Biotecnologia, Brasil). cDNA was synthesized from randomic primers using reverse transcriptase enzyme (Invitrogen). qRT-PCR was performed with SYBR Green (Invitrogen). The reference gene was ribosomal 18S. The mathematical model for analysing gene expression was &Delta &Delta-CT. Samples were divided in two groups, BA (n=26) and IHC (n=9). Mann-Whitney test was performed. Results: The expression of Tie2 by RT-qPCR in BA and IHC was 17.38 (SD 8.7) and 19.78 (SD 3.6), respectively, and there was no significant difference in its expression between groups (P=0.55).
Conclusions:

In the samples analyzed, there was no difference in the expression of Tie2, evaluated by RT-qPCR, in the livers from patients with BA and IHC. (This study is part of the study “Expression of Angiopoietin 1 and 2, and Tie2 receptor in livers from patients with biliary atresia”).

Keywords: BILIARY ATRESIA, LIVERS, NEONATAL CHOLESTASIS, RECEPTOR TIE2

Financial Support: FAPERGS and FIPE (Fundação de Apoio à Pesquisa do Hospital de Clínicas de POA).

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Santos, K. C. 1; Silva, S. J. B. 1; Wanderley, C. W. S. 1; Sousa, L. N. 1; Santos, R. G. S. 1; Silva, C. M. S. 1; Monteiro, F. M. F. 1; Santos, A. A. 2; Palheta Jr, R. C. 1

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2 Departamento de Fisiologia e Farmacologia, UFC

Objectives:

The essential oil of *Carum carvi* possesses pharmacological activities, although the effects of these activities on the gastrointestinal tract are unknown. Carvone, the principal constituent of this oil, is widely used in aromatherapy and alternative medicine (Food chem. 95:413, 2006).

Methods and Results:

In our work, we evaluated the influence of carvone on gastric retention (GR), intestinal transit (IT) and changes in intragastric pressure (IP) of a liquid test meal in awake mice. Male Swiss mice (30–40g, N=115) were randomly treated to oral doses of vehicle (Cremophor 2%, control group) or Carvone with doses 10, 50, 100 or 300 mg/kg (experimental groups). Thirty min later, they were fed with a liquid test meal (0.3 mL phenol-red 0.5 mg/ml in glucose 5%). After 10, 20 or 30 min post-prandial period, the animals were sacrificed by a cervical dislocation, so as to study GR or measure the IT (J Physiol Sci. 60:75, 2010). In addition, we evaluated changes in IP (Neurogastroenterol Motil. 21:430, 2009) after vehicle or Carvone 100 mg/kg treatments. Results: The GR at 10-min post-prandial period in the control group was (40.7 ± 4.0%), a value that is significantly (p>0.05) between the control and the carvone 100 mg/kg group at 20-min post-prandial period. Yet in relation the basal values, the Carvone treatment induced a decrease (p>0.05) in the amplitude of gastric tonus (24.2 ± 3.6 vs. 10.7 ± 2.8 mmHg), which however remained in stable before and after Cremophor treatment in control mice (29.6 ± 2.9 and 24.6 ± 3.0 mmHg, respectively). Besides, Carvone treatment also delayed IT of the liquid test meal (54.5 ± 3.6 %) in comparison to that of the respective control group (72.9 ± 3.1%).

Conclusions:

Carvone inhibits gastrointestinal motility, expressing probable antispasmodic properties; thus could be used in problems involving gut dismobility.

Keywords: Estradiol Cypionate , Gastric emptying, Neurohumoral Pathways
ANAEROBIC TRAINING INCREASES GASTRIC COMPLIANCE BUT DELAYS GASTRIC EMPTYING IN RATS. DEPARTAMENTO DE FISIOLOGIA E FARMACOLOGIA – UFC/FORTALEZA-CE, BRASIL. FACULADE DE MEDICINA – UFC/SOBRAL-CE, BRASIL.

Oliveira, F. G. V. D. ; Silva, M. T. D. ; Okoba, W. ; Campos, C. P. S. ; Pinheiro, A. D. N. ; Mendes, . . S. ; Rocha, F. S. ; Gomes, F. J. ; Graça, J. R. V. D. ; Goiana, S. W. ; Santos, A. A. D.
Depto de Fisiologia e Farmacologia Faculdade de Medicina , UFC-Ce

Objectives:

Introduction: Published data show that anaerobic training (AnaT) in rats promotes adaptive changes in matrix of metalloproteinases (MMPs) (Int. J Sports Med 29:559-63. 2008). However, few studies have investigated the effects of this type of training on the gastrointestinal tract. Thus, we aimed to study the effect of AnaT on the gastric retention (GR) and gastric compliance (GC) in rats. Objectives: To investigate the effect of AnaT on the GR, GC, hemodynamic parameters and weights of organs in rats.

Methods and Results:

Methods: after being approved by the institution ethics committee, N°82/10, male Wistar rats (N= 230-280g) were divided into two main groups sedentary (S) and Anaerobic Training (AnaT). The trained rats were initially subjected to an adaptation session in a water pool. AnaT consisted of rats undertaking jump-exercises (4x10 jump-cycles over 30min. - 5/days/week/4week loaded with 40-70% BW) as specified in Hypertension, 46:1010-5. 2005. Exactly 24h after the last training, rats were anesthetized for the cannulation of femoral blood vessels and subsequent monitoring of the mean arterial pressure, MAP and the heart rate, HR. After recovery from anesthesia, we monitored the hemodynamic parameters over 40-min. before administering by gavage a liquid test meal (SG 5% phenol red) to assess 10min. postprandial GR (J Physiol, 131:452-62, 1956). The gastric compliance was assessed according to Planta Med 77:57-9, 2011. Data was expressed (mean ± SEM) compared by ANOVA and Tukey test.

Results: When compared to S, AnaT rats had lower resting bradycardia (360.1 ± 12.2 vs. 290.4 ± 8.0 beats.min p

Conclusions:

Four-week-anaerobic exercise training is capable of promoting significant resting bradycardia. Such training leads too, to physiological adaptations of heart, liver and colon increasing mass of the same. In relation to the gastrointestinal motility, the AnaT. decreases gastric emptying, suggesting that this phenomenon is due to increased gastric accommodation as evidenced in studies of gastric compliance, where AnaT rats showed increased GC when compared to S.

Keywords: ANAEROBIC TRAINING, GASTRIC COMPLIANCE, GASTROINTESTINAL MOTILITY

Financial Support: CNPq; Capes; Funcap.
Objectives:

The hydroxycitronellal is a compound widely used as fragrance in cosmetics. This compound can be obtained by semi-síntesis from citronellal, a terpenoid isolated from essential oil of citronella (Cymbopogon marginatus) or Eucalyptus (Eucalyptus maculata), and also found in other plants. The aim of this study is to demonstrate the gastroprotective of hydroxycitronellal in GASTRIC ULCER models.

Methods and Results:

Animal handling and experimental protocols were registered on the Institutional Ethics Committee (CEPA) under number 052/2011. Swiss mice were used, were divided into groups of 8 (n = 8), and undergo fasting of 16h, then were treated with HC in doses 0.5; 2.5 and 12 mg/Kg or NAC (750 mg/Kg). After 30 min they received 0, 2 ml of absolute ethanol per oral and after 30 min, the animals were sacrificed and stomachs removed and analyzed the lesion index and dosage of GSH (reduced glutathione).

In order to investigate the involvement of prostaglandins, NO and potassium channels, before treatment with HC animals received L-NAME(20mg/Kg) or L-arginine(600mg/Kg), indomethacin (10mg/Kg) or misoprostol(0.03µg/Kg), Glibenclamide(5mg/) or Diazoxide(3mg/Kg). To investigate the participation of TRPV1 receptors, animals received capsaicin (0,3mg/Kg) or capsazepina(5mg/Kg). In the model of injury by NSAID’s, the animals were treated with HC (12.5; 50 and 200 mg/Kg) or Cimetidine (100 mg/Kg) 30 min before treatment with indomethacin (60 mg/Kg), and after 6h animals were sacrificed and stomachs removed and examined under-rated scores. In model of injury by ethanol, HC at the doses 0, 5; 2.5 and 12 mg/Kg was able to prevent injury in 31.0; 52.9 and 69.3% respectively. HC also restored the GSH levels in mucosa in 31.19% compared to the ethanol group. LNAME, Glibenclamide and Indometacin were able to reverse the protective effect of HC, demonstrating the involvement of Prostaglandins, NO and potassium channels in its mechanism of action. Capsazepine was unable to reverse the effect of HC, thus excluding a possible involvement of TRPV1 receptors. In the model of injury by NSAID’s, HC in tested doses reduces the injury scores in 28.8, 56.3, and 84.1% respectively.

Conclusions:

We can conclude that the HC has pharmacological activity with gastroprotetor effect in the gastric mucosa. This protection appears to be mediated in part by modulation of Prostaglandin/NO/Katp, which is of great importance in mucosal defense and in maintaining blood flow to the stomach.

Keywords: HYDROXYCITRONELLAL, GASTROPROTETOR EFFECT , GASTRIC ULCER

Financial Support: CAPES and CNPq

QuebraPagina

Resumo:04-044

EFFECTS OF EXPERIMENTAL ULCERATIVE COLITIS ON THE P2X7 RECEPTOR AND ENTERIC NEURONS OF THE RAT DISTAL COLON.

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Objectives:

In the digestive tract the ulcerative colitis and Crohn's disease presenting as pathophysiological bowel necrosis. This project aimed to study the P2X7 receptor and chemical coding of the distal colon myenteric plexus of the rats subjected to ulcerative colitis experimental. The chemical code, area and neuronal density were analyzed in the enteric neurons immunoreactive to nitric oxide synthase (NOS), choline acetyl transferase (ChAT), calbindin, calretinin, and the P2X7 receptor.

Methods and Results:

We analyzed the distal colon of a) rats with ulcerative colitis by administration of TNBS (2,4,6-trinitrobenzene sulfonic acid) in the large intestine, for periods of 24 h b) Sham rats were injected with PBS and c) animals with no intervention (control group-CG). The tissues were prepared by immunohistochemical methods of double marking of P2X7 receptor with NOS, ChAT, calbindin and calretinin, anti-Hu. The stain of P2X7-IR receptor (immunoreactive), NOS-IR, ChAT-IR, calbindin-IR, calretinin-IR, and Hu-IR neurons were reduced in the Control, PBS and Colitis groups. In the colitis group neurons exhibited characteristics deformed, mainly in NOS-IR neurons. The colocalizations of P2X7 receptor-IR neurons have shown decreased in the colitis group, with NOS-IR by 4.7% (19.1%±1.7%), ChAT-IR by 9.7% (25.6%±1.4%), calbindin-IR by 10% (9.3%±0.3%) and calretinin-IR by 3.4% (12.4%±2.0%) and Hu by 6.8% (89.0±0.8). The neuronal density showed decreased by 42.28%, 34.9%, 22.87%, 60.57 % and 14.69% of the NOS-IR, ChAT-IR, calbindin-IR, calretinin-IR and Hu-IR neurons, respectively in the colitis groups. In the area of NOS-IR and ChAT-IR decreased 6.8% and 21% respectively. Calbindin-IR neurons increased 20% to the area in the colitis group.

Conclusions:

This study demonstrated that colitis affected the NOS-IR, ChAT-IR, calbindin-IR and calretinin-IR neurons that can cause alterations in gut motility.

Keywords: colitis, P2X7 receptor, myenteric plexus, distal colon, enteric neurons

Financial Support: FAPESP and CNPq

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Resumo:04-045

LUNG AND LIVER DAMAGE IN MICE SUBJECTED TO INTERMITTENT HYPOXIA - A SLEEP APNEA MODEL

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Objectives:

Sleep apnea promotes intermittent hypoxia, leading to oxidative stress and inflammation. Our objective was investigate the extent of pulmonary and hepatic injury in mice subjected to intermittent hypoxia (IH).

Methods and Results:
We exposed two-month old male C57Bl6 mice to IH (n=6) or to sham IH (SIH; n=6) in sealed acrylic chambers. The gas system controlled by a timer insufflated a mixture of N2 (90%) and CO2 (10%) for 30 seconds reducing oxygen concentration from 21 to 6%. Afterwards, room air was insufflated during 30 seconds. The cycles were repeated during eight hours daily, simulating sleep apnea. After 35 days of IH the animals were euthanized. Lung and liver samples were collected for assessment of lipid peroxidation (nmol / mg prot), superoxide dismutase (SOD – USOD/mg prot), catalase (CAT - nmol / prot) and expression of NF-kb by p65 portion by western blotting (AU). The IH group showed significantly higher lipid peroxidation in both lung (mean±SD. IH: 5.1±0.3; SIH: 4.7±0.3) and liver (IH: 3.6± 0.3; SIH: 3.0± 0.4). SOD was significantly lower in lung (IH, 4.6±0.55; SIH: 7.2±2.4) and higher in liver of the IH group (IH, 5.9±1.7; SIH, 3.1±1.3). CAT activity was significantly higher in both lung (IH: 3.5±0.3; SIH: 2.6±0.5) and liver (SIH: 0.9±0.3; IH: 1.9 ± 0.8). In the evaluation of NF-kb by its p65 portion, we found a significantly higher expression in IH in both lung (IH: 4.1 ± 0.7, SIH: 0.5 ± 0.5) and liver (IH: 0.7±0.1 SIH: 0.1± 0.01).

Conclusions:

These results suggest that the IH can induce lung and liver damage possibly by the involvement of oxidative stress and activation of NF-kB.

Keywords: Hipóxia Intermitente, Estresse Oxidativo, Apnéia do Sono

Financial Support: FIPE-HCPA, UFRGS, PIBIC/UFCSAP, ULBRA

THE IMPACT OF HIGH SALT DIET AND PHYSICAL EXERCISE OF MODERATE INTENSITY ON GASTRIC CONTRACTILITY AND OXIDATIVE STRESS

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Objectives:

The effects of physical exercise and high salt diet on gastric oxidative status and contractility are unknown. The understanding of this relationship is extremely important, since these factors have been related to the development of gastric cancer. This study aimed to assess the impact of high salt diet and physical exercise on gastric contractility and redox status.

Methods and Results:

30 male Balb/c mice, 7 weeks old, were randomly ascribed into four groups: sedentary (CT-SED, n=5), exercised (CT-EX, n=5), supplemented with high salt diet and sedentary (DI-SED, n=10), and supplemented and exercised (DI-EX, n=10). Body weight measurements were done every week. The groups CT-SED and CT-EX were fed with a standard diet AIN 76A throughout the procedure; and animals from DI-SED and DI-EX groups were fed with high salt diet (7.5% NaCl) from 8th week of life to animal death (20th week). Animals from groups CT-EX e DI-EX were adapted during 5 days to exercise environment and then performed a moderate exercise protocol, consisting of a treadmill running at 13 m/min. Exercise training was initiated at the 8th week of life and terminated 2 days before the animal death. Gastric contractility was assessed by the construction of concentration- isometric contractile response curves in isolated segments of the gastric fundus, stimulated with either carbachol or KCl for the evaluation of EC50 and Emax. The tissue redox status was assessed by protein oxidation, by measuring carbonyl radicals by the technique of Reznick. It was shown an increase of 3.0% in the body weight of CT-EX group, while the loss body weight of DI-SED and DI-EX groups were around 35%. Gastric maximal contractility (Emax) in response to carbachol was impaired by moderate exercise training independently of the animal diet, while the high salt diet significantly enhanced gastric
contractility in the sedentary group (CT-SED: 1.8 ± 0.1 g; CT-EX: 1.0 ± 0.1 g; DI-SED: 2.3 ± 0.3 g; DI-EX: 0.9 ± 0.1 g). Different result was observed in KCl-induced contraction. Gastric maximal contractility in response to KCl was decreased by exercise independently of the animal diet (CT-SED: 2.7 ± 2.1 g; CT-EX: 0.8 ± 0.1 g; DI-SED: 2.4 ± 0.3 g; DI-EX: 1.2 ± 0.4 g). The modified diet decreased the logEC50 of the carbachol-induced dose-response curves (CT-SED: -6.2 ± 0.1, DI-SED: -6.9 ± 0.3; CT-EX: 6.1 ± 0.3, DI-EX: -7.2 ± 0.7). Regarding KCl-induced contraction, no differences in logEC50 were observed (CT-SED -0.7 ± 0.7 g; DI-SED: -1.3 ± 0.2 g; CT-EX: -1.3 ± 0.3 g; DI-EX: -1.6 ± 1.0 g). The level of reactive carbonyl group was higher in DI-SED (9.09 ± 0.05 nmol/ml), compared to CT-SED (3.55 ± 0.05 nmol/ml), and, there was less radical formation in DI-EX (5.13 ± 0.05 nmol/ml) group, in comparison to the group DI-SED (9.09 ± 0.05 nmol/ml).

Conclusions:

The high salt diet increased gastric contractility and oxidative stress, while the physical exercise of moderate intensity has decreased contractility and oxidative stress.

Keywords: isometric contraction, stomach, high salt diet, exercise, oxidative stress

Financial Support: FAPESP, Centro Universitário São Camilo

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**NITREGIC PATHWAYS INVOLVED IN INTESTINAL TRANSIT BREAKE OF A LIQUID INDUCED BY OXYTOCIN IN RATS.**

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2 Departamento de Fisiologia e Farmacologia, UFC

**Objectives:**

The neurohypophysial hormone oxytocin (OT) causes due to uterotonict and milk-ejecting activity, OT also inhibit the gastric emptying and intestinal transit of a liquid in awake rats (Naunyn Schmiedebergs Arch Pharmacol. 367:406, 2003). In this work, we decided to evaluate the nitrergic pathways involved in intestinal transit break of a liquid induced by exogenous OT.

**Methods and Results:**

Female rats (n = 120, 180-220g) were anesthetized with intraperitoneal (i.p) ketamine hydrochloride (90 mg/kg) plus xylazine (10 mg/kg) to perform the ovariectomy and gastrostomy, and a separated group of animals submitted also a subdiaphragmatic vagotomy (VGX). After seven days they were randomly assigned to pretreatment with (i.p) saline 0.15 M (1 ml/kg), Atosiban (40 μg/kg), Hexamethonium Hydrochloride (10 mg/Kg), L-NAME (3 mg/Kg), L-NAME plus L-arginine (200 mg/Kg) or glibenclamide (1 mg/Kg). After 30 min, the rats were treated with (i.p) vehicle (saline 0.15 M - 0.1 ml/100g, named control) or oxytocin (OT, 5 μg, experimental groups). Twenty min later, they were fed with a test meal (1.2 ml - 0.5 mg/ml - 1 of phenol red in 5% glucose solution). After 10 min post-prandial period, the rats were sacrificed and the small intestine was carefully slightly stretched and removed for obstructive ligatures by determination of the test meal progression through the intestinal segments (Braz J Med Biol Res. 41:78, 2008). The data was expressed as median values within the interquartile range and (p< 0.05) the intestinal progression 2.9 [2.7 to 3.1] in comparison the respective values of control group 3.3 [3.1 to 3.7]. It occurred also in glibenclamide pretreated rats, those who received OT the intestinal transit progression were less 2.9 [2.6 to 3.1] in comparison to the respective values of control rats 3.6 [3.3 to 3.8].

**Conclusions:**
Oxytocin decreases the intestinal transit of liquids via nicotinic ganglionic pathways through Nitric Oxide, but this phenomenon is neither the vagus nerve nor potassium-ATP channels dependent.

Keywords: Intestinal transit, Nitric Oxide, Peptides

Financial Support: CNPq and FACEPE

QuebraPagina

Resumo: 04-048

CO INCREASES H2O AND ELECTROLYTES ILEAL SECRETION IN ANAESTHETIZED RATS

Fisiologia/Faculdade de Medicina, UFC

Objectives:

Hemeoxigenase (HO) enzyme present in many cells, comes in three isoforms (HO-1, inducible, constitutive HO-2 and constitutive HO-3?). Carbon monoxide (CO), biliverdin and free iron are products of the HO activity. Signaling HO/CO represent a pathway, not adrenergic/ non-cholinergic (NANC), in gastrointestinal tract (GI). Enterocytes express HO on the apical and basal membrane. Carbon monoxide with a neurotransmitter GI was shown by retardation of intestinal transit in mice knockout for the HO-2. We investigate the role of HO in the intestinal transport of water and electrolytes.

Methods and Results:

We utilized male Wistar rats, under 24h of fasting (180-240g, n=42) treated (s.c.) with Hemin (3mg/kg) (Heme) or 1mM NaOH buffer vehicle of Hemin (VHeme), DMDC (2.5µmol/kg) or TWEEN 80 (0.5%) vehicle of DMDC (VDMMC) and zinc protoporphyrin IX (ZnPP) (9mg/kg) or vehicle buffer Na2CO3 of Zinc (VZnPP). After 30min of treatment, rats were anesthetized (urethane 1.2g/kg, i.p.). Following laparotomy, a polyvinyl cannulas (OE 50mm and OI=30mm) were inserted into the terminal ileum (30cm). After the ileum was perfused (0.2mL/min) (Tyrode, 0.05mg/mL phenol red (PR) at 37°C during 60min). Concentrations of Na+, K+ and Cl- (mmol/L) and PR were determined in perfusate by flame photometry and colorimetry, respectively. Differences between the values of Na+, K+ and Cl- were used to calculate the ileal transport of electrolytes (uEq/g/min). Differences in concentrations of PR were used to evaluate water transport (mL/g/min). DMDC pretreatments promoted secretion of water and Na+ (0.055±0.05ml/g/min and 2.010±0.55uEq/g/min vs vehicle 0.02±0.02ml/g/min and -0.33±0.5uEq/g/min, respectively, p

Conclusions:

Results indicate the involvement of CO, a product of the activation of HO, as a pro-secretors molecule in the terminal ileum. These findings suggest, on the first hand, an important physiological role of HO in the control of gastrointestinal transport of water and electrolytes.

Keywords: Absorption, Carbon monoxide, Hemin, Ileal

Financial Support: CAPES, FUNCAP, FAMED-Sobral, CCZ-PMS and UFC

QuebraPagina
ANTIFIBROTIC EFFECT OF N-ACETYLCYSTEINE ON HEPATIC SCHISTOSOMAL GRANULOMA


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Objectives:
Assess the effect of N-acetylcysteine (NAC) in combination with praziquantel (PZQ) or not, about the deposit de collagen and morphometric aspects of schistosomal hepatic granuloma in mice infected with Schistosoma mansoni.

Methods and Results:
Swiss Webster mice were infected by 50 S. mansoni cercariae. Three experimental groups were divided in 4 subgroups (n=9): NAC, PZQ, NAC+PZQ and control. Oral administration of NAC (200 mg/Kg/day) was carried out on the first day after infection for the acute phase (G1) and on the 45th for the intermediate (G2) and chronic (G3) phases for 59 and 45, 75 days, respectively. PZQ (100mg/Kg/day), was given orally from the 45th to 49th day after infection. Mice were sacrificed 60 (G1), 90 (G2), and 120 (G3) days post-infection. Liver fragments were fixed and embedded in paraffin to obtain histological sections (5µm) stained with picrosirius red and Masson trichrome. Sections were used to evaluate the degree of fibrosis, classified as mild, moderate or intense, according to the arrangement, amount and size of collagen fibers. The morphometry took into consideration the measurement of the average number and diameter of granulomas. Images were obtained using a computer system (Motic Images Plus 2.0 MLTM). The experiment were approved by Federal University of Pernambuco’s Ethics Committee for Experiments on Animals. For all phases of the infection, the average number of granulomas in the PZQ and NAC+PZQ subgroups was found to be significantly lower (p

Conclusions:
Mice infected with S. mansoni and treated with PZQ have a reduced number of granulomas in liver tissue. Treatment with NAC and/or PZQ significantly reduced granulomas average diameter. In subgroups where the NAC was part of the treatment regimen showed a smaller deposit of collagen and the degree of liver fibrosis in schistosomal granuloma.

Keywords: Schistosomiasis mansoni, N-acetyl-L-cysteine, Praziquantel, Antifibrotic effect, Morphometric aspects

Financial Support: Fundação de Amparo à Pesquisa de Pernambuco – FACEPE and UFPE

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CINTOS

EFFECTIONS OF ORCHIDECTOMY ON THE EXPRESSION AND FUNCTION OF RAT CAUDA EPIDIDYMAL ALPHA1-ADRENOCEPTORS.

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Objectives:

α1-adrenoceptors (α1A-AR, α1B-AR and α1D-AR) are widely expressed in male reproductive tract. α1-AR activation on the peritubular smooth muscle of cauda epididymis (CE) is a key event to propel the sperm towards the vas deferens during ejaculation. Although it is well known that the structure and function of the epididymis depend on the sexual hormone milieu no studies have investigated the modulation of CE α1-ARs by gonadal steroid hormones. This study describes the effects of orchidectomy on the expression and function of α1-ARs in the rat CE as a part of our investigation focusing on the modulation of epididymal α1-ARs by the gonadal hormones testosterone and estradiol.

Methods and Results:

Adult male Wistar rats (120 days) were castrated by bilateral orchidectomy. Eight days after castration the rats were killed and the density (Bmax) of α1-ARs was determined by [3H]Prazosin binding to intact segments of proximal CE duct. In addition, the function of α1-ARs was assessed by conventional contraction studies. Segments (1.5cm length) of distal CE duct were isolated and mounted in 10ml organ baths to record of isometric contractions to noradrenaline. The α1-ARs mediating contractions of CE to noradrenaline were identified by evaluation of affinities (pKb) of selective antagonists Prazosin (α1-selective), Yohimbine (α2-selective), RS 100329 (α1A-selective) and BMY 7378 (α1D-selective) by Schild analysis. Castration by 8 days reduced by 61% (control: 633.7±26.5mg, n=6; castrated: 250.1±17.5mg, n=8; p

Conclusions:

The expression and function of CE α1-ARs are dependent on testicular factor(s). Interestingly albeit the receptor density was significantly reduced the functional subtype mediating contraction of epididymal smooth muscle remained the same. Experiments in progress will determine whether the androgen or estrogen withdrawal is responsible for the alterations observed. If the altered contractility of CE smooth muscle after castration is related to the hormonal disruption the present results highlight the fertility hazard of exposure to environmental compounds with (anti)androgenic/(anti)estrogenic action.

Keywords: ALPHA1-ADRENOCEPTORS, CAUDA EPIDIDYMIS, CONTRACTION, EXPRESSION, ORCHIDECTOMY

Financial Support: CAPES

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Resumo:05-032

OXYTOCIN IMPROVES SEXUAL BEHAVIOR OF MALE MICE

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Objectives:

The purpose of the present study was to investigate the role of oxytocin (OT) on the modulation of the sexual and reproductive behavior in male mice.

Methods and Results:

Male control (WT; C57BL/6) and OT knockout (OTKO) mice (WT n=3 and OTKO n=5; body weights of 26±1g) were genotyped using DNA prepared from tail extracts with a polymerase chain reaction (J Neuroendocrinol.8:847, 1996). They were maintained under a reversed light/dark cycle (12h/12h) at constant room temperature (approximately 22°C) with free access to water and food. For the test, each male was placed with a female for at least a week to gain sexual experience. After that, the males were placed alone and the estrous cycles of control females were done. The sexual test occurred on the evening of
proestrus. 1h after light out, at 5 o'clock, the behavioral tests were started in plexiglas arena (60 cm × 45 cm × 45 cm) under dim red illumination. Males were allowed to acclimate to the testing chamber for 10 min before introducing the female. The males from groups WT and OTKO where tested during 30 minutes. The test was recorded on video and analyzed with an event recording program (The observer version 2.0). Mean (±SEM) of behaviors was analyzed using Mann-Whitney test. Our preliminary results have shown that the latency (2.66 ± 0.88 versus 9.00 ± 1.48, p=0.035) and the duration (235 ± 0.57 versus 128 ± 16.23, p=0.036) of sexual investigation (when the male sniffs the female’s genital) were different between groups (control and OTKO group, respectively) showing a decline of interest in the OTKO group. The ejaculation latency was different between groups (1532 ± 268.3 versus 1800 ± 0.00). In the OTKO group no male ejaculated. However, the latency and frequency of mounts with intromission were not different.

Conclusions:

The results of this study indicate that OT modulates the sexual and reproductive behaviors of male mice, since OTKO males showed a decrease in sexual behavior.

Keywords: behaviour, mice, oxytocin, sexual

Financial Support: PROAP/UFCSPA

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Resumo:05-033

SUPEROXIDE DISMUTASE RESTORES THE ERECTILE DYSFUNCTION IN MIDDLE-AGED RATS.

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Objectives:

Aim: Epidemiologic studies have reported that aging is an independent predictor of erectile dysfunction (ED). We have previously shown that middle-aged rats exhibit ED, as evaluated by the impaired nitrergic-, endothelium-dependent, and endothelium-independent relaxations, along with increased adrenergic-mediated contractile responses (Silva FH et al, SBFTE 2010). The aging process causes an unbalance between reactive-oxygen species (ROS) production and antioxidant capacity of the tissues leading to cell damage. Superoxide anion rapidly reacts with nitric oxide (NO), forming peroxynitrite, causing a reduction on the NO bioavailability. The aim of the presence study was to verify whether oxygen-derived free radicals are involved in the ED of middle-aged rats. Therefore, we examined the effects of the superoxide anion scavenger superoxide dismutase (SOD) in the corpus cavernosum (CC) relaxations from control and middle-aged rats.

Methods and Results:

Methods: The experimental protocols were approved by the Animal Ethical Committee of UNICAMP (Nº 2110-1). Male Wistar rats were divided into two groups: (a) young (14-15 weeks) and (b) middle-aged rats (37-38 weeks). Relaxations induced by acetylcholine (ACh, 0.00001-10 mM), sodium nitroprusside (SNP, 0.00001-10 mM) and electrical field stimulation (EFS) were carried out in isolated corpus cavernosum from rat (RCC) in the absence and presence of SOD (75 U/ml) in all groups. Results: The relaxations induced by ACh and SNP in middle-aged rats were significantly lower in CC (Emax: 37±2% and 89±3%, respectively, P

Conclusions:

Conclusion: Our findings showed that SOD ameliorates the ED in middle-aged rats, suggesting that increased generation of superoxide anion in corpus cavernosum greatly contributes to this disorder.
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5 Centro de Pesquisas, HCPA

Objectives:

Most cancer treatments affects tissues responsible for cell growth and some are extremely gonadotoxic. Often, gonadal functions are irreversibly affected. Busulfan is an alkylating and cytostatic agent that impairs spermatogonial stem cell. The aim of this study was to evaluate the degenerative gonadal effect of busulfan at different periods after dilution in Wistar rats.

Methods and Results:

24 rats (60 days, 280g mean) were divided into control group (n=4) and 4 groups (n=5) treated with busulfan at a 30mg/kg single dose, administered at different periods after dilution (PAD, 7, 14, 21, 28 days). Testicles were weighted and measured. Spermatogenesis analysis included: number of spermatogonia per seminiferous tubule, necrosis degree and diameter of seminiferous tubules. Testicle masses of control group (1.96g±0.34g) were higher than the others (p < 0.05). Masses of groups with PAD of 21 days (1.60g ± 0.11g) and 28 days (1.58g ± 0.14g) were significantly higher than that of PAD of 14 days (1.28g ± 0.16g) and 7 days (1.11g ± 0.09g). Control group (1.53g ± 0.38g) showed testicle volumes significantly higher than groups with PAD of 14 (0.87g ± 0.12g; p < 0.01) and 7 days (0.85g ± 0.19g; p < 0.01). A significant association was detected (p < 0.001) when comparing the number of spermatogonia and the PAD, indicating that “rare spermatogonia” was associated to PAD of 7 days, “few spermatogonia” was associated to the PAD of 14 days, “moderate spermatogonia” was associated to the PAD of 21 and 28 days. Control group was associated with “abundant spermatogonia”. The absence of necrosis was associated with control group, necrosis degree of 1-25% was associated with PAD of 28 days; 26-50% was associated with PAD of 21 days; 51-75% was associated with PAD of 14 days; and 76-100% associated with PAD of 7 days (p < 0.001). Control group (52.6 ± 3.7), and PAD of 28 (39.2 ± 17.1) and 21 days (32.3 ± 11.1), presented mean of seminiferous tubules significantly higher than the groups with PAD of 14 (21.5 ± 11.9) and 7 days (10.7 ± 10.5) (p < 0.001). A positive correlation was detected (r=0.820, p < 0.001) indicating that the lower PAD proved to be correlated to lower mean diameter of seminiferous tubules. The opposite situation was observed in the analysis of necrosis degree, which was lower as the PAD increases (r =-0.926, p < 0.001).

Conclusions:

Results demonstrate that the shorter the period after busulfan dilution, the greater the cell depletion. The effect is inversely proportional to its PAD; the lower the PAD, the greater the action over cell depletion. This study suggests that there should be a standard in the application period after dilution of busulfan, not exceeding the period of 7 days.

Keywords: Busulfan, Spermatogenesis, Wistar rats
SEAFOODS CONTAMINATED BY ORGANO Tin AS RISK FACTOR TO PREGNANCY IN RATS: PRELIMINARY STUDIES


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2 Depto. de Fisiologia/Universidade Federal do Espírito Santo, UFES
3 Depto. de Biologia/Universidade Federal do Espírito Santo, UFES

Objectives:
Organotin compounds (OT) have been widely used as biocides and disinfecting agents in circulating industrial cooling waters, as well as antifouling paints for marine vessels. There are many reports of the toxic effects of OT, for example, OT are potent endocrine disrupters in gastropods, through the inhibition of aromatase, it can disrupt reproductive parameters, and induce sterility in mollusks by the development of a syndrome called imposex. The importance of OT as environmental endocrine disrupters and their potential to adversely affect human health, was previously demonstrated in several comprehensive studies of the reproductive toxicity of OT, however the toxic effects on reproductive cycle induced by OT contaminated seafood is not well understood. We evaluated the potential toxicity of OT, in female rats, and its disruptive capacity on reproduction, regular reproductive cycling and pregnancy, all correlated with changes in rat dam’s estrous cycle and female contamination in uterus.

Methods and Results:
Wistar female rats with regular estrus cycle (±230g, ±3 months age, R. norvegicus) where randomly divided in: 1) Control (CON, n=16, treated with non-contaminated oyster extract C. gigas); 2) Treated with OT contaminated oyster extract, C. brasiliana (MOD, n=16). After 15 treatment days by gavage, female were put together with a rat male, into individual cages, for copulation. They were separated 2 weeks later and the rates of copulation {(number of copulations/number of pairs)x100}, fertility{(number of pregnant/number of copulations)x100} and pregnancy frequency {(number of pregnant/total number of females)x100} where measured. Moreover, female pups from pregnant rats of MOD were treated until pubertal period, to a new copulation and measurement of the same parameters (F1 group). We utilized the qui-square test, whose significance was below p

Conclusions:
These data suggest that intake of contaminated seafood by OT, may affect rat female fertility, and its consumption through chronic periods, as the intrauterine, can be a risk factor to pregnancy.

Keywords: endocrine disruptor, female reproductive organs, pregnancy, triorganotins

Financial Support: UFES e FAPES (n°45446121/09)
Objectives:

SPAG11 (Sperm-associated antigen 11) is an androgen dependent beta-defensin like gene that might be involved in innate immunity and reproductive function. It presents a complex genomic structure and mRNA splicing pattern generating at least 23 isoforms in different species. One of these isoforms, SPAG11C, is known to be expressed in several species, including human and rodents. In the adult rat, SPAG11C expression was observed in tissues from the male reproductive tract, such as testis and epididymis. Herein, we determined SPAG11C expression during embryonic development of male rat in order to gain insights into regulation and potential physiological roles of this protein.

Methods and Results:

Male fetuses, collected at embryonic days E14, 16, 18 and 20 from pregnant Wistar rats, were immersed in Bouin’s fixative for 24 h and embedded in paraffin (ethical approval: UNIFESP-EPM 1563/09). From each age, at least three animals from different litters were analyzed. Spatiotemporal expression of SPAG11C was evaluated by immunohistochemistry and in situ hybridization, using a locked nucleic acid (LNA) probe and then compared with the well-known immunohistochemical distribution pattern of the androgen receptor (AR) at these early stages of sexual development. Immunohistochemistry of AR, used as a positive control, confirmed the expression of this receptor in male reproductive tissues (positive signal in mesenchymal cells in the Wolffian duct, testicular interstitial and peritubular myoid cells, but not in the germinative cell line or Sertoli cells) at the stages of the rat development analyzed. SPAG11C immunostaining was detected in both male reproductive tract and extragenital tissues at all time-points analyzed. No difference in the immunodistribution of SPAG11C was observed among the embryonic ages evaluated. SPAG11C immunoreactivity was detected in the mesenchymal cells, matrix of connective tissue, and epithelium of the Wolffian duct, as well as in the testis cords, especially in the primordial germ cells, Sertoli cells and in the interstitium, including the matrix between cells, but not in the peritubular myoid cells. SPAG11C positive staining was also observed in the kidney, smooth and skeletal muscle, chondrocytes, adrenal, liver, pancreas, epithelia of some organs (such as intestine and lungs), dorsal root ganglia neurons and in the spinal cord. In situ hybridization revealed the presence of Spag11c mRNA in the same sites that were immunoreactive for SPAG11C, confirming the ability of these tissues to synthesize this protein.

Conclusions:

SPAG11C expression throughout the early development of the rat suggests novel physiological roles for this protein, not only in the developing testis and epididymis, but also in the organogenesis of non-reproductive tissues of different embryonic origins. Additionally, the presence of SPAG11C in tissues that are not primarily dependent on androgens also points out to the involvement of regulatory factors, besides this steroid hormone, in Spag11c gene modulation.

Keywords: EMBRYONIC DEVELOPMENT, EPIDIDYMIS, GENE REGULATION, INNATE IMMUNITY

Financial Support: Fapesp, CNPq, CAPES, Fogarty International Center (Unifesp/UNC Chapel Hill)
Objectives:
Characterize morphologically and morphometrically the testis of the bat *Sturnira lilium* during dry and rainy seasons, in order to identify the existence of reproductive seasonality, thus providing more knowledge about the reproductive biology of this species.

Methods and Results:
Adult males were captured in Viçosa-MG, Brasil, during the dry (autumn-winter), \( n = 6 \) and rainy (spring-summer), \( n = 6 \), seasons. After euthanasia, the testes were fixed in Karnovsky, dehydrated, embedded in historesin and analyzed under light microscope. Morphometric analysis were performed using the Image Pro-Plus software and the results were analyzed by ANOVA (STATISTICA 1999), followed by Duncan's test. Our results showed that the testis of *S. lilium* follows the pattern described for other mammals, with a tubular compartment (TC) formed by tunica propria (TP), seminiferous epithelium (SE) and lumen (L). The intertubular compartment (IC) is composed of Leydig cells (LC), blood vessels (BV), connective tissue (CT) and lymphatic spaces (LS). Mean body weight was 22.50±1.32 g in the dry season and 23.77±1.91 g in the rainy season (\( p > 0.05 \)). In the dry season the testicular parenchyma was 84.10±4.67% of TC, which is formed by 70.82±5.55% of SE, 9.40±1.59% of L and 3.87±1.29% of TP. The IC represented 15.90±4.67% of the parenchyma, consisting of 85.27±6.21% of LC, 8.42±2.52% of BV and 6.45±4.08% of CT+LS. These values were not statistically different from those found in the rainy season, where the percentage of TC was 82.85±9.34%, with 66.83±6.75% of SE, 12.16±3.64% of L and 3.86±0.39% of TP. The IC represented 17.15±9.34%, with 84.62±7.54% of LC, 7.38±2.82 of BV and 8.52±4.08% of CT+LS. In both dry and rainy seasons we observed bats at different stages of reproductive activity, but always with sperm in testicular lumen throughout the year. Although the diameter of seminiferous tubules (129.72±18.37 μm - dry season and 139.39±23.16 μm - rainy season) and height of seminiferous epithelium (38.85±5.29 μm - dry season and 40.86±8.38 μm - rainy season) remained unaltered between seasons (\( p > 0.05 \)), the length of seminiferous tubules per testis (0.84±0.24 m² - dry season and 0.50±0.14 m² - rainy season) and per gram of testis (22.06±8.87 m² - dry season and 11.16±6.23 m² - rainy season) was significantly higher in the dry season compared to the rainy season. These results indicate an increased investment in spermatogenesis in the autumn and winter.

Conclusions:
While patterns of pronounced reproductive seasonality are common in bats from temperate regions, our results showed little alterations on testes morphometrical parameters, indicating a continuous annual reproductive patterns in *S. lilium*, often observed in tropical areas.

Keywords: Dry season, Leydig cells, Rainy season, Seminiferous epithelium, Spermatogenesis

Financial Support: CAPES

QuebraPagina

Resumo:05-038

**EXPRESSION OF GLUCOSE TRANSPORTER (GLUT) 4 IN THE CANINE CORPUS LUTEUM**

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Objectives:

GLUT4 is an ‘insulin-responsive’ isoform expressed in tissues, which respond to insulin by acutely increasing their rates of glucose uptake, such as skeletal muscle and adipose tissue (Cell Metab 5; 237-252, 2007). Evidences of the presence of insulin
receptors in the corpus luteum (CL) as well as the established temporal relationship between ovarian-derived hormonal profile and insulin resistance states lead us to investigate the presence of GLUT4 in canine CL covering the periods of its formation, early and late regression.

Methods and Results:

On each of the days 10, 20, 30, 40, 50, 60 and 70 after ovulation (p.o.), four mongrel bitches were ovariohysterectomized. After the onset of pro-estrus bleeding, blood samples were collected every two days and the day of ovulation was considered when plasma progesterone levels reached ≥5 ng/mL. Plasma glucose, insulin, progesterone and 17β-estradiol profiles were determined. The CL were separated from the surrounding ovarian tissue and formalin-fixed or nitrogen frozen. Paraffin-embedded tissue sections (5 µm) were obtained and stained immunohistochemically for GLUT4 and western blotting analysis was done after protein extraction. Progesterone concentration peaked at day 20 p.o. (22.95 ± 3.84 ng/mL), and decreased continuously towards day 70 (0.67 ± 0.51 ng/mL). Both 17β-estradiol and the insulin resistance rate, predicted by the ratio insulin/glucose, peaked at day 40 (22.33 ± 4.90 pg/mL and 0.04, respectively), decreasing gradually thereafter (day 70 = 15.02 ± 1.93 pg/mL and 0.02).

GLUT4 protein (45kDa) was identified in both cytosolic and nuclear luteal homogenates. The luteal cells cytoplasm GLUT4 immunostaining was strongest on days 10 and 20, but signal intensity tended to decrease by day 30. In contrary, the nuclear GLUT4 signal remained intense from the beginning to the end of the diestrus (day 60 p.o.).

Conclusions:

The presence of GLUT4 suggests that insulin-sensitive glucose uptake may occur in the canine CL mediating luteal function and life span.

Keywords: Glucose uptake, GLUT4, Corpus luteum, Canine

Financial Support: FAPESP

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**WNT ANTAGONISTS EXPRESSION IN THE BOVINE ENDOMETRIUM AND PLACENTA**

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Objectives:

In response to embryo implantation, the maternal side of the bovine placentome develops from restricted areas of the endometrium known as caruncle (CAR), a process that includes intense cell proliferation and connective tissue remodeling. Furthermore, placentome establishment, closely resembles epithelial-mesenchymal interactions during embryo digestive and respiratory system organogenesis, which are strictly controlled by morphogens like WNT proteins and its antagonists.

Methods and Results:

This work evaluated the evolution of CAR villous-like projections in the pregnant and non-pregnant (luteal phase, LP) cow (Bos spp) based on morphological analysis and morphogen gene expression by in situ hybridization. For the labelling hybridization index (LI), six or more fields within entire epithelium (E) and subepithelial stratum (SS) for CAR and epithelium (E) and stroma (ST) for IC were utilized to count the number of silver granules/area. By morphological analysis of CAR during implantation, it was established four developmental stages: S1 – trophoblast cells adhesion on uterine epithelium S2 – initial development of CAR villous-like projections, S3 – CAR villous-like projections expansion and anastomosis, S4 – placentome consolidation. Dkk1 and Sfrp2 were widely expressed in LP and S1 to S4 CAR and IC endometrium. Dkk1 was highly expressed on CAR area...
in S1 and S2 to S4 villous-like projections. Sfrp2 expression was observed mainly on myometrium and deep endometrium in S1 to S4 stages.

Conclusions:

The expression of Wnt antagonists in the CAR and IC regions seems to be responsible for the capability of cow endometrium quick remodelling and for the ability to establish the synepitheliochorial placentation. Furthermore, they also attest to the complex synergistic/antagonistic signaling mechanisms of still unknown factors modulating endometrium response during embryo implantation and placentation.

Keywords: Placenta, Cow, Wnt, endometrium, placentome

Financial Support: CNPq, CAPES

QuebraPagina

Resumo:06-012

SOURCEs OF VARIATION IN THE EVALUATION THE CRITICAL TEMPERATURE MAXIMUM (CTMAX)

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Objectives:

The critical thermal maximum (CTmax) was proposed as a link between animal physiology and thermal ecology. Whereas this link is valid in theory, the evaluation of physiological tolerances carries some problems that deserve attention and are the focus of this study. Objectives: Our immediate aim was to investigate sources of variation in CTMax, as repeatability, mass-dependence, daily rhythms, effects of sub-maximal temperature exposure, heating rates and effect of experimental manipulation in the ant Atta sexdens rubropilosa.

Methods and Results:

Methods: Critical Thermal Maximum: We used a Sable Systems (Las Vegas, USA) equipment to heat up 6.2-cm-diameter and 2.4-cm-deep aluminum container where the ant CTmax was measured. The experiment ended when upside-down ants could not turn over. Heating rates. We measured the CTmax of 409 ants at 12 different heating rates, from 1C each 0.5 minute to 1C each 6min. Daily Cycles: The ants were collected and tested at the following times: 0:30, 3:30, 6:30, 9:30, 12:30pm, 3:30pm, 6:30pm and 9:30pm. 232 ants were tested in total. Repeatability: 76 ants had CTmax measured following the standard protocol with a heating rate of 1C every 2 minutes. The ants were painted silver and then were returned to the colony. After 24 hours, 41 silver-marked ants were collected and marked gold, then they had their CTmax measured (1C every 2 minutes). After 24 hours (48 hours from the first test) 24 gold painted ants had their CTmax measured again. As a handling control 70 ant from the same colony were submitted to the same protocol always at room temperature and after 48 hours from the first handling 28 gold-marked ants had their CTmax measured. Effect of sub-maximal temperature exposure: 50 ants were initially tested following the standard protocol with a heating rate of 1C every 2 minutes. However, the equipment was turned off when the temperature of the test containers reached 35C. After 24h, 27 ants painted silver were collected and were subjected to the standard CTmax test. Results: In an analysis that includes all the experiments was observed an influence of the mass in CTMax (R=0.084 p=8.8*e-16), bigger ants tolerated higher temperatures. The mass effect was controlled previously to perform each of the subsequent tests. Heating rates: CTMax was higher for higher rates (faster heating) than for lower rates (slow heating) (R: 0.0856, p=2.047e-09). Daily rhythms: No differences were found in CTMax at various times of day (p=0.61). Repeatability: The average values of CTMax found on the first day were higher than those found on day 2 and day 3, (Tukey Day 1 x Day2 p=0.005014 and Day 1 x Day 3, p=6.13E-05) day 2 and day 3 were not different from each other (Day 2 x Day 3 p=0,505). The control showed no effect of handling (P= 0.862771 when compared to first day) Sub-maximal temperature exposure: There were no differences among treatments (p=0.2994).
Conclusions:

We found that CTmax may be influenced by issues that are intrinsic to methods (heating rate) and also issues such as repeatability and mass-dependent relations. And we found no effects of daily rhythms and of sub-maximal temperature exposure. So, these several sources of variation in CTmax can compromise theoretical considerations made in comparative studies on thermal biology.

Keywords: Thermal Physiology, Insects, CTmax

Financial Support: FAPESP

FREE RADICAL METABOLISM UNDER SUPPRESSION OF GLUTATHIONE AND CATALASE IN A GASTROPOD

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Objectives:

Part of the consumed oxygen through respiration is constantly converted into reactive oxygen species, such as H$_2$O$_2$, which react with macromolecules. The control of their levels at physiological values by antioxidant components avoids excessive cellular damage – oxidative stress. The relevance of each antioxidant was investigated by several studies using different organisms. For example, some of them assessed which enzyme plays a more important role in controlling H$_2$O$_2$ levels, glutathione peroxidase or catalase. It was demonstrated that the function of an antioxidant is, in general, partly replaced by the other. Most studies that inhibited in vivo the action of a particular antioxidant showed significant rearrangements of other parameters and increases of oxidative stress markers. The aim of this study was to investigate the adjustments of free radical metabolism in the land snail Helix aspersa in response to a concomitant depletion of two important antioxidants, glutathione and catalase.

Methods and Results:

The animals were injected with an inhibitor of glutathione synthesis, buthionine sulfoximine (BSO, 1g/kg), and an inhibitor of catalase activity, 3-amino-1,2,4-triazole (ATZ, 1g/kg). Various parameters were measured in the hepatopancreas of animals receiving no injection (control; n = 9), saline-injected animals (n = 10), and BSO- plus ATZ-injected animals (n = 10): ATZ concentration, activities of catalase, glutathione S-transferase (GST), glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G6PDH), and TBARS. BSO/ATZ-injected group showed the presence of ATZ in hepatopancreas. Although ATZ concentration values were lower than those observed in a previous work (a difference that is explained by the longer time of exposition in our present study), catalase activity was suppressed by 92% and 80% in relation to control and saline groups, respectively. GST, GR and G6PDH activities persisted unchanged in response to glutathione depletion and catalase activity. Levels of lipid damages (TBARS) also remained unaltered in BSO/ATZ-injected animals.

Conclusions:

The high suppression in catalase activity in the BSO/ATZ-injected group despite a low presence of its inhibitor indicates that the resynthesis of this enzyme does not occur immediately (or rapidly enough) after the loss of its function. Considering data from previous studies applying BSO or ATZ alone in different organisms, which showed increased H$_2$O$_2$ levels and significant changes in antioxidant parameters, the persistence of GST, GR and G6PDH activities was surprising. In fact, our experiment should cause significant readjustments of antioxidants, since it has been demonstrated (in an insect) that the simultaneous in vivo
administration of BSO and ATZ has additive/synergistic effects on augmenting H$_2$O$_2$ concentration. The maintenance of TBARS levels was also unexpected, since oxidative stress is commonly present in organisms receiving BSO or ATZ, or under higher levels of H$_2$O$_2$. The present results show that Helix aspersa shows an exceptional resistance to strong interferences in its free radical metabolism and that glutathione and catalase functions may be partly compensated by other components of its antioxidant system.

Keywords: Antioxidant, Hydrogen peroxide, Oxidative Metabolism, Peroxidase, Oxygen

Financial Support: Redoxoma-CNPq and FINATEC.

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Resumo:06-014

OXIDATIVE METABOLISM AND REDOX IMBALANCE DURING INSECT DIAPAUSE

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Objectives:

Chlosyne lacinia (Lepidoptera, Nymphalidae) the main sunflower pest in Brazil (An. Soc. Entomol. Brasil. 23; 81, 1994) completes its life cycle from egg hatching to the adult death within 35 days. However, this animal is able to halt its development entering in diapause, in which they endurance up to six months without feeding or moving. Little is known about the relationship between free radical metabolism and diapause. Previous results from our laboratory showed a 20-fold increase in glutathione S-transferase activity in C. lacinia after 20 days of diapause (SBBq 2011, abstract E-17). Thus, our aim is to identify further particularities of the antioxidant apparatus between diapausing and non-diapausing C. lacinia larvae as well as oxidative metabolic capacity.

Methods and Results:

Egg masses were collected from leaves of the wild sunflower (Tithonia diversifolia, Asteraceae), and after hatching, the caterpillars were maintained at 25±2° C, 55±10% R.H. and 16 h photophase in 3.5 L plastic cages and fed daily with fresh wild sunflower leaves until the third instar when they were divided in two groups (in January 2010): non-diapausing (control) and 20 day-diapausing larvae. Whole body homogenates from a pool of three larvae were used in each assay, enzymatic activities (n=3-4), oxidative stress markers (n=7) and glutathione parameters (n=6). Citrate synthase activity - a marker of oxidative metabolism - decreased 80% in 20 day-diapausing animals. Moreover, TBARS (indicator of lipid peroxidation), carbonyl protein (indicator of protein oxidation by free radicals) and total glutathione (GSH-eq) were reduced in diapausing C. lacinia by 24%, 69% and 18%, respectively. During diapause, there was a decrease (82%) in the concentration of GSH, while the amount of GSSG decreased 72%. The ratio GSSG/GSH-eq was increased by 58%. No changes were observed in catalase and glucose 6-phosphate dehydrogenase activities.

Conclusions:

The remarkable decrease in citrate synthase activity indicates low Krebs cycle activity and metabolic depression after 20-days diapause in C. lacinia. Thus, decreased oxidative stress markers levels, as well as total glutathione levels, are coherent with the diminished oxidative metabolic rate after 20 days in diapause. Extremely high GST activity and increased GSSG/GSH-eq in diapause could be related to the modulation of redox signaling and/or the control of oxidative stress. Furthermore, it is clear that a redox imbalance is taking place in diapausing C. lacinia larvae. The analysis of a longer period in diapause (40 days) and other antioxidant enzymes activity/expression are underway, which may compose a clearer picture of oxidative metabolism and redox balance during insect metabolic depression.
DIFFERENT FORAGING STRATEGIES IN ANTS OF DIFFERENT SIZES: OPTIMIZATION?

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Objectives:
A longstanding controversy in evolutionary biology involves the possible role of optimization in natural selection. As optimization is often the main issue in foraging studies, the foraging behavior in ants could provide a basis to understand how natural selection happens. In this context, Wetterer (1994) showed that there was a direct correlation between the mass of the ants and the mass of the material carried by them. This result can be interpreted as an indication that there is an optimization in food intake. Helanterä and Ratnieks (2008) showed that in the foraging of fruit the correlation between the size of ants and the piece cut is even more evident. This would be due to the fact the fruit demand a 3D cut challenge. So, Helanterä conclude that bigger ants do this task better than small ants because they have bigger jaws that facilitate the cutting of 3D materials. The authors interpreted these results as another example of a mechanistic phenomenon of how variation in size between ants enhances the efficiency of division of labor.

Methods and Results:
The purpose of our study was to evaluate in a systematic way the relation between ant size and the size of material foraged. 183 ants were collected over five days. All ants collected belonged to the same natural colony of Atta located at the University of Sao Paulo, and at the moment of collection the ant was carrying a piece of leaf. Both the ant’s head size and the area of the leaf piece that it was carrying were measured using a highly accurate computational tool developed in MatLab. Measurements of head capsule ranged between 1.01 mm and 5.05 mm, average 2.76 mm. The ants were organized schematically, into two groups: small ("S" - brain capsules smaller than 3mm) and large ("L" - brain capsules larger than 3mm). There was a correlation between ant size and size of material foraged in group “S” (rho = 0.5035, p

Conclusions:
Data analysis showed that ants of different sizes (S and L) have different behaviors regarding their foraging activity. Therefore, it is clear that the optimization does not follow a single rule as proposed by (Wetterer, 1994; Helanterä and Ratnieks, 2008). It may have at least two different rules, one for small ants and another for big ones. References: Helanterä H, Ratnieks FLW (2008) Geometry explains the benefits of division of labour in a leafcutter ant. Proceedings of the Royal Society B-Biological Sciences 275(1640): 1255-1260. Wetterer JK (1994) Ontogenic Changes in Forager Polymorphism and Foraging Ecology in the Leaf-Cutting Ant Atta-Cephalotes. Oecologia 98(2): 235-238.
SEROTONIN EFFECTS ON CARBOHYDRATE METABOLISM OF NEOHELICE GRANULATA CRABS FED ON A HIGH PROTEIN (HP) OR CARBOHYDRATE RICH (HC) DIETS

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Objectives:
Serotonin (5-HT) is an important neurotransmitter and neurohormone in most animal species. Serotonin has multiple and complex roles in crustaceans, controlling motor pattern generation, female reproduction, cardiac activity and color changes. In all crustaceans tested, administration of serotonin caused elevation on the glucose levels in the hemolymph and a postural alteration known as 5-HT-like posture. This work aims to investigate the effects of the administration of 5-HT and its antagonists, cyproheptadine (5-HT2) and metiotepin (5-HT1), on carbohydrate metabolism and posture of Neohelice granulata crabs fed on a high protein (HP) or carbohydrate rich (HC) diets.

Methods and Results:
Male crabs were maintained in standardized conditions and fed with raw bovine meat (HP diet) or boiled rice (HC diet) during 15 days. After this acclimatization period, the HC and HP crabs were subdivided in the following subgroups: control (received twice crustacean saline injections with a 30 min interval, n= 21HP, 20HC); 5-HT (received a saline injection followed by 5-HT injection 5,69x10^-3M, 30 min after, n= 26HP 20HC); cyproheptadine-5-HT (received cyproheptadine 3,08 x 10^-3M and 5-HT 5,69x10^-3M after a 30 min interval; n= 23HP 19HC) and metiotepin-5-HT (received metiotepin 2,21x10^-2M and 5-HT 5.69x10^-3M after a 30 min interval, n=11HP 10HC). Hemolymph samples were collected 3 hours before the beginning of the experiment and 60 and 120 min after the second injection in order to measure glucose circulating levels (mg/dL). At the end of the experiment, the crabs were crioanesthesized to collect samples of hepatopancreas and mandibular muscle to measure glycogen concentration (mg/g of tissue). The crabs were photographed before and after the injections to register the postural alterations. Serotonin administration caused elevation on glucose circulating levels 60 min, (from 5,41 to 14,31, p<0.05) at 120 min on crabs fed on both diets. Metiotepin treated groups had similar effects as 5-HT treated groups on hemolymph glucose levels (p>0.05). Glycogen concentration on mandibular muscle were higher in HC fed crabs than HP crabs (p<0.05). As in muscle, glycogen levels in the hepatopancreas were higher in HC than in HP fed crabs (p<0.05). In HC crabs, all tested groups presented similar glycogen values (p>0.05). Cyproheptadine administration did not prevent the development of the 5-HT-like posture, but metiotepin administration immediately caused an antagonist posture and interfered in 5-HT-like posture.

Conclusions:
These results suggest that: 1) serotonin effects on carbohydrate metabolism of N. granulata are influenced by the diet; 2) as suggested in other crustacean species, this crab may have more than one type of 5-HT receptor.

Keywords: Carbohydrate, Metabolism, Neohelice granulata, Serotonin

Financial Support: CAPES and CNPq.
Objectives:

To develop instrumentation and record the electrocardiogram (ECG) and systolic heart movement in awake, non-anesthetized geckos (*Hemidactylus mabouia*).

Methods and Results:

Adult geckos of either sex were used (weight 5.5 ± 0.3 g, snout-vent length 67 ± 5 mm, N=12). Animal capture (IBAMA, 14566-3) and experimental protocols (CEUA - UNICAMP, 2331-1) were previously approved. ECG was measured using conventional electrodes (circular, 10 mm diameter, positive on the neck, negative on the abdomen, and ground on the tail) connected to a portable differential amplifier (gain: 2000; low-pass filter: 80 Hz; high-pass filter: 0.5 Hz) developed at the Center for Biomedical Engineering/UNICAMP. Animals were kept under three different conditions: a) manual immobilization (MI); b) spontaneous tonic immobility after being placed dorsal side down on an instrumented platform (TI); and c) non-restrained inside an instrumented (16-electrode array) plastic tube (TB), which is used as a refuge by the animals in the laboratory terrarium. Under in TI, heart movement (HM) was also recorded via optical detection. It was possible to identify in the ECG signal the QRS complex, positive P and T waves, short T-P interval and long S-T segment. Following each QRS complex, HM was discernible from the optical signal (0.52 ± 0.004 duration, peak coincident with T wave). The heart rate (HR) was significantly higher (P

Conclusions:

To our knowledge, this is the first description of the ECG and HR in geckos. The methodology and instrument developed are useful for non-invasive, *in vivo* studies of the heart of small reptiles without the need of physical restraint or anesthesia.

Keywords: Gecko, ECG, reptile, heart, biomedical instrumentation

Financial Support: CNPq (Grant 300632/2005-3).

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Resumo:06-018

PRIMARY STRUCTURE COMPARISON OF TRANSFERRIN RECEPTOR OF *HOMO SAPIENS* AND *TRYPANOSOMATIDS*

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Objectives:

In blood plasma, ferric ions are linked to transferrin (Tf) which forms a complex with a membrane protein called transferrin receptor (TfR), allowing the internalization of Fe³⁺. The *Homo sapiens* TfR is a homodimer that presents three extracellular domains: an apical domain, a protease-like domain, and a helical domain; the latter show the main amino acid residues that interacts with the Tf. Human protozoan parasites of the genus *Trypanosoma*, organisms phylogenetically distant of humans, also have a transferrin receptor which allows the iron the internalization from the host to the parasite. In order to verify the presence of Tf and TfR human binding sites in trypanosomatids, a comparison "in silico" between TfR primary structure of the *Trypanosoma brucei*, *Trypanosoma evansi* and *Homo sapiens* was made.
Methods and Results:

The human and trypanosomatids TfR amino acid sequences were obtained from GenBank databases and the alignment of primary structures was made with T-Coffee, an application implemented in Expasy, on EBI website (European Bioinformatics Institute). Amino acid sequences were compared two by two building a global alignment and a series of local alignments using BLOSUM (62) matrices, the best to seek similarities between these kind of sequences. The consensus areas of the alignment were analyzed by the Jalview application implemented in Expasy. Alignments revealed low levels of similarity among primary structure of the TfR of these organisms, including the main sites of Tf-TfR interaction. In human TfR, the amino acids L (leucine), R (arginine) and Y (tyrosine) in positions 619, 629 and 643, respectively, are essential for Tf binding (Int. J. Biochem.Cell Biol. 36:2137, 2004). On the other hand, TfR of parasites presented on positions 619 and 629, the amino acids E (glutamic acid) and Y (tyrosine), respectively, which are amino acids with very different chemical characteristics. The alignment showed a gap in the residue 643, possibly related to deletion or insertion mutation event occurred during the evolutionary process.

Conclusions:

Primary structure analyses revealed important differences between protozoan and human TfR, meaning that probably the transferrin binds to TfR of trypanosomatids in a different way. Future studies will investigate the three-dimensional structure of the receptor looking for possible implications of the differences identified in the affinity of the TfR by Tf.

Keywords: transferrin receptor, trypanosomatids, bioinformatic, T-coffee, alignment

Financial Support: PIIC/ UFSJ

Resumo:06-019

IN SILICO INVESTIGATION OF EVOLUTIONARY RELATIONSHIPS BETWEEN ZIP (ZINC REGULATED TRANSPORTER, IRON REGULATED TRANSPORTER – LIKE PROTEIN FAMILY) OF TRYPANOSOMATIDS AND THEIR VERTEBRATE HOSTS.

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Objectives:

Parasites belonging to genus Leishmania require high levels of intracellular iron for a successful infection since this element is important for the metabolism function and proliferation of the parasite as well as to the protection of the parasite from the oxidative attack of host. To attain these goals, the parasite needs to manipulate the iron balance mechanisms of the hosts in order to incorporate their intracellular iron. The transporter protein Nramp1, located in the lysosomal membrane of macrophages, works as a proton-dependent channel that removes Fe$^{2+}$ and Mn$^{2+}$ ions from intracellular compartment and represents a key-mechanism of defense from intracellular parasites. On the other hand, it was identified during the intracellular stage of some species of Leishmania, a specific iron transporter protein (Leishmania Iron Transporter - LIT) that compete with Nramp1 to uptake the iron into the parasite cell. LIT was the first iron transporter identified in trypanosomatids and it is a member of a protein family called ZIP, which also occurs in vertebrates, plants, yeasts and nematodes. There are evolutionary relationships proposed to ZIP family, including the corresponding gene of Trypanosoma brucei in the subfamily I, the same as plants and yeasts while nematodes, invertebrates and vertebrates were proposed to be clustered in the subfamily II (Biochim Byophys Acta: 1465, 190, 2000). The increased data about ZIP family brought the opportunity to review the phylogenetic relationship of their member’s family, allowing the investigation of evolutionary processes between parasites and their hosts. The aim of this study was to analyze the evolutionary relationships between ZIP transporters of parasites from genus Leishmania and their vertebrate hosts, looking for confirmation of the phylogenetic relationship already proposed with the additional data from public genetic
databases.

Methods and Results:

Amino acid sequences of vertebrates hosts (Homo sapiens, Mus musculus, Rattus norvegicus, Bos taurus, Gallus gallus) and Leishmania (L. braziliensis, L. infantum, L. major, L. mexicana) ZIP proteins were obtained from NCBI databases (National Center for Biotechnology Information) and TritrypDB (Kinetoplastid Genomics Resource). Multiple aligned sequences (n = 36) were analyzed to estimate the most appropriated evolutionary model to conduct the phylogenetic reconstructions. Neighbor Joining and Maximum Likelihood trees were obtained by using JTT (Jones-Taylor-Thornton) model and complete deletion option. Phylogenetic trees reconstructed by both methods defined monophyletic groupings for each kind of ZIP protein of vertebrates and the LIT of Leishmania. Similarity of the Leishmania’s LIT carriers and ZIP1, ZIP2 and ZIP3 of vertebrates was highly supported by bootstrap probability values in consensus trees.

Conclusions:

This study contributes to the understanding of ZIP phylogeny, suggesting a new clustering of protozoan proteins with subfamily II. More analyses, including other taxa’s sequences, are necessary to confirm this initial investigation and to purpose the evolutionary processes involved.

Keywords: ZIP-family, alignment, phylogeny, Leishmania, bioinformatic

Financial Support: PIIC / UFSJ

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Resumo:06-020

EFFECT OF HYPOXIA AND POST-HYPOXIA RECOVERY ON ATP CONCENTRATION IN TISSUES OF CRAB NEOHELICE GRANULATA

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Objectives:

In the estuarine environment, the habitat of crab Neohelice granulata, the concentration of O2 dissolved in water can range from 2.80 mg O2/L to 11.78 mg O2/L. This study was designed to determine the effect of hypoxia and of the post-hypoxia recovery phase on the concentration of ATP in muscle (M), hepatopancreas (HEP), and anterior (AG) and posterior (PG) gills of N. granulata.

Methods and Results:

Male crabs (n=21) collected from the Tramandai Lagoon (RS) were kept in aired fish tanks (155 Torr of O2 dissolved in water) and fed ad libitum for 10 days with raw bovine meat. After this period, the group was transferred to a tank with 38 Torr of O2 dissolved in water where they remained for 1 (n=12) or 8 (n=14) hours. After this hypoxic stress some of these animals were placed in another tank in normoxic conditions for 3 (1h/n=4, 8h/n=6) or 24 hours (1h/n=5, 8h/n=3), these being the post-hypoxia recovery groups. The control group (n=5) was maintained in normoxia (155 Torr de O2). Upon completion of the experimental periods, the animals were cryoanesthetized and tissues (~100mg) were sampled and immediately frozen in liquid nitrogen. The tissues were homogenized with lysis buffer (TCA 6%, NaF 20mM, Gelatin 0.4g/L) and the concentration of ATP was determined through the bioluminescent luciferin technique using the Invitrogen kit (A22066). Results were expressed as μmol of ATP.mg-1 of tissue. In the AG, PG and M of the animals submitted to hypoxia for 8 h there was a decrease (p

Conclusions:
These findings suggest that during hypoxic stress ATP concentrations in the AG, PG and M are used as energetic substrate. However, in the HEP, ATP concentration remains unchanged in the studied periods. After, 3-hour of post-hypoxia recovery (after 8h of hypoxia) the ATP values returned to control levels, in the M, PG and AG.

Keywords: ATP, crustacean, hypoxia

Financial Support: CAPES / CNPq

GLYCERONEOGENESIS IN THE HEPATOPANCREAS OF CRAB NEOHELICE GRANULATA SUBMITTED TO DIFFERENT DIETS

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Objectives:
The aim of this work was to analyze the influence of a protein rich or carbohydrate rich diet on the incorporation of [2-{14C}]pyruvate into glycerol, fatty acid and CO2 in the hepatopancreas of crab Neohelice granulata.

Methods and Results:
Male crabs N. granulata in stage C of the intermolt cycle (14 animals) were collected in the Tramandaí Lagoon, Rio Grande do Sul, Brazil (29º58'S, 50º08'W). In the laboratory, the animals were divided into groups and placed in tanks with running water (salinity of 20‰, temperature 25°C under a light-dark cycle of 12L:12D) and fed ad libitum with a HP diet (beef) or HC diet (boiled rice) for 15 days. The animals were then anesthetized by chilling on ice for 5 min and their hepatopancreas was dissected out. The samples (200-300mg of hepatopancreas) were weighed, placed in 2ml tubes with 500μl of crab physiologic solution (CPS) [374mM of NaCl; 10 mM of KCl; 8.8mM of H3BO3; 10mM MgCl2; 10mM of HEPES; 0.1mM of PMSF and 25mM of Cl2Ca (2H2O)], 1 mM (0.2μCi) of pyruvic acid [2-C14] plus 5.0 mM of pyruvate, and aired with carbogen. Incubation was performed for 1 hour at 25°C with constant stirring. Afterwards, the samples were separated from the incubation medium and rinsed with CPS to remove the excess radioactivity. Lipid extraction was then performed according to Folch (J. Biol. Chem. 226: 497-509. 1957) and, later, isolation of fractions containing glycerol and fatty acid. The formation of glycerol and marked fatty acid was quantified in a beta counter. The determination of 14CO2 production was performed according to Marqueze et al. (Exp. Mar. Biol. Ecol. 332,198-205, 2006). For the statistical analysis we used the Student’s t test for non-paired data, and the differences between means were considered significant when P < 0.05. The values of 14C-glycerol in hepatopancreas did not differ across the HP (141.5 ± 34.6 nmol.g-1.h-1) and HC (123.6 ± 22.5 nmol.g-1.h-1) groups. As for the formation of 14CO2, no difference was found between the groups either (HP= 0.137± 0.008 mmol of 14CO2 /g of tissue and HC= 0.12 ± 0.09 mmol of 14CO2 /g of tissue). The values of marked fatty acids were significantly greater in the HC (221.3 ± 44.6 nmol.g-1.h-1) than in the HP group (92.4±16.9 nmol.g-1.h-1).

Conclusions:
The presence of the glyceroneogenic pathway in crab N. granulata was evidenced. The administered diets (HP or HC) failed to change this pathway in the hepatopancreas and did not affect the formation of 14CO2. The HC diet stimulates lipogenesis, which may explain the greater formation of fatty acids observed in this work.

Keywords: gliceroneogênes, dietas, crustaceo, glicerol
CONDITIONED ALARM BEHAVIOR INDUCED INCREASE IN IMMEDIATE EARLY GENES EXPRESSION (IEGS) OF OLFACTORY BULB AND TELENCEPHALON IN PIAÇU FISH

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Objectives:
Piaçu fish adopt antipredator (alarm) behavior when they detect alarm substance (AS) released from an injured conspecific. However, alarm reactions may also occur in response to chemical and visual stimuli that fish learn to associate with release of alarm pheromone. Despite the existence of behavioral data about conditioned alarm behavior in fish, the neural bases are unknown. The current work evaluates if piaçu can learn to associate a visual stimulus with predation risk. In addition, was investigated the involvement of forebrain in this behavior measuring c-fos and egr-1 protein levels, which are the products of IEGs frequently documented for indentify neuronal activity upon stimulus or related activities.

Methods and Results:
In the first experimentation day (training session) the fish were simultaneously exposed to blue light and water (control, n=8) or blue light and AS (n=10). In the second day (test session) the animals were tested using blue light alone. Ninety minutes after the test session the animals were sacrificed and the brains were dissected to perform western blotting assay for the IEGs. Behavioral endpoints (distance traveled, time of locomotion, time spent in the top of the aquarium) were assessed during two consecutive observation periods (baseline and post-stimulus) of 5 minutes each, using the software EthoVision XT 7. Light paired with water did not alter the ongoing behavior or significantly change the behavioral endpoints (p>0.05) relative to baseline during training session. The same was observed in the test session of control group. However, light paired with AS induces freezing behavior characterized by the cessation of swimming activity as the animal settles to a bottom corner of the aquarium and reduced movements of the dorsal and tail fins, and cause a decrease in the behavioral endpoints (p

Conclusions:
Exposure to a single-trial of combined cues shows a strong retention at testing, demonstrating a robust ecological mechanism by which piaçu fish learn to recognize indicators of predation risk. The increase of olfactory bulb and telencephalon IEGs, in the AS conditioned animals, when compared with to control group suggest that the olfactory bulb is probably the first neural substrate required for perceptual memory and corroborates with other studies that have demonstrated that the telencephalon is essential for memory retention.

Keywords: alarm reaction, immediate early genes, forebrain, learning and memory

Financial Support: CAPES and FAPESP
MELATONIN IN ECHINODERMS

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Objectives:
Melatonin, the main secretory product of the vertebrate pineal gland, is suspected to be a ubiquitous molecule principally involved in the transduction of photoperiodic information and protection against free radicals. Besides vertebrates, melatonin has been detected throughout phylogeny in numerous non-vertebrate taxa. In the present study, the occurrence of melatonin in the sea star Echinaster brasiliensis and its possible pathway of production was evaluated.

Methods and Results:
Melatonin was evaluated by HPLC with an electrochemical detector. Presence of isoforms of the genes tryptophan hydroxylase and arylalkylamine N-acetyltransferase from melatonin's pathway of production was evaluated by PCR. Activity of the enzymes was checked by radio assays. Our results indicate the presence of melatonin in the sea star Echinaster brasiliensis, with a circadian pattern of production. This pattern is sustained even in constant darkness, indicating that this melatonin's production is the manifestation of an endogenous clock. We also have find isoforms of the genes of the enzymes tryptophan hydroxylase and arylalkylamine N-acetyltransferase. Together with the results of the activity of these enzymes, in a rhythm near of the melatonin's production, these data indicate that the melatonin's pathway of production in Echinaster brasilienses is similar to the one observed in vertebrates.

Conclusions:
Taken together our results show, for the first time, the presence of melatonin in an echinoderm. More than that, we have demonstrated that the classical pathway melatonin's production is probably the one used in this marine invertebrate. More research are necessary to evaluate possible functions of the melatonin in the sea star.

Keywords: melatonin, echinoderms, invertebrates, aralkylamine N-acetyltransferase, Tryptophan hydroxylase

Financial Support: FAPESP

REGULATION OF ACTIVITY AND EXPRESSION OF THE EFFLUX PROTEIN ABCC1 IN THE MA104 EMBRYO KIDNEY CELLS

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Objectives:
The ABCC1 (MRP1) transporter, involved in the multidrug resistance (MDR) phenotype, is responsible for glutathione (GSH) secretion, which appears to be involved with the kidney’s urine concentration mechanism. As this protein is present in straight
distal tubule and collecting duct cells, it’s possible that its role in kidney cells is related to GSH transport and protection to oxidative damage caused by osmolarity variations. The goal of the present study was to study the effects of hyperosmolarity on the activity and expression of ABCC1 in Ma104 kidney embryo cell line.

Methods and Results:

Cells were incubated with crescent concentrations of NaCl, Urea or NaCl + Urea during 4 days, until final concentrations of 100 mM NaCl, 200 mM Urea and 50 mM NaCl + 100mM Urea were reached, keeping the final osmolarity of the medium at approximately 500 mOsm/L. At the end of the incubations, activity assays were performed using CFDA as a fluorescent substrate and MK571 as an inhibitor. Alternatively, cells were processed for protein expression assays using the rabbit polyclonal antibody A23 as a primary antibody. Both assays were performed using flow cytometry techniques. NaCl treatment resulted in approximately 80% inhibition of ABCC1 activity, whereas urea lead to a 70% inhibition. The expression of ABCC1 was reduced by 35% by NaCl treatment, while urea did not change its expression. Treatment with NaCl also caused noticeable change in cell complexity, with side scatter values being 25% higher than in control conditions.

Conclusions:

Treatment with sodium seems to downregulate ABCC1 expression and also reduces CFDA efflux capacity. Urea produces similar results in the activity assay, although concomitant treatment of NaCl and urea has failed to produce the same results. It is possible that the reduction of ABCC1 activity in those cells is important to allow survival in the hyperosmotic NaCl and urea rich medium of kidney medulla.

Keywords: Ma104, ABCC1, expression, activity, osmolarity

Financial Support: CAPES, CNPq, FAPERJ

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Resumo:07-022

EFFECTS OF TEOCINOBUFAGIN IN THE ISOLATED PERFUSED RAT KIDNEY

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Objectives:

Bufadienolides are cardiotonic steroids structurally related to the clinically relevant cardenolides, such as a digoxin. Some bufodienolides, including telocinobufagin, were detected in plasma and urine of human and mammalian subjects and its pathophysiological significance is under intense investigation. The production/secretion of these endogenous steroids increases during saline volume expansion and other states such as end stage renal disease, preeclampsia, uremic cardiomyopathy and salt-sensitive hypertension. The evaluation of the renal effects of bufadienolides in vivo is difficult due to its potent actions on the vascular system. Therefore, this work was undertaken in order to study the effects of telocinobufagin (TBG) on glomerular and tubular function of the isolated kidney of normotensive rats.

Methods and Results:

Male wistar rats (250-300g) were anesthetized with xylazin (10 mg/Kg; ip) and ketamine (90 mg/Kg; ip) and the renal artery was cannulated through the superior mesenteric artery and the right kidney was immediately perfused in situ. Thereafter the organ was transferred to a closed perfusion system. The kidney was perfused with a modified Krebs-Henseleit solution (kept at 37°C and gassed with 95% O2 and CO2) enriched with 6% albumin and 0.05% insulin for glomerular filtration rate (GFR) estimation. The fifty 30 minutes was an equilibration period and then during more 90 minutes with collection of perfusate and urine samples every 10 minutes as an internal control period. Thereafter, DMSO (vehicle) and TCB (1, 3 or 10μM) was added to the perfusate
and then tubular and vascular effects were evaluated. All data were compared with control group and with each experimental internal control. TBG at 1 μM showed no significant effect, however in all protocols, TBG at 3 and 10μM showed promising effects. GFR was increased at 3μM (GFR120 = 1,35 ± 0,08) and 10μM (GFR120 = 1,97 ± 0,05), measured in ml/g/min, compared with control DMSO (GFR120 = 0,65 ± 0,02). The records of urinary flow (UF), in ml/g/min, showed the same behavior: 3μM (UF120 = 0,31 ± 0,050); 10 μM (UF120 = 0,44 ± 0,050) and DMSO (UF120 = 0,14 ± 0,002). About the renal vascular resistance (RVR) measured in mmHg/ml.g⁻¹.min⁻¹, was significant increased only in 90 minutes at 3μM (RVR90 = 7,53 ± 0,68) and 10 μM (RVR90 = 7,12 ± 0,48), and control DMSO (RVR90 = 5,53 ± 0,68). The sodium excretion (µmEq/g/min) was increased in these two concentrations, 3μM (Na120 = 44,30 ± 2,60); 10 μM (Na120 = 62,11 ± 5,98) and DMSO (Na120 = 17,58 ± 1,02) showing a significant natriuretic effect. The potassium excretion (µmEq/g/min) at 3 μM was increased only at 120 minutes (K120 = 1,24 ± 0,08) and at 10 μM the greatest effect was also at 120 minutes (K120 = 1,83 ± 0,16), DMSO (K120 = 0,95 ± 0,09).

Conclusions:
TCB showed diuretic, natriuretic and kaliuretic activity probably due to its effect on vascular resistance and GFR.

Keywords: telocinobufagin, steroid cardiotonic, renal perfusion

Financial Support: CNPq, CAPES

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Resumo:07-023

THE INFLUENCE OF EXTRACELLULAR CALCIUM SENSING RECEPTOR (CAR) ON H+-ATPASES ACTIVITY IN MICE KIDNEY

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Objectives:
The kidneys are important organs for the maintenance of fluid and electrolyte homeostasis and also in acid-base balance. Recent studies have shown the presence of an extracellular calcium sensing receptor (CaR) along the nephron segments, being present from proximal tubule to collecting duct. Recent reports suggest that extracellular calcium (Ca2+o) can modulate Na+ independent proton secretion in intercalated cells of mouse cortical collecting duct (CCD) and outer medulla collecting duct (OMCD). The major goal of this project was to investigate the interaction between CaR and proton extrusion by the vacuolar H+-ATPase (H+-ATPase) and gastric H+/K+-ATPase in mice nephron.

Methods and Results:
All methods used in this research was approved by Comitê Setorial de Ética em Experimentação Animal of UFPR at the process nº 354. The animals used in this research was male adult swiss mice. The determination of biochemical activity of H+-ATPases was performed using fractions partially purified from cortex or outer medulla plasma membranes obtained from mice kidneys, in a protocol modified from that employee by Boumendil-Podevin (Biochimica el Biophysica Acta, 735:86-94, 1983) and Caruso-Neves (Biochem. et Biophys. Acta, 1431:483-491, 1999). The inorganic phosphate (Pi) released in the reaction was quantified by a colorimetric reaction adapted from the method described by Fiske and Subbarow (Can J Physiol Pharmacol., 87:653, 2009). This method is based on calculations involving the difference between the Pi liberated in the absence or presence of specific inhibitors of H+-ATPases, 10⁻⁷M bafilomycin and 10⁻⁸M concanamycin for H+-ATPase and 10⁻⁵M Schering 28080 for gastric H+/K+-ATPase. ATPase activity is expressed in nmol Pi.mg⁻¹.min⁻¹. The data are presented as mean values ± SEM. Statistical comparisons were made by analysis of variance followed by the Student-Newman-Keuls contrast test. Differences were considered significant if p < 0.05. The H+-ATPase activity from cortical and outer medullary region was significantly stimulated by increasing the Ca2+o, in a dose-dependent pattern. In the same way, gastric H+/K+-ATPase activity was sensitive to changes...
in Ca²⁺o levels. A significant increase of H⁺-ATPase activity was also observed when the CaR was stimulated with agonists such as 300μM Gd³⁺ and 200μM neomycin, both in cortex: 9.2 ± 1.2 basal vs 16.5 ± 2.9 nmol Pi.mg⁻¹.min⁻¹ with Gd³⁺, and 12.5 ± 2.2 nmol Pi.mg⁻¹.min⁻¹ with neomycin, n=10; and outer medulla: 12.3 ± 1.4 basal vs 28.5 ± 3.3 nmol Pi.mg⁻¹.min⁻¹ with Gd³⁺, and 17.9 ± 2.4 nmol Pi.mg⁻¹.min⁻¹ with neomycin (n=10). The cortical and outer medullar gastric H⁺/K⁺-ATPase activity was also stimulated by Gd³⁺ and neomycin. Finally, cortical H⁺-ATPase activity was significantly stimulated by 10⁻⁹M angiotensin II (16.6 ± 2.1 basal vs 22.0 ± 2.8 nmol Pi.mg⁻¹.min⁻¹ with angiotensin, n=7), and the stimulation of CaR in the presence of angiotensin enhanced significantly these effect: 27.77±2.16 nmol Pi.mg⁻¹.min⁻¹ with angiotensin plus Gd³⁺, n=7, suggesting an interaction in the intracellular signaling pathways involved.

Conclusions:

These data allow us to conclude that CaR enhance proton secretion in both cortical and outer medulla regions of mice kidney, by activation of H⁺-ATPase and gastric H⁺/K⁺-ATPase.

Keywords: calcium sensing receptor, vacuolar H⁺-ATPase, gastric H⁺/K⁺-ATPase, Angiotensin II

Financial Support: The authors are grateful to CNPq (Brazil) for financial support.

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Resumo:07-024

RELATIONSHIP BETWEEN RETINOL LEVELS AND OXIDATIVE STRESS MARKERS IN HAEMODIALYSIS PATIENTS

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Objectives:

Haemodialysis treatment is the main resource for patients in end stage of renal disease. These patients by treating have an imbalance of vitamins and some minerals. Also with hemodialysis causes an increase in formation of species reactive oxygen amplifying the oxidative stress. Retinol, the major circulating form of vitamin A, was shown to have some antioxidant properties although recent studies demonstrated that at higher doses it is a pro-oxidant. Any way, the mechanism by which retinol can act as a pro-oxidant is not well elucidated yet. The δ-aminolevulinatedehydratase (δ -ALA D), a zinc metalloenzyme of the haem biosynthesis pathway, requires reduced thiol groups for its activity. For this reason, ALA-D has been suggested as a biomarker for oxidative stress. It is important to clarify that δ - ALA-D activity is decreased in CRF, especially during HD treatment. Furthermore, malondialdehyde (MDA) is a more specific and sensitive biomarker for the evaluation of the lipid peroxidation status in many pathologies, including in patients under chronic HD treatment. The aim of this study was to verify the possible influence of plasma retinol levels on erythrocyte ALA-D activity AND plasma MDA levels the most used lipid peroxidation biomarker and on in HD patients.

Methods and Results:

Twenty-nine patients with diagnosis of CRF (19 men and 10 women) under going regular HD treatment at Caridade and Casa de Saude Hospitals, located in Santa Maria, RS, Brazil were included in the study. The study protocol was approved by the Human Ethics Committee of the Health Science Center from the Federal University of Santa Maria (protocol nº: 091/2003). Plasma retinol quantification was realized by method of Murata et al. with modifications. Lipid peroxidation was estimated by measured
malondialdehyde (MDA). The measurement of plasmatic MDA was determined by high performance liquid chromatography with visible detection (HPLC-VIS), according to the method of Grotto et al. The δ-ALA-D activity was determined in the total blood, with heparin as anticoagulant, according to the modified method of Sassa. Results: Hemodialysis patients have levels of retinol and MDA increased significantly compared with healthy subjects (6.86 ± 0.60 mmol/L versus 2.41 ± 0.12 mmol/L and 6.92 ± 0.35 versus 4.53 ± 0.16 mmol/L). The activity ALA-D enzyme was 20.00 ± 1.49 UI versus 11.57 ± 0.55 UI and the ALA-D reactivation 21.05 % ± 2.88 and 74.74 % ± 7.35 in control and HD patients respectively.

Conclusions:

Retinol and ALA-D were negatively correlated, showing that high levels of retinol may be directly influencing the inhibition of this enzyme. So, with this work we demonstrate that retinol, in HD patients, may be acting as an oxidant / pro-oxidant agent.

Keywords: Hemodialysis, Malondialdehyde, Oxidative stress, Retinol, &-ALA D enzyme

Financial Support: Capes/DAAD, Institute of Biological Chemistry and Nutrition (Germany) and CNPQ.

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Resumo:07-025

CLC-5 AND PROTEINURIA IN DIABETES: WHAT THE RELATIONSHIP?

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Objectives:

Proteinuria is one of the main characteristic of diabetic nephropathy. CLC-5 is an important protein involved in the mechanism of endocytosis of low molecular weight proteins in the kidney proximal tubule. In the present study, we investigated the renal function, the protein excretion profile and the CLC-5 protein expression in rats subjected to diabetic nephropathy.

Methods and Results:

8 weeks-old male Wistar rats (170-210 g) were divided into control (CTRL; minimal n=4) and diabetic (DM; minimal n=4) groups. Diabetes was induced intraperitoneally by a single injection of streptozotocin (45 mg/Kg). The presence and maintenance of hyperglycemia was monitored in blood tail using a glucometer. Urine samples (24h urine) were collected 4 (CTRL4, DM4) weeks after diabetes induction and the rats were sacrificed. Glomerular filtration rate (GFR) and protein excretion was determined. Urine samples were dialyzed against water using dialyze bags (cut off 10000) and then lyophilized. Protein concentration was determined by Bradford assay. Proteins were submitted to standard SDS-PAGE loading equal amounts of protein per lane (30ug) using 10% SDS-polyacrylamide gel. The proteins in the gels were stained by comassie blue and analyzed by the program Image J. Then the expression of CLC-5 in kidney of diabetic rats and controls were analyzed by Western Blot. Statistical analysis was performed by One-Way ANOVA (Newman-Keuls post-test). Differences were considered significant when p

Conclusions:

The GFR and the protein clearance were higher in DM group. Diabetic Wistar male rats showed a different pattern in the urinary proteins of low molecular weight and a lower expression of CLC-5 compared to controls rats. Therefore, this reduction in the expression of CLC-5 protein may be a factor involved in the genesis of the changing in protein profile of low molecular weight
Changes in Renal Function and Blood Pressure During the Aging

Nogueira, L. C. 1; Feltran, G. S. 1; Oliveira, I. S. C 1; Oliveira, H. D. 2, 1; Santos, B. T. 2, 1; Castiglione, R. C. 2, 1; Barbosa, C. M. L. 2, 1; Morales, M. M. 2; Souza-menezes,j. 1, 2

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2 Nucleo de Pesquisas em Ecologia , NUPEM/UFRJ

Objectives:

In this work it was investigate the changes in renal function and blood pressure in Wistar male rats subject to aging.

Methods and Results:

7 weeks-old male Wistar rats (170-210 g) were divided into Young (Y; n=8), Adult (A; n=7) and Old (O; n=7). The rats were kept into the cages until 8 (Y), 24 (A) and 40 (O) weeks of age with free access of water and food. One day before de sacrifice the systolic, diastolic and mean blood pressure were measured. Urine samples (24h urine) were collected in all experimental groups. Food and water intake and urinary flow were measured. After urine collection (in metabolic cages) the animals were sacrificed and the kidneys were excised where their weight were determined. Glomerular filtration rate and clearance of sodium, potassium, chloride, urea, glucose and protein were calculated. Statistical analysis was performed by One-Way ANOVA (Newman-Keuls post-test). Differences were considered significant when p

Conclusions:

The renal function of Wistar male rats changes significantly during the aging. Also it was observed a reduction in GFR and a rising in blood pressure. The last one can be associated with the increasing in sodium and chloride reabsorption observed in 40 weeks old Wistar rats.

Keywords: Aging, Renal function , Clearance, Blood pressure

Financial Support: FAPERJ, CNPq, CAPES, FUNEMAC

EFFECT OF L-TRYPTOPHAN ON RENAL MORPHOLOGY OF RATS EXPOSED POSTNATAL PROTEIN RESTRICTION.

Rêgo, L. C. 1, 2; Silva, B. N. 1; Meira, G. L. D. S. 2; Medeiros, J. M. B. 2; Manhães-de-castro, R. 3; Deiró, T.
Objectives:

Restricting protein in the maternal diet in rats, depending on the period, has been linked to the onset structural changes and diseases in adult life. It has been shown that serotonin can modulate the development of various organs, and nervous system. Thus, the purpose of this study was to investigate the effects of supplementation of L-Tryptophan (TRIP), serotonin precursor, in renal morphology and possible target organs of rats subjected to protein restriction during lactation.

Methods and Results:

Wistar male newborns whose mothers were submitted to protein restriction (with 8% protein) were treated with TRIP (15mg/kg/day subcutaneously, n = 6) or placebo (distilled water, n = 8) during lactation. At 180 days of age, animals were sacrificed and the vascular system was perfused with saline and then fixed. The heart, liver, both kidneys, and retroperitoneal fat mass (RFM) were removed and weighed. The right tibia was dissected and its length was obtained to evaluate and standardize their organ masses and adipose tissue in relation to the length of the animal. The heart, liver and both kidneys masses and the RFM were similar in both groups. The organ masses: tibia length (TL) and RFM: TL ration were also similar. However, we found a significant decrease (P

Conclusions:

Preliminary results suggest that tryptophan supplementation in critical lactation period for kidney development in rats may change renal morphology in animals submitted to protein restriction.

Keywords: kidney, lactation, malnutrition, morphology, Tryptophan

Financial Support: CAPES, FAPESB, PIBIC.

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Resumo:07-028

ACETYLCHOLINE AND RENAL FIBROSIS.

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2 Departamento de Ciencias Biologicas, UNIFESP

Objectives:

To evaluate the effect of acetylcholine in renal fibrogenesis.

Methods and Results:

Female Vacht homozygous, heterozygous and wild type knockout mice, aged 6-8 weeks were used. Animals were subjected to unilateral ureter obstruction (UOU) and killed after 7 days. Urine from the bladder and from the pelvis were collected for
proteinuria and creatinuria measurements. Pro and anti-inflammatory cytokines were measured by qPCR. Statistical analyses were performed (parametric t-test) and p value of 0.05 was considered significant. Protocol was approved by ethic committee on animal research. Vacht homozygous presented a significant reduction in body weight 7 days after surgery. Furthermore, these animals showed a significant increase in bladder and pelvic proteinuria as compared to heterozygous (p

Conclusions:

Absence of acetylcholine signaling is associated with worsen renal injury after unilateral obstruction of the kidney.

Keywords: fibrose renal, acetilcolina, inflamação

Financial Support: CNPq

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Resumo:08-064

COMPARISON OF ANTHROPOMETRIC MEASURES, APNEA-HYPOPNEA INDEX AND MINIMUM O2 SATURATION BETWEEN MEN AND WOMEN UNDERGOING POLYSOMNOGRAPHY.

Passos, V. M. M. 1; Brasileiro-santos, M. S. 3; Santos, A. C. 3; Soares, A. F. 2; Tenório, L. H. S. 1; Lima, A. M. J. 2

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2 DMFA/Universidade Federal Rural de Pernambuco, UFRPE
3 Educação Física/ Universidade Federal da Paraíba, UFPB

Objectives:

Obstructive Sleep Apnea (OSA) is common disorder characterized by cyclic and repeated episodes of upper airways collapse. The obstruction could be partial or total, associated with oxygen desaturation and carbon dioxide elevation. Polysomnography is considered the “gold standard” for OSA diagnosis, through the apnea-hypopnea index (AHI), and provides other disease-related parameters. The aim of this study was to compare anthropometric measurements, AHI and minimum O2 saturation between men and women undergoing polysomnography.

Methods and Results:

Cross-sectional study approved by the Ethics Committee for Research on Human Beings of the Universidade Federal de Pernambuco (CAAE - No. 0367.0.172.000-10). A total of 311 patients of both genders referred to the sleep laboratory of Clínica Neurológica Dr. Luiz Ataíde, Recife, PE, were enrolled between November/2010 and February/2011. The variables assessed for gender (M/F) were age, weight, height and body mass index (BMI), neck circumference and, in the polysomnographic report, AHI and minimal oxygen saturation of O2. The Student t test was used to compare each variable between groups and the results are shown as mean ± standard deviation. P

Conclusions:

According to the results of this work, we suggest that in men, the symptoms of OSA may be present in younger subjects, leading them to seek early diagnosis. Other variables such as AHI and minimum O2 saturation did not appear to be influenced by gender in the study population in question.

Keywords: Anthropometric Measures, Sleep APnea, Polysomnography
HYPEROXIA-INDUCED REDUCTION OF ALVEOLAR NUMERICAL DENSITY IN BALB/C LUNG TISSUE

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Objectives:
The present study aims to analyze the pulmonary histarchitecture in adult mice, newborn mice exposed to room air and newborn mice exposed to hyperoxia.

Methods and Results:
Balb/c adult mice (A) (n=10), newborn mice (N) (n=10) was exposed to normoxia (21% oxygen) and newborn mice (NH) (n=10) were exposed to hyperoxia (100% oxygen) in a (length= 30cm, width= 20cm, and height= 15cm) chamber for 24h, with a 2l/min flow. Groups A and N were exposed to normoxia in the same type of chamber. After exposure they were euthanized by cervical dislocation (adult group) and decapitation (newborn groups), right and left lungs were removed for histological analysis and processed according to the laboratory routine. Sections (3 im thick) were stained with hematoxylin and eosin (H&E). Morphometric analysis was performed using a video microscope and a cycloid test system superimposed on the monitor screen to analyze the surface density of gas exchange (Sv [ge]). The volume density of parenchyma (Vv[par]) was estimated by using the test points system. The alveolar numerical density (Nv[alv]) was estimated by using a test area system. We have also quantified macrophages located in the alveolar lumen and atelectasis areas. We observed macrophages decrease in NH group (p=0.04) in 24h when compared to N group. The volume density of parenchyma (Vv[par]) in exposed animals (NH) also decreased (p<0,0001).

Conclusions:
Our results indicate that hyperoxia exposure could promote histoarchitecture changes in newborn mice, decreasing volume density of parenchyma, surface density of gas exchange and alveolar macrophages, and increasing atelectasis areas. We could also find that the alveolar numerical density is sensitive to changes induced by hyperoxia.

Keywords: HYPEROXIA, LUNG, MORPHOMETRY

Financial Support: FAPERJ AND FUNADESP

PRESSURE SUPPORT VENTILATION IMPROVES OXYGENATION WITH LESS LUNG INJURY AND IS FURTHER IMPROVED BY RANDOM VARIATION OF PRESSURE SUPPORT

Uhlig, P. C. ¹; Spieth, P. M. ¹; Carvalho, A. ¹; Güldner, A. ¹; Kasper, M. ²; Spieth, S. ³; Wiedemann, B. ⁴; Uhlig, S. ⁵; Pelosi, P. ⁶; Abreu, M. G. D. ¹

Financial Support: FACEPE
Objectives:

In acute lung injury (ALI), pressure support can be advantageous over pressure controlled ventilation (PCV), and benefit from noise, i.e. random variation in pressure support. We investigated whether conventional pressure support ventilation (PSV) improves lung function and attenuates the pulmonary inflammatory response compared to PCV and random variation of pressure support (noisyPSV) adds further beneficial effects to PSV.

Methods and Results:

This study comprehended two protocols: A and B. In both protocols, juvenile female pigs (26.6-46.6kg) were anesthetized intubated and mechanically ventilated in volume controlled mode. ALI was induced by surfactant depletion with saline lavage. In protocol A, 24 animals were randomly assigned to three groups (n=8 per group) undergoing 6 hours of mechanical ventilation with: 1) pressure controlled ventilation; 2) pressure support; 3) noisy pressure support, with a coefficient of variation of 22% (normal distribution). We determined respiratory variables, post-mortem lung histological damage, mechanical stress and inflammation. In protocol B, all modes of ventilation were applied in 12 animals in random sequence (1 hour each). Driving pressure was set to achieve 6 ml/kg mean tidal volume, according the ARDS Network. We determined respiratory variables, distribution of lung aeration with static and dynamic computed tomography, and distribution of pulmonary blood flow by means of i.v. administered colored microspheres. Compared to PCV, pressure support: 1) improved oxygenation (p<0.05).

Conclusions:

Pressure support with and without noise improved respiratory function by redistribution of lung perfusion from dorsal to ventral zones and reduced lung injury compared to controlled ventilation. Variable pressure support added further benefits to gas exchange, reduced inspiratory effort and improved features of lung injury compared to traditional pressure support ventilation.

Keywords: ACUTE LUNG INJURY, BIOLOGICAL VARIABILITY, MECHANICAL VENTILATION, VENTILATION-PERFUSION, VENTILATION ASSOCIATED LUNG INJURY

Financial Support: by a grant from the German Research Council
Objectives:

To test the hypothesis that a single early intravenous dose of glutamine is associated not only to the improvement of lung morpho-function, but also the reduction in kidney, liver, and small intestine villi injury in septic undernourished rats.

Methods and Results:

In undernourished groups, male Wistar rats (n=6/group) received 1/3 of their usual daily food consumption for 4 weeks. In the control group (C), rats received food ad libitum for 4 weeks. Undernourished rats were divided into two groups: in CLP group, sepsis was induced by cecal ligation and puncture surgery, while a sham operated group (Sham) was used as control for animals undergoing CLP. One hour after surgery, Sham and CLP groups were further randomized into subgroups receiving intravenous saline (Sal) (1 ml) or glutamine (Gln) (0.75 g/kg body weight). At forty-eight hours, lung static elastance (Est,L) was computed by end-inflation occlusion method. Lung morphometry was analyzed and the degree of apoptosis on lung, kidney, liver, and small intestine villi as well as the amount of collagen and elastic fibers were quantified. Heat shock protein 70 (HSP70) and heat shock factor-1 (HSF-1) expressions were analyzed in lung tissue (Western Blotting). Undernutrition led to an emphysema-like lesion, lower elastic fiber content, and higher degree of apoptosis on lung and distal organs compared to C. Comparing to Sham-Sal, Sham-Gln animals presented: 1) reduced polymorphonuclear cell infiltration (40%, p

Conclusions:

In undernourished septic animals, a single intravenous dose of glutamine modulated the inflammatory process reducing the risk of lung and distal organs injury. These beneficial effects were not related to HSP70 and HSP-1, which are the usual glutamine mechanism of action in sepsis. Thus further studies should be performed to evaluate the mechanisms of glutamine in undernourished septic rats.

Keywords: acute lung injury, apoptosis, glutamine, lung mechanics, undernutrition

Financial Support: CNPq, PRONEX-FAPERJ, FAPERJ, CAPES

QuebraPagina

Resumo:08-068

EFFECTS OF LASSBIO596 ON LUNG MECHANICS AND HISTOLOGY IN A MURINE MODEL OF EMPHYSEMA.

Padilha, G. A. 1; Guimarães, I. H. 1; Lopes-pacheco, M. L. 1; Crossetti, J. 1; Lima, L. M. 2; Barreiro, E. J. 2; Xisto, D. G. 1; Rocco, P. R. M. 1

1 Laboratório de Investigaçao Pulmonar / IBCCF, UFRJ
2 Laboratório de Avaliação e Síntese de Substâncias Bioativas, UFRJ

Objectives:

The present study investigated the effects of LASSBio596, a hibrid of thalidomide and sildenafil which exhibits potent inhibitory effects on TNF-α and TGF-β, on lung mechanics and histology in a murine model of emphysema.

Methods and Results:

Twenty-two BALB/c (20-25 g) mice were randomly assigned into two groups (n = 11/each): control (CTRL) and emphysema (ELA). CTRL animals were intratracheally (it) instilled with saline (50 microl) while ELA mice received porcine pancreatic
elastase (0.1 UI, it). Saline and elastase were intratracheally instilled once a week during 4 weeks. After the last intratracheal instillation, animals were further randomized into 2 subgroups to receive DMSO [10 mg/kg, 0.02 ml, intraperitoneally (ip)] or LASSBio596 (10 mg/kg, 0.02 ml ip) for eight consecutive days. Animals were then anesthetized, tracheotomized and mechanically ventilated. Lung static elastance, airway resistance and viscoelastic/inhomogeneous pressure were analyzed by end-inflation occlusion method. Lungs were fixed and stained for histological analysis. ELA group presented lower lung static elastance (19%), higher alveolar hyperinflation (81%) and mean alveolar diameter (33%) compared to CTRL. LASSBio596 led to increased lung static elastance, as well as decreased alveolar hyperinflation and mean alveolar diameter.

Conclusions:

In the present experimental model of emphysema, the treatment with LASSBio 596 improved lung mechanics and histology.

Keywords: Emphysema, Inflammation, Pharmacological therapy, TNF-alpha

Financial Support: INCT-INOFAR, CNPq, PRONEX, FAPERJ, CAPES.

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Resumo:08-069

OPPOSITE ROLES OF BRADYKININ RECEPTORS IN BLEOMYCIN-INDUCED PULMONARY INFLAMMATION AND FIBROSIS IN MICE

Russo, R. C. 1,2; Cordeiro, B. F. 2; Garcia, C. C. 2; Tavares, L. P. 2; Lopes, G. A. O. 2; Lima, B. H. F. 2; Soriani, F. M. 2; Cassali, G. D. 3; Teixeira, M. M. 2

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Objectives:

Bradykinin (BK) is the principal effector of the plasma kinin system. Released from high molecular weight kininogen by the action of plasma kallikrein, BK is a peptide that promotes multiple pro-inflammatory effects such as cellular migration, cytokines release, pain and edema. BK acts through two GPCRs: B2R is constitutively expressed in healthy tissues and B1R is induced upon tissue damage and inflammation. Bleomycin (BLEO) is an anticancer drug, which main side effect is lung toxicity correlated with fibrosis. In mice, BLEO is used as a model to study pulmonary fibrosis pathogenesis. Here, we evaluated the role of BK receptors in the context of lung inflammation and fibrosis induced by BLEO in mice.

Methods and Results:

B1R, B2R and B1B2R deficient mice (B1RKO, B2RKO and B1B2RKO) or C57Bl6/j (WT) were intra-tracheally instilled with (BLEO 3.75U/Kg) (approved by the animal ethics committee CETEA/UFMG, protocol number: 146/06). We evaluated weight loss and lethality during 21 days, lung inflammation and fibrosis at 7 and 21 days post-BLEO instillation. WT mice showed 30% of survival (d=21) and 20% of weight loss when challenged with BLEO. B1RKO mice succumbed with 100% of mortality (d=14), however, B2RKO mice were protected from lethality and weight loss induced by BLEO. The B1B2RKO mice showed the same phenotype as WT mice after BLEO injection. BLEO induced marked neutrophil influx into lung and airways with protein leakage (d=7) in WT, B1RKO and B1B2RKO mice, but not in B2RKO mice. B2RKO mice displayed reduced blood hematocrit than WT mice, B1RKO mice and B1B2RKO mice post-BLEO (d=7). Lung levels of IL-1β, IL-6 and CXCL1 were increased in WT, B1RKO and B1B2RKO mice, but not in B2RKO mice after BLEO injection (d=7). TGF- β 1 was elevated only in WT and B1B2RKO mice, and B2RKO mice showed increased IL-10 lung levels induced by BLEO (d=7). Real-time PCR did not show differences in the kinetic of B1R expression between WT and B2RKO mice challenged with BLEO, however, B1RKO mice presented a marked increase in B2R expression if compared to WT mice (d=4,7). iNOS mRNA expression was
progressively increased in B1RKO compared to WT mice (d=4,10), but it was blunted in B2RKO mice exposed to BLEO. B2RKO mice also showed a reduced expression of eNOS, von Willebrand Factor, α-SMA and Coll α 1 (d=10) compared to WT or B1RKO mice when challenged with BLEO. WT and B1B2RKO mice presented increased lung hydroxyproline content, but not B2RKO mice (d=21). Histopathology (H&E and Gomori’s Trichrome) confirmed lung integrity of B2RKO mice when compared to WT or B1B2RKO mice 21 days post-BLEO administration.

Conclusions:
We showed the paradoxical role of “constitutive vs. inducible” bradykinin receptors in the context of pulmonary inflammation and fibrosis in mice. We conclude that the constitutive receptor B2 is more important for lung inflammation and fibrosis than the inducible receptor B1, suggesting that B2R inhibitors may be beneficial for pulmonary fibrosis treatment.

Keywords: Bleomycin, Bradykinin, Lung fibrosis, Lung inflammation, Neutrophils

Financial Support: CNPq and FAPEMIG

QuebraPagina

Resumo:08-070

EFFECTS OF CORTICOSTEROID AND MONTELUKAST TREATMENT ON DISTAL LUNG PARENCHYMA AND AIRWAY WALLS IN INFLAMMATION IN GUINEA PIGS WITH CHRONIC ALLERGIC INFLAMMATION

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Objectives:
The effects of montelukast or dexamethasone in asthma pathophysiology are barely understood. So, we evaluated the inflammation in distal lung parenchyma and airway walls in guinea pigs (GP) with chronic allergic inflammation.

Methods and Results:
GP (Harley, male, 250-300g) were inhaled with ovalbumin (OVA group-2x/week/4weeks). After 4th inhalation, GP were treated with montelukast (M group-10mg/Kg/PO/day) or dexamethasone (D group-5mg/Kg/IP/day). After 72 hrs of 7th inhalation, GP were anesthetised, lung strips were submitted to histopathological evaluation. On distal parenchyma both montelukast and dexamethasone were effective in reducing RANTES and NF-kB positive cells compared to OVA group (p<0.05).

Conclusions:
In this animal model, both corticosteroid and montelukast treatments contribute to the control of the inflammatory response in distal lung parenchyma and airway walls. Dexamethasone treatment induced a greater reduction of NF-kB expression in airway walls which suggests one of the mechanisms that explains the higher efficacy of this therapeutic approach.

Keywords: corticosteroid, montelukast, inflammation, guinea pigs, chronic allergic inflammation

Financial Support: FAPESP, CNPq, LIM-20-HC-FMUSP

QuebraPagina
COMMERCIAL MATE TEA BENEFICIAL ACTION IN ACUTE PULMONARY INFLAMMATION MODEL BY CIGARETTE SMOKE – EFFECTS AS AN ANTIOXIDANT

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Objectives:
The Ilex paraguariensis herb has been commonly used in alternative medicine. After toasting process, the infusion known as mate tea (MT) that contains many bioactive compounds that are cofactors in metabolic reactions. As cigarette smoke (CS) is the main cause of chronic obstructive pulmonary disease (COPD), we analyzed the effects of mate tea on inflammatory and oxidative lung damage caused by CS.

Methods and Results:
C57BL/6 mice were exposed to CS and treated with mate before, during and after acute exposure. Groups were formed as follows (n=10/group): I) Concomitant treatment = animals exposed to room air (Control), animals exposed to room air and treated with mate (mate), animals exposed to cigarette smoke (CS - The animals were exposed to six commercial cigarettes per day for five days) and animals exposed to cigarette smoke and treated with mate (mate + CS). II) Pre-treatment = animals pre-treated with mate and exposed to ambient air or animals pre-treated with mate (Control Pre-mate) and exposed to cigarette smoke (CS + Pre-mate). III) Post-treated= animals exposed to cigarette smoke and euthanized 5 days after the exposure protocol (Post-CS) animals exposed to cigarette smoke and post-treated with mate (CS + Post-mate). Cells counts were done in the bronchoalveolar lagave fluid (BAL). Lung homogenates were analyzed regarding the levels of total reactive oxygen species production (ROS), pro-inflammatory cytokines, thiobarbituric reactive species (TBARS), protein carbonyls, nitrite, hydroxyproline and total polyphenols were measured. Also, glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities were measured. Mate administration both blocked and prevented the influx of leukocytes into the lungs of mice exposed to CS compared with the control group (p

Conclusions:
We conclude that the concomitant administration of mate was more efficient in protecting mouse lungs exposed to CS since mate administrations presented significant anti-inflammatory effects, accelerated the synthesis of collagen fibers, and reduced both ROS production and oxidative damage.

Keywords: chá mate, fumaça de cigarro, antioxidantes, inflamação pulmonar, estresse oxidativo

Financial Support: FAPERJ, Capes, Mate Leão S.A
Objectives:

We have recently observed that the effects of early bone marrow-derived mononuclear cell (BMDMC) therapy [before ovalbumin (OVA) challenge] on lung morphofunction were more pronounced after intratracheal instillation compared to intravenous administration. However, so far, no study has analyzed any beneficial effects of BMDMC therapy after ovalbumin challenge, when the remodeling process is already installed. Thus, this study investigated the impact of both routes of bone marrow-derived mononuclear cell administration on lung inflammation and remodeling in a murine model of chronic allergic asthma.

Methods and Results:

36 female C57BL/6 mice were randomly assigned into two groups. In the OVA group, mice were sensitized and challenged with ovalbumin while control group (C) received saline using the same protocol. C and OVA groups were further randomized into subgroups receiving saline (50 µL, SAL) or bone marrow-derived mononuclear cell (BMDMC, 2x10^6, CELL) intravenously or intratracheally, 24 hours after the last challenge. Airway and lung parenchyma remodeling were evaluated by quantitative analysis of collagen fiber, and electron microscopy. Furthermore, airway resistance, viscoelastic pressure, static elastance were analyzed. BMDMC therapy, independent of the route of administration, led to a reduction in alveolar collapse, eosinophil infiltration, subepithelial fibrosis, myocyte hypertrophy and hyperplasia, and the amount of myofibroblasts in airways and lung parenchyma compared to OVA. Nevertheless, airway resistance and viscoelastic pressure were significantly more reduced after BMDMC through intratracheal instillation (80% and 47%, respectively) compared to intravenous (27% and 30%, respectively).

Conclusions:

In the present model of chronic allergic asthma, BMDMC therapy was effective at modulating the inflammatory and fibrogenic processes independent of the route of cell administration. However, lung mechanics presented a greater improvement after intratracheal instillation of BMDMC.

Keywords: Inflammation, Collagen fiber, Lung mechanics, Stem cells, Asthma

Financial Support: PRONEX-FAPERJ, CNPq, FAPERJ, CAPES, INCT-INOFAR

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Resumo:08-073

**EFFECTS OF REGULAR AND MODERATE AEROBIC EXERCISE ON AIRWAY AND LUNG PARENCHYMA REMODELING IN A MURINE MODEL OF CHRONIC ALLERGIC ASTHMA.**

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Objectives:

The present study investigated whether regular and moderate aerobic exercise might prevent airway and lung parenchyma
remodeling in experimental chronic allergic asthma.

Methods and Results:

48 BALB/c mice were assigned into 2 groups: sedentary (S) and trained (Tr). Tr group ran on a motorized treadmill at moderate intensity (8-12 m/min), 5% grade, 30 min/day, 3 times a week for 8 wks. At 8 wks, animals were further randomized into 2 subgroups to be immunized and challenged with ovalbumin (OVA) or to receive saline using the same protocol (C). Aerobic exercise continued until the end of the protocol. Echocardiographic analysis was done before, at 4 and 8 weeks of training, and after asthma induction. Twenty-four hours following the last challenge, lung mechanics and histology were analyzed. Airway and lung parenchyma remodeling were evaluated by quantitative analysis of collagen and elastin, immunohistochemistry (smooth-muscle actin expression) and electron microscopy. Additionally, total and differential cell count in the bronchoalveolar lavage fluid (BALF) and lung tissue were measured. Interleukin (IL)-5, IL-13, IL-10, interferon (IFN)-gamma, and transforming growth factor (TGF)-beta were analyzed in BALF and blood.

Trained, compared to sedentary mice, presented: 1) an increase in systolic output, left ventricular mass, and end-diastolic volume; 2) a reduction in airway resistance (23%), viscoelastic pressure (12%), static elastance (23%), airway hyperresponsiveness, eosinophil infiltration, smooth-muscle actin expression, and collagen fiber content in airways and lung parenchyma; 3) a decrease of TGF-beta levels in BALF and blood; 4) an increase in IFN-gamma in BALF and blood; 5) an augment of IL-10 in blood but a reduction in BALF; and 6) a decrease in IL-5 and IL-13 only in BALF.

Conclusions:

Regular and moderate aerobic exercise was effective in preventing airway and lung parenchyma remodeling in the present murine model of chronic allergic asthma, improving lung function.

Keywords: ASTHMA, EXERCISE, INFLAMMATION, REMODELING

Financial Support: CAPES, PRONEX, FAPERJ, CNPq, INCT-INOFAR

QuebraPagina

EFFECTS OF GLUTAMINE THERAPY IN EXPERIMENTAL PULMONARY AND EXTRAPULMONARY ACUTE LUNG INJURY.

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3 Laboratory of Inflammation/IBCCF, UFRJ

Objectives:

To test the hypothesis that a single intravenous dose of glutamine is able to protect the lung independent of the etiology of acute lung injury (ALI). For this purpose, the effects of glutamine therapy on lung mechanics and histology were analyzed in experimental pulmonary and extrapulmonary ALI with similar degrees of mechanical compromise.

Methods and Results:

Thirty-six male BALB/c mice (n=6/each group) were randomly divided into two main groups. In control (C) groups, the animals were subdivided to be treated with a single intravenous dose of saline (Sal, 0.2 mL) or glutamine (0.75 g/kg body weight). In the ALI groups, mice received E. coli lipopolysaccharide (LPS) (10 microg i.t. in ALIp and 125 microg i.p. in ALIexp). At six hours, animals were treated either with a single intravenous dose of saline (Sal, 0.2 mL) or glutamine (0.75 g/kg body weight). Twenty-
four hours after ALI induction, lung static elastance (Est,L) was computed by end-inflation occlusion method. Lung histology (light microscopy) was analyzed. Survival rate, levels of IL-6 and TGF-beta in the bronchoalveolar lavage fluid (BALF), and reduced glutathione (GSH) levels and glutathione peroxidase (GPx) activity were also investigated. Survival rate was 94% (ALI-p-Sal) and 78% (ALI-exp-Sal), and 100% in both ALI groups treated with glutamine (p

Conclusions:

The treatment with a single intravenous dose of glutamine reduced morphofunctional changes independent of the etiology of ALI. These beneficial effects were not related to the modulation of these cytokines or increased GSH levels. Thus further studies should be performed to evaluate these beneficial effects.

Keywords: CYTOKINES, EXTRAPULMONARY ACUTE LUNG INJURY, GLUTAMINE, LUNG MECHANICS, PULMONARY ACUTE LUNG INJURY

Financial Support: INCT-INOFAR, CNPq, PRONEX-FAPERJ, FAPERJ, CAPES

QuebraPagina

Resumo:08-075

VENTILATORY PATTERN, PULMONARY AND INTRABUCAL GASES OF THE SOUTH AMERICAN LUNGFISH LEPIDOSIREN PARADOXA

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Fisiologia / Faculdade de Medicina de Ribeirão Preto - USP, FMRP - USP

Objectives:

Our objective was to characterize respiratory pattern along with pulmonary and intrabuccal gases in the South American lungfish Lepidosiren paradoxa (Dipnoi).

Methods and Results:

Experiments were performed with five lungfish Lepidosiren paradoxa (n=5). Animals were submitted to surgical procedures to implant a cannula into pulmonary and buccal compartments for gases sampling (PO2 and PCO2). Pulmonary Ventilation (Ve) was measured using pneumotachograph method. The mean value of pulmonary ventilation (Ve) was 90.38 ± 24.59 ml -1 BTPS Kg h -1. The respiratory cycle of Lepidosiren paradoxa begins with expiration followed by inspiration. The expiratory phase is marked by a series of small events. The PCO2 in the lung and buccal compartments had mean values of 2.78 ± 0.3% and 3.14 ± 0.35% respectively. PO2 values were 13.78 ± 0.72% for pulmonary and 12.57 ± 1% for the buccal compartment.

Conclusions:

These results suggest a close similarity of breathing pattern between Lepidosiren paradoxa and Amphibian group, since Lepidosiren paradoxa is dependent on the pulmonary ventilation and has obligatory air breathing like amphibians.

Keywords: Intrabuccal gases, Lepidosiren paradoxa (Dipnoi), Pulmonary gases, Ventilatory pattern

Financial Support: CNPq and FAPESP

QuebraPagina
LEPTIN TREATMENT WITHIN RVLM/RTN REGIONS ENHANCES VENTILATORY RESPONSE TO CO2 IN OB/OB MICE.

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Objectives:
Studies have demonstrated that leptin-deficient (ob/ob) mice have attenuated ventilatory responses to CO2 which were reversed by leptin replacement either peripheral or centrally. We hypothesized that leptin modulates ventilatory response by acting within rostral ventrolateral medulla and/or retrotrapezoid nucleus (RVLM/RTN), both important central regions for breathing control.

Methods and Results:
Male ob/ob mice (42±2 g) with a stainless steel guide cannula implanted in the RVLM/RTN were used. We evaluated pulmonary ventilation (VE), tidal volume (VT) and respiratory frequency (f) under normocapnic or hypercapnic conditions (4% CO2 and 7% CO2) using a plethysmographic method. Ventilatory responses were analyzed in ob/ob mice after leptin or vehicle microinjections into the RVLM/RTN (1 µg/100 nl, n=7/group) for 3 days. Baseline ventilation increased significantly after leptin (2398±176 ml.min⁻¹.kg⁻¹) compared to vehicle treated group (1572±160 ml.min⁻¹.kg⁻¹). Leptin increased tidal volume (8.83±0.69 ml.kg⁻¹, P

Conclusions:
These data suggest that leptin modulates ventilatory responses to CO2 by acting in the RVLM/RTN region.

Keywords: central chemoreception, leptin, obesity

Financial Support: FAPESP, CNPq and Capes

QuebraPagina

EFFECTS OF CORTICOSTEROID OR MONTELUKAST ASSOCIATED TO INOS INHIBITION ON INFLAMMATION AND REMODELLING OF DISTAL LUNG IN ANIMALS WITH CHRONIC INFLAMMATION

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Objectives:
Aims: Distal lung parenchyma alterations have been recently addressed on asthma pathophysiology. We evaluated oxidative stress, actina content and IL5 and MMP9 positive cells after montelukast or dexamethasone treatments associated or not to an iNOS inhibitor (1400W) in guinea pigs (GP) with chronic lung.
Methods and Results:

Methods: GP were inhaled with ovalbumin (OVA group-2x/week/4weeks). After 4th inhalation, GP were treated with montelukast (M group-10mg/Kg/PO/day) or dexamethasone (D group-5mg/Kg/IP/day). 1400W (W group-1mg/kg/day) was given daily in the last 4 days (W, DW and MW groups). After 72 hrs of 7th inhalation, GP were anesthesised, lung strips were submitted to histopathological evaluation. Results: Isoprostane was reduced in M(9.6±0.4%), D(7.4±0.2%), MW(6.1±0.1%), DW(5.5±0.2%) and W(6.9±0.4%) compared to OVA(14.5±0.2%, p<0.05).

Conclusions: In this animal model, corticosteroid or anti-leukotriene associated to iNOS inhibition contributes to the reduction of the oxidative stress, actin and was efficient to attenuate Th2 inflammatory response in distal lung parenchyma.

Keywords: asthma, corticosteroids, iNOS, inflammation, remodelling

Financial Support: FAPESP, CNPq, LIM-20-HC-FMUSP

QuebraPagina

Resumo:08-078

EFFECTS OF CENTRAL INJECTIONS OF LEPTIN OR MELANOCORTIN ANTAGONIST ON ARTERIAL PRESSURE AND VENTILATORY RESPONSES TO HYPERCAPNIA.

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2 Fisiologia/ Facul. Medicina de Ribeirão Preto, FMRP-USP
3 Physiology and Biophysics/Univ. Mississippi Medical Center, UMMC

Objectives:

Melanocortin 3/4 receptors (MC3/4R) are suggested to play a pivotal role in the mediation of metabolic and cardiovascular actions of leptin. We hypothesized that MC3/4 receptors may also mediated leptin effects on ventilatory response to CO2. Therefore, in the present study, we evaluated the changes in pulmonary ventilation (VE) to hypercapnia (7% CO2), mean arterial pressure (MAP) and heart rate (HR) in rats treated with daily injections of leptin or SHU9119 (MC3/4R antagonist) into the lateral ventricle (LV) for 7 days.

Methods and Results:

Male Holtzman rats (n=6/group) with stainless steel cannula implanted into the LV were used. Ventilation was recorded by plethysmographic method and MAP and HR through a catheter inserted into the femoral artery. Leptin (5 lg/5 day) injected into the LV increased MAP and HR (127± 4 mmHg and 422± 21 bpm, respectively) compared to saline treatment (106± 6 mmHg and 355± 21 bpm, respectively). Leptin treatment also increased tidal volume (11± 0.67 ml.kg-1, vs. saline: 7.79± 0.70 ml.kg-1) and the ventilatory response to CO2 (1796± 140 ml.min-1.kg-1, vs. saline: 1213± 126 ml.min-1.kg-1) and reduced food intake by 70% and body weight by 17%, without changing respiratory frequency. The blockade of MC3/4R with antagonist SHU (1 nmol/5 il/day) promoted opposite effects on food intake and body weight (increases of 27% and 13%, respectively), without changing MAP and HR (106± 6 mmHg and 360± 17 bpm, respectively) or the ventilatory response to CO2 (1185± 53 ml.min-1.kg-1).

Conclusions:
Central administration of leptin increases MAP, HR and ventilatory responses to hypercapnia, whereas MC3/4R blockade does not alter MAP and respiratory responses.

Keywords: obesity, central breathing control, leptin, MC3/4 receptors

Financial Support: FAPESP, CNPq and Capes

MODULATION OF ORAL TOLERANCE ON THE OXIDATIVE STRESS RESPONSES IN DISTAL LUNG PARENCHYMA OF GUINEA PIGS WITH CHRONIC ALLERGIC INFLAMMATION

Departamento de Clínica Médica/ Faculdade de Medicina, FMUSP

Objectives:

We previously had shown that oral induced tolerance contributes to reduce distal lung responsiveness, inflammation and remodelling (Nakashima et al., 2008) in a model of chronic inflammation in guinea pigs (GP). In the present study, we evaluated if these responses were associated to alterations on the oxidative stress responses in distal lung.

Methods and Results:

Methods: GP were submitted to multiple inhalations of ovalbumin (OVA) or normal saline (NS) (2x/wk/4wks). At the same period oral tolerance was induced by offering GP ad libitum 2% ovalbumin in sterile drinking water during 4 weeks (OVA-T1) or starting oral ovalbumin after the 4th inhalation of ovalbumin (OVA-T2). Afterwards, lungs were removed, strips of distal lung were stained for iNOS and PGF2alfa (isoprostane) and analysed by morphometry. Results: In OVA group there was an increase in the iNOS positive cells (20.7±1.0/104ìm2) and PGF2alfa content (1.7±0.25%) compared to NS group (p

Conclusions:

Conclusion: Oral tolerance attenuates the oxidative stress responses in distal lung in this animal model of chronic pulmonary inflammation. These results may clarify the mechanisms involved in the attenuation of mechanical responsiveness, inflammation and remodeling of distal lung by oral tolerance, as previously shown in this animal model.

Keywords: oral tolerance, oxidative stress, guinea pigs , distal lung, chronic allergic inflammation

Financial Support: FAPESP, CNPq, LIM-20-HC-FMUSP.

ENHANCED AIRWAY SMOOTH MUSCLE REACTIVITY TO CHOLINERGIC PROVOCATION IS ASSOCIATED TO MAST CELLS IN A/J MICE.

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Objectives:

Prior studies show that genetic strain variations may exist with regard to respiratory mechanics and airway hyperresponsiveness in mice. It has been shown that A/J mice are more sensitive to cholinergic-mediated bronchial spasm as compared to Balb/c mice. The current study is undertaken in order to test the hypothesis that the number of mast cells in the target tissue underlies the relative hyperreactivity expressed by mice of the A-J strain.

Methods and Results:

Age-matched mice of strains A/J and Balb/c were employed as donors of tracheal and bronchial tissues for functional and histological approaches. Mast cell population was evaluated by histomorphometry in carnoy-fixed and paraffin-embedded sections stained with alcian blue-safranin. Tracheal and bronchial rings were mounted in isolated organ bath for assessment of carbachol response (0.01-100 µM) in presence or absence of sodium cromoglicate (SCG). We found increased mast cell numbers in tracheal (16.2 ± 1.7 cells/section) and bronchial tissues (36.7 ± 4.1 cells/section) (mean ± SEM, n=5) recovered from naive A/J mice as compared to those from BALB/c tracheal (9.8 ± 0.7 cells/section) and bronchial tissues (15.4 ± 3.7 cells/section). Both tracheal and bronchi rings obtained from A/J mice were clearly more responsive to carbachol-induced contraction, as compared to those obtained from BALB/c mice. Following 1 mM SCG treatment, there was a marked attenuation of the tracheal response to carbachol irrespective to the mouse strain; however a higher sensitivity of A/J mice tracheal rings to the mast cell stabilizer was noted.

Conclusions:

Our findings indicate that there are more mast cells in the airway smooth muscle tissue of A/J mice as compared to Balb/c mice and that this mast cell tissue enrichment may be critical in the airway hyperresponsiveness exhibited by A/J mice following cholinergic provocation.

Keywords: A/J mice, hiperresponsiveness, mast cell

Financial Support: FAPERJ, CNPq

QuebraPagina

Resumo:08-081

TIDAL VOLUME IMPAIRED LUNG AND DISTAL ORGAN DAMAGE IN THE PRESENCE OF INTRA-ABDOMINAL HYPERTENSION DEPENDING ON THE ETIOLOGY OF ACUTE LUNG INJURY

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Objectives:

Pulmonary (p) and extrapulmonary (exp) acute lung injury (ALI), associated or not with intra-abdominal hypertension (IAH), are similarly treated with protective ventilation. However, ALIp and ALIexp present different morphological aspects and respond differently to ventilator strategies. This study compared the effects of protective tidal volume (VT) (6 ml/kg, VT6) with higher
VT (10 ml/kg, VT10) on lung and distal organs in experimental models of ALIp and ALIexp with and without IAH.

Methods and Results:

Male Wistar rats (n=48) were randomly allocated to receive Escherichia coli lipopolysaccharide intratracheally (200 µg, ALIp) or intraperitoneally (1 mg, ALIexp). After 24 hours, they were randomized into subgroups with or without IAH, which was induced by inserting gauze into the abdominal cavity for a maximum stabilized intra-abdominal pressure of 15 mmHg. They were then ventilated with PEEP=5 cmH2O and VT6 or VT10 during 1 hour. Static lung elastance (Est,L), arterial blood gases, lung and distal organ histology, and interleukin (IL)-1β, IL-6, caspase-3, and type III procollagen (PCIII) mRNA expressions in lung tissue were analyzed. In the presence of IAH: 1) VT6 and VT10 improved oxygenation independent of ALI etiology. In ALIexp, PaO2 increase were lower with VT6 than VT10; ALIp: 63% (VT6) and 129% (VT10), ALIexp: 81% (VT6) and 135% (VT10) and led to increased PCIII, IL-1β, IL-6, and caspase-3 mRNA expressions in lung tissue, diaphragmatic Z-disk edema and mitochondrial injury, as well as liver, kidney, and vill cell apoptosis independent of ALI etiology; 2) in ALIexp, VT10 was associated with decreased Est,L, alveolar collapse, as well as IL-6 and caspase-3 mRNA expressions in lung tissue; and 3) in ALIp, VT10 increased IL-6 mRNA expression.

Conclusions:

In ALIexp with IAH, VT10 improved lung morphofunction with less inflammatory process in lung tissue compared to VT6. Conversely, in ALIp with IAH, VT10 increased IL-6 expression in lung tissue. Therefore, in the presence of IAH, higher VT may minimize lung injury in ALIexp, but not in ALIp.

Keywords: acute lung injury, intra-abdominal hypertension, Mechanical ventilation

Financial Support: CNPq, PRONEX-FAPERJ, FAPERJ, CAPES

QuebraPagina

Resumo:08-082

LUNG MECHANICAL IMPAIRMENT IN C57BL/6 MICE INFECTED WITH DIFFERENT STRAINS OF PLASMODIUM

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Objectives:

Malaria presents a significant morbidity and mortality in the tropics causing more than 1 million deaths every year. Animal models of infection are indispensable tools to better understand the dynamic host/parasite interactions that lead to different outcomes. We aimed to analyze lung mechanics in mice exposed to different strains of Plasmodium.

Methods and Results:

Male C57BL/6 mice (17-25 g) were intraperitoneally (ip) inoculated with red blood cells (RBC) infected with P. berghei ANKA (PBA group), P. berghei NK65 (PBNK group), P. chabaudi AS (PCh group) or received only RBC (CTRL group). Lung mechanics was determined 3 or 7 days after inoculation. At the 3rd day there was no significant difference between the analyzed groups. At the 7th day PBNK (n = 4) group showed higher static elastance (87.6 ± 11.0 cmH2O/ml) than CTRL (n = 5), PBA (n = 3) and PCh (n = 2) groups (36.7 ± 3.9, 39.9 ± 0.7 and 38.2 ± 1.9 cmH2O/ml, respectively). PBNK group also showed higher viscoelastic component of elastance and viscoelastic/inhomogenous pressure (15.4 ± 3.1 cmH2O/ml and 2.5 ± 0.4 cmH2O, respectively) than PBA group (5.6 ± 0.2 cmH2O/ml and 1.2 ± 0.1 cmH2O). There was no difference in resistive and total resistive pressures among the analyzed groups.
Conclusions:

Exposure to *P. berguei* NK65 led to impaired pulmonary function with increased elastic and viscoelastic components of lung mechanics 7 days after inoculation. The other models did not result in models of lung injury due to malaria. However, further experiments are necessary to improve the power of our results.

Keywords: lung mechanical, plasmodium, malaria

Financial Support: CNPq, FAPERJ, MCT

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Objectives:

The present study investigated the effects of LASSBio596, a hybrid of thalidomide and sildenafil which exhibits potent inhibitory effects on TNF-alpha and TGF-beta, associated with surfactant on lung and distal organ injury in an experimental sepsis-induced acute lung injury (ALI).

Methods and Results:

32 BALB/c mice were randomized to undergo cecal ligation and puncture surgery (CLP) or sham surgery (C). At one hour, C and CLP groups were further randomized into subgroups to receive saline [0.2 ml, SAL, intraperitoneally (i.p.)], LASSBio 596 (10 mg/kg i.p., 596), surfactant (Survanta® 4 ml/kg, 0.1 ml intratracheally, S) or LASSBio596 associated with surfactant (596-S). 24 h after the surgery, pulmonary mechanics (airway resistance, viscoelastic pressure, and static elastance), lung histology (light and electron microscopy), and bronchoalveolar and peritoneal lavage fluid, and blood cellularity were measured. Additionally, blood biochemistry [urea, creatinine, aspartate aminotransferase (AST), and alanine aminotransferase (ALT)] was analyzed. The survival rates of all mice were monitored for 7 days after CLP. LASSBio596 associated or not with surfactant significantly improved survival (p)

Conclusions:

In the present experimental model of sepsis induced ALI, a single early dose of LASSBio596 associated or not with surfactant may modulates the inflammatory process reducing not only the risk of lung injury, but also distal organ impairment. These results suggest that LASSBio596 may represent a promising novel therapeutic strategy for the treatment of sepsis induced ALI.

Keywords: Acute Lung Injury, sepsis, treatment, pharmacotherapy, surfactant

Financial Support: CAPES, INCT-INOFAR, FAPERJ, PRONEX, CNPq
THERE IS A CORRELATION BETWEEN MAXIMAL RESPIRATORY PRESSURES AND DIAPHRAGMATIC MOBILITY IN MORBID OBSESE SUBJECTS?

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Objectives:

On the morbidly obese the accumulation of fat in the thorax and abdomen areas engenders a mechanical compression on the respiratory muscles, lungs and thoracic cavity, which leads to changes in the maximal respiratory pressures (MRP). Moreover, compromised respiratory mechanics may to initiate diaphragmatic dysfunction and thus affect their mobility. Therefore, the aim of this study was to evaluate the correlation between MRP with the diaphragmatic mobility in patients with morbid obesity.

Methods and Results:

The diaphragm mobility (DM) was assessed by the ultrasound Philips HD7 (Philips Medical Systems, Bothell, WA, USA) with a convex transducer of 2-5MHz of high resolution in B-mode. The DM was observed in three moments: quiet breath (QB), voluntary sniffing (VS) and deep breathing (DB). The inspiratory and expiratory maximal pressures (PImax and PEmax) were obtained by the digital manovacuometer MVD-300 (GlobalMed, Rio Grande do Sul, Brazil). Data distribution was analyzed by the Kolmogorov-Smirnov test, the relationships between ultrasonographic measurements and MRP were by linear regression analysis to produce a correlation coefficient. Results are expressed as means±standard deviation and differences were considered significant at p<0.017 and DB versus PEmax (r=0.155, p<0.407).

Conclusions:

There is no linear association between the maximal respiratory pressures and diaphragm mobility in morbidly obese, which suggests that respiratory pressure changes do not interfere with the biodynamic of the diaphragmatic muscle.

Keywords: DIAPHGRAM, MAXIMAL RESPIRATORY PRESSURES, OBESITY

DOUBLE HUMP PRESSURE-VOLUME INFLATION CURVE OF THE RAT LUNG

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Objectives:

Pressure-volume (PV) curve presents a double hump in some mice species. A putative role of the chest wall in limiting lung expansion during PV maneuver has not been clarified, which we aimed at studying in rats.

Methods and Results:

14 male Wistar rats (295–390 g) were anesthetized, paralyzed and mechanically ventilated (VT = 6 ml/kg, ZEEP, RR = 90 bpm, I:E ratio = 1:2 in room air) during 5 min for stabilization. To assess the role of chest wall mechanics, animals were divided in 2 groups: Open Chest group (OC, n=7) without the anterior chest wall and Restricted Chest group (RC, n=7) with a pressure cuff around the chest filled to 11.5 (11.0-12.0) cmH2O. Five quasi-static PV curves (VT = 30 ml/kg, ZEEP, RR = 6 bpm, I:E ratio = 4:1 in room air) were done before (Closed Chest = CC) and after the removal or restriction of the chest wall. Volume (V) was calculated by numerical integration of flow and analyses were extracted from the third curve. Differences between groups were assessed by Wilcoxon test (p< 0.05). Data were expressed as median and range. CC and RC groups presented PV curves with a sigmoidal profile with maximal Paw = 24.0 (23.0-28.3) cmH2O and 29.3 (27.0-31.8) cmH2O, respectively. In OC group the PV curve presented a double hump plus a low inflection point with a maximal Paw = 20.9 (20.0-23.8) cmH2O. Interestingly, after the occurrence of the second inflection point at a Paw of 17.0 (16.5-20.0) cmH2O, inflation continued linearly with increasing Paw, and no fall in compliance ensued.

Conclusions:

We conclude that the final portion of the respiratory system PV curve in closed chest rats is flattened by a restriction imposed by the chest wall.

Keywords: Pressure-volume curve , Mechanical Ventilation, Elastance of the Respiratory System

Financial Support: PRONEX/FAPERJ, FAPERJ, CNPq, MCT

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Resumo: EXERCISE ATTENUATES THE INFLAMMATORY PROCESS REDUCING THE RISK OF LUNG AND DISTAL ORGAN INJURY INDUCED BY ABDOMINAL SEPSIS.

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Objectives:

Physical activity, inflammation and immunity are closely linked. The aim of this study was to investigate whether regular and moderate exercise attenuates the risk of lung and distal organ injury in experimental abdominal sepsis.

Methods and Results:
Forty BALB/c mice (25-30 g) were randomly assigned into 2 groups (n=20/each): sedentary (S) and trained (Tr). Tr group ran on a motorized treadmill, at moderate intensity (8-12 m.min⁻¹), 5% grade, 30 min/day, 3 times a week for 8 weeks. Echocardiographic analysis was done before training protocol, and at 4 and 8 weeks, at which time systolic output and left ventricular mass were increased in Tr group compared to S. At 8 wks, sepsis was induced by cecal ligation and puncture surgery (CLP) while a sham operated group was used as control (C). Twenty-four hours later, animals were anesthetized, and the following parameters were measured: lung mechanics and histology (light and electron microscopy), the degree of kidney, liver, and small intestine villi cell apoptosis, and the number of total and differential cells and levels of cytokines in bronchoalveolar (BALF) and peritoneal lavage (PL) fluids as well as blood. Tr-CLP animals, compared to S-CLP, presented: 1) a significant improvement in survival [53% to 83% (p

Conclusions:

Regular and moderate exercise attenuated the inflammatory and fibrogenic processes, reducing the risk of lung and distal organ injury, thus increasing survival.

Keywords: Exercise, Inflammation, Sepsis

Financial Support: CAPES, INCT-INOFAR, FAPERJ, PRONEX, CNPq

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Resumo:08-087

EFFECTS OF NEBULIZED TERBUTALINE IN RESPIRATORY MECHANICS AFTER BRONCHOCONSTRICTION WITH METHACHOLINE IN FEMALE MICE

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Objectives:

Cholinergic system plays a role on lung dysfunction observed in asthma and COPD leading to airway hyperresponsiveness (AHR) and mucus secretion. Several models of lung diseases show that some bronchodilators, used to relieve the respiratory deterioration caused by excessive bronchoconstriction also might alter structure and lung function. Terbutaline is a β2-adrenoceptor agonist often used as bronchodilator in humans but data regarding its effects on mice lung mechanics are yet poorly reported. Thus, the purpose of this study was to assess the effects of nebulized terbutaline in respiratory mechanics after bronchoconstriction with methacholine (MCh) in order to verify how useful it would be in a murine model study protocol.

Methods and Results:

Naïve female mice (C57BL/6, 6-8 wk old, mean body weight = 21 g) were anesthetized with ketamine (100 mg/kg ip) and xylazine (20 mg/kg ip), tracheostomized with a metal cannula (18G) and subsequently connected to a computer-controlled small-animal ventilator (flexiVent, SCIREQ, Canada). The mice were ventilated with 450 breaths/min, tidal volume of 10 mL/kg, and positive end expiratory pressure of 3 cmH2O. Bronchoconstriction was induced with MCh (Acetyl-β-Methylcholine Chloride, Sigma-Aldrich, USA) in phosphate-buffered saline (PBS) at a concentration of 50mg/mL nebulized by an ultrasonic nebulizer (Aeroneb Lab, Aerogen, Ireland) delivered to the airway opening for 10s. After 90s (peak of MCh response), respiratory resistance was assessed using the single compartment model, and terbutaline in PBS (different concentrations) was nebulized for 10s. Respiratory mechanics was assessed following terbutaline (Sigma-Aldrich, USA) nebulization after 90s. The animals were divided into five groups, each group received a different concentration of nebulized terbutaline diluted in PBS: 0mg/mL (Control Group) (n = 3), 0.1mg/mL (n = 4), 1mg/mL (n = 5), 2mg/mL (n = 3), and 10mg/mL (n = 5). The study was approved by the local Ethics Committee on Animal Experimentation (Certification no. 113, 2009). The difference between resistance in peak response
of MCh and resistance in terbutaline response were (mean ± SD): 26.6% ± 24.4% (Control Group), 33.5% ± 6.4% (0.1 mg/ml), 25.5% ± 29.1% (1 mg/mL), 42.7% ± 14.4% (2 mg/mL), 50.9% ± 6.7% (10 mg/mL).

Conclusions:

Our results showed that acutely terbutaline reduced the increased respiratory resistance due to MCh challenge. This effect was observed in a dose-dependent manner and may suggest that terbutaline might be considered as pharmacological tool to study the putative changes of lung function induced by β2-adrenoceptors.

Keywords: Respiratory mechanics, Terbutaline, Methacholine, Bronchodilation

Financial Support: FAPESP (2009/52782-7) and CNPq

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Resumo: 09-036

EVALUATION OF LQB173, A SYNTHETIC AURONE, AS NA+/K+ATPASE INHIBITOR.

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Objectives:
The aim of the present work was to characterize the interaction between Na/K-ATPase and the synthetic aurone LQB173, comparing with ouabain and the previous described inhibitor, the original synthetic coumestan PCALC36 (Biochem Pharmacol, 66:2169, 2003). LQB173 was elected since it has structural similarities with PCALC36, but a simple synthetic route.

Methods and Results:

Rat brain and kidney fractions enriched in Na/K-ATPase were used to measure inhibition of the enzymatic activity using the colorimetric method of Fiske and Subbarow. Inhibition curves revealed that unlike ouabain, that is a thousand times more potent to inhibit rat brain than kidney isoforms, LQB173 had a similar affinity for both isoforms (IC50=25.07 ± 3.09 µM and 20.91 ± 5.10 µM, kidney and brain, respectively). Moreover, the addition of 5 mM dithiothreitol, decreased the inhibitory effect of LQB173. The results express the mean of three experiments performed in triplicate. As was observed for PCALC36, the presence of dithiothreitol seems to protect the sulphydryl groups of Na/K-ATPase from the oxidation promoted by the aurone (Bioorg Med Chem., 16:8801, 2008).

Conclusions:

We conclude that LQB173, a non-steroidal molecule, has a mechanism of inhibition different from the cardiac glycosides, but a similar mechanism comparing with the coumestan PCALC36, with the advantage of a more viable synthetic route.

Keywords: aurone, coumestan, Na+/K+ATPase, ouabain

Financial Support: CNPq, FAPERJ
P2X PORE FORMATION IS INHIBITED BY COLCHICINE: A POSSIBLE MECHANISM FOR ITS ANTI-INFLAMMATORY ACTIONS

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Objectives:
The two longest C-termini of the purinergic P2X receptors belong to P2X2 and P2X7. This region of the channel is thought to interact with multiple cytoplasmatic proteins, which are important for the function of these channels. Among the proteins are members of the cytoskeleton, including microtubules. In this work we asked whether disrupting the microtubule cytoskeleton might affect these channels functions.

Methods and Results:
P2X2, P2X4 and P2X7 were expressed in Xenopus oocytes and/or HEK293 cells and evaluated with electrophysiology and dye uptake following ATP, after treatment with colchicine. Disrupting the microtubule network did not affect currents generated by ATP in P2X2 and P2X7-expressing oocytes. However, it affected YO-PRO-1 uptake, by inhibiting it in approximately 72 and 89% in oocytes expressing P2X2 and P2X7, respectively. In HEK293 cells expressing P2X2, P2X4 and P2X7, the same was observed and the kinetics of YO-PRO-1 uptake was reduced by colchicine pre-treatment. Resident peritoneal mouse macrophages were plated and assayed for permeabilization and reactive oxygen species (ROS) and other pro-inflammatory agents formation induced by ATP with or without colchicine treatment. Macrophages freshly plated or cultured also showed less ATP-induced permeabilization to ethidium bromide in the presence of colchicine (58% and 39% reduction, respectively). In addition, plated macrophages produced less ATP-evoked ROS when treated with colchicine (55%), but the treatment did not alter phorbol ester-induced ROS formation. Colchicine also inhibited IL-1β (-34%) and nitric oxide (NO) (-43%) release by macrophages incubated with LPS and ATP. As shown with oocytes, colchicine did not affect ATP-gated currents in cultured macrophages. Finally, mice were inoculated with LPS and ATP, followed by treatment with or without colchicine, for subsequent ROS and cytokine production measurements. In vivo treatment with colchicine also diminished ROS (-45%), IL-1β (-60%), INFγ (-90%) and nitric oxide (-60%) production.

Conclusions:
In search for the function of cytoskeletal interactions with P2X receptors, we found out a new tool to study the biophysics of these channels, since colchicine specifically blocked pore formation and known cellular consequences of this pore and not other functions of these channels. Colchicine has known anti-inflammatory actions and is currently used to treat several conditions known to involve P2X receptors, such as gout and familial Mediterranean fever. Here we show that perhaps some of the anti-inflammatory actions of colchicine, microtubule dependent or not, might be due to inhibition of pore formation by P2X receptors.

Keywords: Inflammation, Electrophysiology, Microtubule, P2X7, Xenopus oocyte

Financial Support: FAPERJ, CNPq, Pronex.
EFFECTS OF NERVE GROWTH FACTOR ON EXTRACELLULAR LEVELS OF GLUTAMATE AND THIOLS IN THE CHICK RETINA

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Objectives:
Nerve growth factor (NGF) represents an important factor for nerve differentiation and survival. Recently, NGF had been implicated in neurotransmitter release in different brain areas. In the present study we investigated whether NGF could also induce alterations in the normal pattern of glutamate and thiol compounds (-SH) release in the retinal tissue. We also evaluated the cellular mechanism involved in these phenomena.

Methods and Results:
Retinal tissues from chick embryo (E10/11) were incubated in Hank’s solution containing NGF (10, 50 and 100 ng/ml) for 15, 30 or 120 min. NaCl was replaced by LiCl in the experiments in media without sodium and we used EGTA (2 mM) in Ca²⁺-free condition. To block the glutamate/aspartate transporter (GLAST), retinas were incubated with 100 µM ZnSO₄. Extracellular levels of glutamate and thiols were determinate by high performance liquid chromatography and colorimetric assay (using DTNB), respectively. Results were normalized per mg of protein and the values expressed as means ± SD of percent of control, n ≥ 4. Total protein was quantified by Bradford’s method and cellular survival was measured through lactate dehydrogenase release. Our results demonstrated that when retinas were incubated with NGF 50 ng/ml for different periods a significant increase in extracellular glutamate levels after 15 min (335 ± 83%) and 30 min (385 ± 124%) could be observed, whereas increased thiol levels (33 ± 2%) in the medium were noticed only after 120 min of NGF treatment. In its turn, elevation of thiols in the medium was followed by a reduction of about 17 ± 2% of its intracellular levels. When retinas were incubated in Ca²⁺-free buffer during 15 min of NGF incubation no effect was observed in the glutamate release induced by NGF (128 ± 19% Cont vs. 123 ± 8% NGF + EGTA). However, at 30 min the NGF-induced increase of glutamate levels in the medium occurred in a partially Ca²⁺-independent fashion, since NGF led to an increase of extracellular glutamate in about 110 ± 7%. Furthermore, treatment with 100 µM ZnSO₄, a known GLAST inhibitor, did not change glutamate (231 ± 32% NGF vs. 215 ± 8% NGF + ZnSO₄) and thiol content (132 ± 7% NGF vs. 138 ± 3% NGF + ZnSO₄) in the medium. NGF effect on extracellular glutamate and thiol was Na⁺-independent as it induced a significant increase of glutamate in the medium at 30 min (85 ± 24% Cont vs. 189 ± 10% NGF - LiCl) and thiol in 120 min (117 ± 17 Cont vs. 163 ± 1% NGF - LiCl) even when Na⁺ ions were absent, suggesting a participation of an Na⁺-independent component (possibly system Xc⁻) in both processes. Interestingly, NGF was able to induce survival of retinal cells after 120 min incubation (100 ± 3% Cont 15 min vs. 146 ± 30% Cont 120 min vs. 82% ± 10 NGF 120 min).

Conclusions:
NGF rapidly enhances extracellular glutamate levels and it can induce an increase in thiol content in the medium in a more prolonged time of incubation. Initially, there is a prominent Ca²⁺-dependent component of extracellular glutamate increase but a non-vesicular and Na⁺-independent component appears at 30 min. Extracellular thiol increase also was Na⁺-independent at 120 min, indicating a possible involvement of system Xc⁻ in both processes.

Keywords: Retina, Glutamate, Thiol, Neurotrophins, System Xc⁻

Financial Support: CNPq, CAPES, Makarú-Ltda
Objectives:

Angiotensin II (Ang II), which elicits Ca2+-dependent intracellular signaling pathway, is found at high concentrations in renal tubule. Therefore this luminal Ang II could be responsible to trigger an effect on cellular Ca2+ homeostasis. The aim of this study is to elucidate the molecular mechanisms involved in this process.

Methods and Results:

LLC-PK1 cells were cultivated in DMEM medium with 10% FBS at 37°C with 5% CO2. 5 x 105 cells were seeded in a 25 cm2 culture bottle. After reaching 90% of confluence, cells were treated with different conditions, in serum free DMEM medium, harvested and lysed in a buffer solution (1 mM EDTA, 20 mM Hepes-Tris pH 7.0, 250 mM Sucrose and 0.15 mg/mL Trypsin inhibitor). Protein concentration was assayed as in J. Biol. Chem. 193: 265, 1951. Ca2+-ATPase activity was measured with colorimetric method and calculated by difference between absence and presence of 2 mM EGTA (J. Biol. Chem. 202: 675, 1953).

It was observed that Ang II at low concentrations stimulates total Ca2+-ATPase activity at 65% (10^-10 M) and does not modulate at high concentration (10^-6 M). Ang II effect on SERCA and PMCA activities showed that SERCA is the target ATPase for luminal Ang II to regulate Ca2+ homeostasis in renal cells, with a rapid (30 sec) and persistent (30 min) stimulus. In the presence of Ang II AT1 and AT2 receptors antagonists, 10^-10 M losartan and 10^-7 M PD123319, demonstrated that luminal stimulus of SERCA by Ang II is blocked by this two antagonists, and immunoprecipitation followed by Western blotting assays confirmed the formation of AT1/AT2 functional heterodimers induced by Ang II. The PLC inhibitor (2 x 10^-6 M U73122) and 5 x 10^-8 M Calphostin C (PKC inhibitor) blocked SERCA activation, and treatment with phorbol ester [phorbol 12-myristate 13-acetate (PMA)], which stimulates PKC activity, mimicked luminal Ang II effect on SERCA activity.

Conclusions:

These results indicate that luminal Ang II induce the formation of AT1/AT2 heterodimer to activate PLC/PKC signaling pathway, increasing SERCA activity to regulate calcium homeostasis in renal cells.

Keywords: Angiotensin II, Calcium, Luminal Membrane, Heterodimers, PLC/PKC

Financial Support: CNPq, FAPERJ.
gated K+ (Kv) channels on trigeminal ganglion neurons and the human Kv1.5. As shown here, the phenolic derivative also inhibits the isoform Kv1.3, stably expressed in the cell lineage L-929. The prospects are a fully evaluation of the mechanism of interaction drug-channel protein.

Methods and Results:

The L-929 cells stably transfected with human Kv1.3 were cultivated on poli-L-lysine treated glass slips. The cells were kept in MEM/EBSS + 10% equine serum at 37°C and under 5% CO2 atmosphere. A vertical puller PB-7 (Narishigue Co. Ltd) was used to produce patch micropipettes from borosilicate capillaries. After Silgard® coating (Silgard 184, Dow-Corning) the tips were heat polished - microforge MF-9 (Narishigue Co. Ltd). K+ currents were observed in the whole-cell configuration of the patch-clamp technique, using an Axopatch 200B amplifier (Axon Instruments Inc.) and the pClamp suite, 10.2 (Axon Instruments Inc.) for data acquisition and analysis. The K+ currents were characterized as passing through Kv1.3 analyzing the I-V curves and kinetics of the current. For evaluating eugenol effects, time series of square depolarizing pulses from a holding potential were applied. The inhibitory effects were dose-dependent and reversible. A preliminary dose response gave an IC50 of 3.82 mM. The inhibition settling and wash-out were relatively fast (49.2 ± 6.1 seconds, n=6).

Conclusions:

we demonstrated that eugenol inhibits the human isoform Kv1.3 in a dose-dependent and reversible fashion. Although not proved, the effect seems to be due to a low affinity to a site in the channel protein.

Keywords: analgesic agent, eugenol, patch-clamp, voltage-gated potassium channels (Kv1.3)

Financial Support: FAPESP

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Resumo:09-041

EFFECT OF SUB-LETHAL MICROCYSTIN-LR DOSE IN RENAL PROXIMAL TUBULE OF RATS

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Objectives:

Microcystin (MCYST) is a toxin synthesized by cyanobacteria, which induces damages in many tissues, including kidney. Molecular mechanisms involved on renal dysfunction are not clear. Microcystin-LR is the most toxic and studied toxin. Therefore, the aim of this study is to elucidate microcystin-LR effect on renal Na+ transporters and the signaling pathway involved.

Methods and Results:

One sub-lethal dose of microcystin-LR (50 µg/Kg) was injected intraperitoneally in Wistar male adult rats. Animals were sacrificed after 24 h. Left kidneys of control and MCYST groups were used for histological analyses performed by classical method of fixation and dehydration. Sections of renal cortex were fixed on slides and stained with haematoxylin-eosin (H/E) for structural assessment of the tissue, picrosirius red to measure collagen and PAS to allow visualization of the basement membrane. The fields were photographed at random and quantification was obtained using Image-Pro Plus (Media Cybernetics). Histological analyses from kidney exposed to MCYST-LR showed a significant increase of interstitial space, when compared with control group. The presence of MCYST in renal tissue caused an interstitial infiltrate, characterized as interstitial edema and/or formation of fibrosis. After one single dose of MCYST-LR, the interstitial space showed an increased collagen deposition, compared to control group in both cortex and medulla. Right kidneys were used to obtain cortex tubules by homogenization and centrifugation for 10 min at 2500 rpm 4°C to remove cell debris. Protein was assayed with the phenol reagent and ATPase activity was
measured by evaluating the Pi released from ATP. It was observed that in MCYST group, Na+/K+-ATPase activity was inhibited by 20% and analysis by Western blotting showed no alteration in enzyme expression. Na+-ATPase activity was inhibited by 35% in MCYST exposed kidneys. Microcystin-LR did alter neither PKC or PKA protein expression nor activity, indicating that the inhibition of both ATPases is not induced by increased kinases-mediated phosphorylation.

Conclusions:

Only one sub-lethal dose of microcystin-LR was able to alter renal structural features, as increased collagen deposition and interstitial space. Renal function was attained by decreased Na+ reabsorption, due to sodium pumps inhibition. It is known that microcystin-LR is a phosphatase inhibitor, thus the effect on the ATPase are to maintain a sustained regulatory phosphorylation state of both Na+,K+ ATPase and Na+ ATPase.

Keywords: Microcystin, ATPase, kinases

Financial Support: CNPq, FAPERJ, CNPq- PIBIC

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Resumo:09-042

EFFECT OF TEMPERATURE ON WATER AND GLYCEROL PERMEABILITY AND HYPOTONIC SWELLING OF ERYTHROCYTES FROM FREEZE-TOLERANT COPE’S GRAY TREEFROGS

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Objectives:

Cope's gray treefrog, Hyla chrysoscelis, is an amphibian from eastern USA that tolerates freezing in the winter. The animals accumulate high concentration of glycerol during the cold months before freezing. During cold acclimation, freezing and thawing, as ice forms and melts, cells are exposed to changing osmotic environments. At these times, water and glycerol transport are important for maintaining homeostasis. Thus, the objective of this project is to assess the ability of nucleated erythrocytes from gray treefrogs to transport water and glycerol and to regulate cell volume. We tested the effects of acclimation temperature and assay temperature on those processes.

Methods and Results:

Animals captured near Dayton, OH, were divided in two groups. Warm-acclimated frogs were kept at 21ºC with unrestricted food and water and 12:12 light dark circle. Cold-acclimated animals were progressively cooled to 4ºC over a period of 2 months; light was reduced to a dim light for 8h/day, and animals ceased feeding as they cooled. Freezing was induced by further cooling to -2.5ºC, and frozen animals were thawed by return to 4ºC. We collected blood samples from warm- and cold-acclimated animals and from animals frozen and then thawed for 1 or 3 days. To assess responses to hypotonic challenge, cells were placed under a Nikon inverted microscope, rapidly exposed to bathing medium of interest at either 4ºC or 20ºC, and photographed at 5-30 sec intervals for up to 15min at 40X using Metamorph software. Changes in cell shape were subsequently assessed using ImageJ image analysis software. Bathing media were either dilute PBS (70 mOsm) or, to directly assess glycerol permeability, isosmotic glycerol. Erythrocytes from warm animals subjected to hypotonic swelling (70 mOsm bathing solution) at 20ºC changed shape rapidly. In contrast, cells from cold-acclimated or thawed animals changed shape more slowly. For cells from animals in all conditions, assay in hypotonic PBS at 4ºC was markedly slower than at 20ºC. In contrast, the effect of glycerol immersion was greater in cells from cold animals than from warm, and assay temperature had little effect on these changes. In the time course that we examined, cells did not swell to the point of lysis in any of the assays, and viability at the end of the assays remained high (live/dead assay).
Conclusions:

We conclude that water uptake (permeability) is higher in cells from warm-acclimated than cold-acclimated animals. In contrast, it appears that glycerol permeability is elevated in erythrocytes from cold-acclimated animals. These results may reflect a combination of changes in glyceroporin expression (up-regulated in the cold) and in membrane lipid properties. Erythrocytes from gray treefrogs appear to have robust volume regulatory capacity in both warm- and cold-acclimated animals.

Keywords: aquaporin, difference in temperature condition, glycerol and water, osmosis, volume regulation

Financial Support: CAPES/FIPSE

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Resumo:09-043

GABA UPTAKE IN SYNAPTOSOMES FROM BRAIN REGIONS OF DYSTROPHIC (MDX) MICE.

Campos, D. V.; Parames, S. F.; Lima-landman, M. T. R.; Lapa, A. J.; Souccar, C.
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Objectives:

Aim: Mutations of the dystrophin gene and lack of the protein expression in Duchenne muscle dystrophy (DMD) and in the mdx mouse result in progressive muscle degeneration. Approximately 30% of DMD patients also present cognitive impairments attributed to synaptic disfunctions (Anderson et al., Brain 125: 4, 2002). In the normal brain, dystrophin is localized at the post-synaptic membrane of GABAergic synapses. Declustering of GABA receptors and altered probability of spontaneous transmitter release have been reported in brain regions of the mdx mouse (Kueh et al., Clin. Exp. Pharm. Physiol. 35: 207, 2008), indicating abnormalities of GABAergic synapses. This work was aimed to evaluate the effect of dystrophin absence on GABA uptake in brain regions enriched in dystrophin from control and mdx mice.

Methods and Results:

Methods: Crude synaptosomes suspensions were prepared from cortex (Cx), hippocampus (H) and cerebellum (Cb) of male control and mdx mice at 4 months of age. For time course studies, synaptosomes (0.05-0.1 mg/mL protein) were pre-incubated at 25°C for 15 min in Tris-Krebs buffer (pH 7.4), and the uptake was initiated by adding 45 nM [3H]-GABA. For saturation analysis, uptake was measured in the presence of different GABA concentrations (0.01-30 μM). In both assays, the reaction was stopped after 5 min by adding ice-cold solution of 0.15 M NaCl + 0.05% bovine serum albumin and rapid filtration. The retained radioactivity in the filters was counted in a scintillation counter. Specific uptake was defined as the total uptake minus that obtained in the presence of 10 mM nipecotic acid (Protocol CEP/UNIFESP 1178/10).

Results: Uptake of [3H]-GABA in synaptosomal preparations from all three brain regions was related to the protein content and time of incubation. Maximal uptake was obtained in samples containing 100 μg protein/mL after 5 min incubation. Maximal [3H]-GABA uptake determined in all three regions did not differ among control (Cx: 91.8 ± 3.9; H: 101.9 ± 4.1 and Cb: 23.5 ± 1.5 pmol/mg protein, n=3-4) and mdx mice. Incubation of Cx, H and Cb synaptosomes with increasing concentrations of GABA (0.01-30 μM) resulted in hyperbolic concentration-uptake curves. Kinetic analysis of these curves showed increase in the Vmax (70%) with no alteration in affinity of the transport proteins for GABA in the cortex region of mdx mice. In contrast, the decrease in Km value detected in the cerebellum of mdx mice indicates

Conclusions:

Conclusion: The results indicate increase in the number of uptake sites with no alteration in affinity of the transport proteins for GABA in the cortex region of mdx mice. In contrast, the decrease in Km value detected in the cerebellum of mdx mice indicates
increase in affinity of the transport proteins for GABA. These data are probably related to the altered GABA release reported in brain regions of dystrophin-deficient mice, supporting a role of the dystrophin-glycoprotein complex on synaptic function.

Keywords: central nervous system, dystrophic mouse, dystrophin, GABA uptake, synaptosomes

Financial Support: CAPES, CNPq, FAPESP

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**SPIDER VENOM TOXINS, TX3-4 AND PH&ALPHA;1&BETA;, HAVE DIFFERENT ACTIONS ON N-TYPE CALCIUM CHANNEL SPLICE ISOFORMS**

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Objectives:

Cell-specific alternative splicing resulting in the expression of unique Cav2.2 calcium channel splice isoforms in specific neuronal populations. Cav2.2 N-type calcium channels are important targets of analgesics but inhibition of N-type calcium channels elsewhere in the nervous system leads to unwanted responses including hypotension. Thus there is interest in knowing if Cav2.2 splice isoforms, particularly those in nociceptors, are pharmacologically unique. Here we focus on a pair of mutually exclusive exons, e37a and e37b because exon 37a-containing CaV2.2 channels are enriched in nociceptors. Compared to e37b-containing channels, those that contain e37a are more sensitive to inhibition by G protein coupled receptors. Two peptide toxins from the venom of the Brazilian “armed” spider, Tx3-4 and Phα1β, are known to inhibit voltage-gated calcium channels in cells expressing cloned Cav2.2 and Cav2.1 channels. Furthermore, Phα1β acts as an analgesic in animal models of neuropathic pain. We aimed to compare the ability of these two ω-phonetoxins to inhibit e37a and e37b splice isoforms.

Methods and Results:

We used tsA201 cells to express Cav2.2-e37a or e37b channels and test the inhibitory actions of the toxins. Both Tx3-4 (130 nM) and Phα1β (500 nM) inhibit N-type calcium currents over a range of voltages. Phα1β inhibited N-type currents evoked by a test step to 10 mV by 75.2 ± 4% (n=7) and 64.7 ± 5% (n=7) in cells expressing Cav2.2-e37a and Cav2.2-e37b, respectively while Tx3-4 inhibited by 79.9 ± 2.4 % (n=18) and 69.6 ± 2.9 % (n=18) e37a and e37b, respectively. Inhibition of 37a by Tx3-4 persisted even when depolarizing pulses were higher, while inhibition of Tx3-4 in 37b was partially retrieved due to stronger depolarizing pulses. The constant tau for inhibitory action of Tx3-4 was 61+8 s and 107+24s for 37a and 37b, respectively. This suggest that Tx3-4 inhibit 37a more strong and with fast kinetics than in 37b N-type channels. Next, we evaluated Tx3-4 effect in calcium currents of DRG neurons from mutant mice expressing only the 37b isoform of N-Type chanels and compared it to wild type mice. Tx3-4 exerted similar inhibition of those currents in terms of averaged efficiency. However wild type mice showed 2 distinct populations of cells inhibited by Tx3-4 while in bb mice the distribution of cells inhibited by Tx3-4 was normal (single Gaussian) confirming the hypothesis of greater inhibition of Tx3-4 on 37a containing channels.

Conclusions:

Our data suggest that Tx3-4 is more effective at inhibiting N-type currents that contains 37a splice variant which is mainly found in nociceptors.

Keywords: Phoneutria Toxins, Alternative splicing, N-Type channels, DRG, TsA201 cells
OSMOTIC STIMULATION INDUCES CALCIUM-INDEPENDENT GLUTAMATE RELEASE IN HYPOTHALAMIC PREPARATION

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Objectives:
In the hypothalamus, glutamate is responsible for the majority of fast excitatory neurotransmission. We recently reported that hypothalamic neurons were able to release glutamate in response to hyperosmotic stimulus, although, the mechanisms involved in this phenomenon were still not elucidate. Several studies demonstrated that glutamate release can be mediated by calcium dependent (vesicular pathway) and calcium independent mechanisms (reversion of glutamate transporters). Thus the aim of the present study is to evaluate the cellular mechanisms of glutamate release under hyperosmotic stimulation of the hypothalamus.

Methods and Results:
Adult male Wistar rats, weighting 230-280 g, were housed in individual cages in a temperature-controlled environment (23 ± 2 °C) with a 12: 12-h light/dark cycle with free access to rat chow and tap water. After decapitation, the brain was quickly removed and hypothalamic fragments were immediately dissected out from an area 1.0 mm lateral to the midline at the anterior border of the optic chiasm anteriorly and the anterior border of the mammillary bodies posteriorly. Total dissection time was less than 1 min after decapitation. Each hypothalamic fragment was transferred to perifusion chambers containing Krebs Ringer bicarbonate buffer isotonic pH 7.4, 280 mOsm with 1% glucose (KRBG), Chambers connected to a peristaltic pump were perifused at flow rate of 0.5 ml/min. After 30 minutes stabilization, medium effluent was collected every minutes during 10 min. The hypertonic stimulation (340 mOsm) was performed during 3 minutes. The same was performed in calcium-free KRBG, for both isotonic and hypertonic solution. Glutamate was determined on a high-performance liquid chromatograph (HPLC) system (C18 reverse-phase column) and by fluorescent detection. Pre-column derivatization was performed with o-phthalidialdehyde. The excitation wavelength of the fluorimetric detector was set at 340 nm and the emission cutoff filter was set at 460 nm. Our results demonstrated that glutamate released by hypothalamic fragments perifused with isotonic KRBG was about 9.8 ± 1.4 pmol/mg ptn and in the fragments perifused with hypertonic KRBG was about 20.3 ± 1.0 pmol/mg ptn (p< 0.05) and 29.2 ± 2.3 pmol/mg ptn (p< 0.01) in 5 and 6 minutes respectively. When the hypertonic stimulus was realized in a calcium-free KRBG medium, the glutamate release was significantly increased to 30.6 ± 2.4 pmol/mg ptn (p< 0.01) in 6 minutes, suggesting that hyperosmotic stimulus could be evoke a glutamate release mediated by reverse transport in the glutamate transporters.

Conclusions:
Hypertonic stimulation induces glutamate release by calcium independent mechanism in the hypothalamic preparations. These results suggest that hyperosmolarity evokes a non vesicular glutamate release in the hypothalamus.

Keywords: Hypothalamus, Hyperosmolality, Glutamate, calcium

Financial Support: EGPA, UFPA, CNPq
UNDERNUTRITION ALTERS THE BIOACTIVE LIPID PATTERN AND CHOLESTEROL CONTENT IN RENAL PROXIMAL TUBULES

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Objectives:

Undernutrition occurs due to insufficient nutrient and energy intake, which is caused by different factors. In Brazil the highest rates of undernutrition are either in northeast of the country and in the periphery of the big cities. In the eighties Teodósio and coworkers developed an experimental diet named Basic Regional Diet (BRD) that is capable of provoking in animal model alterations as a result of undernutrition. Several studies using the BRD diet for different periods showed that it affects the renal tissue in diverse levels, inducing arterial hypertension and changes in Na+ renal transporters’ activities (Na++K+-ATPase and Na+-ATPase). Previous results from our laboratory showed that the Na+ transporters can be modulated by bioactive lipids such as phosphatidilinositol-4-phosphate (PtdIns(4)P), diacilglycerol (DAG), phosphatidic acid (PA), lisophosphatidic acid (LPA) and ceramides (ceramide and ceramide -1-phosphate). The aim of this work was to study the formation of bioactive lipids in and the cholesterol content in the renal tissue from undernourished (BRD-fed) rats.

Methods and Results:

Wistar male rats were separated into control and undernourished group (BRD). Total membranes from kidney proximal tubules were obtained by differential centrifugation. This membrane fraction was used to study the generation of bioactive lipids and cholesterol content. Total cholesterol was measured by colorimetric method, while the production of bioactive lipids was accessed by thin layer chromatography. Our results showed fewer formation of PtdIns(4)P in BRD animals compared with control (5.1 ± 0.6 vs. 7.9 ± 0.7 pmol.mg-1.min-1, P

Conclusions:

The reduction on the total cholesterol content probably induces a disruption in the lipid rafts on BDR group, since these membrane microdomains are stabilized by cholesterol. This hypothesis is reinforced by the observed reduction in the PMCA content, which is known to be localized and active in the rafts. Together these results showed for the first time that BDR alters the generation of important bioactive lipids which can explain the physiological alterations already reported for Na+ transporters in the kidney.

Keywords: bioactive lipids, cholesterol, kidney

Financial Support: CNPq, FAPERJ and CAPES
Objectives:

ABC superfamily of transporters is the largest family of membrane proteins described in literature. These proteins act as hydrophobic efflux pumps, having an important role in cell protection from xenobiotics. ABCB and ABCC subfamilies have been widely studied because they are associated with the phenomenon of multidrug resistance in tumor cells, a major obstacle in cancer treatment. Reversin 205 and MK571 are compounds capable of modulating the transport mediated by ABCB1 and ABCC1 proteins, respectively. Studies about the expression and activity of ABC proteins have been focused mainly on somatic cells. The knowledge about the physiological role of ABC proteins in gametes and embryonic cells is still incipient. Our group recently characterized the activity of ABCB1 and ABCC1 transporters in gametes and embryonic cells in early stages of embryonic development (first cleavage, second cleavage and morula) of sea urchin Echinometra lucunter. Until morula stage both proteins showed a homogeneous pattern of distribution in whole embryos [Biosci. Rep., 30:257, 2010]. In the present work we investigate ABCB1 and ABCC1 proteins activity in an advanced stage of the E. lucunter sea urchin development: pluteus larva.

Methods and Results:

Animals were collected at Cabo Branco beach (João Pessoa, PB - Brazil) and maintained in filtered sea water under frequent air flow. Gametes were collected by intracoelomic injection of KCl (0.5 M). After fertilization, embryos were transferred to erlenmeyers in a final concentration of 20 embryos/mL and kept at constant temperature (26 ± 2 degree Celsius). Larvae were treated with reversin 205 (10 μM) and MK571 (10 μM) for 20 minutes and incubated for 1 hour with the fluorescent dye calcine-AM (250 nM), an ABC protein substrate. Calcein fluorescence was analyzed under confocal microscopy and subjected to Z-series. Both modulators promoted an increase in calcine intracellular accumulation, indicating that ABCB1 and ABCC1 transporters have functional activity in the larval stage. Although, the pattern of distribution for both proteins was distinct: ABCB1 transporter activity was notably present in the gastrointestinal tract of larvae while ABCC1 protein showed a more ubiquitous distribution in the organism.

Conclusions:

Our results suggest a strong conservation in the pattern of distribution of ABCB1 and ABCC1 proteins among evolutionarily distant organisms, as ABCB1 transporter exhibits high activity in human gut cells while ABCC1 protein has a ubiquitous distribution, like observed in the sea urchin E. lucunter larvae. This work is the first report of ABC transporters activity in larval stage of development.

Keywords: ABC proteins, Echinometra lucunter, Embryonic development, Pluteus larvae, Sea urchin

Financial Support: CNPq

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Resumo:09-048

EVALUATION OF GENDER DIFFERENCES IN OUABAIN-SENSITIVE NA'K+-ATPASE ACTIVITY AND IN CONTRIBUTION OF POTASSIUM CHANNELS TO ACH-INDUCED RELAXATION

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Objectives:
Studies have shown that endothelium dependent vasorelaxation is higher in female than in male rats being abolished by endothelium removal or by ovariectomy. Potassium channels and Na\(^{+}\)K\(^{-}\)-ATPase are essential to maintain membrane potential and regulation of vascular tone and they may be directly related to the genesis of these differences. The objective of the present study is to analyze the involvement of potassium channels and Na\(^{+}\)K\(^{-}\)-ATPase in vascular relaxation in male and female rats.

Methods and Results:

Methods: Wistar rats (25 males and 30 females), 9 ± 1 week, 290 ± 18 g were anesthetized and the hemodynamic measurements were performed. The evaluation of the involvement of potassium channels in the vascular reactivity was performed by means of relaxation induced by acetylcholine (ACh) in the presence of the inhibitor of the synthesis of nitric oxide (100 μM, L-NAME) and blockers of potassium channels: tetraethylammonium (TEA, 2 mM), 4-aminopyridine (4-AP, 5 mM),iberiotoxin (IbTX, 30 nM), apamin (0.5 μM) and charybdotoxin (ChTX, 0.1 μM). The assessment of functional activity of Na\(^{+}\)K\(^{-}\)-ATPase sensitive to ouabain (OUA) was performed by means of potassium induced relaxation in rings with intact endothelium (E+) and after incubation with L-NAME. All values are expressed as mean ± S.E.M. For each concentration-response curve, the maximal effect (Emax, g) and the concentration of agonist that produced 50% of the maximal response (pEC\(_{50}\)). Differences were analyzed using Student’s t-test, one or two-way ANOVA followed by a Bonferroni test. *P< 0.05 was considered significant. Experiments were approved by the Institutional Ethics Committee (CEUA-EMESCAM 003 and 004/2007). Results: In E+ rings males animals showed decreased relaxation induced by potassium in the presence and absence of OUA, compared with females. When the endothelium was removed or in the presence of L-NAME relaxation induced by K\(^{+}\) was not different in males and females. However, the OUA’s capacity to inhibit Na\(^{+}\)K\(^{-}\)-ATPase was higher in males before and after endothelium removal. Females had higher sensitivity and Emx to ACh in the presence of endothelium (Males: pEC\(_{50}\) -6.75 ± 0.08; Emx -94.25 ± 1.19 g, n= 17 vs. Females: pEC\(_{50}\) -7.10 ± 0.12*; Emx -96.56 ± 2.14 g*, n= 25). After incubation with L-NAME the relaxation was inhibited similarly in males and females (Emax Males: -3.47 ± 1.28 g, n= 6; Female: -1.03 ± 0.51 g, n= 8). In the presence of 4-AP the Emx and pEC\(_{50}\) of the females was higher than in males (Males: pEC\(_{50}\) -5.01 ± 0.17; Emx -47.87 ± 5.44 g, n= 7; Female: pEC\(_{50}\) -5.5 ± 0.14*; Emx -81.97 ± 4.23 g*, n= 11). Similarly, the three calcium-activated potassium channels inhibitors reduced the Emax in females compared with males (Emax IbTX Males: -95.26 ± 2.60 g, n= 9; Female: -82.24 ± 3.97 g*, n= 12; Emax ChTX Males: -94.65 ± 5.45 g, n= 7; Female: -75.00 ± 4.17 g*, n= 15; Emax Apamina Males: -93.53 ± 2.02 g, n= 9; Female: -77.86 ± 4.25*g, n= 14).

Conclusions:

These results suggest that the vascular functional activity of Na\(^{+}\)K\(^{-}\)-ATPase is higher in males than in females. The ACh-induced relaxation of female is greater than in males. The K\(^{+}\) channels that appear to be involved in differences of gender in the vascular relaxation are the BK\(_{Ca}\) in females and K\(_V\) channels in males. Thus, it is possible to speculate that differences in vascular reactivity between genders may be related to involvement of potassium channels and Na\(^{+}\)K\(^{-}\)-ATPase.

Keywords: Gender differences, Na+K+-ATPase activity , potassium channels

Financial Support: CAPES; CNPq; FAPES/FUNCITEC

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Resumo:09-049

CA2+ INFLUX IS REQUIRED TO THE SEA URCHIN ECHINOMETRA LUCUNTER FERTILIZATION.

DEPARTAMENTO DE BIOLOGIA MOLECULAR , UFPB

Objectives:

Fertilization is an essential event for perpetuation of species in sexual reproduction. Despite being extensively studied, molecular aspects of fertilization remain poorly understood. Historically, studies of marine invertebrates stand out in the elucidation of the
basic principles governing fertilization once these animals exhibit structural and physiological analogy between its gametes and of humans. Calcium (Ca2+), an inorganic ion essential for a range of physiological events, has been extensively associated to fertilization processes described in several species (Reprod. Biomed. 21:1, 2010). In this perspective, the objective of the present study was to investigate the relevance of extracellular Ca2+ and the involvement of voltage-gated calcium channels (Cav) in the fertilization of the sea urchin *Echinometra lucunter*, a species widely found in the Brazilian coast but rarely studied.

Methods and Results:

*E. lucunter* sea urchins were collected in Cabo Branco Beach (João Pessoa, Brazil) and maintained in aquarium with filtered seawater under constant aeration. Gametes were collected via KCl (0.5 M) intracoelomic injection. Fertilization was obtained by dilution of spermatozoa suspension in artificial seawater (1:5,000) containing 100,000 eggs/mL. Gametes were incubated in medium containing different Ca2+ concentrations, unspecific Cav blockers (verapamil - VP and diltiazem - DT) or L-Type Cav blocker (nifedipine - NF). One hundred eggs were evaluated for each treatment and percentage of fertilization was estimated by fertilization envelope elevation under optical microscopy. Fertilization was completely blocked on a low calcium medium (100% inhibition at 25 μM). In addition, it was found that unspecific Cav blockers VP and DT were more efficient than the L-Type Cav blocker NF on the inhibition of the fertilization. Fertilization was fully abolished with VP and DT treatment (150 μM) while NF (200 μM) blocked 11.28% of fertilization.

Conclusions:

These results show that fertilization process in *E. lucunter* requires extracellular Ca2+ and Ca2+ influx through the Cav. Our data also suggest the involvement of several Cav in *E. lucunter* fertilization and support a promising research around the universality of the key events associated with fertilization among animal species.

Keywords: fertilization, calcium channel, sea urchin, *Echinometra lucunter*, calcium channel blockers

Financial Support: CAPES

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Resumo:09-050

**CEREBRAL MALARIA INDUCE IMPAIRMENTS OF GLUTAMATE TRANSPORT AND GSH LEVELS IN MURINE RETINAL TISSUE**

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Objectives:

Cerebral malaria (CM) is the major life-threatening complication resulting from *Plasmodium falciparum* infection. Several reports have already showed an intense relation between CM and retinal dysfunction. Works with human CM described a decrease in the visual field and loss of visual acuity, although the underlying mechanisms involved in this process remain incompletely understood. Glutamate and other metabolites such as lactate and alanine have been implicated in the cerebral complications (behavioral symptoms) resulting from this pathological condition, however the neurochemistry mechanisms involved in this process remain unknown. Therefore, in the current work we investigated the role of glutamate transporters in the retinal tissue of animals susceptible to CM condition.

Methods and Results:

6-8 week old male and female C57Bl/6 pathogen-free mice, weighting 20-25g, were inoculated with *Plasmodium berghei* ANKA(PbA) by intraperitoneal route using a standardized inoculation of six millions parasitized red blood cells (pRBC) suspended in 0,1 ml PBS. Control animals received the same volume of PBS. Survival and parasitemia rates were determined,
showing a pattern of infection in which the majority of mice die within 7 days displaying cerebral malaria symptoms (paralysis, ataxia and coma) with low rates of parasitemia (15%). 3H-glutamate uptake was measured (scintillation counting) in retinal explants obtained from control and inoculated mice at the time of 2, 4 and 6 days after PbA inoculation (d.p.i). Results demonstrated that glutamate uptake rate (in NaCl - Hank’s solution) were higher in animals with 4 and 6 d.p.i. (130±10% and 164±15%, respectively) when compared to the control groups (100±8%). The glutamate uptake rate at the 2º d.p.i did not show any significant difference (100±5%; 95±10%). When the retina explants of infected animals were incubated with NaCl-free medium, glutamate uptake rate demonstrated the same pattern of increase showed in the NaCl-medium. Additionally, glutamate release was evaluated at the 6º d.p.i by the high-performance liquid chromatograph (HPLC) system, using a fluorimetric detection. The results showed that the glutamate release was inhibited (51±5%) in the animals with 6 d.p.i when compared to the control mice (100±5%). In order to demonstrate whether the increase of glutamate uptake and the decrease of glutamate release are associated with the oxidative conditions of PbA inoculated mice, we performed the measure of glutathione in the retinal tissue. Data reported in this study demonstrated that in the 2º d.p.i the glutathione amount was highly decreased (52±9%) in infected animals, although at 4º and 6º d.p.i. this decrease was not so evident(25±8% and 20±5%, respectively), when compared to control (100±8%)suggesting that the increase in the glutamate uptake could be responsible for the increase in the glutathione levels.

Conclusions:
In conclusion, in this work we characterized the involvement of oxidative stress conditions and the glutamate transporters in the pathophysiology of cerebral malaria, suggesting that xCG-transport system could play a role in this process since the removal of NaCl from the medium did not alter the rate of glutamate transport.

Keywords: cerebral malaria, glutamate transport, glutathione, retinal tissue

Financial Support: UFPa, FAPESPA, CNPq

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Resumo:10-041

NON-INVASIVE TELEMETRY AS INSTRUMENT FOR HEART RATE PARAMETERS IN AN OLD WORLD PRIMATE.

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2 Universidade Federal Rural da Amazônia, UFRA
3 Centro Nacional de Primatas, CENP
4 Universidade Federal de São Paulo , UNIFESP
6 Centro Universitário do Estado do Pará, CESUPA

Objectives:
The biotelemetry allows measurement and remote of sensing functions, activities and conditions of humans and animals using wireless data by receivers connectors, getting real data about the physiological parameters without influence of stress on physical restraint or anesthesia, demonstrating reliable values. The National Primate Center of the Evandro Chagas Institute (CENP / IEC / SVS / MS) has the first non-invasive telemetry equipment for non-human primates installed in Brazil, being used to obtain physiological parameters (H.R., B.P., R.F., Activity, T°, ECG) of primates, enabling more reliable clinical data in biomedical experiments

Methods and Results:
Eight male specimens of Chlorocebus aethiops, aged 15 to 21 years, kept in indoor cages in the CENP/IEC/ SVS/MS were used in this experiment. These animals were sedated with a combination of tiletamine and zolazepam hydrochloride (10 mg/kg) for placement of ECG electrodes and other connectors of the EMKA TECHNOLOGIES ® non-invasive telemetry transmitter system (V. 1.5). After this procedure the animals returned to their home cages and 18 hours of data were recorded continuously. The mean heart rate was obtained by the software ECG AUTO2 every 1 hour of records for each animal and the average and standard deviation obtained were calculated. The mean heart rate observed in animals while maintaining normal activities in captivity, was 110.74 bpm (SD 27.34). We also observed that animals with 21 years old (n = 3) tend to have lower values of heart rate (mean = 96.04 bpm, SD = 3.06) when compared to younger animals (15 a 17 years old) (n = 5) (mean = 119.55 bpm SD = 31.36).

Conclusions:

The values of heart rate observed for the samples studied differ from the average observed for the same specimens on anesthetic condition, measured with a stethoscope (91 bpm (± 29)), which confirms that the use of non-invasive telemetry allows a more reliable data of the physiological values of animals under normal and/or during experimental condition.

Keywords: biotelemetry, heart rate, non human primates

Financial Support: INCTC/CNPq e IEC/SVS/M.

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Resumo:10-042

EFFECT OF PUNICA GRANATUM HIDROALCOHOLIC EXTRACT IN MICE INITIAL PREGNANCY AND SPONTANEOUS CONTRACTILE ACTIVITY IN ISOLATED UTERUS.

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Objectives:

In an ethnobotanical survey made in Alfenas region showed that Punica granatum (romã) was cited as abortifacient and/or emmenagogue. In the academicals literatures nothing was discover that supporting this practice. Because it the aim of this work was investigated the action of hidroalcoholic extract of this plant during initial pregnancy and contract uterine musculature in mice.

Methods and Results:

Swiss female mice were mated with males and first day of pregnancy (dop) was considered the day of vaginal plug presence was observed. The pregnant females were treated by gavage with 100 or 300 mg/kg of Punica granatum extract in 1st to 4th dop and animals were sacrificed at 8th or 11th dop. The number of corpus Luteum, observed was compared to the number of total implantation sites (IS) found in the uterine horns to calculate the implantation rate (IR).The animals treated with 100mg/kg in 8th dop showed no significant difference compared to control and when treated with 300mg/kg an IR of the 71.4% (Punica granatum extract increased the force of spontaneous contraction to 170.7% (dose of 100 mg/mL) compared with the control period, with-logEC50 (pD2) = 9.48 mg/mL. Doses of 10, 30 and 100mg/mL promoted an increase in spontaneous contractions (16.4 ± 2.2, p

Conclusions:

The morphologicalanalysis of pregnant uterus showedan important prejudicial effect in embryo development associated with hemorrhagic and embryo reabsorptions and increased the strength and frequency of contraction suggesting potent uterotonic property. In conclusion this work on mouse showed that an special attention is required when this plant are used during women
pregnancy.

Keywords: contractile, granatum, pregnancy, punica, uterus

Financial Support: FAPEMIG and UNIFAL-MG

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Resumo:10-043

EFFECT OF AQUEOUS EXTRACT OF EQUISETUM PYRAMIDALE IN DIABETIC RATS INDUCED BY STREPTOZOCIN: A HISTOCHEMICAL STUDY

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Universidade para o Desenvolvimento do Estado do Pantanal, Anhanguera-Uniderp

Objectives:

To describe the organization of tissue connective of the blood wall in control rats and diabetics induced by streptozotocin (STZ) and treated with the extract of the plant *Equisetum pyramidale*.

Methods and Results:

A total of 21 male rats *Wistar* were divided into four groups which were treated as follows: control group with saline (SS), control group with the extract of *Equisetum pyramidale* (SSExEp), diabetic group with a solution salt (DSS) and the diabetic group with extract of *Equisetum pyramidale* (DExEp). Diabetes was induced by a single intraperitoneal injection of streptozotocin (55 mg / kg). The saline and the extract of *Equisetum pyramidale* was administered by gavage, 1ml/dia for 40 consecutive days. During euthanasia, the rats were anesthetized with ketamine and xylazine and placed on their backs to the liver resection. Then the organs were stored in formalin, and after, there was histological processing. Were measured the weight (g), longitudinal and transverse length (mm) of the livers of animals SS, SSExEp, DSS and DExEp. Has been calculated the average assessment of blood glucose (mg / dl) in groups SSExEq (120.5), DSS (471.3) and DExEq (497.5). Then samples were obtained from liver connective tissue stained with Masson Trichrome. The analysis of histological sections by the use of histochemical method by Masson Trichrome allowed to characterize the tissue present around the wall of blood vessels, bile ducts and hepatocytes among the four different groups of rats. In the group of SSExEp the connective tissue was more evident and thick compared to the group of diabetic animals treated with *E. pyramidale* (DExEq), also showing the largest diameter of the blood vessel wall in relation to other treatments. The measures of the variables of weight (g) of the organ were expressed as mean ± standard deviation. SS groups (13.0 ± 1.2); SSExEq (11.7 ± 1.7); DSS (10.0 ± 1.3); DExEq (10.1 ± 0.9). Information for the longitudinal and transverse length (mm), respectively, in the same four groups: SS (29.4 ± 1.9 and 43.0 ± 4.1); SExEq (28.0 ± 3.2 and 43.6 ± 4.0); DSS (32.4 ± 3.0 and 40.2 ± 1.7); DExEq (29.5 ± 5.0 and 40.2 ± 1.7). There were no significant differences between the various groups.

Conclusions:

The organization of the connective tissue surrounding blood vessels and bile ducts of the liver tissue were more evident in the SSExEq group toward the group DExEq. The dimensional analysis of the livers from the four groups did not show any relevant structural changes in the morphology of liver tissue we treat with *E. pyramidale* and saline.

Keywords: diabetes, Equisetum pyramidale, fígado, tecido conjuntivo, vasos sanguíneos

Financial Support: PIC-AESA, University Anhanguera - UNIDERP
QUALITY ASSURANCE IN CENTRAL ANIMAL HOUSE OF THE BUTANTAN INSTITUTE
Instituto Butantan, IBU

Objectives:
Knowing the importance of conserving the quality standard of animal facilities, which reflects directly on animal welfare and therefore the result of scientific studies, we adopted a system of “Good Conduct” with the implementation of the Good Manufacturing Practices (GMP) and quality requirements facing the Quality Assurance process and organizational planning, implementation, monitoring and records of all the established routine in the Central Animal House. The main purpose of quality management is Central Animal House make processes more efficient, improving the results obtained with the animals and the welfare of workers. For this, we proposed a program of continuous quality, aiming to produce better quality results in the production and maintenance of laboratory animals.

Methods and Results:
In preparing the program was awarded the Quality Assurance: Monitor stock of materials; Validation of equipment; Implementation, development and monitoring of laboratory quality control; Preparation of documentation, flow chart of activities, animal handling and cleaning; Establishment, implementation and monitoring of internal audits regularly; Qualification of suppliers and development and implementation of training programs; The results of the ten-year period showed that one can obtain higher reliability throughout the process of reproduction and handling of animals, as well as the rationalization of work. The new program has resulted in the deployment of the course "Laboratory Animals - A special", offered twice a year, which highlights the motivational and educational work, both for employees and for the Central Animal House other institutions; After the courses and training could reduce the staff since they were qualified and demonstrating better performance in their activities in providing optimization management techniques, such as reducing the number of players getting the same or higher monthly demand of animals. Through the implementation of health control of laboratory animals and environmental control of breeding areas, there was improvement in the quality of cleaning of areas including health and welfare of animals. In addition, we designed a system for online solicitation of laboratory animals, which facilitated the attendance to the users of the institution and, to the animal house and obtaining a greater control of these animals along with the Ethics Commission.

Conclusions:
The Quality Management Program in Bioterium makes processes more efficient, more reliable results, less skilled labor enabling the optimization of production and well-being of both animals and workers.

Keywords: Animal Laboratory, Quality Assurance, Good Manufacturing Practices

A RAPID NON-INVASIVE METHOD OF GENOTYPING LEPTIN-DEFICIENT MUTANT MICE BY ORAL SWAB SAMPLING AND RESTRICTION DIGESTION
Objectives:

Obesity is a widespread epidemics intimately related to the development of type 2 diabetes mellitus (T2DM). Hence, in vivo models of obesity are of value for the study of T2DM. The spontaneous mutant obese ob/ob mouse, which does not produce leptin (Lep−/−), presents an attractive model of insulin resistance and adipose tissue inflammation, typical of T2DM. However, if Lep−/− animals are easily distinguished from the normal mice because of the evident obesity presented already in the early weeks after birth, heterozygous Lep−/+ are phenotypically indistinguishable from normal Lep−/+ animals. Moreover, Lep−/− mice are infertile so that laboratory matings should be performed by using only heterozygous animals, which implicates genotyping. Since the excision of a piece of mouse tail or venipuncture for obtaining DNA samples are difficult to be employed in newborns, we developed a simple non-invasive method based in oral epithelial cells for genotyping the animals.

Methods and Results:

By using oral swabs or polyethylene microspatulas, the oral mucosa of 18 mice (2 heterozygous controls, 1 Lep−/− control and 15 unknown animals) was scrapped twice. The cells were resuspended in 500-µL microtubes containing 200 µL of TES solution (10mM Tris-HCl pH 7.6; 1mM EDTA; 0.6% SDS) and vortexed for 30 s. Then, 10 µL of a 10 mg/mL Proteinase K solution was added to each tube, which were incubated for 120 min at 42°C. Afterwards, proteins were precipitated with 15 µL of 6 M NaCl and samples were centrifuged at 15,000 x g (4 min, room temperature). Then, supernatant fractions were transferred to a fresh 1.5-mL microtube and mixed with 450 µL of absolute ethanol and then centrifuged at 15,000 x g (4 min, room temperature) again. The pellets containing DNA were resuspended in 300 µL of 70% (v/v) ethanol solution, and the tubes were centrifuged again under the same conditions. After supernatant discard, the pellets were dissolved in 15 µL of Tris-HCl pH 8.0; and DNA contents were spectrophotometrically determined (average yield: 14.5 ±7.6 µg/µL). Aliquots of 50 µg DNA were run for real-time PCR amplification (50 cycles) with Stratagene Brilliant® II SYBR® Green qPCR Master Mix and primers (FW: TGT CCA AGA TGG ACC AGA CTC; RV: ACT GGT CTG AGG CAG GGA GCA) yielding typically 715 ± 137 µg/µL DNA. Then, 900 ng of PCR product were enzymatically cut with 5 units of Ddel restriction enzyme (2 h, 37°C) and DNA fragments were resolved in 4% agarose. Whereas digests from wild mice presented a characteristic single 155 bp band, mutant Lep−/− showed 2 bands (55 and 100 bp) and heterozygous samples showed 3 bands (55, 100 and 155 bp) allowing for the rapid characterization of any littermate.

Conclusions:

With this simple, not time-consuming technique, mouse DNA may be obtained and analyzed in less than 6 h allowing the genotyping of leptin mutant mouse newborns without the need of venipunctures and the risk of infections or mutilations. The method is safe, relatively inexpensive, time-sparing and reliable.

Keywords: genotyping, non-invasive, leptin, oral swab, restriction digestion

Financial Support: CNPq, CAPES, Propesq-UFRGS and INCT Hormones and Women’s Health.

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Resumo:10-046

PERINATAL PROTEIN RESTRICTION INDUCES ALTERATION OF FOOD PREFERENCE IN ADULT RATS
Objectives:
The feeding behavior of animals is an adaptive response, arising from the demands of the internal environment, and is modulated by limitations imposed by the external environment. Animals undernourished in early life show deficits in satiety and exhibit a strong preference for fatty foods. Assess food preference in rats subjected to perinatal malnutrition.

Methods and Results:
We used male Wistar rats with 270 days of life. The animals were divided into two groups according to the diet offered to the mother during pregnancy and lactation. The Control Group (C, casein 17%) and Malnourished Group (D, low-protein diet 8%). The two experimental groups were offered for five consecutive days: Standard Diet (SD) and 10% sucrose solution or palatable diet (PD) and 10% sucrose solution. From the diets offered to animals were formed the following experimental groups: control animals with access to standard diet (C/SD), control with access to palatable diet (C/PD), malnourished with access to standard diet (D/SD) and malnourished with access palatable diet (D/PD). Was recorded daily consumption of diet and sucrose. Only the last three days were used for statistical analysis. Regarding the intake of sucrose, the malnourished and control animals with access to standard diet ingest more sucrose than the controls (C/SD= 38.4; C/PD= 28.62, P=

Conclusions:
All animals, regardless of the experimental group who were eating a diet low in fat, drank a higher amount of sucrose solution. Malnutrition caused perinatal malnourished animals' preference for fat compared to sucrose. Thus, the rats invariably prefer to attach the flavor with more calories.

Keywords: feeding behavior, FOOD PREFERENCE, palatable diet, perinatal malnutrition , sucrose

Financial Support: FACEPE; CnPq.
insertion and, therefore, is not indicated as a root canal sealer. In an attempt to combine the physicochemical properties of a root canal sealer with the excellent biocompatibility of the MTA, some modifications have been proposed in the MTA to allow its use as a root canal sealer. Thus, the tissue reaction promoted by an experimental MTA Sealer in the rat subcutaneous was investigated by morphological, immunohistochemical and quantitative analysis of inflammatory cells.

Methods and Results:

In the Wistar rats from each group (n=20), a polyethylene tube filled with MTA Sealer (MTA-SG), Portland cement (PCG) or MTA (MTAG) was implanted in the dorsal subcutaneous. In the control group (CG), empty tubes were implanted. After 7, 14, 30 and 60 days of implantation, the specimens containing the tissues surrounding the implanted tubes were removed, fixed and embedded in paraffin. In the HE-stained sections, the numerical density of inflammatory cells in the capsule was estimated. The differences between the groups were statistically analysed by the SigmaStat 2.0 software; the data were submitted to ANOVA and Tukey test. The significance level accepted was $p \leq 0.05$. The expression of proteins involved in the bone calcification, osteopontin (OPN) and osteocalcin (OC), was evaluated by immunohistochemistry in the capsule adjacent to the implanted tubes. Moreover, sections were also submitted to the von Kossa histochemical method for detection of calcified structures in the capsule. At 7 and 14 days, a moderate inflammatory process in the capsule which surrounded the implanted tubes was seen in all groups. However, in the period of 60 days, the capsule was formed by a typical dense connective tissue containing numerous bundles of collagen fibers arranged between the fibroblasts. A significant reduction in the number of inflammatory cells was verified in comparison to initial periods (7 and 14 days). In addition, significant differences in the numerical density of inflammatory cells were not verified among the groups. Dense structures strongly von Kossa-positive were often observed in the capsules adjacent to all materials implanted at 7, 14 and 30 days. On the other hand, positive structures were not frequently seen in the capsule surrounding the materials after 60 days. In addition, von Kossa-positive structures were never found in the capsule of the CG. OPN and OCN immunolabeling were detected in some fibroblasts of the capsule juxtaposed to all materials implanted in the subcutaneous. However, an enhanced immunolabeling was evident in the capsule surrounding the implants containing the experimental MTA Sealer.

Conclusions:

Thus, the results strongly indicate that the experimental MTA Sealer exhibits biocompatibility similar to MTA and Portland materials and stimulates the formation of calcified structures in ectopic sites. Moreover, the immunolabelling of OPN and OCN in cells of the capsule suggest that some cells of the subcutaneous can express the “osteoblast-like” phenotype and, thereby, the MTA Sealer may play an osteoinductive role.

Keywords: Dentistry , Endodontics , Materials Testing , Subcutaneous Tissue


QuebraPagina

Resumo:10-048

DIABETES MELLITUS DETERMINES OXIDATIVE STRESS IN THE LIVER OF DIABETIC RATS INDUCED BY ALLOXAN.

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Objectives:

Clinical and experimental evidence suggests that cellular oxidative stress plays a crucially important role in the genesis and progression of chronic diabetic lesions, particularly the non-alcoholic fatty liver disease (NAFLD) and its development forms. In this study, it was investigated whether diabetes mellitus chemically induced in rats by alloxan is capable of changing, in the long term, the oxidative balance in the liver tissue of such animals. We also reviewed the main intracellular mechanisms of
mitochondrial dysfunction and alteration in the metabolism of free fatty acids, mediated by reactive oxygen species (ROS), advanced products of non-enzymatic glycosylation (AGE) and by the molecular pathways of polyol, hexosamines, protein-kinase C (PKC) and proinflammatory cytokines.

Methods and Results:

Sixty male Lewis rats weighing 250-280g were randomly distributed into 2 experimental groups: NG - 30 non-diabetic control rats; DG - 30 diabetic rats without treatment. Each group was later divided into 2 subgroups comprising 10 rats which were sacrificed after 1, 3 and 6 months of follow-up. Blood glucose, urinary glucose, glycosylated hemoglobin and insulin were determined in the plasma of all animals at the beginning of the experiment and prior to all sacrifice periods. The concentrations of lipid hydroperoxides (LH) and the activity of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) were also measured in the liver tissue of all animals. Rats in the DG group showed high levels of blood glucose and glycosylated hemoglobin, with significantly lower plasma insulin levels than those observed in NG rats (p<0.01).

Conclusions:

It was shown, in an animal model, that diabetes determines oxidative stress in the liver, which is characterized by increased concentration of reactive oxygen species and significant reduction in the antioxidant defenses in that organ. Such oxidative unbalance in the liver cells may play a relevant role in the genesis of the non-alcoholic fatty liver disease observed in diabetic patients as well as in its occasional progression to steatohepatitis and cirrhosis.

Keywords: Alloxan, Diabetes Mellitus, Liver, Oxidative Stress, Rats

Financial Support: Sources of research support FAPESP

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Resumo:10-049

EFFECTS OF PERINATAL PROGRAMMING ON THE BEHAVIOUR RELATED ANXIETY IN RATS.

Universidade Federal de Pernambuco, UFPE

Objectives:

We used the elevated plus-maze (EPM) as a measure of anxiety of malnourished rats.

Methods and Results:

Wistar rats were divided into two groups according to diet to the mother during pregnancy and lactation: control group (normal protein diet C17% casein) (n=9) and malnourished group (low-protein diet D8% casein) (n=9). The animals aged 180-200 days were tested using the EPM. The sections were videotaped for later analysis of input frequency in the open arms (OA) and closed arms (CA) and the time that remained in the OA and CA. The malnourished group had increased frequency in OA (D17%= 0.77 ± 0.43; D8%= 3.00 ± 0.76, p<0.05)

Conclusions:

Malnourished animals showed a decrease in the level of anxiety and increased impulsivity compared to control group. Malnutrition during pregnancy and lactation can modify the behavior of rats in models of anxiety.
EFFECTS OF OBESITY ON ANXIETY AND DEPRESSION BEHAVIORS IN RATS (RATTUS NORVEGICUS)

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2 Curso de Nutrição - Universidade de Ribeirão Preto, UNAERP

Objectives:

To investigate the effects of obesity on anxiety and depression behaviors in rats.

Methods and Results:

The animals were divided into three experimental groups each, according to the nutritional condition. 1) Control Group (C, n=10); 2) Obesity induced by “Cafeteria Diet” Group (CD, n=11); and 3) Obesity induced by Hyperlipidic Diet Group (HD, n=10). Once a week the body weight, abdominal circumference and length were recorded. The behavioral tests used were: open field, forced swimming and elevated plus maze (EPM). Behavioral tests were performed at 50 days of life. The measures of weight and size were treated by an analysis of variance (ANOVA) followed by the multiple comparison test of Newman-Keuls (p

Conclusions:

Obesity induced by CD caused increased weight and abdominal circumference; however the animals from the HD group had lower weight, lower abdominal circumference and lower length, when compared to C rats. CD and HD rats were more impulsive and presented lower risk-assessment behaviors in the open field and EPM tests. Finally, it is also suggested that CD rats presented higher depressive behavior, contrasting with lower depressive behavior in HD rats, both compared to C.

Keywords: ANXIETY, DEPRESSION, OBESITY

Financial Support: CAPES

EFFECTS OF EARLY PROTEIN AND PROTEIN-CALORIE MALNUTRITION ON SPATIAL LEARNING AND MEMORY PROCESSES IN ADULT RATS TESTED IN TWO DIFFERENT WATER MAZES TASKS.

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Objectives:

To investigate the effects of early protein and protein-calorie malnutrition on spatial learning and memory processes in adult rats.

Methods and Results:

The animals were divided into three experimental groups, according to nutritional condition: Control Group (C, n=12), fed with a 16% protein diet; Protein Malnourished Group (PM, n=12), fed with a 6% protein diet; Protein-Calorie Malnourished Group (PCM, n=12), fed with a 16% protein diet, restricted to 50% of the amount consumed by C group by day. The rats were fed with the experimental diets until the weaning at 21 days of life. After weaning rats from the three groups were located in pairs, water and lab chow were available all the time until the end of the experiments. Once a week, the body weight was recorded. The learning tasks used to evaluate spatial learning and memory were the Morris Water Maze (MWM), at 70 days of life during four consecutive daily sessions and the Water Radial Arm Maze (RWAM), one week later, during 12 consecutive daily sessions. The last memory retention test in the MWM occurred at 102 days of life, 28 days after the fourth learning session. Body weight measures and behavioral data were treated by an analysis of variance (ANOVA) followed by the post hoc multiple comparison Newman-Keuls test (p

Conclusions:

Protein and protein-calorie malnutrition caused long-lasting damages to body development evidenced by lower weights in malnourished rats from both groups. Protein malnutrition led to learning and working memory deficits as demonstrated in the MWM. Nevertheless, PM rats exhibited better performance in the WRAM, with lower latencies and fewer working memory errors at the first sessions. Protein-calorie malnutrition led to reference memory deficits in the MWM however no differences were observed on learning and spatial memory processes in the WRAM test.

Keywords: Learning, Memory, Protein malnutrition, Spatial task

Financial Support: CAPES

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Resumo:10-052

PERINATAL MALNUTRITION ALTERS LOCOMOTOR ACTIVITY AND DEPRESSIVE STATE IN RATS

UNIVERSIDADE FEDERAL DE PERNAMBUCO, UFPE

Objectives:

During growth and development, the nervous system of mammals shows phases of intensive functional and structural changes. Several environmental factors can modify, modulate or direct this development. Epigenetic factors focusing on that stage, impacting the development leading to structural and physiological changes causing problems such as depression and changes in locomotor activity.

Methods and Results:

Wistar rats aged 60 (n=14) and 400 days (n=14) were divided into two groups according to the diet provided during perinatal life: malnutrition (D8%) (Low protein diet) and control (C17%) (Normal diet). We performed the forced swim test (FST) to assess behaviors related to depression and open-field test (OFT) to analyze locomotor activity. In statistics we used the Student t test, data expressed as mean±SD. FST in the malnourished animals at 60 days old, showed a decrease in latency (D17%= 88.90±8.15;
D8% = 69.30±4.71) and increased frequency of diving (D17% = 21.41±3.51; D8% = 44.0±5.48). At 400 days of life, there was a decrease in latency (D17% = 33.73±4.23; D8% = 47.84±5.13) with significant increase in the frequency of diving (D17% = 30.46±3.50; D8% = 46.73±4.50). In the OFT, the malnourished animals at 60 days showed an increase in distance (D17% = 18.60±1.99; D8% = 23.70±1.16), average speed (D17% = 0.060±0.0057; D8% = 0.079 ± 0.0039) and number of stops (D17% = 68.39±4.25; D8% = 84.23±2.78), with a decrease in the time of stops (D17% = 216.86±7.96; D8% = 191.90±5.23). At 400 days, the malnourished animals had a decrease in distance (D17% = 20.09±1.26; D8% = 15.33±1.03) and average power (D17% = 4.57±0.44; D8% = 2.52±0.23) compared with controls.

Conclusions:

When young malnourished rats have behavioral characteristics used in the tests related to depression, but with higher performance in the open field compared to controls. Malnourished rats of middle-age had higher rates of depression-related behaviors and changes in locomotor activity. Perinatal malnutrition alters the behavior related to depression. These effects widen with advancing age.

Keywords: DEPRESSIVE , LOCOMOTOR ACTIVITY, malnutrition , PERINATAL MALNUTRITION, RATS

Financial Support: FACEPE; CnPq

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Resumo:10-053

PREDICTIVE FACTORS FOR BACTERIAL TRANSLOCATION AND SEPSIS IN SPLENECTOMIZED RATS.


Universidade Federal de Pernambuco, UFPE

Objectives:

To investigate the presence of bacterial translocation (BT) and sepsis in splenectomized rats and to determine predictive factors, such as weight, sex, changes in gut microbiota.

Methods and Results:

A total of forty 125-days-old Swiss webster mice (Mus musculus) were divided into two distinct groups: total splenectomized (10 male/10 female) and control (10 male/10 female). After seven days of the splenectomy, mice were anaesthetized and euthanised in order to evaluate BT, intestinal morphometry and microbiota. Weighing was performed since the day of splenectomy until the euthanasia. To determine the microbiota, feces were collected from the middle region of the small intestine. For the morphometric analysis, segments from the same area were transversally and longitudinally sectioned. To study BT, samples of peripheral and portal blood, as well as mesenteric lymph nodes and liver were cultured. Male and female splenectomized mice showed a reduction in the ponderal weight evolution from the 125th up to 132nd day of birth (p

Conclusions:

The results suggest that asplenia increases the susceptibility to the bacterial translocation and consequentially to sepsis. Sex and duodenal morphometric changes may particularly exert an effect on such phenomenon.

Keywords: Splenectomy, Bacterial translocation, Sepsis, Mesenteric lymph nodes, Intestinal microbiota
THE LIGHT-DARK CYCLE MODULATION OF MOTHER-INFANT INTERACTION IN RATS

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1 Neurociências e comportamento / Instituto de Psicologia, USP

Objectives:
This study was designed to investigate the emergence and maintenance of maternal behavior and how it is controlled by the interaction of environmental and neural factors. In order to detect characteristic behavioral patterns lactating rats were video recorded during day and night time. Specifically, it was tested if mother rats would show typical sequential patterns of behavioral responses according to the time of the day.

Methods and Results:
Female rats were tested for maternal behaviors. Subjects were adult Wistar nulliparous female rats weighing 180-200 g and approximately 90 days of age at the beginning of the experiments. The females were time-mated by placing them with sexually experienced males. The day on which sperm was observed in the vaginal lavage was designated day 1 of pregnancy. Pregnant females were individually housed in opaque polypropylene cages (30 x 40 x 18 cm) in rooms with constant ventilation system (23 ± 2 °C) and light/dark cycle of 12 hours (0600 to 1800h). Food and water were available ad libitum. Animals were maintained in accordance with the guidelines of Committee on Animals of the Colégio Brasileiro de Experimentação Animal (COBEA). From days 3 to 5 of lactation, the animals (n = 7) were monitored by camera (SONY DCR-SR220 with night shot) and the occurrence, frequency and time spent on each behavior were recorded: licking, nest building, foraging, self-grooming, grouping, nursing, kyphosis and full maternal behavior (FMB). The behavioral parameters were assessed using specific software The Etholog 2.2 (Ottoni, 2002). According to the results, it is observed that the rats remain a contiguous period of time, whether in daylight, or darkness, nursing. During the daylight course, rats spend more time taking care of the offspring, e.g., grouping of pups, nest building. While in the dark period, they exhibit more general activities such as self-grooming, drinking water or stand still.

Conclusions:
The results suggest that, during lactation, rats express a sequential pattern of behavioral parameters according to the time period. It is possible that sensorial signals emitted by the pups or the environmental clues serve as conditioned stimuli for these animals.

Keywords: maternal behavior, pregnancy, lactation

Financial Support: FAPESP (2009/51276-0)
Objectives:

The present study investigates the mechanisms involved in the antidepressant-like effect of EEH. Moreover, we investigated the antidepressant potential of podoandin (POD), a sesquilactone isolated from the EEH of this plant.

Methods and Results:

using as animal model the Open field test and forced swim test in male mice (N:8-13; 25-30g;e months) we found that EEH(50 mg/kg,i.p.) and POD (10mg/kg,i.p.) decreased (EEH -65,12±3,6S;POD -32,34,0±4,28S;VEIC- 120±3,24S) the immobility time in the forced swimming test without accompanying changes in ambulation in the open-field test. The anti-immobility effect of EEH (63,34±3,12S) was prevented by pre-treatment of mice with ondansetron (a 5HT3 selective receptor antagonist)(131±8.2S), NAN190 (a 5-HT1A selective receptor antagonist) (127,23±6.11S), Pindolol (a 5-HT1A/1B receptor antagonist) (142,8±4.17S), prazosin, (a α1-adrenoceptor antagonist)(142,1±5.12S),yohimbine(aα2-adrenoceptor antagonist)(183,34±5.2S), haloperidol (a nonselective dopaminergic receptor) (112,±2.83S), SCH23390 (a dopamine D1 receptor antagonist)(112±4.24S), sulpiride (a dopamine D2 receptor antagonist) (99,35±3.12S). On the other hand, the pre-treatment of mice with: p-chlorophenylalanine (PCPA, an inhibitor of serotonin synthesis, 4 consecutive days), ketanserin (a serotonin 5-HT2A/2C receptor antagonist), naloxone (a nonselective opioid receptor antagonist), naltrindole (a selective δ opioid receptor antagonist), bieculline, (an antagonist of GABAA receptor), phaclofen, (an antagonist of GABAB) or L-arginine (a nitric oxide precursor) did not blocked the antidepressant-like effect of EEH. In addition, pretreatment of animals with methylene blue (an inhibitor of nitric oxide synthase and soluble guanylate cyclase), NG-nitro-L-arginine (an inhibitor of nitric oxide synthase) or 7-nitroindazole (a specific neuronal nitric oxide synthase inhibitor), at subeffective doses, did not caused a synergistic effect with EEH at an effective dose in the forced swimming test. The anti-immobility effect of POD (42,28±4.5S) also was prevented by pre-treatment of mice with NAN-190 (168,85±10,2S), ondansetron (136,42±21S), prazosin (85.4±10,50S), yohimbine (91.42±6.22S), sulpiride (106,28±15,54S) and haloperidol (115,33±15.2S).

Conclusions:

The results indicate that antidepressant-like effect of EEH and POD is dependent on the serotonergic ,noradrenergic and dopaminergic systems but not GABAergic, opioid and oxidonitrergic systems.

Keywords: ANTIDEPRESSANT, ADRENERGIC, DOPAMINERGIC, SEROTONERGIC

Financial Support: CNPq - ProPEC - UNIVALI

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Resumo:10-056

IN VIVO ANTIMALARIAL ACTIVITY OF NOVEL HYDROXYETHILAMINES

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Objectives:

Malaria is one of the most severe infectious diseases, primarily affecting the world’s most disadvantaged populations. There is a growing need for effective drugs with new mechanism of action, due to the high rate of mutation of the parasite, which leads to the development of resistance. Our group reported recently the antimalarial activity of novel hydroxyethylpiperazine-based compounds (HBC) in vitro against W2 clone of P. falciparum. The aim of this study was to evaluate the effect of treatment with HBC in experimental malaria.

Methods and Results:

Male C57BL/6 mice were intraperitoneally (i.p.) inoculated with 5x10(6) P. berghei-GFP pRBCs withdrawn from a previously infected mouse. The HBC (10 mg/Kg/day diluted in 5% DMSO) or artesunate (10 mg/kg/day diluted in 10% ethanol and 90% propylene glycol) were orally administered (p.o.) daily. Eighteen HBC were used to evaluate the antimalarial activity, however only treatment with PVMD07D led to a 51% of inhibition of parasitemia in treated P. berghei-infected mice. We also observed that PVMD07D improved the survival of infected mice when compared with non-treated mice. The in vivo antimalarial activity of PVMD07D suggest that such substance is distributed by systemic circulation. In order to discard the inhibition of parasitemia as results of hemolytic activity, the hemoglobin leakage induced by PVMD07D treatment was evaluated. Nevertheless, erythrocytes exposition to drug solution containing serial twofold dilutions of PVMD07D in PBS (range = 31,2 to 1000 μg/ml) did not present hemolytic activity, suggesting that PVMD07D acts directly on parasite. We also evaluate the ability of different doses of PVMD07D to impair in vitro differentiation of P. berghei. Non-treated parasites were able to differentiate to schizonts stage after 24 h in culture, however, neither artesunate- nor PVMD07D-treated (1000 µg/ml) parasite were able to differentiate, supporting that hydroxyethylamine derivatives are able to impair parasite metabolism.

Conclusions:

Our results suggest that PVMD07D were able to inhibit parasites growth in vivo, probably by inhibit Plasmodium differentiation. Despite evidences presented herein, further experiments should be conducted to determine the hydroxyethylamine target on Plasmodium sp.

Keywords: MALARIA, Antimalaricos, HYDROXYETHILAMINES

Financial Support: Cnpq, FAPERJ

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Resumo:10-057

**ROLE OF NEUROCHEMISTRY IN THE ADAPTIVE VALUE OF BEHAVIOR: COMPETITION BETWEEN MATERNAL BEHAVIOR AND THE SICK DURING LACTATION**

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Objectives:

In mammals, the amount of caring a mother offer to her offspring is defined as maternal behaviour(MB). MB is a complex behaviour with specific characteristics for each species, which are determined by physiological changes which happens a bit before or right after labour (Numan 1994; MATTSON et al,2001). During this special period , the main objective is to ensure hers and her offspring’s survival. Of according to Hart (1988), the changes found in sick animals, were defined in group as sick behaviour(SB), which corresponds to an organized group of behavioural and physiological changes. Infections caused by LPS during pregnancy might cause mental diseases and among many other problems(Have et al, 2006). By knowing little about the
possible relationship between MB and SB, we used the LPS, an endotoxin derived from the wall of a bacterium, as an inducer of SB.

Methods and Results:

For the study of MB and MB aggressive, 40 rats were divided in 1 control and 3 experimental groups. The experimental group received 100ug/kg LPS by ip, 48h after endotoxin administration began the observations of general activity and MB and MB aggressive. For choice these days, were used 20 virgin female rats where they received ip 100ug/kg measured body weight, water and feed consumption, and measured body temperature for 120h. Females in the control group were observed in the same way, but were treated with vehicle of LPS. The results showed that: 1)In the virgin treated with LPS altered the body temperature 48h after application, 2)In the period of lactation were observed increase in frequencies of raise after application of LPS 3)Changes were observed in MB and MB aggressive.

Conclusions:

We conclude, therefore, that females rats exposed to LPS during lactation have a greater care for the offspring with respect to females only treated with the vehicle of LPS.

Keywords: maternal behaviour, LPS, rats

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Resumo:10-058

LOW LASER THERAPY INCREASE ANGIOGENESIS IN A MODEL OF RANDOM SKIN FLAP

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Objectives:

Different studies have shown that Low Level Laser Therapy (LLLT) is able to increase skin flap viability by decreasing the necrotic area and increasing neoangiogenesis. However, the mechanisms by which laser acts on cells are not fully understood. The present study investigated the effects of two different laser wavelengths (660 nm e 780 nm) at 30 and 40J/cm² on angiogenesis process, hypoxia-inducible factor 1 alpha (HIF-1α) and vascular endothelial growth factor (VEGF) mRNA expression.

Methods and Results:

Sixty male Wistar were used in this study. The animals were divided into the following groups (n=12 per group): control group, group irradiated with 660nm at fluencies of 30J/cm² and 40J/cm², group irradiated with 780nm, at 30J/cm² and 40J/cm². The skin flap (10X4 cm) was performed on the back of the animals, with a plastic sheet interposed between the flap and the donor site. A low-energy AsGaAl laser, 660nm e 780nm (MM Optic Ltda®) CW, 0,04 cm² beam diameter,40 mW, was performed immediately after the surgery and on days 1, 2, 3, and 4 post-surgery, the irradiation was made punctually, on 24 points on the skin surface. The percentage of the necrosis area of the flap was calculated by the paper template method at the postoperative day 7. A sample of skin flaps was collected of each group, to perform the count of blood vessels. HIF-1α protein and VEGF mRNA were evaluated by western blot and qRT-PCR, respectively. Results: No differences were found in the necrotic area among the groups; however laser irradiation 660 and 780 nm, had a stimulatory effect, increasing the number of blood vessels. In the group irradiated with 660 nm, there was an increase at fluence of 40J/cm² (p

Conclusions:
LLLT was able to induce angiogenesis in random skin flap, although we could not observe improvement on the viability of the skin flap. In our model, the increase of the number of vessels seems to be mediated by VEGF production. In conclusion, our data suggest that LLLT with 660 nm induces angiogenesis dependent of VEGF secretion and HIF-1α activation.

Keywords: Angiogenesis, Laser Therapy, Skin Flap

Financial Support: CNPq

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EVALUATION OF LACTOBACILLUS RHAMNOSUS GG IN ZEBRAFISH INTESTINES AFTER ETHANOL EXPOSURE

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Objectives:

Our aim is the colonization of LGG in zebrafish intestines under ethanol exposure.

Methods and Results:

Materials and Methods: Thirty wild-type, adult zebrafish were divided into 3 groups (n=10): C – control; P – probiotic; PE – probiotic and ethanol. During 2 weeks the group C was fed with fish food and the groups P and PE received fish food supplemented (1%) with 10^10 CFU of lyophilized probiotic LGG (Culturelle, Amerifit, USA). Ethanol (0.5%) was added in the PE group tank. At the end of the trial fish were sacrificed after cryoanesthesia and the intestinal contents excised. The contents were diluted in PBS and spread on plates with MRS agar, a selective media for acid lactic bacteria. The plates were incubated at 37°C in microaerophilia. After 48h the bacteria cells were collected, suspended in buffered solution and submitted to thermal lyses, PCR was carried out with species-specific primers and Gram staining performed. This study was approved by the HCPA Ethics Committee. Results: The plates from group C did not show development of LGG colonies, but the P and PE plates showed a significant growth. The PCR confirmed the LGG colonies. Gram staining showed the lactobacilli in the fish gut (P and PE).

Conclusions:

This study confirms the persistence of LGG in the zebrafish intestines during ethanol exposure.

Keywords: Zebrafish, Lactobacillus rhamnosus GG, Ethanol

Financial Support: CNPq
BIFLORIN INDUCES DOWN-REGULATION OF N-CADHERIN AND INHIBITS IN VITRO MELANOMA CANCER CELL INVASION

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Objectives:
Most cancer deaths are due to the development of metastasis and/or therapeutical failure. To invade, cancer cells of epithelial origin have to move from the primary tumor mass by breaking their cell–cell contacts, known as adherens junctions. Tumor cells that losses E-cadherin function acquire an invasive or metastatic phenotype. The down-regulation or loss of epithelial markers (E-cadherin) is accompanied by mesenchymal markers neoexpression (N-cadherin). This process is called epithelial-mesenchymal transition (EMT) and is wellknown to enhance cell motility. E-cadherin generally suppresses invasiveness, whereas N-cadherin promotes invasion and metastasis in vitro. Biflorin is an ortho–naphthoquinone isolated from Capraria biflora with proven anticancer properties. In this way, the aim of this work is to investigate the role of biflorin in MDA-MB-435 invasive melanoma cell in vitro and the N-cadherin protein status.

Methods and Results:
Biflorin (1, 2.5 and 5 μM) were tested against MDA-MB-435 (melanoma), MCF-10A (normal mammarian epithelial cell) and Melan-A (normal melanocyte) using the Alamar Blue assay, after 12h incubation. Cell growth was quantified by the ability of living cells to reduce resazurin to a pink product. Crystal violet assay was also performed to evaluate cytotoxicity. Adhesion assay was performed after 12h treatment using fibronectin, collagen I and IV as substrate. Invasion assay and westernblot analysis for N-cadherin were performed after 12h treatment. Biflorin inhibited MDA-MB-435 cell invasion in a dose-dependent manner (p 5μM) were observed to all cells used in this study at this time course. After 12h, biflorin display citotoxicity towards MDA-MB-435 cells with IC50 of 2.10 μM (1.7 - 3.4) after 72h, but not to normal cells (IC50 > 5 μM). Likewise, biflorin down-regulated N-cadherin expression in a dose-dependent manner. In the other hand, biflorin did not inhibit MDA-MB-435 cell adhesion to all tested substrates.

Conclusions:
Biflorin inhibited cell invasion and N-cadherin expression in a concentration-dependent manner. Since no toxicity was observed to cancer and normal cells, N-Cadherin down-regulation may be involved in invasion inhibition. Biflorin can be a prototype to new anticancer drugs design however further studies have to be performed to elucidate biflorin mechanism in vivo.

Keywords: Biflorin, N-cadherin, invasion inhibition

Financial Support: CNPQ, CAPES, FAPEAM
Objectives:
Considering the high occurrence of cervical cancer, reaching the rank of second most common cancer worldwide, and side effects of currently available treatments, it is important to consider studies resulting in new therapies for this type of tumor. Therefore, the propose of this study is to investigate the effects of different fractions of extracts from a plant of southern and southeastern Brazil with some biological effects described in the literature (Baccharis articulata) on the viability of human cervical cancer cells.

Methods and Results:
The powdered aerial parts of B. articulata were extracted using soxhlet and fractionated. Human tumor cell lines (SiHa and HeLa) were grown in Dulbecco’s modified Eagle’s medium (DMEM)/10% fetal bovine serum (FBS) and seeded in 96-well plates at densities of 2,000 cells/well. Culture cells were maintained in 5% CO2 at 37°C. Treatment with the fractions of extracts from B. articulata (dichloromethane, ethyl acetate, n-butanol, aqueous and crude) at concentrations of 100-1,000 ug/mL was started 36 hours after the plating. The fractions were dissolved in DMSO except the aqueous fraction. Wells containing DMEM and DMSO were used as control treatment. After 24 and 48 hours of incubation, the cells containing extracts and controls were subjected to MTT (5 mg/mL) for analysis of cell viability. Different concentrations of the fractions used to tested extracts significantly inhibited the viability of tumor cells studied after 24 hours of incubation (between 24 and 80% for SiHa and 5 and 50% for HeLa) and 48 hours of incubation (between 20 and 90% for SiHa).

Conclusions:
Inhibition of viability of tumor cells by fractions of B. articulata contributes to the generation of prospects for the use of brazilian native plant species as new therapies for human cervical cancer.

Keywords: human cervical cancer, Baccharis articulata, cell viability

Financial Support: PROBITEC; FAPERGS

ANALYSIS OF THE UNFOLDED PROTEIN RESPONSE (UPR) IN EMBRYONIC STEM CELLS

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Objectives:
It has been described that early embryogenesis occurs in a hypoxic environment [J. Reprod. Fertil. 99:673, 1993]. Hypoxia triggers the accumulation of misfolded proteins in the endoplasmic reticulum lumen, leading to the activation of the unfolded protein response (UPR) pathways [FEBS Lett. 582:2521, 2008]. In order to analyze if UPR occurs during embryo formation, we used embryoid bodies (EB) as a model of study. It has been recently reported that hypoxic conditions favor EB formation [Tissue
In this work, we analyzed activation of UPR and Oct 3/4 expression in naive pluripotent stem (ES) cells and in ES cells undergoing spontaneous differentiation (EB).

Methods and Results:

Embryonic stem cells (R1) were maintained in culture as pluripotent colonies or as embryoid bodies (2 and 4 days). Both mouse embryonic stem cells (R1) and human embryonic stem cells (H9) were treated with an UPR inducer (tunicamycin) for 24h. Proteins were then extracted for western blot. We analyzed Oct3/4 (pluripotency marker) and UPR activation marker (CHOP). Moreover, we have also evaluated the effect of the increase in phosphorylation of eIF2alpha in human embryonic stem cells (H9). CHOP increases during the EB formation. CHOP levels are lower in pluripotent stem cells than in EBs after two days and four days. In naive embryonic stem cells (R1 and H9), the treatment with tunicamycin, which triggers UPR activation and increases CHOP, levels of Oct3/4 are reduced. In addition, we verified that the treatment with an inhibitor of eIF2alpha dephosphorylation leads to a decrease in Oct3/4 in human embryonic stem cells (H9) during EB formation.

Conclusion:

These results indicate that the UPR activation occurs during spontaneous differentiation of embryonic stem cells as embryoid bodies. These data are in agreement with the hypothesis that UPR activation favors degradation or reduces expression of Oct3/4 and consequently differentiation.

Keywords: CHOP, Embryonic Stem Cells, Oct3/4, Spontaneous Differentiation, Unfolded Protein Response

Financial Support: CNPq, FAPERJ, CAPES, UFRJ-PIBIC

Objectives:

The existence of loss and gain of chromosomes, known as aneuploidy, has been previously described within the central nervous system (J Neurosci. 25:2176, 2005; Dev Neurobiol 67: 1334, 2007). During development, at least one-third of neural progenitor cells (NPCs) are aneuploid (Proc Natl Acad Sci. 98: 13361, 2001). Notably, aneuploid NPCs may survive and functionally integrate into the mature neural circuitry (Proc Natl Acad Sci USA. 102: 6143, 2005). Given the unanswered significance of this phenomenon, we tested the hypothesis that neural differentiation induced by all-trans retinoic acid (RA) in pluripotent stem cells is accompanied by increased levels of aneuploidy, as previously described for cortical NPCs in vivo.

Methods and Results:

In this work we used embryonal carcinoma (EC) cells, embryonic stem (ES) cells and induced pluripotent stem (iPS) cells differentiated into NPCs by RA treatment. Ploidy analysis revealed a 2-fold increase in the rate of aneuploidy, with the prevalence of chromosome loss, in RA primed stem cells when compared to naive cells. In an attempt to understand the basis of...
neurogenic aneuploidy, micronuclei formation and survivin expression was assessed in pluripotent stem cells exposed to RA. RA increased micronuclei occurrence by almost 2 fold while decreased survivin mRNA and protein expression by 50%, indicating a possible mechanism by which stem cells lose their chromosomes during neural differentiation. DNA fragmentation analysis demonstrated no increase in apoptosis on cells differentiated with RA, indicating that cell death is not the mandatory fate of aneuploid NPCs derived from pluripotent cells. In order to exclude that the increase in aneuploidy was a spurious consequence of RA treatment, not related to neurogenesis, mouse embryonic fibroblasts were treated with RA under the same conditions and no alterations in chromosome gain or loss were observed.

Conclusions:

Our findings indicate a correlation amongst neural differentiation, aneuploidy, micronuclei formation and survivin downregulation in pluripotent stem cells exposed to RA, providing evidence that somatically generated chromosomal variation accompanies neurogenesis in vitro.

Keywords: aneuploidy, neural differentiation, pluripotent stem cells, retinoic acid, survivin

Financial Support: Faperj,CNPq, Fapesp, Ministério da Saúde do Brasil

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Resumo:12-043

HUMAN EMBRYONIC STEM CELLS CULTURED ONTO A MEF-DERIVED ORGANIZED MATRIX FORM SYMMETRIC EMBRYOID BODIES PRONE TO THE NEURAL PHENOTYPE

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Objectives:

Human embryonic stem (hES) cells have been exhaustingly cultured on mouse embryonic fibroblast (MEF) feeder layers. Although this approach maintains hES cells pluripotency and proliferative properties for long-term periods, embryoid bodies (EBs) derived from this system are morphologically irregular, varying in size and symmetry. Here we describe a strategy for the formation of symmetric EBs by culturing hES cells on ECM coats without the requirement of MEF feeder layers. This system abolishes feeder-stem cell contact, maintaining the organized characteristic of feeder-produced matrices, which differs from unorganized matrix systems, such as matrigel.

Methods and Results:

ECM was obtained from MEFs by chemical treatment. H9 cells cultured onto plates previously coated with MEF-derived ECM (MEF-ECM) were compared with H9 cells cultured on mitomycin C-inactivated MEFs (control) for 100 days. Cells were analyzed for the expression of pluripotency markers revealing that H9s cultured on either control MEFs or MEF-ECM expressed similar levels of Oct-4, SOX-2, TRA-1-60 and SSEA-4. EBs derived from MEF-ECM H9 cells were smaller (136 ± 32 μm) compared to control embryoid bodies (202 ± 65 μm) and more symmetric, with a longer/shorter diameter ratio closer to 1 (1.14 ± 0.13) when compared to the control group (1.33 ± 0.28). In addition, when stimulated to the neural phenotype, MEF-ECM derived EBs presented higher levels of the neural markers SOX-2 and β-III tubulin compared to the control EBs.

Conclusions:

In conclusion, we show here that hES cells differentiation dynamics can be altered by changing colony substrate to a MEF-ECM coat, and this alteration leads to a change in EB morphology and specific lineage differentiation.
Objectives:

The bladder cancer is the most prevalent tumor in the genitourinary tract and the current treatments are not efficient to prevent recurrence and progression tumor cases, then new and more effective treatments have been tested. Resveratrol is found in a variety of plant species and have demonstrated many effects as antioxidant, cardioprotective, anti-inflammatory and antitumor activities, including in bladder cancer cells. Quercetin is a flavonoid widely distributed in the plant kingdom, and among other effects has been described as a potential anticancer agent, also including its effects in bladder tumor cells. Considering that, resveratrol and quercetin showed anticancer effects in cell growth of bladder cancer lines, the aim of present study was to investigate whether these compounds together have a synergic effect on decrease cell growth in T24 human cancer cell.

Methods and Results:

The T24 human bladder cancer cell line was grown in RPMI culture medium supplemented with 10% fetal bovine serum (FBS) and maintained in 5%CO2/95% air at 37 0C. Cells were treated with 10 or 30µM quercetin or resveratrol alone or in cotreatment for 24, 48 or 72 hours. Treatment with 0.5% DMSO (dimethylsulfoxide) was taken as control. For the evaluation of the antitumoral activity, the cell proliferation assay was performed; cells were trypsinized after the end of treatment time and counted in a hemocytometer. To measure cell viability, the MTT assay was performed, and optical density of each sample was evaluated in ELISA reader. Furthermore, to the cell cycle evaluation cells were stained with IP (Propidium Iodide) followed by flow cytometer analysis. Results shown that in proliferation assay the treatment with 30 µM quercetin and 30 µM resveratrol decreased by 47% ± 10 and 74% ± 4 SD, respectively, while the cotreatment with both at 30 µM reduced 79% ± 3.8 SD of cell proliferation in 48 hours. Results of MTT assay showed that, in 48 hours, 30µM quercetin and 30µM resveratrol decreased 17% ± 14 SD and 14% ± 9.7 SD, respectively, while the cotreatment with both at 30 µM reduced 37% ± 5.8 SD of cell viability. Cell cycle analysis showed that 30 µM resveratrol was able to enhanced G0 and G2 phases and decreased G1 while 30 µM quercetin did not have any significant effect, and the cotreatment with 30 µM resveratrol plus 30 µM quercetin decreased G1 phase (from 58.6 ± 4.3 SD to 19 ± 3.6 SD), and also enhanced G2 phase, (from 25 ± 3.9 SD to 65.8 ± 2.0 SD).

Conclusions:

Taken together these results showed that both resveratrol and quercetin alone or in cotreatment have antitumor effects in T24 bladder cancer cell line, and despite it was not observed synergic effects, these natural substances have a potential antitumoral effect on bladder cancer and their use needs to be better investigated.

Keywords: Bladder cancer, Resveratrol, Quercetin

Financial Support: UFRGS and CNPq
ENDOPLASMIC RETICULUM STRESS INCREASES APE/REF-1 EXPRESSION IN MELANOMA CELL LINE (B16F10)

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Objectives:

Accumulation of unfolded proteins in the lumen of endoplasmic reticulum (ER) leads to ER stress and activation of Unfolded Protein Response (UPR). Adaptation to ER stress and activation of UPR have been associated with solid tumor formation, malignancy and resistance to therapy. However, the molecular mechanisms that lead to tumor survival in this context have not been entirely elucidated. Moreover, inducers of ER stress have emerged as an alternative to trigger cell death in tumors. Apurinic/apyrimidinic endonuclease-1/redox factor-1 (APE/Ref-1) is a multifunctional protein involved in DNA base excision repair and redox regulation of many transcription factors. Upregulation of expression of APE/Ref-1 was described in different types of solid tumors, including melanoma. In melanoma, the upregulation of APE/Ref-1 have been associated to resistance of tumor cells to death induced by DNA damage. The purpose of this study was to test the effects of activation of UPR upon APE/Ref-1 expression.

Methods and Results:

In this study, we used tunicamycin, an inhibitor of N-glicosylation, which induces accumulation of unfolded proteins in the lumen of the ER and activates UPR. Melanoma cell line (B16F10) was maintained for 24 hours with distinct concentrations of tunicamycin (0.03 – 3ug/mL). After that, protein extracts were prepared to further analysis by western blot for both APE/Ref-1 and markers of activation of UPR (CHOP/GADD153 and BiP/GRP78). The effect of tunicamycin upon programmed cell death were analysed by TUNEL assay, identification of cleaved caspase-3 and growth curve. Treatment of tunicamycin induced an increase of CHOP/GADD153 and BiP/GRP78 in a dose-dependent manner. We found that lower doses of tunicamycin induce both an increase of APE/Ref-1 content and an increase of the number of cells. In addition, we found that higher concentrations of tunicamycin decrease the number of melanoma cells while increase the incidence of both cleaved caspase-3 and TUNEL positive cells. Double staining for APE/Ref-1 (by immunofluorescence) and DNA fragmentation (by TUNEL assay) showed that APE/Ref-1 is located in the nuclei of healthy cells while TUNEL positive cells do not show any immunostaining for APE/Ref-1.

Conclusions:

We found that activation of unfolded protein response induces an increase of APE/Ref-1 content. These data are in agreement with our hypothesis that moderate endoplasmic reticulum stress (as induced by low doses of tunicamycin) activates APE/Ref-1 expression, which favors survival of melanoma cells. Furthermore, we found that during ER-stress-induced cell death (as induced by high doses of tunicamycin) APE/Ref-1 protein is no more detected in the nuclei of the dead cells. These data indicates that even melanoma cells, that present high levels of APE/Ref-1 protein, can be susceptible to a degeneration induced by endoplasmic reticulum stress.

Keywords: APE/Ref-1, Endoplasmic reticulum stress, Melanoma

Financial Support: CNPq, CAPES, FAPERJ
AMBLYOMIN-X: A NEW INHIBITOR KUNITZ TYPE IS ABLE TO INDUCE DEATH IN HUMAN BREAST CARCINOMA CELLS MCF-7

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Objectives:
The aim of this study is to evaluate the effect of Amblyomin-X (Ambly-X), a Kunitz type inhibitor with ~ 14 kDa obtained in recombinant form (Toxicon 823:34, 2008), in cell cultures of human mammary MCF-7 through cytotoxicity assays and microinjection technique.

Methods and Results:
The MCF-7 cells were grown and maintained in RPMI 1640 supplemented with 10% fetal calf serum (FCS) and Penicillin - Streptomycin 1%. Cells were treated with 0.1, 0.5, 1 and 2μM of Ambly-X. As negative control was used only medium supplemented with 10% FCS and as positive control 1μM of Paclitaxel for 24 hours. The mitochondrial potential, indicative of cell viability, was measured by MTT assay. Another test performed for evaluation of death, was the microinjection of 0.5μM Ambly-X in MCF-7 cells with a micromanipulator InjectMan NI 2 (Eppendorf, Hamburg, Germany) connected to the microinjector FemtoJet (Eppendorf, Hamburg, Germany. After 2 and 4 hours of injection, the cells were stained with Hoechst (1μg/ml) for evaluation of morphological alterations of nuclei. The MTT assay showed that concentrations of 0.1, 0.5, 1 and 2μM led to a decrease in cell viability after 24h of treatment, to 57.5%, 53.5%, 41.2% and 40.4 %, respectively, as compared to control (ANOVA, Dunnett's Multiple Comparison Test P

Conclusions:
The molecule Ambly-X was able to reduce the population of tumor cells in vitro causing clinical signs of death. The microinjection of the molecule suggests that this needs to be internalized to produce its cytotoxic effects, that treatment are time-dependent and that death can be caused by the apoptosis mechanism.

Keywords: Amblyomin-X, Cell death, Kunitz, MCF-7

Financial Support: CAPES, CNPq, FAPESP

THE NOVEL CYTOKINE PANDER/FAM3B INHIBITS CELL DEATH IN PROSTATE TUMOR CELLS

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Objectives:

Resumo:12-047
PANDER (Pancreatic-derived factor) is a novel cytokine that induces apoptosis in insulin-secreting $\beta$-cells. Since gene expression analysis in paired clinical tissue samples revealed that PANDER mRNA levels are increased in late stage prostate carcinoma, we set out to evaluate the role of this cytokine in tumor progression.

**Methods and Results:**

As shown by cell growth analysis, DU145 prostate tumor cells overexpressing PANDER increase cell mass 56±8% more than vector alone-transfected cells. Comparison of cell viability by MTT assay, DNA fragmentation and phosphatidyl serine externalization analysis revealed that PANDER also provides a 42±13% relative survival advantage to the DU145 transfected cells, protecting them from apoptosis triggered by different stimuli such as staurosporine, TNF-$\alpha$ + cycloheximide and serum deprivation. These survival advantages provided by PANDER are accompanied by an increased expression, at both the mRNA and protein levels, of the anti-apoptotic genes Bcl-2, Bcl-XL and XIAP. Moreover, a diminished caspase-3, -8 and 9 activity was also observed.

**Conclusions:**

In contrast to its role in pancreatic $\beta$-cells, PANDER was capable of activating pro-survival mechanisms in DU145 prostate tumor cells. This novel function may be exerted by Bcl-2-mediated inhibition of programmed cell death pathways, highlighting a putative role for this cytokine in tumor progression.

**Keywords:** Prostate cancer, cell death, cytokines

**Financial Support:** FAPESP, FINEP, CNPq, PRP-USP

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**EVALUATION OF DIFFERENT STIMULI OF CELL GROWTH AND DEATH**

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**Objectives:**

This is a study of interaction through in vitro stimulation of smooth muscle cells (A7R5) with the supernatant fluid from cultured cells of Multiple Myeloma, rich in growth factor, to analysis the growth and/or cell death.

**Methods and Results:**

For the quantitative and qualitative study the major factors of proliferation were quantified trough the cultivation of Multiple Myeloma cells and the vascular smooth muscle cells from thoracic aorta of rat embryo were cultured in RPMI 1640 supplemented with 10% Fetal Bovine Serum (FBS), 100U/mL penicillin, 100mg/mL streptomycin, 24 mM NaHCO3 until confluence for the experiments. This cells are being kept in CO2 humid incubator (5%) at 37°C. The quantification of growth factors VEGF (Vascular Endothelial Growth Factor), Interleukin-6 (IL-6), Transforming growth factor-beta (TGF-beta) and Fibroblast Growth Factor (FGF) were quantified by an enzyme-linked immunosorbent assay (ELISA) following the recommendations of the Kit RayBiotech. The reading was done using a spectrophotometer at 595nm with reference filter at 655nm. The results of the growth factors quantification using the ELISA technique presented as mean and standard deviation: 0.2473 ± 0.03296pg/mL of VEGF; 0.0775 ± 0.008963 pg/mL of IL-6; 11.98118 ± 5.80917 pg/mL of TFG-beta; and 0.098333 ± 0.003512 pg/mL of FGF. The cell proliferation and viability were evaluated by MTT assay, where 4x104 A7R5 cells were plated into each well and maintained under culture conditions in 96-well strip plates in triplicate and different amounts of supernatant of MM were added in ascending order (5%, 10% and 30%). Cells were incubated for 24, 48 and 72 consecutive hours. The
quantification of viable cells was done daily. 0.5mg/mL of MTT reagent was added to each period, with 4 hours of incubation (37°C), then dissolved in 100mL of DMSO. This reaction occurs only in metabolically active cells through the mitochondrial enzyme succinate dehydrogenase. It was observed an increase in the rate of cell proliferation with p

Conclusions:

The evaluation of stimuli secreted by the tumor parenchyma on the other cells present in the tumor microenvironment will help to improve the knowledge about neoplasias and then new therapeutic strategies can be developed.

Keywords: ANGIgenesIS, GROWTH FACTORS, TUMOR MICROENVIRONMENT

Financial Support: Fundação Araucária

Objectives: Anabolic-androgenic steroids (AAS) are synthetic molecules similar to the male sex hormone testosterone. The classical therapeutic uses of these substances are associated with treatment of hypogonadism, bone marrow failure syndromes, bone mineralization and some muscle–wasting disorders. Thus, the aim of this study was to determine the impact of nandrolone decanoate superdosage on lipid profile, liver glycogen and body composition in a rat model.

Methods and Results:

Adult male Wistar rats weighing 200-250 g (60 days) were divided in two groups: normal control rats (submitted to vehicle injection; peanut oil with 10% of benzoic alcohol) and rats treated with nandrolone decanoate (Deca Durabolin (50 mg/mL-1Organon)) 1 mg.100 g-1 b.w. Steroid and vehicle were administered by a single intramuscular injection in the hind limb once a week for 8 wk. After euthanasia, blood, liver and carcass was collected. Liver and carcass was weighed. After collection, blood was centrifuged (15 minutes, 3000 g) and serum was stored at -20°C. Total cholesterol, serum triglycerides, HDL and LDL-cholesterol were measured by commercially available kits. Liver glycogen was measured as described by Casimiro-Lopes et al. (2009). Body composition was determined by carcass analysis, as described by Leshner AL & Litwin VA (1972). The results are expressed as the mean ± SEM. All data were analyzed by unpaired t test. A value of p ≤ 0.05 was considered statistically significant. Our result observed an increase in serum cholesterol (C=164.6 ± 6.1 mg/dL, n=10; T=188.9 ± 8.5 mg/dL, n=10) and in triglycerides (C=176.7 ± 3.5 mg/dL, n=10; T=193.7 ± 4.1 mg/dL, n=10) in DECA-treated group in comparison with control group. Serum HDL significantly decreased in the treated group in comparison to control (C=45.8 ± 3.5 mg/dL, n=10; T=34.9 ± 3.7 mg/dL, n=10) and serum LDL increased with the treatment (C=83.39 ± 6.7, n=10; T=115.2 ± 10.5, n=10). Liver glycogen content did not change between treated (96.06 mM glycogen/ g liver ± 8.39, n=13) and control group (86.52 mM glycogen/ g liver ± 7.41, n=12). Treatment with nandrolone decanoate significantly decreased body fat mass (2.33 ± 0.13 g/ 100g carcass, n=12) when compared to control group (2.82 ± 0.18 g/ 100g carcass, n=11) (Figure 1A). Body protein mass significantly increased in treated group (27.61 ± 0.69 mg/ 100g carcass, n=11) in comparison to control (23.81 ± 1.19 mg/ 100g carcass, n=11) (Figure 1B), moreover, water content increased in the treated group (42.92 ± 0.78 g/ 100g carcass, n=12) when compared to control (40.65 ± 0.56 g/ 100g carcass, n=11) (Figure 1C).

Conclusions:
In supraphysiological doses, nandrolone decanoate can invoke some physiological disturbs in relation to lipid profile, pointing to the potential danger of this drug to health.

Keywords: Deca durabolin, lipid profile, liver glycogen, rats, body composition

Financial Support: PRONEX, CNPq, FAPERJ, CAPES

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Resumo:13-103

CHANGES IN SKELETAL MUSCLE AND BROWN ADIPOSE TISSUE IN OVARIECTOMIZED WISTAR RATS AND COLD EXPOSURE EFFECTS.

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Objectives:

Aim: Menopause is usually linked to an increase in visceral adiposity (Endocrinology 150; 2161, 2009) and diminished muscle function (J. Nutr. Health Aging 4; 156, 2000). Ovariectomy is a surgical procedure used to model the post-menopause condition. Ovariectomized animals have decreased metabolic rate measured by indirect calorimetry but the underlying molecular mechanisms are not well understood (Endocrinology 150; 2161, 2009). The aim of this work was to evaluate possible causes for the differences in metabolic rate and muscle function in ovariectomized rats.

Methods and Results:

Methods and Results: Female Wistar rats weighing approximately 200 g were divided into two groups: sham-operated and ovariectomized (ovx). The animals were sacrificed either 21 days or 13 days after surgery. At the 10th day of the 13 days protocol, the rats were divided into 2 groups: maintained at 22°C or exposed to 4°C during 3 days. In the 21 days protocol, body weight gain was significantly greater in ovx group (30.6±2.6g; n=10) compared to sham (14.9±3.1g; n=10) even though differences in food intake were not observed. The amount of uncoupling protein 1 (UCP1), a mitochondrial protein important for BAT (brown adipose tissue) thermogenesis, did not differ between groups. The same result was observed for the sarcoplasmic reticulum Ca2+-ATPase (SERCA1a), an enzyme involved in both muscle relaxation and heat production. In the 13 days protocol, both groups were able to maintain body temperature when exposed to cold. The differences in body weight between sham and ovx persisted. In spite of the differences in body weight gain, oxygen consumption and heat released by mitochondria isolated from BAT were similar in sham and ovx maintained at 22°C. Cold exposure promoted an increase in these parameters in both groups.

Conclusions:

Conclusion: Taken together, these data suggest that increased body weight gain in ovariectomized rats cannot be explained by decreased BAT thermogenesis.

Keywords: Ovariectomy, Mitochondria, cold, heat

Financial Support: FAPERJ, PRONEX, CNPq
SEXUAL DIMORPHISM IN THYROID REDOX BALANCE OF RATS

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Objectives:

A member of the NADPH Oxidase (NOX) family called Dual Oxidase (DUOX) produces H2O2 to sustain thyroperoxidase activity and another member of this family, NOX4, is also expressed in the thyroid gland. Thus, the thyroid is exposed to large amounts of hydrogen peroxide (H2O2). Cellular dysfunctions are associated with increased ROS levels. In order to protect cells against ROS, antioxidant enzymes, such as catalase and glutathione peroxidase (GPx), act as detoxifying mechanisms. As thyroid diseases are more prevalent in women than in men, the objective of this study was to evaluate the existence of a sexual dimorphism in thyroid redox balance.

Methods and Results:

H2O2 generation was measured in thyroid particulate fraction of 3 months old male and female Wistar rats by Amplex Red assay. GPx activity was assayed following NADPH oxidation at 340 nm, while catalase activity was evaluated by H2O2 decomposition at 240 nm. DUOX1, DUOXA1, DUOX2, DUOXA2, NOX4, p22phox, catalase and GPx mRNA expression were measured by real time PCR. In order to evaluate protein oxidation, the level of carbonyl group generation was measured by a colorimetric method. We observed a higher thyroid H2O2 production in the thyroids of females (F=46.76 ± 2.33 nmol H2O2/h/mg protein, n=10; M=36.06 ± 2.82 nmol H2O2/h/mg protein, n=10), as well as 50% more NOX4 mRNA expression, with no differences in the expression of the other NOX components. Catalase expression and activity (F=1913 ± 64.2 U/mg protein n=5; M=1385 ± 105.5 U/mg protein n=5) were lower in females, and although females had higher GPx mRNA expression, its activity, as well as carbonyl content, were not different between sexes.

Conclusions:

Here we show that female thyroid glands are submitted to a higher oxidative stress level. These results can explain the higher prevalence of thyroid diseases in women related to a redox imbalance during life span.

Keywords: thyroid, reactive oxygen species, antioxidant defence

Financial Support: FAPERJ, CNPq

DIABETES MELLITUS ALTERS MYOSIN-VA EXPRESSION ON THE BRAIN

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Objectives:

Diabetes mellitus is a disease characterized by an increase in circulating glucose in the blood. This hyperglycemia produces damage in brain tissue, which contributes to changes in synaptic transmission. Several proteins are involved in neurotransmitter function, such as myosin-Va. This protein is related to synaptic vesicle transport to the active zone. Our study investigated the expression and distribution of myosin-Va in the diabetic rat brains.

Methods and Results:

After 20 days of diabetes induction by streptozotocin, the brains were removed, homogenized, and analyzed by western blotting, qRT-PCR, and immunohistochemistry. The results show that the diabetic brains have a decrease in the myosin-Va level (protein and mRNA) compared to non-diabetic. Moreover, neuronal and glial cells of the occipital and frontal cortex exhibited a decrease in immunostaining for myosin-Va in diabetic rat brains.

Conclusions:

In conclusion, diabetes mellitus alters the expression and distribution of myosin-Va in the brain, and this finding may contribute to the basic understanding of this myosin role in brain function related to diabetes-associated neurological disorder.

Keywords: Diabetes mellitus, brain, myosin-Va

Financial Support: FAPEMIG, CNPq, CAPES, UFU and PROBIOTEC

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Resumo: 13-106

ACTION OF RESISTANCE TRAINING AND RALOXIFENE ON DENSITOMETRY AND BIOCHEMICAL PARAMETERS OF RATS ACYCLIC.

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Objectives:

Important advances have occurred in the understanding of bone cells and interactions with local and systemic factors that regulate the activity of these cells. The estrogen deficiency that affects women during menopause is essential in the development of osteoporosis. Thus, this study aims to examine whether resistance training (RT) and raloxifene (RLX), as well as their association, improves bone quality in rats acyclic.

Methods and Results:

The work consisted of eight experimental groups: (1) Sham + NaCl, (2) Sham + NaCl + RT, (3) Sham + RLX, (4) Sham + RLX + RT, (5) OVX + NaCl, (6) OVX + NaCl + RT, (7) OVX + RLX, (8) OVX + RLX + RT. The animals received RLX (1mg/Kg/day) or saline by gavage. For the RT protocol, maximum strength using steel balls in tubes attached to the animal's tail until it reached the concentric failure, then it was estimated at 20% of the maximum force of animals and weekly load was increased by 10% to 80% of maximum force. Earlier the 3rd and 4th month the maximum strength test was revised to suit the load. On the first day of the experiment, the rats were anesthetized and underwent bone densitometry in DPX-Alpha instrument.
for measuring bone mineral density (BMD) baseline. After 120 days of the beginning the TR or RLX, the animals were anesthetized, blood collected and processed for analysis of markers of bone metabolism and the femurs were removed for analysis of final BMD. Multiple comparisons of results were performed by analysis of variance (ANOVA) followed by Tukey test. The level of significance was P

Conclusions:

These results suggest that the action resulting from regular exercise in preventing postmenopausal bone loss is not enhanced by RLX.

Keywords: bone, exercise, menopause, raloxifene, resistance training


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Resumo:13-107

EFFECT OF INTRACEREBROVENTRICULAR (ICV) DEXAMETHASONE ON THE GLUCOSE METABOLISM OF MSG-OBESE RATS.


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Objectives:

Glucocorticoids hormones secreted by adrenals cortex exert effects on several metabolic process such as glucose homeostasis. It has been showed that peripheral insulin sensitivity and glucose uptake are decreased, while the gluconeogenesis is raised by glucocorticoids action. Thus, hyperglucocorticoid levels contribute to appearance of peripheral insulin resistance and hyperglycemia which is markers to metabolic syndrome. Independent of species, human or any experimental animal model, such as obese rats, causes impairment of insulin secretion and function, which allows contributing to fasting hyperinsulinemia associated with insulin resistance. However, there is no enough studies about the peripheral effects of glucocorticoids mediate by its actions in the central nervous system. Therefore, the present work aimed to study the central effect of dexamethasone (DEXA) on glycemic control.

Methods and Results:

At 90-day-old rats were submitted to implant of an intracerebroventricular (icv) cannula in the lateral ventricle. After 5 days of the surgery, icv injection of DEXA (5µg/day) was administrated during consecutive 4 days in the rats (DEXA), while saline solution was injected in the control animals (C). To evaluate the central effect of DEXA on glucose metabolism a silicone cannula was implanted into the right jugular vein to achievement of intravenous glucose tolerance test (ivGTT). Blood samples were collected immediately before glucose load (zero time) and at 5, 15, and 30 minutes after that. Plasma was stored at -20 °C to posterior dosage of blood glucose and insulin concentrations by the glucose oxidase and radioimmunoassay methods, respectively. Icv injection of DEXA promotes hyperglycemia (DEXA, 150.3 ± 2.86 vs C, 90.1 ± 2.45mg/dL, p

Conclusions:

Our results suggest that the changes caused by DEXA on glucose homeostasis are mediated, at least part, by the central nervous system through hypothalamic pathways.
Objectives:

Thyroid disorders are three times more prevalent in women than in men, with higher incidence of thyroid cancer during fertile life, which suggests a possible role of sexual steroids. Estrogens can affect thyroid function indirectly, interfering with thyroid-pituitary axis, or directly modulating thyrocyte function and proliferation. During thyroid hormone biosynthesis, the enzyme dual oxidase (DUOX) produces H$_2$O$_2$ to sustain thyroperoxidase activity. However H$_2$O$_2$ can stimulate cell proliferation, oxidation of cellular components and DNA strand breaks which can affect cellular function and predispose to mutation, transformation and carcinogenesis. Thus, the aim of this study was to evaluate the influence of estrogen on thyroid H$_2$O$_2$ production.

Methods and Results:

To study the estrogen effect on thyroid H$_2$O$_2$ production, we used in vitro and in vivo models. Rat thyroid cell line PCCL3 was treated with 10-9M, 10-8M and 10-7M 17$\beta$ estradiol (n=6 for each experimental condition) during 48 hours and then extracellular H$_2$O$_2$ production was measured by amplex-red/HRP (molecular probes) assay. Results were expressed as nmols H$_2$O$_2$.h$^{-1}$.mg$^{-1}$ protein. When compared to control, 10-9M 17$\beta$ estradiol was able to raise extracelular H$_2$O$_2$ production (control= 1.81± 0.46; 10-9M estradiol=2.78±0.39), but the other concentrations were not (10-8M=1.10±0.18; 10-7M=2.05 ±0.26). As in vivo model, we used two months aged female Wistar rats. The estral cycle phase was assessed through vaginal smears for three consecutive cycles and analyzed directly on microscope. To evaluate the effect of estrogen on thyroid H$_2$O$_2$ production, ovary resection was performed (OVX), followed by administration of vehicle (OVX) or physiological dose of 17$\beta$ estradiol (Eb) (0.7µg/100g body weight, sc). H$_2$O$_2$ production was measured by the amplex red / HRP (Molecular Probes) and mRNA expression was evaluated by real time PCR. Even though H$_2$O$_2$ production did not differ among groups (n=8 for each experimental condition), the activity tends to be reduced in the OVX group, what was reversed by the treatment with Eb (control=33.18±1.26; OVX=28.66±2.15; Eb=36.47±2.08). Moreover, DUOX2 mRNA content was decreased in OVX thyroid rats when compared to control group (n=5 for each experimental condition), what was reversed by estradiol replacement (control=1.00± 0.10; OVX= 0.68± 0.08; Eb= 0.90± 0.18). On the other hand, NOX4 mRNA was increased in OVX and normalized in Eb group (control=1.00± 0.11, n=7; OVX=1.65± 0.16, n=5; Eb=1.43± 0.10, n=7). Thyroid H$_2$O$_2$ production was also evaluated in the four phases of rat estrous cycle. In the estrus phase, we found greater levels of H$_2$O$_2$ production when compared to diestrus 2 (estrous=72.09±6.11; diestrus 2= 62.62±5.21), what could be related to estradiol peak during the previous phase (proestrus).

Conclusions:

Estrogen acts regulating H$_2$O$_2$ production and modulating the transcription of the thyroid NADPH-oxidases (DUOX2 and NOX4) what could be related to the greater prevalence of thyroid diseases in women during reproductive phase.
ASSOCIATION BETWEEN HORMONAL LEVEL AND COGNITIVE FUNCTION IN MENSTRUAL LUTEAL PHASE

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Objectives:

Sex hormones are related with Hypothalamo-Pituitary-Adrenal Axis (HPA) and this axis is known to be involved in stress responses, by the action of cortisol. If sex hormone levels do influence the modulation of this axis, they can, indirectly, determinate affective factors, like depression and anxiety symptoms. Taking menstrual cycle as an example, a physiological situation with a huge hormone variation, is possible to investigate if complains are related to the influence of sex hormones, by the modulation of HPA. Therefore, we hypothesized that menstrual cycle should express sex hormone variation correlated with psychometric tests and cognitive functions, in different phases.

Methods and Results:

Participants comprised 9 men (mean of age: 21.87±0.63) and 6 women (mean of age: 21.72±0.32). Individuals were included in the present sample if they did not have a current major Axis I psychiatric disorders. In addition, women had their menstrual cycle analysed and they were excluded if any irregularity was found. To investigate symptoms, we used psychometric tools: Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI) and Barrat Impulsiveness Scale (BIS). Moreover, to assess cognitive function, we used Mini-Mental State Examination (MM). The biological parameters were plasmatic progesterone, estradiol and cortisol. The blood collections were realized at morning, after four saliva samples (after awakening, 30 and 60 minutes later, and before the test). The menstrual phases were divided in early follicular (1-5 day), peri-ovulatory (12-14 day) and midluteal (21-23 day). Women were evaluated at each phase and men were evaluated at once. Statistical analyse of estradiol, progesterone and cortisol was performed by ANOVA one-way, and Bonferroni test was used for post-hoc analyse. Pearson’s Correlated Test was calculated to see associations between hormones and psychometric and cognitive parameters. An initial analyse of our results showed correlated variables. BIS (62.0±4.0) was positively correlated with estradiol (166.9±35.8) [r= 0.94, p=0.005] and negatively correlated with plasmatic cortisol (19.0±0.9) [r= 0.83, p= 0.04], at luteal phase, but not in other phases, nor in men. Also in this phase, exclusively, it was found that BDI (5.0±1.6) and BAI (7.7±1.95) had a positive association among them [r=0.96, p=0.003] and with progesterone (6.7±2.3) [Pearson’s correlated coefficients, respectively, 0.84 and 0.83; p= 0.038 and 0.04]. MM (29.6±0.21) and salivary cortisol of 30 minutes after waking (0.83±0.04) had a statistic correlation [r=0.83, p= 0.04], positively, also in this phase.

Conclusions:

These data demonstrate that, in luteal phase, but not in other moment of menstrual cycle and not in men, biological variables, like gonadal steroids and cortisol, have significant correlation with psychometric and cognitive factors. Further longitudinal studies with larger populations and evaluation of hormonal changes are needed to confirm these data.

Keywords: cognitive function, cortisol, menstrual cycle, sex hormone

Financial Support: FAPESP; CNPq
HMGCOA REDUCTASE AND ABCG1 GENE EXPRESSION VARY ACCORDING TO THE PLASMA HDL-C CONCENTRATION AND CONTRIBUTE TO THE INTRACELLULAR CHOLESTEROL HOMEOSTASIS IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS

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Objectives:
The antiatherogenic functions of high density lipoprotein (HDL) include its role in reverse cholesterol transport, but to what extent HDL-C interferes with the cellular cholesterol metabolism is unknown. Therefore, we evaluated the cellular cholesterol content, the gene expression of HMGCoA reductase and of the receptors involved in the cholesterol transport across the cellular membrane in peripheral blood mononuclear cells (PBMC) in healthy subjects that differ according to their plasma HDL-C concentration.

Methods and Results:
Healthy participants (both genders) were recruited for presenting low HDL-C (< 40mg/dL, n=37, 21 male and 16 female) and high HDL-C (> 60mg/dL, n=36, 20 male and 16 female), BMI< 0,01; 2) ABCG1: 0,89 ± 0,13 vs. HDL-C 1,00 ± 0,15, P < 0,01 (Student’s t test).

Conclusions:
We hypothesize that low HDL-C display low reverse cholesterol transport rate. Consequently less cholesterol from peripheral tissues reaches the liver explaining elevated cholesterol synthesis rate which in the PBMC cells is mirrored by HMGCoA reductase gene expression. The latter is compatible with a diminished ABCG1 gene expression. PBMC cholesterol content, that expresses the liver cell cholesterol, did not differ between the two groups because their reduced input of cholesterol is perfectly balanced by an increased synthesis rate.

Keywords: HMGCOA REDUCTASE, ABCG1, HDL, ATHEROSCLEROSIS, MONONUCLEAR CELLS

Financial Support: FAPESP 06/60585-9 and 08/50185-9

ENDOPLASMIC RETICULUM STRESS TEMPORALLY CORRELATES WITH APOPTOSIS INDUCED BY PALMITATE IN HEPATOCYTES

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Objectives:
Excess of free-fatty acids (FFA), are described to contribute to hepatic steatosis and insulin resistance in obese patients. Recently, it was demonstrated that the activation of Unfolded Protein Response by palmitate in pancreatic beta cells plays an important role in pancreatic lipotoxicity. The UPR is activated when an excess of nutrients in the body causes an increase in protein unfolded in the lumen of the endoplasmic reticulum. The cellular response of ER stress is mediated by three distinct ER transmembrane proteins including the kinase 1, PKR-like ER kinase (PERK), activating transcription factor (ATF)-6, and serine/threonine-protein kinase/endoribonuclease Ire1. The aim of this study is to assess the time course of activation of UPR and apoptosis in hepatocytes treated with palmitate.

Methods and Results:
HepG2 cell line originated from human hepatoma were maintained in culture medium (DMEM plus 5.6 mM glucose, 10% fetal calf serum, 100 U / ml penicillin and 0.1 mg / ml streptomycin) in atmosphere with 5% CO2 at 37 ° C. Upon reaching approximately 90% confluence, cells were treated with 0,5 mM palmitate pre-conjugated with albumin and the parameters of UPR were evaluated in parallel with DNA fragmentation. UPR activation was assessed by western blot and DNA fragmentation was measured by flow cytometry. Palmitate-induced apoptosis was maximal after 12h of treatment. CHOP, a marker of ER-stress-dependent apoptosis was also modulated by palmitate and peaked 12h after beginning of treatment but was already enhanced as early as 6h after exposition to palmitate. Processed ATF6 was upregulated 3h after palmitate exposition and these levels were sustained up to 12h of treatment with this FFA. In contrast, PERK phosphorylation and ATF4 expression were not altered along the treatment period. GADD34, a eiF2a phosphatase was reduced by palmitate.

Conclusions:
Altogether, our results show a temporal concordance between ER stress and apoptosis stimulated by palmitate in hepatocytes. Activation of ER stress seems to be partial and possibly plays a role in hepatic steatosis.

Keywords: Endoplasmic reticulum stress, Unfolded Protein Response, Free-fatty acids , Hepatic steatosis, Insulin resistance

Financial Support: Fapesp

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Resumo:13-112

DIETARY CALCIUM SUPPLEMENTATION PREVENTS OBESITY, INSULIN RESISTANCE AND IMPROVES LIPID PROFILE IN ADULT RATS PROGRAMMED BY EARLY WEANING

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Objectives:
It is well known that obesity is a worldwide epidemic and an adequate nutrient supply during early life is essential to establish the future endocrine and metabolic status. Malnutrition only during lactation can program for metabolic and endocrine disorders of the progeny at adulthood such as obesity (Horm Metab Res, 41; 866, 2009). Recently, our group evidenced that, early weaning caused by lactation interruption with breast banding, the adult progeny showed higher adiposity, higher triglycerides and insulin resistance programming for metabolic syndrome parameters, such as obesity, type 2 diabetes and dyslipidemia (Br J Nutr, 1, 2011). Some studies have shown that a diet rich in calcium reduced body weight (J Am Diet Assoc, 106; 1066, 2006) and adiposity (J Am Coll Nutr, 24; S537, 2005), and improved both insulin sensitivity (Diabetes Care, 30; 980, 2007) and lipid profile.
(Am J Clin Nutr, 91; 131, 2010). Considering that dietary calcium therapy could have an anti-obesity action, in the present study we studied the possible effect of dietary calcium supplementation on these endocrine dysfunctions in this experimental model.

Methods and Results:

Lactating rats were separated in: EW (early weaning) - dams were involved with a bandage to interrupt the lactation in the last 3 days of lactation, and C (control) - dams whose pups had free access to milk during all lactation (21 days). At 120 days-old, EW and C offspring were subdivided into 4 groups: 1) C - standard diet; 2) CCa - calcium supplementation (10g of calcium carbonate/kg of rat chow); 3) EW - standard diet, and 4) EWCa - calcium supplementation. Rats were killed at 180 days by decapitation and blood was collected. The visceral fat mass was weighed. Lipid profile and glycemia were determined by colorimetric assay, serum leptin, insulin and adiponectin were measured by radioimmunoassay. All significant data were p

Conclusions:

we reinforced that early weaning leads to late development of some components of metabolic syndrome. Dietary calcium supplementation was effective in prevents obesity development, glucose homeostasis impairment and hypertriglyceridemia. Thus, may represent a possible therapeutic strategy against the endocrine-metabolic disorders development in early weaning offspring.

Keywords: calcium supplementation, early weaning, insulin resistance, lipid profile, obesity

Financial Support: CAPES, FAPERJ, CNPq

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Resumo:13-113

HYPOGLYCEMIC ACTIVITY OF NEW SULFONYLHYDRAZONE DERIVATIVES IN STREPTOZOTOCIN-INDUCED DIABETES

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Objectives:

Type 1 diabetes mellitus is a metabolic disorder characterized by hyperglycemia, consequent to the destruction of pancreatic beta cell. When not properly treated can progress to complications, dysfunction and failure of various organs such as kidney, brain and heart. New sulfonhydrazine derivatives (LASSBio-1471 and LASSBio-1473) were synthesized and tested in animal model of type 1 diabetes induced by streptozotocin (STZ).

Methods and Results:

The protocols were approved by Animal Care and Use Committee at Universidade Federal do Rio de Janeiro. Male Wistar rats (180-220 g) received a single STZ intraperitoneal injection (45 mg/kg) for induction of type 1 diabetes. Seven days after the STZ-induced diabetes, animals with glucose levels above 200 mg/dL measured using Accu-Check ® system were randomly divided in three groups each one treated during 10 days with either vehicle (DMSO) or the derivatives (20 mg/kg, i.p.). During treatment period, glucose level was measured before and after 1, 3, 5 and 10 days of treatment. At the end of treatment period, rats were submitted to the oral glucose tolerance test. Rats were placed in fasting (12-18 h) and control glucose levels were obtained. Then, they received an oral administration of glucose (2 g/kg) and glucose levels were measured after 30 min and 2 h.

Conclusions:
LASSBio-1471 and LASSBio-1473 promote not only a significant hypoglycemic effect in animal model of type 1 diabetes but also prevent glucose intolerance in these animals.

Keywords: diabetes, streptozotocin, glycemia, sulfonylhydrazone derivatives, glucose

Financial Support: Faperj, CNPq, FUJB, CAPES, PRONEX, INCT, PENSARIO

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**Resumo:**

MELATONIN-INDUCED ACTIVATION OF HYPOTHALAMIC INSULIN SIGNALING ACTIVATES AN INTER-ORGAN COMMUNICATION LEADING TO SUPPRESSION OF HEPATIC GLUCONEOGENESIS


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Objectives:

Melatonin, produced by the pineal gland in a circadian fashion peaking at the nighttime, is associated with the control of several parameters of energetic metabolism such as gluconeogenesis, lypolisis and glucose uptake by adipose cells. In this sense, melatonin was demonstrated to activate intracellular insulin signal transmission within hypothalamic areas but the precise relevance for this molecular mechanism has not been settled so far. Among several steps, melatonin was demonstrated to activate the PI3K/AKT pathway. The present study aimed to demonstrate an importance for melatonin-induced hypothalamic AKT activation for the regulation of hepatic gluconeogenesis.

Methods and Results:

Adult male Wistar rats (200-250 g) were used in all experiments in accordance with the guidelines of the Brasilian College for Animal Experimentation (COBEA). The rats were subjected a surgical procedures for cannula implantation into the lateral ventricle, as described by Paxinos and Watson (1986). Seven days after surgery rats were fasted for 12h and received an intracerebroventricular (icv) injection of melatonin and anaesthetized. Two hours after injection, the animals were intraperitoneally challenged with pyruvate (Pyruvate Tolerance Test - PTT) in order to assess whole-body gluconeogenesis. An independent set of rats were sacrificed and hypothalamus and liver were removed for protein extraction and immunoblotting detection of phosphorylated AKT (hypothalamus) and PEPCK, G6Pase and phosphorylated STAT3 (Liver). Icv injection of melatonin efficiently increased AKT phosphorylation in rats hypothalamus. In parallel, this effect of melatonin was accompanied by reduction of hepatic PEPCK and G6Pase. Concordantly, the phosphorylation of STAT3, a repressor of these enzymes, was stimulated by melatonin. These molecular alterations were paralleled by the ability of icv melatonin in reduced gluconeogenesis. In addition, both pharmacological inhibition of hypothalamic PI3K/AKT and subdiaphragmatic vagotomy supressed the effect of icv melatonin on gluconeogenesis.

Conclusions:

The present data suggest a metabolic relevance for melatonin-induced PI3k/AKT pathway activation within the hypothalamus. This mechanism may lead to suppression of hepatic PEPCK and G6Pase expression and, by extesion, abrogate gluconeogenesis. Our data also evidences that this information is transmitted from the hypothalamus to the liver through the parasympathetic autonomic nervous system. These findings might underlie hyperglycemia found in pinealectomized rats.

Keywords: Melatonin, gluconeogenesis, PI3K/AKT pathway
Objectives:

Dual oxidase (DuOx) is the enzyme responsible for the generation of hydrogen peroxide in the thyroid, an essential cofactor for thyroid hormone biosynthesis. However, the possible difference in DuOx regulation between sexes has not been evaluated so far, even though it is well-known that the prevalence of thyroid disease is greater in women than in men. Thus, the objective of the present work was to evaluate the effect of iodide and TSH on thyroid dual oxidase activity in males and females.

Methods and Results:

Wistar rats weighing approximately 200g were divided into six groups: control (males n=6; females n=3) animals treated with methimazole (MMI) 0.03% (female n=4, male n=8); treated with potassium perchlorate (KClO4) 1% (male n=9) and animals treated with both (KClO4+MMI) (male n=10). Both MMI and KClO4 were administered in the drinking water for 10 days. Animals were then sacrificed and thyroids obtained for processing and dosage of DuOx activity by Amplex Red method. Results were expressed as nmols H2O2.h⁻¹.mg⁻¹ protein. Male rats treated with MMI showed a significant reduction of DuOx activity when compared to control group (control=15.73±5.52; MMI=1.25±0.72), while KClO4 (9.79±2.99) and KClO4+MMI (8.28±2.38) did not. Thyroid DuOx activity of control female rats did not significantly differ from that of male rats (male=15.73±5.52; female=38.13±18.69). In MMI treated rats, females had significantly higher DuOx activity than males (males=1.25±0.77; female=8.55±2.74). We have also evaluated whether thyroid iodide uptake could differ between sexes. Male and female rats were divided into two groups: control and MMI (n = 5 for all experimental groups). Na125I (3700 Bq, ip) was administered 15 min before sacrifice. Thyroids were removed, weighed and radioactivity was measured in a gamma counter. Results were expressed as percent of 125I administered/mg thyroid. Control thyroid iodide uptake did not differ among sexes (male=0.032±0.008; female=0.027±0.003). However, the treatment with MMI led to significant increase in thyroid iodide uptake in female but not in male rats (male=0.084±0.004; female=0.118±0.019). Moreover, MMI treatment produced an enhancement in thyroid weight in male rats, which is significantly greater than all groups (male control=15.02±0.53mg; male MMI=36.3±1.00mg; female control=16.66±1.59mg, female MMI=18.98±1.54mg), what was not observed in female rats.

Conclusions:

In animals treated with MMI, which have elevated TSH, the inhibitory effect of iodide seems to overcome the possible stimulatory effect of TSH on the DuOx function. The greater thyroid DuOx activity of female rats might be due to a stimulatory effect of estrogen on the enzyme, since DuOx activity was greater in females even in the presence of higher iodide uptake. On the other hand, the greater capacity of female rats to concentrate iodide might be responsible for the smaller increase in thyroid weight after MMI treatment observed in female rats.

Keywords: Dual Oxidase (DuOx), Iodide Uptake, Sexual Dimorphism, Thyroid

Financial Support: CNPq, FAPERJ, PIBIC
PROLONGED USE OF DERMOSONIC LIPOCLASIA FOR REDUCTION OF SUBCUTANEOUS FAT INCREASES CARDIOMETABOLIC RISK IN HEALTHY RATS: PRECLINICAL STUDIES

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Objectives:
In recent years in Brazil has increased significantly the use of lipoclasia dermosonic (LCD, subcutaneous lipolysis by ultrasound) as a treatment for skin disorders, especially lipodystrophy gynoid. However, the systemic effects of prolonged use of the LCD are not yet well established. In this study the objective was to investigate the cardiovascular and metabolic effects of prolonged use of LCD for reduction of subcutaneous fat in female rats.

Methods and Results:
Healthy female rats (Wistar) were divided into two groups, named control (n=20) and treated with LCD (n=20). After 10 days, ½ of the female rats in both experimental groups were sacrificed and evaluated; the others remained for another 10 days washout period for the effects of the LCD. For the treatment of animals used were 10 applications of LCD on alternate days, the following parameters: ISATA= 3MHz, 1W.cm-2, pulsed mode (2ms ON: OFF 8ms), cycle 30% for 3 minutes in 3cm2 of inguinal subcutaneous adipose tissue. Cardiovascular and metabolic parameters were evaluated during stages of the study. Body composition was measured using bioimpedance and bilateral lipectomy, and biochemical profile and inflammatory response plasma were determined. The LDC reduces (P

Conclusions:
The LCD shows potential as an inducer of subcutaneous lipolysis, however, prolonged use appears to increase the cardiometabolic risk factors in female rats.

Keywords: Lipoclasia dermosonic, Lipolysis, Dyslipidemia, Insulin resistance, Cardiovascular risk

Financial Support: CDV-FACITEC
Objectives:

n-3 polyunsaturated fatty acids (n-3 PUFA) from fish oil (FO) exert important hypolipidemic action by modulating nuclear receptors activity, such as Peroxisome proliferator activated receptor (PPAR). Likewise, thyroid hormones (TH) promote lipid-lowering effects, especially via the hepatic TH receptor β1 (TRβ1). We demonstrated that in euthyroid (EU) rats, FO administration increased TRβ1 protein content and activity of mitochondrial glycerophosphate dehydrogenase (mGPD) in the liver, while reduced serum lipids. However, in hypothyroid (HYPO) rats, the hypotriglyceridemic effect of FO was abolished, as well as hepatic increase in TRβ1 protein and mGPD activity, suggesting TH signaling involvement in n-3 PUFA effects. Therefore, we investigated mechanisms underlying the different responses to FO administration depending on TH status.

Methods and Results:

EU and HYPO male adult rats (methimazole-treated for five weeks) received fish oil (FO - ROPUFA-75, Roche) or soybean oil (control) daily by gavage during three weeks (0.5mL/animal – n=7/group). Hepatic mRNA expression of TRβ1 and mGPD and PPARα protein expression was evaluated by real-time PCR and western blotting, respectively. Differences were considered significant at p

Conclusions:

n-3 PUFA affect differentially hepatic thyroid hormone signaling in EU and HYPO animals acting through transcriptional mechanisms. These findings in addition to PPARα up-regulation by hypothyroidism supports important crosstalk mechanism between those fatty acids and TH signaling in lipid metabolism.

Keywords: Fish oil, PPAR, Thyroid hormone

Financial Support: CNPq, CAPES and FAPERJ

Resumo:13-118

SALIVARY GLANDS ADAPT TO HYPERGLYCEMIA DURING LONG TERM DIABETES MELLITUS.

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2 Departamento de Ciências Morfológicas, UFRGS

Objectives:

The chronic hyperglycemia that characterizes Diabetes Mellitus (DM) affects the homeostasis and function of many organs, including salivary glands and kidneys. Oral manifestations of DM are often secondary to the hipofunction of salivary glands that produce the saliva, which is essential for maintaining oral health due to its several protective functions. We have previously shown alterations in parenchymal basement membrane and TGF-α2 signaling in the parotid gland caused by DM, which might be involved in salivary gland hipofunction. The aim of this study was to evaluate morphology, as well as laminin and collagen type III distribution (extracellular matrix –ECM proteins from basal lamina and stroma, respectively) in the parotid and submandibular glands (SMG) of adult male control and diabetic rats for 30 (D30) and 180 (D180) days. The kidneys from the same animals were also analyzed for comparison, since the effects of DM on kidney ECM homeostasis are progressive.

Methods and Results:
Sixteen adult male Wistar rats were employed. DM was induced by streptozotocin intraperitoneal injection (60mg/kg b.w.), while control rats received only the vehicle. Food intake, body weight, urinary volume, glycosuria and glycemia were measured for the control of diabetic status. On sacrifice the salivary glands were excised, fixed, processed and embedded in Paraplast®. Five \( \mu \)m-sections were submitted to hematoxylin/eosin staining and immunohistochemistry for laminin and collagen type III. Morphometric analysis was performed in the SMG. The results showed that DM promoted vacuolization of acinar cells in the parotid gland, mainly in D30. Animals with glycemia above 600 mg/dl presented a discrete atrophy of striated ducts in D30 (88% of the group) and in D180 (45% of the group). Diabetic rats (D30 and D180) showed a 27% decrease in the volume of convoluted granular ducts and a discrete increase of acinar volume (11%) in SMG. Morphological alterations were not directly correlated with glycemia levels. In both glands there was increased deposition of laminin and collagen III, similar between D30 and D180. The same augment of laminin was observed in renal glomeruli, increasing progressively over time.

Conclusions:

These results suggest that salivary glands are affected by hyperglycemia in the short term, but seem to be capable of adaptation in the long term, especially when compared to kidneys.

Keywords: Hyperglycaemia, Salivary Glands, Laminin, Collagen type III, Kidney

Financial Support: FAPESP AND CNPq

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**QuebraPagina**

**Resumo:**

**INFLUENCE OF ESTRADIOL ON THE LIPOLYTIC ACTIVITY OF DIFFERENT DEPOTS OF ADIPOSE TISSUE IN OBESE AND HYPERTENSIVE FEMALE RATS.**


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**Objectives:**

Estrogen seems to promote and maintain the typical female type of fat distribution. Menopause-related changes in body fat distribution may partially explain the greater risk of cardiovascular disease during the postmenopausal years. However, the effect of the menopause transition on body fat distribution remains unclear. The aim of the present study was to determine the lipolytic activity of estradiol in different adipose tissue depots of ovariectomized and high fat diet-fed spontaneously hypertensive rats (SHR).

**Methods and Results:**

Twenty-four 12-wk-old female SHR were ovariectomized or sham-operated. The animals were divided (6 per group) according to the diet received during 24 weeks: high-fat diet (54.4% of fat) and standard diet (groups: OHFD: ovariectomized high fat diet-fed rats, OSD: ovariectomized standard diet-fed rats, SHFD: sham-operated high fat diet-fed rats and SSD: sham-operated standard diet-fed rats). Body weight was measured weekly. After, the rats were sacrificed by decapitation. Gonadal, mesenteric (visceral depot) and retroperitoneal tissues (nonsubcutaneous nonvisceral) were dissected and weighted. The rate of lipolysis was determined in the collagenase-digested adipocytes obtained from the three fat depots of OHFD rats by measuring glycerol release in the presence and absence of isoproterenol or estradiol. Our results demonstrate that the OHFD group showed an increased in the body weight compared to control group (520±14; 270±6g, p<0.05).

**Conclusions:**

The estradiol in association with the isoproterenol was able to increase the glycerol release in both mesenteric and gonadal fat
Depots. On the other hand, estradiol inhibits the isoproterenol-stimulated lipolysis in retroperitoneal depot. These results suggest that estradiol-stimulated lipolysis is fat depot-dependent in ovariectomized SHR.

Keywords: estradiol, fat depot, lipolysis

Financial Support: CNPq

**QuebraPagina**

**Resumo:**

*T3 RAPIDLY INCREASES PROINSULIN MRNA EXPRESSION IN PANCREATIC BETA CELLS BY ACTIVATING THE PI-3K/GSK3-BETA SIGNALING PATHWAY AND THE TRANSCRIPTIONAL FACTOR PDX-1.*

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Objectives:

Thyroid hormones (THs) modulate gene expression and cell function by transcriptional and posttranscriptional (non-genomic) mechanisms. The latter occurs in a short period of time and in the presence of transcriptional and translational blockers. Normally, these actions are dependent of PI-3K/Akt/GSK3-beta and PKC signaling pathway activation or by THs binding to the alphaVbeta3 integrin receptor, being the latter blocked by previous RGD peptide administration. In this study, we have attempted to investigate whether THs affects proinsulin gene expression and the possible molecular mechanisms involved on it, such as, PI-3K/Akt/GSK3-beta signaling pathway, PKC activity, alphaVbeta3 integrin and the transcriptional factor PDX-1.

Methods and Results:

INS-1E cells were cultured in a medium containing normal fetal bovine serum (FBS) or FBS depleted of TH, for 24 h, treated or not with T3 for 30 min. Before T3 addition, the cells were subjected to actinomycin D (ActD), cycloheximide (CHX), RGD peptide, as well as PI-3K (Wortmannin), Akt and PKC inhibitors treatment, being the proinsulin expression evaluated by real time PCR, afterwards. Phosphorylated GSK3-beta and total PDX-1 were evaluated by Western blotting analysis. T3 rapidly increased proinsulin mRNA expression, an effect completely abolished by ActD and CHX, indicating a genomic action established in a short period of time. RGD peptide as well as Akt and PKC inhibitors did not change the stimulatory effect of T3 on proinsulin mRNA expression, excluding the alphaVbeta3 integrin as well Akt and PKC participation of T3 stimulatory effects on proinsulin mRNA expression. Interestingly, PI-3K activity inhibition prevented the increase of proinsulin mRNA induced by T3. The GSK3-beta phosphorylation, which leads to GSK3-beta inhibition and PDX-1 content were increased after T3 treatment, which appears to improve the proinsulin gene expression since GSK3-beta inhibition leads to increase of PDX-1 content and activity.

Conclusions:

Therefore, we conclude that T3 acting firstly by non-genomic stimulation of PI-3K/GSK3-beta signaling pathway, rapidly induces the proinsulin mRNA expression by genomic mechanisms, which appears to involve one of the major transcriptional factors for insulin synthesis, the PDX-1.

Keywords: mRNA proinsulin, Thyroid hormones, PI-3K/GSK3-beta signaling pathway, Transcriptional factor PDX-1

Financial Support: FAPESP

**QuebraPagina**
CHRONIC TREATMENT WITH CB1 RECEPTOR ANTAGONIST INCREASES GLUT4 EXPRESSION THROUGH A NF-KAPPA B DEPENDENT MECHANISM

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Objectives:
Evidences have suggested that the endocannabinoid system is overactive in obesity, resulting in enhanced endocannabinoid levels in both circulation and visceral adipose tissue. The cannabinoid CB1 receptor is expressed in the adipose tissue besides the brain. Few studies in vitro suggest that CB1 activation increases glucose uptake in adipocytes. The objective of the present study was to investigate the CB1 receptor modulation on glucose transporter GLUT4 expression, which is encoded by the SCL2A4 gene, and the related mechanisms.

Methods and Results:
3T3-L1 adipocytes were incubated in the presence of a selective antagonist of CB1 receptor, AM251 [1-(2,4-Dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-1-piperidinyl-1H-pyrazole-3-carboxamide]. After 2, 4 or 24 hours, cells were harvest to evaluate GLUT4 mRNA (Real Time PCR) and protein (Western blotting), and NF-kappaB activation specifically on the promoter of SLC2A4 gene (EMSA). Acute and chronic incubation for 4 or 24 hours with AM 251 expressively increased GLUT4 protein content (P)

Conclusions:
The CB1 receptor stimulation enhances GLUT4 expression in adipocytes. Evidences point out to an important participation of the inflammatory transcriptional factor NF-kappaB on the chronic modulation of the SLC2A4 gene expression by CB1 receptor.

Keywords: CB1 receptor, GLUT4, NF-kappaB, obesity, insulin resistance

Financial Support: FAPESP (08/09194-4 and 07/50554-1)
Methods and Results:

Male adult Swiss mice (45 days old, weighing 30g) were subcutaneous inoculated with solid Ehrlich tumor (SET) and sacrificed 14 after tumor implantation whilst control group received saline alone (CTL). Western blotting was performed in muscle for determination IR/P13K/AKT/MTOR protein expression. The results were expressed as Media±SE, n=6. Significance of the differences was evaluated by test t student. The significance level adopted was P

Conclusions:

Based on present data, we are tempted to believe that cachexia establishment as well as crosstalk between decrease in insulin secretion and decrease in key protein expression of metabolic insulin pathways, as a consequence of the tumor presence. Moreover, mTOR is not altered, indicating that could be other factors involved in these cachectic muscle disorders that still remain unclear. Therefore, this model can contribute to the understanding of others factors associated with muscle atrophy in this cachexia condition imposed by the tumor presence.

Keywords: cachexia, solid Ehrlich tumour, insulin, muscle, protein

Financial Support: FAPESP

Resumo:13-123

RESTRAINT STRESS ALTERS BEHAVIORAL AND HORMONAL RESPONSES IN ZEBRAFISH

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Objectives:

In teleosts, changes in swimming, exploring, general locomotor activity, and anxious state can be a response to stress mediated by the corticotropin-releasing factor system activation and its effects on glucocorticoid levels. Zebrafish has been widely used to study neuropharmacology and has become a promising animal model to investigate neurobehavioral mechanisms of stress. Here we validate a protocol of restraint stress for zebrafish by analyzing its behavior pattern, whole-body cortisol content and corticotropin-releasing factor gene expression, as a way to study the mechanisms involved in stress response.

Methods and Results:

In the present study the animals were submitted to restraint stress for different time lengths (15, 60 and 90 minutes) and the swimming activity was evaluated by ANY-Maze recording software. Restraint stress increased the locomotor activity as evaluated by the number of line crossings (84% and 79% for animals submitted to 60 and 90 minutes of stress, respectively), distance travelled (169% for 90 minutes of stress), mean (134% for 90 minutes of stress) and maximum speed (479% and 557% for 60 and 90 minutes of stress, respectively) in relation to control group. Moreover, this protocol also induced an alteration in the swimming pattern as measured by the absolute turn angle, angular velocity and meandering. Whole-body cortisol content showed a positive correlation with increased behavioral indices of locomotion in zebrafish, with higher cortisol levels at 15 (9.2 ± 0.6 ng.g-1 of tissue), 60 (8.5 ± 0.9 ng.g-1 of tissue) and 90 (9.7 ± 0.4 ng.g-1 of tissue) minutes of restraint stress in relation to control animals (5.2 ± 1 ng.g-1 of tissue). However, corticotropin-releasing factor mRNA expression maintained unaltered during the first 60 min of restraint stress and decreased at 90 min (71 ± 6 arbitrary units) of restraint stress in relation to all other animal groups (154 ± 9.3 arbitrary units).

Conclusions:
Altogether, we present a model of restraint stress in zebrafish, confirmed by elevated cortisol content, as a valid and reliable model to study the biochemical basis of stress behavior, which seems to be accompanied by a negative feed-back of corticotrophin-release-factor mRNA expression.

Keywords: Zebrafish, Restraint stress, Locomotor activity, Cortisol, Corticotropin-releasing factor

Financial Support: FAPERGS; CAPES; CNPq

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Resumo:13-124

EFFECTS OF GROWTH HORMONE TRANSGENESIS ON OSMOREGULATION AND ENERGY METABOLISM IN ZEBRAFISH (DANIO RERIO)

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Objectives:
In fish, additionally to its central role in growth, GH is involved with osmoregulatory control, increases appetite, food intake and metabolic rate. The objective this study was to verify the effects of growth hormone on osmoregulation and energy metabolism in zebrafish Danio rerio.

Methods and Results:
In order to verify whether growth is affected by salinity in zebrafish, we determined the growth rate of wild type zebrafish exposed to different salinities (0, 5 and 7.5) for 15 days. The fishes exposed to salinity 5 had faster growth rates when compared with fishes exposed to salinity 0, while growth rates of fishes reared in salinity 7.5 were not statistically different than controls. These results indicate that salinity can affect growth in zebrafish. After, we determined the zebrafish median lethal salinity (MLS). Salinity tolerance tests were performed in zebrafish and adults male fish (n=3x30) were transferred from salinity 0 to 2.5, 5, 7.5, 10, 12.5 and 15 for 96 h. MSL was calculated as 11.6. In addition, a GH transgenic zebrafish model was used to test the hypothesis that a GH transgenic fish could tolerate higher salinity levels, since GH is supposed to be involved with salinity tolerance. For that, were used G1 transgenic individuals from the F0104 lineage which harbour a transgene comprised by the GH cDNA from marine silverside fish under the transcriptional control of carp β-actin promoter. To verify the GH transgenic fish tolerance to higher salinity, non-transgenic (NT) and GH transgenic zebrafish (T) were exposed to MLS. Adult NT and T males were exposed to salinity 11 for 96 h. The experiments were performed in triplicate (n=3x10). Mortality for NT group was 63.88% (±19.25) while for T it was 100% in all replicates. To clarify these results, we investigate the major proteins involved in iono-osmoregulatory regulation of aquatic animals and energy production. Expression of genes coding for Na+, K+, ATPase, H+-ATPase, carbonic anhydrase 1, carbonic anhydrase 2, Na+/H+ exchanger 3b, lactate dehydrogenase and citrate synthase were analyzed by RT-PCR. Na+, K+, ATPase activity and ATP concentration were also determined. Osmoregulation-related genes were down-regulated in the T group exposed to salinity 11. In contrast, in NT fish genes coding for H+-ATPase, CA1, CA2 were induced after exposure to salinity 11. Exposure to salinity 11 induced LDH gene expression in liver of T fish and decreased CS expression in gills, suggesting the predominance of aerobic metabolism in gills and anaerobic metabolism in liver. Gills of adult males were isolated from NT and T zebrafish submitted to salinity 11 for 24 h (n=3x6) for ATP determination and measure of Na+, K+, ATPase activity, as previously cited. ATP content in gills increased in fishes exposed to salinity 11 in both NT and T groups. Gill Na+, K+-ATPase activity was significantly higher in the T fish exposed to salinity 11 than corresponding controls. In contrast, no changes was observed in the NT fish transferred to salinity 11.
Conclusions:

These results support a role for GH on osmoregulation and energy metabolism in zebrafish. Moreover, this work suggests that GH-transgenic zebrafish are not able to cope with higher salinity, probably because the energy available is used for supporting the continuous growth imposed by the GH excess.

Keywords: Growth hormone, transgenesis, zebrafish, osmoregulation, energy

Financial Support: CNPq, CAPES and PPGCF-FAC/FURG

EFFECTS OF GROWTH HORMONE SUPEREXPRESSION ON REPRODUCTIVE PERFORMANCE FROM ZEBRAFISH (DANIO RERIO)

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Objectives:

Evaluate the reproductive performance between growth hormone (GH) transgenic zebrafish and non-transgenic zebrafish.

Methods and Results:

GH-transgenic zebrafish (T) used in this study were from the F0104 lineage, which harbour two transgenes: one comprised by the GH cDNA from marine silverside fish (Donthesthes argentinensis) and another the green fluorescent protein (GFP) gene expression used as a transgenesis label. Both of them are under transcriptional control of carp (Cyprinus carpio) β-actina promoter. Transgenic and non-transgenic (NT) adult individuals (6 – 8 months) were used in the experimental procedures. Animals were maintained in a closed farming system under temperature of 28 °C, near oxygen saturation, with pH near 7, 14h light/ 10h dark photoperiod and constant water flow. All individuals were fed twice a day with commercial ration with high protein content (47.5%; ColorBits, Tetra). Four females and two males were kept in aquariums with traps built using a netting covered plastic open box which only eggs could went through. The trap had artificial vegetation on the top which was recognized as reproductive territory by fishes. Experimentation was made in triplicate. During 30 days, the aquariums were monitored and eggs were collected one hour after spawning for evaluation of fertilization rate under a stereoscopic microscope. Eggs reaching the 4–8 blastomere were considered fertilized. Subsequently eggs were counted and incubated under temperature of 28 °C up to hatching. Hatching rate was determined about three days after and it was expressed as the percentage of hatched larvae in relation to the total number of eggs incubated. Frequency spawning was calculated as the number of spawning for each group during the 30 days of experiment. Results were analyzed by paired t-test and expressed by mean±standard error. There were no significant differences on fertilization rate between the two groups (T= 71.37±13.27 and NT=89.96±1.37; p≤0.05).

However, hatching rate was significantly lower in transgenic group (31.53±6.91) than non transgenic (46.22±9.15), as well as spawning frequency (T= 3.66±1.33 and NT= 9.66±1.33; p ≤0.05).

Conclusions:

Many references have shown that GH-transgenic fishes presented accelerated growth, significant elevation in metabolic rates and oxygen consumption. All these issues have elevated energetic costs to animals. Therefore, the studied individuals might be
lacking energy to supply all metabolic demands, which is reflected over reproductive parameters reduction such as descendant production and larval viability.

Keywords: growth hormone, linhagem F0104, superexpression, transgenic, zebrafish

Financial Support: PPGCF; FAC/FURG, Capes and CNPq

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Resumo:13-126

THYROID STATUS MODULATES JAK/STAT SIGNALLING PATHWAY IN RATS THYROID GLAND.

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Objectives:

Leptin is a hormone secreted mainly by adipose tissue, which reduces food intake and increases energy expenditure maintaining the body energy homeostasis. Beside this, leptin acts as an important neuroendocrine regulator of the hypothalamus-pituitary-thyroid axis. We have previously demonstrated that the thyroid status reduces the content of proteins of the leptin signaling (JAK/STAT) at the hypothalamic-pituitary level. This reduction is probably related to the loss of hypothalamic and pituitary responsiveness to leptin effects on TSH secretion in hypothyroidism. The thyroid gland is also a target of leptin direct action, and together with several cytokines, leptin may directly alter thyroid hormone synthesis and thyroid function via JAK/STAT signaling. Thus, the aim of this study is to evaluate the expression of ObRb and effectors proteins of the leptin pathway STAT3, phosphorylated STAT3 (p) and SOCS3 in the thyroid of hypo- and hyperthyroid animals to detect a possible modulation of the expression of these proteins by the thyroid state of animals.

Methods and Results:

The experiments were performed with hypo- and hyperthyroid Wistar rats with approximately 3 months of age. Hypothyroidism was induced by treatment with methimazole (0.03% in the drinking water/21 days), and hyperthyroidism was induced by subcutaneous daily single injections of thyroxine (T4 - 5μg/100g b.w./5 days). Serum TSH, T3, T4 and leptin were measured by radioimmunoassay at the end of the treatment. The expression of leptin signaling proteins was assessed by Western blotting technique. At the thyroid level, hypothyroid rats showed increased expression of STAT3 (75% increase; p

Conclusions:

Thus, we showed that, at the thyroid level, the thyroid status modulates JAK/STAT pathway in the opposite direction that was previously observed at the hypothalamic-pituitary level. This work shows an increase of the STAT pathway in hypothyroidism and a reduction in hyperthyroidism at the thyroid level. The physiologic relevance of those alterations is still unknown, but they may modify the response of thyrocytes to leptin and several cytokines.

Keywords: thyroid status, JAK/STAT signalling pathway, thyroid gland

Financial Support: CNPq, FAPERJ

QuebraPagina
BAROPODOMETRY IN TYPE 2 DIABETIC AND PRE-DIABETIC PATIENTS WITH AND WITHOUT PERIPHERAL NEUROPATHY

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Objectives:

To compare peak plantar pressure and pressure-time integral among individuals with type 2 diabetes and pre-diabetes, with and without neuropathy, no foot deformities and no history of plantar ulceration, in 5 plantar regions: heel, midfoot, metatarsals heads, toes and hallux. Increase in the value of these variables is correlated with occurrence of plantar ulcers, especially in presence of neuropathy and plantar deformity. Pre-diabetes is a condition that can lead to neuropathy. Previous studies evaluating these plantar pressure variables in pre-diabetes were not found.

Methods and Results:

After Ethics Committee approval (n. 09-445) 85 subjects were evaluated and separated in three groups: 30 type 2 diabetic (DG), 15 pre-diabetic (PDG) and 40 healthy controls (CG). Sensory-motor polyneuropathy was investigated by means of electroneuromyography. Neuropathy was defined by clinical score > 3 on Michigan Neuropathy Screening Inventory (preservation of protective sensation, signs and symptoms). Autonomic neuropathy was investigated by Heart Rate Variability Test. Baropodometry was performed with pressure platform (Emed X-Novel) using first-step protocol, subjects were barefoot walking at their self selected speed. Ten valid attempts were recorded of each foot contact with the platform. Data analysis of plantar pressure was performed by the average of each plantar region to both feet. ANOVA one-way was applied to compare the three groups, followed by post hoc Dunnet (significance level α = 0.05). Both groups were similar for the parameters of age, gender and plantar arch height. Neuropathy was found in 60% of DG and 46% of PDG. DG and PDG showed significant higher peak pressure in the midfoot region (p=0.001) and metatarsal heads areas (p=0.004) compared to CG. Pressure-time integral was significant higher in DG and PDG compared to the CG in midfoot (p=0.001) and metatarsals heads areas (p=0.001). No significant differences between groups in other plantar areas were found. The presence of neuropathy in DG and PDG coincided with increased values of plantar pressure variables evaluated.

Conclusions:

Values of plantar pressure corroborate previous studies that have been found increases in anterior plantar region in diabetic patients. The same pattern of plantar pressure distribution was observed in PDG which points to the need of preventive care as well as the possibility of plantar disorders in this population.

Keywords: Baropodometry, Diabetic, Peripheral neuropathy

Financial Support: CAPES

ABSENCE OF EIF2 ALPHA PHOSPHORYLATION COULD RESULT IN INSULIN RESISTANCE IN L6 MYOTUBES TREATED WITH PALMITATE

Silva, P. E. ; Poletto, A. C. ; Anhê, G. F. ; Machado, U. F.
Departamento de Fisiologia e Biofisica, ICB/USP

Objectives:

To compare peak plantar pressure and pressure-time integral among individuals with type 2 diabetes and pre-diabetes, with and without neuropathy, no foot deformities and no history of plantar ulceration, in 5 plantar regions: heel, midfoot, metatarsals heads, toes and hallux. Increase in the value of these variables is correlated with occurrence of plantar ulcers, especially in presence of neuropathy and plantar deformity. Pre-diabetes is a condition that can lead to neuropathy. Previous studies evaluating these plantar pressure variables in pre-diabetes were not found.

Methods and Results:

After Ethics Committee approval (n. 09-445) 85 subjects were evaluated and separated in three groups: 30 type 2 diabetic (DG), 15 pre-diabetic (PDG) and 40 healthy controls (CG). Sensory-motor polyneuropathy was investigated by means of electroneuromyography. Neuropathy was defined by clinical score > 3 on Michigan Neuropathy Screening Inventory (-preservation of protective sensation, signs and symptoms). Autonomic neuropathy was investigated by Heart Rate Variability Test. Baropodometry was performed with pressure platform (Emed X-Novel) using first-step protocol, subjects were barefoot walking at their self selected speed. Ten valid attempts were recorded of each foot contact with the platform. Data analysis of plantar pressure was performed by the average of each plantar region to both feet. ANOVA one-way was applied to compare the three groups, followed by post hoc Dunnet (significance level α = 0.05). Both groups were similar for the parameters of age, gender and plantar arch height. Neuropathy was found in 60% of DG and 46% of PDG. DG and PDG showed significant higher peak pressure in the midfoot region (p=0.001) and metatarsal heads areas (p=0.004) compared to CG. Pressure-time integral was significant higher in DG and PDG compared to the CG in midfoot (p=0.001) and metatarsals heads areas (p=0.001). No significant differences between groups in other plantar areas were found. The presence of neuropathy in DG and PDG coincided with increased values of plantar pressure variables evaluated.

Conclusions:

Values of plantar pressure corroborate previous studies that have been found increases in anterior plantar region in diabetic patients. The same pattern of plantar pressure distribution was observed in PDG which points to the need of preventive care as well as the possibility of plantar disorders in this population.

Keywords: Baropodometry, Diabetic, Peripheral neuropathy

Financial Support: CAPES

ABSENCE OF EIF2 ALPHA PHOSPHORYLATION COULD RESULT IN INSULIN RESISTANCE IN L6 MYOTUBES TREATED WITH PALMITATE

Silva, P. E. ; Poletto, A. C. ; Anhê, G. F. ; Machado, U. F.
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Objectives:

Obesity and insulin resistance are tightly associated with the onset of type 2 diabetes mellitus (T2DM), possibly due to increased circulating levels of glucose and free fatty acids (FFAs). Recent investigations suggested that endoplasmic reticulum (ER) stress plays an important role in the pathogenesis of T2DM and insulin resistance (IR). The most immediate response to ER stress is the translational attenuation signaled through phosphorylation (Ser51) of the alpha subunit of eukaryotic initiation factor 2 (eIF2alpha) by the PKR-like ER kinase (PERK). However, whether this translation attenuation is connected with SFA-induced reduction in GLUT4 is not established. Therefore, this work aimed to verify the effects of palmitate on GLUT4 and endoplasmic reticulum (ER) stress proteins in L6 muscle cells.

Methods and Results:

GLUT4 expression and eIF2alpha phosphorylation were analyzed in L6 myotubes treated with 0.75mM of palmitate (DMEM+BSA1%) during different time intervals. GLUT4 and Phospho-eIF2alpha proteins expression were detected by Western Blotting-ECL. Data analysis was performed using ANOVA followed by Bonferroni post-test. The results showed a significant reduction of the GLUT4 protein content since the second hour of treatment with the fatty acid (control: 99.99±4.3, 2 hours: 66.37±7.2*, 4 hours: 65.03±8.0*, 6 hours: 67.16±8.8*, 12 hours: 58.88±5.5*, results expressed in arbitrary units, n=4 to 8, *P<0.05).

Conclusions:

These data showed that palmitate impaired GLUT4 expression what is preceded by a decrease in phosphorylation of eIF2alpha. The conditional loss of eIF2a phosphorylation is sufficient to disrupt ER function and cause oxidative damage. This data reinforces the proposition that SFA-induced ER stress is associated with insulin resistance probably by reducing GLUT4 content through mechanisms not fully identified.

Keywords: eIF2 alpha, endoplasmic reticulum stress, insulin resistance, GLUT4

Financial Support: Fapesp 2010/09984-5

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Resumo:13-129

EFFECTS OF ESTROGEN REPLACEMENT THERAPY AND EXERCISE IN SARCOPENIA

Universidade Federal do Pará, UFPa

Objectives:

Sarcopenia is the degenerative loss of skeletal muscle mass associated with aging and hormonal disturbance. Several reports have shown that decrease in the estrogen plasmatic levels induces severe sarcopenia process. Added to this, studies also reveal that low muscular activity can promote sarcopenia. Thus, the aim of the present work is verify if the sarcopenia inducted by castration could be reverted by hormonal replacement therapy and moderate physical activity.

Methods and Results:

Adult female Wistar rats, weighting 200-220 g, were housed in individual cages in a temperature-controlled environment (23 ± 2 C°) with a 12:12-h light/dark cycle with free access to rat chow and tap water. Three experimental protocols were realized: One,
bilateral ovariectomy, this was performed by abdominal-medial incision to access to ovaries in peritoneal cavity. Two, estrogen replacement through the estradiol pellet (cipionato estradiol 0.05 mg) dorsal subcutaneous implant. Three; Moderate exercise, rats were placed in motorized treadmill speed 10 meters/min during 15 minutes, three times a week on alternate days for eight weeks. According to each experimental protocols, the animals were divided into 5 groups: F (Sham), C (only castration), Ex (castration + exercise), E2 (castration + estrogen), Ex+E2 (castration+ exercise + estrogen). Were evaluated plasmatic estradiol levels, urinary creatinine, total muscle protein. These were realized in three different moments (0, 90 and 150 days), zero day begin when bilateral ovariectomy was realized. Our results showed the bilateral ovariectomy decrease plasmatic estradiol levels about 90% (5.72 ± 3.36 pg/dl after 90 days and 1.9 ± 0.2 pg/dl 150 days when compared with the control group 22.3 ± 3.66 pg/dl) and the estradiol pellet implant was able to revert these levels (19.14 ± 1.4 pg/dl after 150 days E2 group), confirming the efficacy of surgical removal ovaries and estradiol replacement. The castrated rats (group: C) the urinary creatinine was decrease about 25% (0.65 ± 0.07 mg/dl) and 30% (0.61 ± 0.04 mg/dl) after 90 and 150 days respectively when compared with the control (0.89 ± 0.08 mg/dl) additionally hormonal replacement was able in avoid these effects promoted increase 105% in estradiol associated with exercise after 150 days (0.92 ± 0.02 mg/dl). The total muscle protein was decrease in C group (castrated rats) about 50% (4.792 ± 1.6 mg/dl/g) after 150 days when compared with the control (8.4 ± 1.2 mg/dl/g) and that decrease was reverted by hormonal replacement (group: Ex+E2, 7.52 ± 1.3 mg/dl/g). These results suggesting estradiol and moderate physical activity have a role in protein metabolism and skeletal muscle mass

Conclusions:
The association between moderate physical activity and estradiol replacement could be the most effective intervention to prevent or reverse sarcopenia

Keywords: sarcopenia, estrogen, exercise

Financial Support: EGPA, UFPA, CNPq, UEPA

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Resumo:13-130

EFFECT OF MATERNAL OBESITY ON THE ADRENAL MEDULLARY FUNCTION AND CATECHOLAMINE SENSITIVITY OF THE OFFSPRING AT WEANING

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1 Instituto de Biofísica Carlos Chagas Filho, UFRJ
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Objectives:
Obesity is characterized by high body fat content and consequent hyperleptinemia. We have demonstrated that hyperleptinemia during the first ten days of lactation results in higher adrenal catecholamine content in the end of leptin treatment and at adulthood. These changes had relevant impact on cardiovascular parameters that may contribute to the development of cardiovascular diseases associated to obesity. In the present study, we evaluated the effect of maternal high fat diet during gestation and lactation on the leptinemia and adrenal medullary function and catecholamine sensitivity in rats at weaning.

Methods and Results:
Wistar female rats were fed with normal (9% fat; C group) or high fat diet (29% fat; HF group) for 8 weeks before mating, and during pregnancy and lactation. Body composition was evaluated before mating by dual emission X-ray absorptiometry (DEXA) to confirm the accumulation of body fat. Pups were killed at 21 days old (weaning). Glycemia was measured with a glucosimeter and leptinaemia by radioimmunassay. Left adrenal glands were collected for catecholamine content quantification by trihydroxyindole fluorescence method. Right adrenals were used for analysis of the tyrosine hydroxylase (TH) content by western
blotting. Catecholamine sensitivity was analyzed through the expression of beta adrenoreceptor in liver and adipose tissue. We have also measured the liver glycogen content and adipose fat pads of the pups. HF group presented higher total fat body content after 8 weeks (+27%, p

Conclusions:
Maternal high fat diet induced early development of obesity in male pups, confirmed by the higher fat depots of the visceral and subcutaneous compartments. Since adipocytes are the major site of leptin production, HF offspring displayed hyperleptinemia. Leptin may stimulate adrenal medullary function and HF offspring showed higher catecholamine content. Besides store energy as fat, HF offspring present larger reserve of glycogen and hyperglycemia that may be a consequence of increased gluconeogenesis. We suggested the lower content of beta2-adrenoreceptor may contribute to the accumulation of glycogen in liver given that adrenaline induces glycogenolysis. These early changes may contribute to development of metabolic syndrome at adulthood.

Keywords: Maternal Obesity, Leptin, Catecholamine, Programming

Financial Support: CAPES, FAPERJ, CNPQ

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Resumo:13-131

BIOCHEMICAL PARAMETERS IN DIABETIC TYPE I RATS TRAINED AND UNTRAINED WITH RESISTIVE EXERCISE ASSOCIATED WITH LOW-LEVEL THERAPEUTIC LASER (LLTL)

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Objectives:
Diabetes mellitus comprises disorders of the metabolism of carbohydrates, proteins and lipids, caused by absolute or relative deficiency of insulin. Thus, the objective was to determine muscle glycogen concentrations (soleus and tibialis anterior) and liver, blood lactate and glucose after resistance exercise in rats associated with low intensity laser therapy (LTBI) to evaluate the biochemical changes in front of the training diabetic animals.

Methods and Results:
We used 40 male Wistar rats, initially weighting 300 to 350g, 30 animals underwent an experimental model of insulin dependent diabetes mellitus (streptozotocin, Sigma ® S-0130, 65mg/kg), these animals were divided into three groups: laser (GL), the trained group (TG) and group training and laser (GTL) and 10 animals without diabetes formed the control group. Treatment protocols with LTBI (model DMC Portable Laser class 3B Ga-Al-As diode with a wavelength of 830 nm, continuous emission, output power of 100 mW, power density of 3.57 W/cm2, area beam 0, 028cm2, divergence of 1.5 °, the fluence of 120J/cm2 and irradiation time of 34s) and the training program, which consisted of an exercise program with resistance in climbing apparatus with weights safe for the tail, was performed three times per week for 6 weeks. One week after inoculation and at the day of euthanasia, blood glucose and lactate levels were determined by the method of tape, by puncturing the tail (Accu-Chek Performa ®). Was determined by spectrophotometry muscle glycogen (antronaE) and was also made analysis for body mass. Statistically significant differences (p>0.05)were detected when the results GL, GT and GLT were compared with GC, such as higher body mass late in GT and GLT in relation to the GL (GC = 340,8± 25,2g, GL = 269,1 ±18,0g, GTL = 267,9± 32,0g, GT = 269,5 ±22,4g) but lower body mass in both diabetic groups compared to CG, an increase of lactate in the GT and GLT stronger than in GL and GC (GC = 2,2 ±0,7mmol/L, GL= 4,9 ± 1,1mmol/L, GTL= 4,2 ± 0,6mmol/L , GT = 2,5 ±1,4mmol/L), high dosage of soleus muscle glycogen in GL, GLT and GT compared to CG (GC = 4,6± 0,8mg/mg; GL= 4,2 ± 0,7mg/mg; GTL = 6,4± 2mg/mg; GT = 6,7 ± 2,3mg/mg) and lower glycogen stores in the liver of the diabetic group (CG = 6,2± 0,6mg/mg; GL= 4,6 ±1,5mg/mg, GTL = 3,2± 0,7mg/mg, GT = 3,4 ±1,3mg/mg).
Conclusions:

In conclusion with these results: an increase of more intense muscle glycogen of rats trained and its less intense depletion after exercise, showed the effect of training on glycogen save this site, the highest lactate at the year end the group trained indicates greater exercise tolerance, and confirms the anaerobic character of the experimental model and increased body mass in the trained group suggests the effect of training in increased muscle mass. Therefore, resistance training was associated with LTBI effective in promoting greater resistance to load of climbing, in both training entente were able to keep the glycogen content in soleus muscle, no differences in lactate levels, and both groups were hyperglycemic after training.

Keywords: BLOOD GLUCOSE, DIABETES TYPE 1, GLYCOGEN, LACTATE

Resumo:13-132

ROLE OF PURINERGIC RECEPTORS ON CARDIAC FUNCTION OF STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Objectives:

Recent studies showed the importance of purinergic neurotransmission in the control of cardiac function. In isolated atria, purines (ATP and UTP) have a biphasic effect that is mediated by purinergic receptors - type P1 (negative inotropic effect) and P2 receptor (positive inotropic effect). Alterations in the purinergic neurotransmission are related with the development of several pathological conditions, such as hypertension. Cardiovascular changes are responsible for 50% of deaths among diabetics. Therefore, the aim of this study was to evaluate the purinergic neurotransmission in atria isolated from Streptozotocin-induced diabetic rats.

Methods and Results:

We used Wistar rats and the diabetes was induced by streptozotocin at dose of 60 mg.Kg-1. We considered diabetic those rats with glycemia above 160 mg.dL-1. All experimental procedures were approved by Ethical Committee of Faculdade Integrado de Campo Mourão (protocol number: 58970/2010). The animals were divided into three groups: control group (CG), diabetic group with 30 days (D30) and 60 days (D60) after Streptozotocin-induced diabetes. The left (LA) and right (RA) atria were isolated and mounted in isolated organ bath. The LA were electrically stimulated (2 Hz; 5 ms, 20-40 V). On the parameters of strength and heart rate, we tested ATP (P1 and P2 receptor agonist) and UDP (preferentially P2y receptor agonist), both used in a concentration of 10-4M. Cumulative concentration-response curve was also performed for Adenosine (P1 agonist). ATP produced a negative inotropic effect (NIE) with 1 min of duration followed by a positive inotropic effect (PIE) which reached the plateau at about 7 min. The NIE (mediated by P1 receptor) in LA decreased 25% in D30. However, no changes were observed in RA. The next step was the study of the Adenosine effect. We performed cumulative concentration-response curves for this agonist and we found that pD2 decreased in D30 (3.4 ± 0.08; n=5), while the pD2 of D60 increased (4.71 ± 0.09; n=5) when compared to CG (3.70 ±0.08; n=5). Analysing the P2 receptor, we found that D60 showed an increased response to UDP in LA (92%) and RA (17%) indicating a change in function or density of P2 receptor.

Conclusions:

Our data demonstrated changes in the contractile response induced by ATP, UDP and ADO purinergic agonist on
neurotransmission in hearts of diabetic animals. In addition, these changes were different depending on the duration of diabetes, which may be related with the development of secondary pathological conditions, such as hypertension.

Keywords: isolated atria, purinergic neurotransmission, purinergic receptors, Streptozotocin-induced diabetic rats

Financial Support: CAPES, CNPQ, FAPESP

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**Resumo**

**NANOENCAPSULATION OF LOVASTATIN FOR THE HYPERCHOLESTEROLEMIA TREATMENT IN WISTAR RAT**

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**Objectives:**

The Hypercholesterolemia is a risk factor for development of coronary artery disease (CAD). Thus, for this pathological condition is necessary a pharmacological intervention. The statins are the most used drugs for the treatment of Hypercholesterolemia. This drug type decreases the synthesis of cholesterol by inhibition of HMG-CoA reductase. However, statins cause many adverse reactions, been necessary the utilization of specific pharmaceutical formulation (PF), such as liposomes. The liposomes are PF of modified liberation that encapsulate drugs and increases the absorption as well as the drug biodisponibility because are formed by phospholipids. Therefore, the aim of this study was to evaluate the action of nanoencapsulation of Lovastatin for the Hypercholesterolemia treatment.

**Methods and Results:**

The nano coatings (nanoliposomes) were prepared to according of reverse phase evaporation (RPE), technique describe by Szoka and Papahadjopoulos (1978). We used male Wistar rats obtained from biotery of Faculdade Integrado de Campo Mourão. The animals were divided into 4 groups (n=5): control group (CG) that received Novilab feed, hypercholesterolemic group (HG) that received hypercaloric diet standardized by Estadella (2004), hypercholesterolemic group treated with Lovastatin in the PF usual (HGU) and hypercholesterolemic group treated with nanoencapsulate Lovastatin (HGN). At the end of the experiment the animals were sacrificed and then biochemical analysis were made for total cholesterol, LDL-C, HDL-C, AST and ALT using commercial kit. All experimental procedures were approved by Ethical Committee of Faculdade Integrado de Campo Mourão (protocol number: 58184/2010).After 30 days of hypercholesterolemia induction, we found an increase in plasma cholesterol for the HG (19.9%) when compared with CG. The biochemical tests showed a decreased in plasma cholesterol for the HGU and HGN when compared with HG (P

**Conclusions:**

Therefore, the treatment with nanoencapsulation of Lovastatin showed pharmacological efficacy in the decrease of plasma cholesterol and increase of HDL-C. These effects are considered protective for coronary artery disease.

Keywords: hypercholesterolemia, liposome, lovastatin, nanoencapsulation
THYROID DUAL OXIDASE 2 PROMOTER ACTIVITY REGULATED BY TTF-1, PAX 8, NKX 2.5 AND THE CO-ACTIVATOR TAZ.

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2 IBCCF, UFRJ

Objectives:
In the thyroid, the source of H2O2 essential to thyroid iodide organification, involves the oxidation of NADPH by a NAD (P)H-dependent flavoprotein, named Duox2. Duox 2 expression is first detected by E15.5, concomitant with iodide organification. Transcription factors have been shown to be expressed in the early steps of thyroid morphogenesis, including TTF-1, PAX8 and NKX2.5. Both TTF-1 and PAX8 is essential for proper development of the gland while NKX2.5 is expressed in cardiac and thyroid progenitor cells, but it is no longer present when Duox 2 expression levels peak. Mutations in NKX2.5 are related to thyroid dysgenesis and cardiac defects, and It has been shown to positively modulate Tg and TPO promoters. Recently, it has been shown that TTF-1, Pax8 and the co activator TAZ are co-expressed in embryonic E14.5 and adult thyroid cells, regulating thyroglobulin expression. To study the regulation of Duox2 promoter activity, we have investigated the role of TTF-1, PAX 8, NKX2.5 and the general co-activator TAZ on Duox 2 promoter activity. Here we analyzed the role and relationships of these transcriptional factors and their ability to regulate Duox2 promoter in transient transcriptional assays and showed that those factors can be reactivated in tumor cells lines.

Methods and Results:
2x105 HEK 293 cells, used as heterologous system were cultured in DMEM, containing 5% penicilin and 20% bovine fetal serum. To study thyroid Duox 2 promoter activity regulation by transcriptional factors. The 0.6 kbp fragment of the human Duox 2 was co-transfected with plasmids encoding wild-type TTF-1, PAX 8, Nkx2.5 and TAZ, using lipofectamine (Invitrogen). After 24 h, cells were collected for Luciferase and Renilla activity measurement and was analyzed using the Dual - Luciferase reporter assay system (Promega). Results are shown relative to controls activity in each experiment. The data are shown as mean ± S.D. of at least three independent experiments in triplicate. Western blots were carried using 40-100 mg of total protein using antibodies and developed by ECL. Total RNA was extracted using TRIZOL (Invitrogen).cDNA synthesis was performed using M-MLV RT (Promega) and random primers and PCR was done using GAPDH and NKX 2.5 primers.

Conclusions:
We have found a synergic stimulation of DUOX 2 promoter activity in the presence of both TTF-1 and PAX 8, up to six time of the control level. NKX 2.5 alone increased DUOX 2 promoter activity as TTF-1 and Pax. -8 did, although the co-expression of NKX 2.5 with TTF-1 or PAX 8 were able to increase 9 times Duox 2 promoter activity. TAZ increased DUOX 2 promoter activity induced by NKX 2.5. Northern Blot and RT-PCR experiments showed that tumor thyroid cells can express NKX 2.5. So, as NKX 2.5 induced DUOX 2 promoter activity induced by TTF-1 and PAX 8, we suggest that the re-expression of this factor is able to stimulate Duox 2 expression. Sources of research support: CAPES, FAPERJ.

Keywords: Duox 2, NKX 2.5, PAX 8, TTF-1, TAZ

Financial Support: CAPES, FAPERJ

Alternative Model for Teaching Vascular System in Veterinary Anatomy

QuebraPagina

ALTERNATIVE MODEL FOR TEACHING VASCULAR SYSTEM IN VETERINARY ANATOMY
Objectives:

In studies of Veterinary Anatomy, there is enough material related to the vascular system of the regions and components of the locomotor system, as well as the internal organs of the thoracic and abdominal cavities of domestic mammals. The knowledge of the circulatory system is of great importance for professionals working in the field of clinical medical scrubs, as for performing surgeries in veterinary medicine, practitioners must possess a good knowledge of anatomy of the vascularity of the region to be imaged. With the growing number and complexity of these operations, that knowledge becomes even more important. Furthermore, alternative materials that can be used to complement and assist education professionals to recognize the region to be imaged without the need to use animals for this formaldehyde, has been a search in Veterinary Anatomy given the precepts of animal welfare.

Methods and Results:

This study aims to develop a three dimensional model of the equine pelvic limb with the system of blood vessels, so as to obtain a model closer to reality, allowing students a deeper knowledge of this subject, facilitating their understanding and assimilation. This model also serves as an alternative resource use parts in formalin, since they have adverse health effects. To make the model, we used real bones of a horse-size, previously prepared by soaking in water, and subsequent bleaching with hydrogen peroxide (H2O2). The pelvic limb was mounted and fixed according to the anatomic position of the live animal in season. Blood vessels are represented by copper wires used in electrical installations, cloaked in red, symbolizing the main trunks of the arteries: femoral, popliteal, cranial tibial, caudal tibial, lateral plantar, medial plantar, dorsal metatarsal III, and lateral plantar digital (dorsal and plantar). After fixation in bone, these wires (blood vessels) were correctly numbered to permit identification of each artery, with their names, according to the bibliographic description of text-books of classics and mandatory consultation. The acceptance by the students was very satisfactory, since the 38 students interviewed, 97.37% (37 students) have adopted this model as a resource, facilitator of learning, being effective in teaching-learning process.

Conclusions:

The option to be able to visualize structures in three dimensions, unlike the two-dimensional images in literature, generates greater interest among students for the study of Veterinary Anatomy. Today we have pieces fixed in formalin in most of the Anatomical country in which we can observe aspects such as size and thickness of vessel walls. However, in this work the focus is to offer guidance and direction of the vessels in the pelvic limb of greatly facilitating their understanding. Moreover, this model can be used in the classroom in support of the lecture, not requiring specific laboratories.

Keywords: Anatomy, Morphology, Alternative model
Objectives:

The integration of scientific knowledge presents challenges to students grappling with the difficulties encountered in learning pharmacology. This study outlines a collaborative project developed to assist undergraduate students to learn and understand pharmacology. The innovative approach taken included the development of a CD-ROM entitled “Farmaco Quiz” incorporating clinical questions about autonomic nervous system, with an accompanying teaching guide.

Methods and Results:

This is an observational study of 78 students enrolled in third or second year of graduation of University of Fortaleza, who was asked to respond anonymously to a questionnaire of satisfaction comprising 11 questions. Seventy eight students were interviewed, 98.7% regarded the program as an interesting tool and 93.59% felt egged on the subject by answering the questions as a game. 93.5%, showed that the material has stimulated the reasoning and 87.1% thought the game effectively to improve the content studied. 70.5% of students reported positive learning and 97.4% agreed that the CD-ROM is an effective method. 74.3% used the CD-ROM before the assessment; 96.1% reported that CD-ROM offers a highly interactive, flexible and student-centred resource, which has received positive feedback on initial use. The clinical cases were considered that the CD-ROM could be useful in the preparation of the university examination, competition in career.

Conclusions:

It seems that teaching traditional academic lectures of pharmacology is not the preferred method of teaching students. They prefer the education provided by the new methodologies, in the form of clinical cases, objective questions, even if the courses offered by the faculty try to adapt in this form. The book remains the primary medium of work, but many students believe that a computer could replace or supplement the traditional media. The Farmaco Quiz, seems helpful in learning the specialty including in the context of the examination class, and effective learning and teaching. Our innovative CD-ROM resulted in significant improvements in behaviours associated with effective pharmacological teaching.

Keywords: Education, Pharmacology, Recreational activities

Financial Support: PROMON AND PROMOV/ UNIFOR
acquisition and development of the oral language, including to hearing memory and the hearing sequential memory, turning in the hearing training: (1) hearing conscience - reaction to the resonant stimuli; (2) hearing attention - concentration of the activity in the hearing stimuli; (3) hearing location - location of the resonant fountain; (4) hearing identification - recognition of the sounds, meaning them; (5) hearing discrimination - differentiation of sounds; (6) evocation - to recall quickly the kept vocabulary; (7) hearing analysis/synthesis - identification of the characteristics of the sounds inside an all, bringing together them. The aim of this workshop had been with priority students from K-12 and high school (private and public). We offer information regarding anatomical auditory structure, auditory function, sound memory and good practices for auditory health.

Methods and Results:

This study purposes a phenomenological approach to identify, in qualitative basis, how visual and sound stimuli help students to perceive the auditory memory phenomena. It was collected information and perceptions through inductive methods as discussions and participant observation. The workshop was developed for the II Brain Awareness Week in Rio de Janeiro (II Semana do Cérebro, 15-19/03/2011), an event that looks for the brain sciences popularization and the recognition of the neurosciences as a basic tool for education. This workshop explored the sound memory of twenty instruments of orchestra enclosing all classes of the orchestra using earphones and a notebook. We elaborated twenty cards with the drawing of each musical instrument utilized for sound memory, promoting the association of visual stimuli with the auditory stimulation. Amplified anatomical models representing auditory system and brain, as well as an expositive panel were used to explain sound detection and perception processes for K-12 and high school students. The practice begins with a brief explanation about the auditory system function and structure. The next step was presentation of the drawings (cards) and of the sounds, following by the questions about sound recognition. The sequence could be repeated.

Conclusions:

The students speech reveals that cards significantly contributed as subsumers for the cultural complementation and correct identification of the musical instruments sounds. Some participants associated the instruments timbre to format, material and/or size of the instruments figures presented in the cards. Students perceived how the sound stimuli contribute to the auditory memory rescue. They were encouraged to explore how auditory memory could be stimulated by other sensory data. The awareness about the relation among sensory stimuli and auditory memory was report for many students. We conclude that the practice-based activity of this nature contributes significantly to stimulate the interest of the participants to explore the scientific basis of formal educational contents in K-12 and high school.

Keywords: AUDITORY MEMORY, HEARING, SENSORY, LEARNING, EDUCATION

Financial Support: Organização Ciências e Cognição (OCC) e FAPERJ.
Methods and Results:

In the first activity (Adv. Physiol. Educ. 34:156, 2010), the aim is to make students intuitively experience the probabilistic nature and nonorientated motion of diffusing particles. Although students easily grasp the notion that random processes are capable of giving rise to emergent behavior, such as the net directional movement of molecules, they frequently confound a macroscopic ballistic view of movement with a microscopic diffusive one. It appears to arise from a deep-seated misconception about random processes. The correct understanding of this kind of phenomena allows, for instance, students to realize why diffusion works so well over short distances and becomes increasingly and rapidly less effective as the distances involved become progressively larger. In the second activity (Adv. Physiol. Educ. 35:97, 2011), we describe a quite simple way for students to figure out the physical forces involved in Laplace’s law (tension is proportional to the product of radius x pressure). It can provide valuable insights into the function of many of the body’s systems, and has a broad implication in physiology and medicine. However, it often leads to counterintuitive predictions about the behavior of hollow structures that work under pressure. Students’ difficulty in understanding the law of Laplace frequently lies in the arid way it is presented: hastily in respiratory physiology courses, in which the only emphasis is given on the role of surfactant in alveolar collapse. It has been shown that students also have serious misconceptions about this kind of behavior. The novelty proposed here is to make students somatically “feel the law,” by means of touch and proprioception. These active-learning strategies create circumstances that push students to develop skills necessary for physiology reasoning. Furthermore, they promote cooperative learning, increasing of motivation and enthusiasm. We have been performing these activities in our introductory physiology courses with great results and extremely positive feedback from the students.

Conclusions:

We believe that these activities are in agreement with the urge for the adoption of teaching approaches that involve the student more actively in the learning process, focus on problem solving, and lead to more meaningful learning.

Keywords: hands-on activities , diffusion , law of Laplace

Financial Support: FAPESP

QuebraPagina

Resumo:14-012

WORKSHOP "WHAT IS DONE OUR BRAINS: A MICROSCOPIC VISION"

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Objectives:

The teaching of science practiced in Brazil is purely theoretical without links with the practical reality, assuming a passive attitude of the students. Running workshops on practical activities provides scientific literacy through the interface between academia and the community promoting the popularization of science education. In the study of biological sciences, the use of the microscope has been particularly important since it allows observations that are outside the scope of direct visibility of the human eye. The workshop "What is done our brains: a microscopic vision" was to awaken the scientific curiosity, popularize basic knowledge in neuroscience and demystify the scientific knowledge by observation of anatomical structures of the nervous system through microscopy.

Methods and Results:
This workshop consisted of demonstration of histological slides containing slices of brain and retina of rodents stained with hematoxylin-eosin and cresyl violet and analysed in six conventional binocular microscopes. We also used a banner to illustrate the anatomical location of the brain and a three-dimensional model of the brain. The workshop was presented in two editions of the event Semana do Cérebro: "Uma NeuroAventura Sensorial" and "Desvendando a memória" and had the participation of I coordinator, 1 organizer and 10 monitors. The audience consisted of students and teachers of basic education, children from 3 years-old and public in general. When the audience was children and young people in school age we need to explain briefly what is the brain, its location and functions, what was achieved with the assistance of the banner and detachable three-dimensional model. Although the activity of microscopy does not have the same appeal playfulness that the other, the presence of the microscope was able to motivate the public to interact with the monitors and observe the specimen. The first edition of the event had the participation of a group of students with hearing disabilities and others with mental disabilities, forcing us to rethink how to approach the subject without the use of spoken language. Thus, for the second edition were printed boards containing the image exposed in the microscope, which greatly facilitated the correct visualization of structures and cells, making the practice of microscopy as a tool of inclusive education. Learning was not confined to the visitors because many monitors were young undergraduates and had little practice in the handling of microscope and the interaction with monitors graduate students encourage them to continuous their education. One of the biggest challenges for monitors was to adapt the specific language obtained in undergraduate and graduate to a diverse audience, constituting an example of indeseparability between education, research and extension.

Conclusions:

Overall, the workshop enabled the contact with the academic and scientific universes, stimulating the study of science. Working with the disabled group revealed that nobody should be excluded or prevented from obtaining knowledge and that we must seek new alternatives to the teaching-learning process.

Keywords: Inclusive education, Neuroscience, Science popularization, Teaching-learning

Financial Support: FAPERJ, PROEX-UFF, OCC

Objectives:

Memory games are known by the majority of the population and have simple rules, which enable people of different age groups to play it. However, as it has a lot of visual information, memory games are directed to non visual-disabled people, harming the educational premise of social inclusion. Thus, this work aims at presenting the development and application of a type of memory game designed for both blind and non-disabled people. This activity was developed during an event of scientific publicizing called “II Semana do Cérebro: desvendando a memória” (15-19/03/2011). Some aspects of the operational, short-term and long-term memories were part of the activity as a way of contextualizing it with the theme of the event, besides being an important tool in the teaching-learning process as it exploits the playful element of the task.
Methods and Results:

It was built a memory game with 32 cards of 7x9cm with pictures of different animals representing the classes of chordates (vertebrates). These cards were printed for non-disabled people and people with low vision, and also were written in Braille to be interpreted by the visually impaired people. Over these cards others 32 cards of 8x10cm numbered from 1 to 32 identified with Braille writing were put. All cards were made by paper magnetized. Another part of the game was a metal tray 45x75cm with 32 partitions, made of magnet to form the recessed spaces where the cards would be allocated, and a panel explaining the rules of the memory game and the memory types worked, with pictures of parts of the brain that were involved in these processes. Before starting the activity, participants were divided into two groups and chose two number cards to be withdrawn in accordance with the corresponding numbers, so that the cards below, with animal pictures, appeared. If the cards were equal, the participants withdrew them from the board and continued the game, when it did not happen, they had to put them back on the charts of the animals, and it was the turn of the other group. The winner was who had the largest number of cards and in the end were explained the types of memories involved during the game. Among the results was noted that the game has fulfilled its role as inclusive as playful element that enabled content explanation concerning the operating, short-term and long-term memories and the proposed curricular theme (chordates). Since the cards were also written in Braille we were able to observe the role of the game in the diffusion of this language code.

Conclusions:

The game was a great tool to focus on the concept of memory with people of different age groups, allowing an active participation of the players. The theme of the game, chordates, attracted them and showed that it may be an interesting topic for Science and Biology classes. Although visual disabled people did not take part in the activity, the use of Braille language proved to be an important instrument for the spread of the idea of social inclusion among non-disabled people.

Keywords: Educational Inclusion , Memory , Memory Game , Neuroscience , Visual Impairment

Financial Support: FAPERJ, Organização Ciências e Cognição e Espaço Ciência Viva

Resumo:14-014

SCIENCE ON WHEELS: THE UNIVERSITY GOES TO SCHOOL


Anatomia/ UFRJ/Instituto de Ciências Biomédicas, UFRJ

Objectives:

“Science on Wheels” (Ciência Sobre Rodas) aims at promoting science in schools, sensitizing students and teachers to apply the scientific method, and creating tools for science education in Rio de Janeiro schools. Through an itinerant laboratory-car, the group has made regular visits to public schools of 4a Coordenadoria Regional de Educação/RJ (4ª CRE), conveying updated knowledge and new educational resources to middle-level school teachers. In addition, using playful and interactive science promotion activities, this project has obtained strong contact with teachers and students directly within the school environment.

Methods and Results:

Activities started in 2008, offering two workshops – “Brain and Perception” and “Locomotion System” – for 80 middle school students (8th grade) of the Francisco José de Oliveira Vianna School. The “Brain and Perception” workshop starts with an activity with cloth caps and colored pens. At fist, students are encouraged to draw the human brain as they think it looks like, at one side of the cap. Then, a slideshow presentation about the brain and their functions is shown. After a detailed explanation, our group shows a model of a real human brain to students. Finally, the students are encouraged to draw the human brain at the other
side of the cloth cap, now knowing better how it is. The “Locomotion System” workshop has also playful and interactive activities and uses artificial human skeletons and visceras models showing the organs anatomy. At the first moment, the class is divided into two groups and each group is asked to mount the skeleton parts spread on the table. Then, the monitor of each group explains the correct positions and name of the bones and organs. At the end of the workshop, the students answer a search questionnaire, suggesting new themes for workshops. This project so far has been conducted in more than 50 schools, reaching about 1500 students. In addition to weekly visits at schools, the project has recently made an closer association with Tenente Antônio João School located within UFRJ campus. The aim of this association is to turn this school into a model in science education. In this case, weekly activities are performed with 8th grade students in accordance to the study plan proposed by the teacher. The project contributes with plastinates organs, artificial human skeleton, magnifying glasses, microscopes and other teaching materials offered by the institute. Today, the group includes 12 undergraduate students and 4 associate professors from the institute. In 2011, we also received 6 graduate students who complement the contents of the workshops.

Conclusions:

We hope to expand this project to a greater number of schools, always with news activities, taking updated knowledge and new educational resources to the public schools of Rio de Janeiro.

Keywords: rodas, universidade, escola

Financial Support: FAPERJ, CNPq, PIBEX/ PIBIC – UFRJ

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Resumo:14-015

“ENCruzilhadas. O JOGO DA SUA VIDA”: CREATION AND IMPACT-EVALUATION OF THE ROLE-PLAYING GAME BOOK AS A TOOL IN CANCER PREVENTION

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Objectives:

Create an alternative way to bring information about risk factors and cancer prevention to young people, using a vehicle that would not cause disaffection and could also demystify the development of the disease, trying to soften the feeling of “this is not going to happen to me” that is common among young people. This project aims to show adolescents the importance of cancer prevention, so simple measures to prevent the disease could be taken by them, leading to fewer cases of cancer in the future.

Methods and Results:

We opted for the development of a role-playing game book (RPG book), in which the player creates a character which will be submitted to several situations in what we call “the best year of their lives”, between the vestibular (ending school) and the first year of college. During this game, the player chooses the path that the character must take, following the course of the history in an interactive way. The book “Encruzilhadas. O jogo da sua vida” narrates more than 300 situations that occur in day-to-day life of most adolescents, putting them ahead with major risk factors in cancer and offering the opportunity to choose the paths that they would follow. By the end of the book, the characters know the importance of prevention throughout their lives. As future prospects, we will adapt the book for deaf people, reducing the number of situations and then recording these references in Libras, the Brazilian sign language. Besides that, we will evaluate the impact of the book among adolescents of private school, using focus groups, and then analyze the use of the book as an alternative educational instruments.

Conclusions:
According to the National Cancer Institute (INCA), about 80% of cancers are related to the environment, in which we find a large number of risk factors such as diet, physical activity, alcohol consumption, smoking, sexual health and some others. Studies implemented throughout the country show that over 40% of adolescents between 14 and 16 years old have bad eating habits, 16.1% of Brazilian youths tried alcoholic drinks when younger than 12 years old and 23.4% have already tried cigarette (6.3% of them did it before 12 years old). In addition, the research also revealed that 35.8% of adolescents were not virgins, and of these, 77.4% said they used condom at the last time they had sexual relations. Other studies show an almost complete lack of information among adolescents in relation to cancer and the risk factors associated with the development of the disease, indicating the need for disclosure of the issue, which is only assimilated by the youth when presented in an interesting and objectively form. Because of all these data we assumed it would be important to create a different way to educate and show all information about cancer prevention and the risk factors to adolescents. Using this material we developed, we hope to show the importance of the day-to-day habits to cancer prevention and also turn the youth into a more conscious group, decreasing, as much as possible, the number of cancer cases.

Keywords: Câncer, Fatores de risco, Jovens, Role-Playing Game

Financial Support: Faperj, Fundação do Câncer

Resumo: 15-044

EFFICACY OF CUMARU SYRUP AS COMPLEMENTARY THERAPY IN MILD PERSISTENT ASTHMA: A DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED STUDY.


Depto. de Fisiologia e Farmacologia, UNIFAC-UFC

Objectives:

Amburana cearensis A. C. Smith, Fagaceae, is a medicinal plant commonly known as “cumaru.” It is used in Northeast Brazil as an anti-inflammatory and spasmyltic agent and also in the treatment of respiratory tract diseases. The aim of the study was to investigate the therapeutic efficacy of cumaru syrup as complementary therapy in mild persistent asthma.

Methods and Results:

This was a randomized, double-blind, placebo-controlled study that consisted of 3 phases, pre-treatment, treatment and post-treatment. The primary efficacy outcome was comparison of the changes reported by patients in the cumaru and placebo groups after treatment, using the “Asthma Quality of Life Questionnaire” (AQLQ). The secondary outcome was the effect of cumaru syrup on lung function based on spirometry. The research project, with the experimental protocol and the term of free and informed consent, were submitted to the Research Ethics Committee of the Federal University of Ceará, which approved the protocol of nº 169/07. Of the 67 patients initially evaluated, only 47 met the inclusion criteria. Twenty-five patients were randomized to receive cumaru syrup and 22 to receive placebo. Four patients in the cumaru and one in the placebo group were lost to follow-up and so were withdrawn from the trial due to a deviation from the experimental protocol. After the phases of the clinical trial, in the cumaru group, the proportion of patients who had global improvement in asthma symptoms was significantly greater (61.90%, P=0.0009) than in the placebo group (9.52%), with a relative risk of 6.500. Only the spirometric parameters forced vital capacity (FVC) and expiratory volume in 1 second (FEV1), both in liters, showed significant intergroup differences in post-treatment (P

Conclusions:

This clinical trial is the first study to evaluate the efficacy of cumaru syrup in humans, and it is concluded that this
phytotherapeutic medication has a beneficial effect, representing an alternative for the complementary therapy of asthma.

**Keywords:** ASTHMA, THERAPEUTIC EFFICACY, CLINICAL TRIAL, HERBAL MEDICINE

Financial Support: FINEP, CAPES, CNPq, MS-RNPC-UNIFAC-HM, Instituto Claude Bernard-INCB.

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Resumo:15-045

NEUROPROTECTIVE EFFECTS OF ACID FOLIC PLUS α-TOCOPHEROL AGAINST BETA-AMYLOID-INDUCED NEUROTOXICITY IN MICE

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Objectives:

In the current study, we assessed the molecular mechanisms, mainly the modifications in the activity of mitochondrial complexes, whereby the association of folic acid and α-tocopherol protects mice against the Aβ-induced neurotoxicity.

Methods and Results:

Oral treatment with folic acid (50 mg/kg) plus α-tocopherol (500 mg/kg), once a day during 14 consecutive days, protected mice against the Aβ1–40-induced cognitive decline, as demonstrated by higher time spent in the right quadrant (73% of increase when compared with animals treated with vehicle and Aβ1–40) during the probe test session in the MWM test (n=8–12 mice per group, p ≤ 0.05). The synaptic loss and neuronal death induced by Aβ1–40 were also prevented by oral treatment with folic acid (50 mg/kg) plus α-tocopherol (500 mg/kg), as detected by higher levels in synaptophysin hippocampal expression (congr 3.3 fold) when compared with vehicle plus Aβ1–40 animals-treated (N= 5–7 mice/group, p ≤ 0.05). However, chronic treatment comprising folic acid plus α-tocopherol was ineffective on Aβ-induced glial cell activation, suggesting that the effect of this treatment is independent of anti-inflammatory features (N= 5 mice/group, p > 0.05). Oral treatment with folic acid (50 mg/kg) plus α-tocopherol (500 mg/kg) was able to prevent the increase both in neuronal and inducible oxide nitric sintase (NOS) expression induced by Aβ in hippocampus of mice (n=5 mice/group, p ≤ 0.05). Interestingly, the results obtained in our study suggest that mitochondrial energy metabolism is impaired by the Aβ peptide, and upregulation of mitochondrial genes may be a compensatory response, as demonstrated by the increase in mitochondrial complexes I, II and IV activity, in the hippocampus of mice, after Aβ1-40 injection (n=5 mice/group, p ≤ 0.05). Of note, the chronic treatment comprising folic acid plus α-tocopherol prevented the increase in the activity of mitochondrial complexes I and IV (25% and 12.5% of decrease, respectively) induced by Aβ1-40 (n=5 mice/group, p ≤ 0.05).

Conclusions:

Together, these results show the antioxidant effect of the combination of folic acid and α-tocopherol, as observed by the decrease in NO generation from iNOS and nNOS, preventing an increase in the activity of mitochondrial complexes, mainly I and IV, and the neuronal death induced by the Aβ1-40 peptide.

Keywords: alpha tocopherol, amyloid beta, cognition, folic acid, mitochondrial complexes

Financial Support: Biola (Pharmaceutical Laboratory), CNPq, FAPESC and CAPES.

QuebraPagina
EVALUATION OF WOUND-HEALING ACTIVITY OF PSIDIUM SP. (MYRTACEAE) IN RABBITS

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Objectives:
Araçuna (Psidium sp.) is a medium-sized shrub from Myrtaceae family that reaches 2–6 meters tall. The name is of indigenous origin (tupi guarani): araça means "fruit that has eyes" and una means "black" because of the blackish purple color of the fruit. It occurs in Brazil and it is common all over the Paraná River basin and Atlantic Forest from Espírito Santo to Rio Grande do Sul states. It presents high tannin content and plants rich in this group of secondary metabolites are used by traditional medicine as remedies for treating diarrhea, hypertension, rheumatism, bleeding, wounds, burns, heartburn, nausea, gastritis and ulcer gastric, urinary system disorders and general inflammatory processes. This study evaluated the therapeutic effects of topical cream made from extract of araçuna (Psidium sp.) leaves in rabbits (Oryctolagus cuniculus).

Methods and Results:
The protocols employed for the collection of biological samples from the studied rabbits followed the ones approved by the Ethics Committee for Animal Experimentation (FAFIA). The crude vegetal extract was obtained by maceration process, with hydroethanolic solution at 60%, filtered and evaporated in a rotary evaporator. The extract was used for the preparation of the cream, with 1% concentration. After antisepsis, 20 rabbits with average weight of 1.124 g underwent general anesthesia with ketamine at a dose of 20 mg/kg, by intramuscular injection, and then they were submitted to two surgical skin failures, with diameter of 3 cm each, on the right and left dorsal lumbar. The dressings were performed twice a day, at the same time of day, applying 1.0 g of the formulation on each right wound of all animals (treatment group), and cleaning the left wounds with saline (control group). The skin lesions were evaluated under the clinical, morphometric and histological aspects. The measurements were taken daily after cleansing and always at the same time of application. Four animals were euthanized with sodium pentobarbital in days 3, 9, 15, 21 and 28 after surgery and the lesions were removed for histopathological analysis, being the first samples used for the histology of normal skin. There was no difference in lesion size (p < 0.0001). The average lesion size of the treatment group was 1.65±1.28 cm and the control group 1.33±0.98 cm. The histopathological evaluations were scored with (-) zero; (+) one; (++) two and (+++) three, and the average was calculated. Crusts occurred until day 21 in both experimental groups. Inflammation process occurred in both groups, and more intensively in the treatment group. Bleeding, tissue granulation, edema, vascular dilation and reepithelialization occurred in equal intensity. It was not detected any presence of fibrin or necrosis at any stage of the experiment.

Conclusions:
No significant difference in the wound healing process between animals in the treatment group (araçuna extract 1% cream) and the control group (saline).

Keywords: Healing activity, Psidium sp., Tannin, Aracauna, Rabbits

Financial Support: This work was financially supported by FAFIA and UFES.
Objectives:

The irrational use of antibiotics coupled to the rapid ability of bacteria to acquire resistance is a perfect combination for the reduction of treatment options in infectious diseases. Among bacteria, \textit{S. aureus} has highlighted as being a very microorganism associated with nosocomial infections, with high rates of morbidity and mortality, oxacillin and vancomycin antibiotics commonly used for treatment against this pathogen. This study aims to determine the resistance of \textit{Staphylococcus aureus}\textsuperscript{1} to oxacillin and vancomycin in a university hospital (HUWC) in the period February 17, 2009 to February 16, 2010.

Methods and Results:

This is a retrospective and descriptive study of the results the profile of resistance to antibiotics oxacillin and vancomycin produced by 285 strains of \textit{S. aureus} isolated from specimens in the Clinical Laboratory of HUWC. Resistance to oxacillin in HUWC was 41%, while all strains were sensitive to vancomycin. It was found the largest numbers of oxacillin-resistant \textit{Staphylococcus aureus} (ORSA) in specimens obtained from male patients (56%) than females (44%). The clinical samples in which \textit{S. aureus} was more prevalent were the blood (169/285) and catheter tip (52/285), which was also observed a higher incidence of \textit{S. aureus} resistant to methicillin / oxacillin, 40.8% and 61.5% respectively. The largest number of \textit{S. aureus} was isolated from the General Infirmary Medical Clinic (125/285), surgical wards (67/285) and ICU (33/285). Recovery Room Units (66.7%) and ICU (57, 6%) had the highest percentages of isolation of strains of \textit{S.aureus} resistant to oxacillin, 66,7% and 57,6%, respectively. The antibiotic oxacillin is no longer the same efficiency against \textit{S. aureus}, while vancomycin was effective against all \textit{S. aureus}.

Conclusions:

Thus, although the frequency of the ORSA strains HUWC is on average compared with other studies conducted in hospitals in various locations, the results show that the rate of ORSA should be controlled, because vancomycin is one of the last alternatives in combating these strains.

Keywords: oxacillin, ORSA, bacterial resistance

Financial Support: Funcap

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**QuebraPagina**

**Resumo:**15-048

**INTRACAVERNOSAL INJECTION OF THE LIGNAN LACTONE “CUBEBIN” FACILITATES GANGLIONIC-INDUCED ERECTILE RESPONSE IN RATS**

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Objectives:

Cubebin, a compound belonging to the dibenzylbutirolactone lignan group, which is widely distributed in the plant kingdom, was isolated from the crude ethanolic extract of the seeds of Piper cubeba L and has been associated with analgesic and anti-inflammatory activities. Previous observations from this group have indicated that cubebin and some related compounds hold an interesting ability to induce erections (priapism) in anesthetized mice when injected subcutaneously. The purpose of this study was to evaluate the potential effect of cubebin in facilitating the ganglionic-induced erectile response in anesthetized rats.

Methods and Results:

Male Wistar rats (280-320 g) were anesthetized by ketamine/xylazine (100/14 mg/100g), had the major pelvic ganglion isolated and electrically stimulated. Intracavernosal pressure (ICP) and mean arterial pressure (MAP) were measured and presented as an index of erection (ICP/MAP). Frequency-response curves (1-12 Hz, 4V, 5ms pulse and 30 seconds for each frequency) were obtained from rats of all groups. Penile erection was evaluated in vehicle and cubebin nanoemulsion treated animals. Statistical analysis was performed with ANOVA followed by Bonferroni test. A value of p < 0.05 was considered significant. Cubebin (25 mg/ml) was added to the oil phase of the nanoemulsion (particle sizes between 100-150 nm). A volume of 0.1 ml of the nanoemulsion containing 2.5 mg of cubebin (n=4) or vehicle (n=4) was injected into the cavernosum tissue and held there for 5 minutes until the blood flow was restored. Erectile function was not affected by vehicle injection, but significantly potentiated after cubebin containing nanoemulsion. The ICP/MAP erection index (mean ± SE) at frequencies ranging from 2, 4, 8 and 12 Hz were 0.35 ± 0.1134, 0.65 ± 0.0634, 0.74 ± 0.0921 and 0.71 ± 0.0997 compared to values obtained from vehicle control rats of 0.21 ± 0.0249, 0.32 ± 0.0117, 0.41 ± 0.0487 and 0.36 ± 0.0513 respectively. The average potentiation calculated was 66, 103, 80 and 97%, respectively, for the following frequencies evaluated.

Conclusions:

Our data indicate that intracavernosal injection of the lactone lignan “cubebin” by a mechanism still to be established potentiates the erectile response induced by ganglionic stimulation in anesthetized rats. This result suggest that either cubebin and/or related compounds show a great potential to be used in the development of new approaches to treat male erectile dysfunction.

Keywords: CUBEBIN, ERECTILE RESPONSE, GANGLIONIC-INDUCED

Financial Support: FAPEMIG, CNPq, UFOP

Diclofenac Determination in Human Plasma by High-Performance Liquid Chromatography Coupled to Tandem Mass Spectrometry (HPLC-MS-MS): Application to Pharmacokinetic Study

Departamento de Farmacologia e Fisiologia/ Unifac, UFC

Objectives:
Diclofenac inhibits prostaglandin synthesis by inhibition of cyclooxygenase (COX) which results in an anti-inflammatory effect. The aim of the present study was to develop and validate a liquid chromatography (LC) method for the determination of diclofenac in human plasma supporting a pharmacokinetic and bioequivalence study.

Methods and Results:

Diclofenac (internal standard) was extracted from plasma using ether/hexane (80/20; v/v) as solvent and separated on a Genesis C8 120A 4µm (150 x 4.6 mm) maintained at 50°C, with acetonitrile:water (80:20 v/v) + 1mM acetic acid as mobile phase. The flow rate was 1200 µL/min and the detection was carried out by mass spectrometry. The bioequivalence study was an open, randomized, two period crossover design with a one week washout interval between the doses. Twenty four healthy volunteers aged between 18 and 50 years and within mass body index between 19 and 30 were selected by clinical evaluation and laboratory tests. The clinical protocol was approved by the local Ethic Committee (Protocol n° 156/08) and the volunteers given written informed agreement to participate in the study. During each period, a single oral dose of diclofenac (1 tablet-50 mg) was given after an overnight fast of at least 10 hours, and the blood samples were collected up to 48 hours post dosing. The method validation investigated the parameters recommended for the bioanalytical methods and yielded good results with limit of quantification of 10 ng/mL. The chromatographic separation was obtained within 2.06 min, and the response was linear in the concentration range of 10 – 4000 ng/mL. The mean extraction recoveries were 91.6% for diclofenac. The intra-day precision for the quality controls low (QCL), middle (QCM) and high (QCH) were respectively 5.4, 3.1, and 1.2%. The inter-day precision for QCL, QCM and QCH were respectively: 5.1, 4.6 and 3.3%. The intra-day accuracy for QCL, QCM and QCH were 91.5, 99.6 and 95.3% respectively. The results of the inter-day accuracy QCL, QCM and QCH were respectively and 92.1, 94.7, 92.5% respectively. The proposed method was successfully applied for the bioequivalence study of two tablet formulations (test and reference) of diclofenac 50 mg after single oral dose administration to 24 healthy volunteers. The geometric means ratios of Cmax, AUC(0-t) and AUC(0-inf) were 75.74, 91.46 and 91.46% with 90% confidence intervals of 66.69 – 86.02%, 86.80-96.37% and 86.91-96.25% respectively.

Conclusions:

Since the 90% CI for Cmax, AUC(0-t) and AUC(0-inf) were not within 80-125% interval proposed by ANVISA and FDA, it was concluded that the two formulations of diclofenac were not bioequivalents, according to the rate of absorption.

Keywords: HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY, DICLOFENAC, PHARMACOKINETIC, BIOEQUIVALENCE

Financial Support: CNPq, InCB, MS-RNPC-UNIFAC-HM, FINEP.

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Resumo:15-050

EFFECT OF PREOPERATIVE MEDICATIONS ON ANXIETY AND EMERGENCE DELIRIUM IN CHILDREN UNDERGOING OUTPATIENT SURGERY: A DOUBLE-BLIND, RANDOMIZED, CLINICAL TRIAL.

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Objectives:

Fear and anxiety in the preoperative holding area and during the induction of anesthesia produces aggressive reactions, increased patient discomfort, and makes the postoperative pain management more difficult. To reduce its negative impact, the standard and
most common premedication is midazolam because of its efficacy on amnesia, anxiolysis in the holding area, and separation anxiety. However, midazolam increases the agitation of children at the induction of anesthesia as well as the incidence of emergence delirium (ED) compared with melatonin. ED occurs in 80% of children after sevoflurane and desflurane anesthesia. Factors such as age, preoperative anxiety, anesthetic (e.g., desflurane), and postoperative pain have been suggested as possible etiologies. Objectives: Compare the effects of melatonin, clonidine and ketamine with midazolam in reducing anxiety in the preoperative course (preoperative holding area, operation room, and at the introduction of the anesthesia mask) and ED in children undergoing outpatient surgery. The null hypothesis was that there was no difference in anxiety and ED among the four treatments.

Methods and Results:

This randomized, double-blind, controlled trial consisted of 113 children aged 2-6 with ASA physical status I-II, scheduled to undergo general anesthesia. Patients were assigned to receive oral melatonin 0.5 mg/kg (n=28), clonidine 4 μg/kg (n=29), ketamine 6 mg/kg (n=29) or midazolam 0.5 mg/kg (n=27). The modified Yale Preoperative Anxiety Scale (MYPAS) and Pediatric Anaesthesia Emergence Delirium (PAED) instruments were utilized. The midazolam-treated patients presented superior anxiolytic effects compared to the other three treatments within 45 minutes after medication and at entrance into the operating room. At the introduction of the mask ketamine-treated showed higher anxyolisis. There is an effect of treatment (P= 0.04), an interaction between group and time (0.010) and an effect over time (P= 0.001). Compared with midazolam, the number needed to treat (NNT) to prevent severe ED using melatonin and ketamine was 3.25 (CI 95%, 1.88 to 22.47) and 2.83 (CI 95%, 1.77 to 10.61), respectively.

Conclusions:

Midazolam was more effective than the other three treatments to produce anxiolysis until the introduction of the anesthesia mask, although it resulted in higher risk of severe postoperative ED. Presumably the associations of preoperative medication could improve the perioperative outcomes.

Keywords: emergence delirium, anxiety, surgery, melatonin, midazolam

Financial Support: CAPES - PNPD/CAPES, CNPq, FAPERGS and Postgraduate Program UFRGS
days: groups 1 and 2 received water; groups 3 and 4 received ethanol (2.0 g/kg) and water; groups 5 and 6 received baclofen (5.0 mg/kg) and water; and groups 7 and 8 received baclofen and water at the previously specified doses. After this treatment, each group received a challenge as follows: G1: water; G2: ethanol; G3: ethanol; G4: baclofen and ethanol; G5: water; G6: ethanol; G7: water; and G8: ethanol. Group 1 was defined as negative control, receiving only water throughout the entire treatment, whereas group 3 was defined as positive control, receiving both water and ethanol throughout the entire treatment. At the end of these proceedings, mice were submitted to euthanasia and liver samples were collected and kept at ~70°C in order to analyze the activity of the following enzymes: superoxide dismutase (SOD), glutathione-S-transferase (GST), and levels of glutathione (GSH). All values were correlated with the amount of protein measured in each sample by the Bradford method. The data was analyzed by two-way ANOVA, followed by Newman-Keuls as a post-hoc test. Results suggest that 24 days of treatment with ethanol may be insufficient as to achieve oxidative stress in the liver, because no statistically significant differences were observed between the control groups. An important increase in the activity of the antioxidant enzymes SOD when compared to the positive control (G3; numerical result of 4.2 ± 1.15 UnSOD / mg of protein) was nonetheless observed as a result of the baclofen challenge treatment in the face of ethanol pre-treatment (G4; numerical result of 6.04 ± 1.48 UnSOD / mg of protein). Similar results were found for the enzyme GSH: its activity was increased in G4 (10.4 ± 3.25 nmol.mg of protein-1) when compared to G3 (6.36 ± 0.5 nmol.mg of protein-1). The absence of such results when baclofen is not administrated (G1; numerical result of 4.95 ± 0.65 UnSOD / mg of protein for SOD and 5.48 ± 1.55 nmol.mg of protein-1 for GSH) indicates a role of baclofen as an acute modulator of the redox cycles and reactions when co-administered with ethanol. In addition, treatment with baclofen since the beginning of the ethanol administration did not produce increase in the activity of the studied antioxidant enzymes, further reinforcing the hypothesis of a predominantly acute effect of baclofen. No statistically significant differences were observed regarding the activity of GST.

Conclusions:

Present results show that baclofen may stimulate acute beneficial effects against alcohol induced liver damage. More studies are necessary to assess any other possible alterations in normal organic functions that may be consequential of such treatment.

Keywords: BACLOFEN, ALCOHOLISM, LIVER, OXIDATIVE STRESS

Financial Support: CAPES, REUNI, Fundação Araucária

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Resumo:15-052

DETERMINATION OF THE PHARMACOKINETIC PROFILE OF KETOROLAC TROMETHAMINE 30MG TABLET ADMINISTERED SUBLINGUAL ROUTE IN HEALTHY VOLUNTEERS.


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Objectives:

Ketorolac tromethamine (ketorolac) is a non-steroidal anti-inflammatory drug (NSAID) used in the treatment of pain. The use of a sublingual analgesic has several advantages, such as increased bioavailability and easy administration, especially in patients who have difficulty swallowing. Thus, the aim of this study was to evaluate the pharmacokinetic profile of a formulation of ketorolac in Brazilian male volunteers.

Methods and Results:

This was an open, non-randomized, 01 period, 01 treatment, single dose under fasting conditions study where was administered 30 mg sublingual tablet formulation of ketorolac. Healthy Brazilians male were eligible for inclusion. After an overnight fast, subjects received a single sublingual dose of ketorolac. Plasma samples were obtained over a 24-hour period after administration.
Plasma ketorolac concentrations were analyzed by High Performance Liquid Chromatography coupled Mass Spectrometry for analysis of pharmacokinetic properties, including Cmax, AUC0-24, and AUC0-∞. Tolerability was assessed by vital sign monitoring, laboratory analysis results, anamnesis and physical examination. A total of 14 male subjects were enrolled and completed the study. The pharmacokinetics parameters values (mean ± standard deviation, except median for time to Tmax) calculated for formulation were as follows: AUC0-∞ (9682 ± 1908 ng*h/mL); AUC0-24 (9346 ± 1789 ng*h/mL); Cmax (2605 ± 465 ng/mL); Tmax (0.58 ± 0.22 h); t1/2 (5.76 ± 0.69 h) and Ke (0.12 ± 0.02 h-1). The ketorolac formulation was well tolerated at the administered dose and no adverse reactions were observed.

Conclusions:
The advantage of the use of the sublingual formulation of ketorolac is its practical administration. It can be the treatment of choice for moderate and severe pain, especially in patients where parenteral route is undesirable or impracticable, or those who have difficulty of swallowing. The methods were successfully applied, it is possible to analyze the pharmacokinetic parameters and assess the safety of the tablet formulation of 30 mg of ketorolac tromethamine administered sublingually in healthy volunteers.

Keywords: ketorolac, sublingual, pharmacokinetic, clinical trials

Financial Support: CNPq, CAPES, FUNCAP, FINEP, MS-RNPC-UNIFAC-HM, Instituto Claude Bernard

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Resumo:15-053

SPECIFIC MATRIX METALLOPROTEINASE 9 (MMP-9) HAPLOTYPE AFFECT THE CIRCULATING MMP-9 LEVELS IN WOMEN WITH MIGRAINE

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2 Farmacologia, FMRP/USP
3 Neurologia, FMRP/USP

Objectives:
We investigated whether three clinically relevant polymorphisms in the MMP-9 gene (C−1562T, -90 CAn and R279Q), isolate or combined in haplotypes are associated with enhance in the susceptibility to migraine attacks, and whether affect the circulating levels of MMP-9 in studied groups

Methods and Results:
In this study were included a total of 187 women with migraine and 105 healthy women without history of migraine. Study groups were divided into 4 groups: 105 healthy women without migraine (control group), 187 migraine patients without and with aura (migraine group), 141 women with migraine without aura (MWA group), and 46 women with migraine with aura (MA group). Venous blood samples were collected and plasma samples were used to measure plasma MMP-9 concentrations. Indeed, aliquots of whole blood were separated and stored for genomic DNA extraction and genotype analyses. We found no significant difference in the genotype and allelic distribution for the three polymorphisms neither when migraine groups were compared with control group nor when MWA group were compared with MA group (p>0.05). There was no significant influence of MMP-9 genotypes on plasma MMP-9 levels when were compared healthy volunteers and migraine groups (p>0.05). Moreover, we found no significant differences in the distributions of haplotype frequencies when all study groups were compared (p>0.05). Indeed, we analyzed the contribution of the different haplotypes on MMP-9 plasma concentrations. Interestingly, we found that the H6 haplotype in migraine group was associated with higher plasma MMP-9 concentrations when compared with H2 and H3.
haplotypes (p<0.05).

Conclusions:

Our findings showed that the MMP-9 haplotype “L C G” was associated with higher MMP-9 plasma levels in migraine group. However, the definition of the exact role of MMPs in migraine pathophysiology remains unclear and poorly understood, certainly because migraine is a complex and multifactorial disorder.

Keywords: Migraine, Matrix Metalloproteinase 9, Haplotype

Financial Support: CNPq, Fapesp, Capes

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**Resumo:**

EFFICACY AND SIDE EFFECTS OF ALPRAZOLAM AND MIDAZOLAM IN THE REDUCTION OF SURGICAL STRESS IN IMPLANTODONTICS: A RANDOMIZED CLINICAL TRIAL.

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Objectives:

Aim: The aim of this study was to evaluate the clinical efficacy and safety of alprazolam, compared to midazolam in reducing stress in surgical dental implants.

Methods and Results:

Methods: Twenty volunteers, ASA I or ASA II, with indication for placement of dental implants, were divided into two equal groups (n=10), treated differently with alprazolam 0.5 mg or midazolam 7.5 mg, orally, in random order and double blind. The efficacy of sedation was assessed by the investigator, using a CGI (Clinical Global Impression) with a score of 7 points, and the volunteers themselves, through a range of 100 mm visual analogue (VAS) in two different times. The systolic and diastolic arterial blood pressure (SBP and DBP), heart rate (HR) and arterial oxygen saturation (PaO2) were monitored before, during and after the intervention. Side effects were investigated by verbal contact, 24 hours after surgery. Data were analyzed by one-way Anova (Tukey test) and Kruskal-Wallis (Dunn test). Results: The results (mean ± standard deviation) for alprazolam and midazolam, before, during and after treatment were, respectively: SBP (134±23; 127±20; 136±25mmHg) (122±16; 113±15; 113±10mmHg), DBP (88±11; 79±11; 85±11mmHg) (77±11; 70±12; 72±8mmHg), HR (69±11; 65±9; 67±10bpm) (73±11; 70±8; 72±9bpm), and PaO2 (97±0.9; 96±2; 96±1%) (96±1; 96±1; 96±2); and VAS before and after treatment (22±22; 7±1mm) (21±14; 7±5mm). Only 20% and 10% of patients showed moderate level of anxiety with alprazolam and midazolam, respectively. No differences were observed between the treatments (p >0.05) for the parameters evaluated and all of them remained within the normal range. Sleepiness was reported by 3 patients in each group.

Conclusions:

Conclusion: It can be concluded that alprazolam is an effective and safe alternative to midazolam in the control of anxiety in surgical dental implant.

Keywords: Alprazolam, Midazolam, Benzodiazepines, Dental implant
THE IMPORTANCE OF THE SENSITIVITY TEST ON THE THERAPEUTIC APPROACH IN TUBERCULOSIS PATIENTS AND ITS RELATIONSHIP WITH THE TREATMENT ABANDON.

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Objectives:
The use of sensitivity test in tuberculosis patients has important role in the implementation of therapy and thus influences the outcome of treatment. In this study we investigated the relationship between sensitivity test application, therapeutic adopted and abandonment of treatment in tuberculosis patients.

Methods and Results:
The study was based on data obtained from medical records of 78 patients who were admitted to the Reference Centre for Respiratory Diseases - Octavio Mangabeira Specialized Hospital (HEOM), Salvador, Bahia, in 2010 with a diagnosis of tuberculosis. They all had at least 18 years old. This study was approved by the ethics committee of the University of Bahia, protocol n. 0603100102672 as well as the Center for Research in Pneumology Hospital. Despite the concern about dropout rates, a factor that difficult the eradication of the disease, only 4 patients left the therapy during the study period. Among them, three (3.85%) were using the IR reinforced scheme, one (1.28%) was treated with individualized regimen (different doses in relation to the treatment protocols recommended by the ministry of health) and all had side effects secondary to therapy. It is also possible to observe that 58 (74.4%) patients had no history of dropouts in previous treatments. In the 78 patients studied, only 24 (30.8%) were submitted to sensitivity analysis previous to treatment. It is important to mention that only 6 (7.7%) patients in the study were undergoing the first treatment.

Conclusions:
Our results show that the sensitivity test was performed in only part of the sample. This fact may influence the more adequate and effective protocol to each individual.

Keywords: Pharmacological treatment, Sensitivity test, Tuberculosis

Objectives:

Streptokinase (STK) is obtained from culture filtrates of certain strains of haemolytic Streptococcus group C, interacts with and activates human plasminogen to form an activator. The recombinant STK has been expressed in E.coli. It consists of a 415 amino acids polypeptide chain, with a molecular mass of 47 kDa. Clinical use worldwide includes thrombolytic therapy of acute myocardial infarction, deep vein thrombosis and arterial thrombosis. The aim of this study was to perform the in vitro plasminogen activator assay to assess the biological potency of streptokinase in pharmaceutical formulations available for clinical use.

Methods and Results:

Samples of pharmaceutical formulations lyophilized containing 1.5 million IU per vial of STK were evaluated against the 3rd International Standard for Streptokinase (WHO 00/464). Standard and samples were diluted to final concentrations of 5 to 40 IU/ml and the assay performed on the 96-well plate at 37°C. Reconstituted chromogenic substrate (S-2251) was transferred to each well and then the human plasminogen was added. The reaction was allowed to proceed for 12 min. The absorbances were measured in the plate reader at 405 nm, and the analysis of variance calculated to demonstrate the validity of the assay. The potencies varied within 98.10 to 110.30% with fiducial intervals (P=0.05) between 80 and 125%, following the Pharmacopeial specifications.

Conclusions:

The results obtained demonstrated the application of the optimized in vitro assay to assess the biological potency of STK specified between 90 – 111%, of the stated potency, and the quality of the biological medicine available for clinical use, contributing to assure the safety and therapeutic efficacy.

Keywords: STREPTOKINASE, PHARMACEUTICAL FORMULATIONS, IN VITRO ASSAY, BIOLOGICAL POTENCY, SAFETY AND THERAPEUTIC EFFICACY

Financial Support: CNPq

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Resumo:16-053

PRIOR USE OF STATIN IN ACUTE MYOCARDIAL INFARCTION: ANALYSIS OF OXIDATIVE STRESS ASSOCIATED WITH ECHOCARDIOGRAPHIC PARAMETERS 48H POST-INSULT.

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Objectives:

Acute myocardial infarction (AMI) leads to a loss of homeostasis with an overexpression of reactive oxygen species. The use of statins could be an alternative for the preservation of cardiac function after AMI due to its pleiotropic effects that include a poorly understood antioxidant potential. Thus, in this study we aimed to investigate the role of prior statin therapy on antioxidant and echocardiographic parameters 48 hours after AMI.
Methods and Results:

Male Wistar rats (60 days old) were divided into two groups: Treated Group (TG): atorvastatin 20 mg/kg (n=8) and placebo (PG): NaCl 0.9% (n=8). Both groups were treated for 14 days prior to AMI by gavage. The animals were subjected to the AMI by left anterior descending coronary occlusion. Forty eight hours after AMI the animal were anesthetized and echocardiographic measurements (fractional shortening, ejection fraction and infarct size) were evaluated. After, the animals were killed and the left ventricles were collected. Analysis of oxidative stress was performed by measuring the activity of antioxidant enzymes (catalase, superoxide dismutase and glutathione peroxidase) and the ratio of reduced/oxidized glutathione (GSH/GSSG), representing the total oxidative stress, damage to proteins and lipids in infarcted (IZ) and non-infarcted zones (INZ) of the hearts. Echocardiographic and enzymatic analyses do not show statistically significant differences. However, INZ in the TG showed less protein damage than INZ-PG group (p = 0.0203).

Conclusions:

Statin therapy protects against oxidative damage to proteins in non-infarcted myocardium. This study suggests that prior use of statins may be beneficial in the remodeling process after AMI.

Keywords: Myocardial infarction, Oxidative Stress, Statin Therapy

Financial Support: : FIPE/HCPA and CNPq

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EFFECTS OF RESISTANCE TRAINING ON HEMODYNAMIC FUNCTION, PULMONARY AND HEPATIC CONGESTION AND STRENGTH GAIN IN HEART FAILURE RATS.

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Objectives:

Currently, the benefits of physical exercise in heart failure (HF) are primarily based on the interventions of aerobic training. However, in recent years, the resistance training (RT) has been considered a possible strategy for primary and secondary prevention of different heart diseases. Therefore, the purpose of this study was to evaluate the effect of RT on hemodynamic function and maximal strength gain in chronic heart failure rats.

Methods and Results:

For this study we used male Wistar rats weighing between 220 to 270g (~90 days old) from Federal University of Health Sciences of Porto Alegre - UFCSPA, animals were divided into four groups: Sham sedentary (Sham Sed, n=6), Sham trained (Sham Tr, n=6), Chronic heart failure sedentary (CHF Sed, n=6) and Chronic heart failure trained (CHF Tr, n=6). Six weeks after occlusion of the left coronary artery, the animals were submitted an 8-weeks of resistance training (4 days/week, 65 to 75% of one repetition maximum (1RM) intensity). To determinate the work load and strength gain was realized a 1RM test before and after training protocol. An electrical stimulation (4-15 miliamperes (mA), 1-s duration, at 3-s intervals) was applied to the rat’s tail to perform the movement. Twenty-four hours of the last exercise day, the animals were anesthetized to hemodynamic variables acquisition and tissue collection. There are no difference in animals body weight at the begin of study. The 8-week of RT decrease the left ventricular end diastolic pressure (LVEDP) in CHF trained group when compared to CHF sedentary group (CHF Sed: 23.27±5.12 vs CHF Tr: 7.68±5.11 mmHg; P<0.05).

Conclusions:
The 8-week RT improves hemodynamic function by decreases in LVEDP and increases in LVSP, +dP/dt and -dP/dt. Moreover, RT was able to reduce the pulmonary and hepatic congestion and to promote strength gain in chronic heart failure rats.

Keywords: Heart failure, exercise, resistance training

Financial Support: CAPES and UFCSPA

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Resumo:16-055

N-ACETYLCYSTEINE NORMALIZES CARDIAC AUTONOMIC IMBALANCE AFTER MYOCARDIAL INFARCTION IN RATS


Objectives:

Some studies showed that myocardial infarction induces an increase in sympathetic nerves density and activity on the heart. N-acetylcysteine (NAC) seems to reduce both of them, by acting on autonomic and enzymatic mechanisms. The purpose of this study was to evaluate the influence of N-acetylcysteine on myocardial infarction-induced autonomic imbalance assessing heart rate variability (HRV) both in time and frequency domain.

Methods and Results:

Nineteen infarcted (IF) (by left coronary artery ligation) and 15 Sham (SH) female Wistar rats were daily treated with water or NAC (250mg/kg) by oral gavage during 4 weeks, starting 24h after surgeries. Thereafter, cardiac function was assessed by echocardiography (ECHO) and a 600 seconds tachograms were generated to evaluate the HRV in time domain. To frequency domain tachograms were resampled to equal intervals and the power spectrum was obtained with a fast Fourier transform based method (Welch's periodogram: 512 points, 50% overlap, and Hamming window). Three frequency bands were determined: very low frequency (VLF: 0.0-0.2 Hz), low frequency (LF: 0.2-0.75 Hz), and high frequency (HF: 0.75-3.0 Hz). All animals underwent cytology analysis to indentify the hormonal cycle during the protocol. The statistical analysis performed was two-way ANOVA with the Bonferroni post-hoc, and the significance adopted was p<0.05).

Conclusions:

The treatment with NAC during four weeks normalized autonomic imbalance induced by myocardial infarction in female rats. Further studies are imperative to investigate if the beneficial effect of NAC on autonomic balance observed herein can improve cardiac function later.

Keywords: N-acetylcysteine, Myocardial Infarction, Spectral Analysis, Autonomic Imbalance

Financial Support: CAPES / FAPERJ

QuebraPagina

Resumo:16-056

ANALYSIS OF THE FUNCTIONAL AGING OF LARGE ARTERIES IN INDIVIDUALS WITH DOWN SYNDROME:
PRELIMINARY STUDIES

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Objectives:

Down syndrome (DS) is known to present characteristics of premature aging in several organs or systems. However, it remains unclear whether this aging also affects the structure and function of the large arterial trunks. In this study, possible alterations due to aging of the large arteries in patients with DS compared to non-carrier individuals were evaluated.

Methods and Results:

Eighty-two individuals of both sexes were selected. The DS group had 41 active subjects consisting of 19 males and 22 females (mean age: 21±1 years, range: 13-42 years) without cardiovascular complications or use of vasoactive drugs. The control group consisted of 41 healthy individuals without trisomy 21 of the same sex and age as the DS group and who did not use medication. Carotid-femoral pulse wave velocity (PWV) was obtained by an automatic non-invasive method and used as an index of aortic stiffness. Individuals with DS had significantly lower blood pressure than individuals in the control group. SBP for the DS and control groups were 106±2 vs. 117±2 mmHg (P

Conclusions:

Despite evidence in the literature that patients with DS undergo early aging, this process does not seem to affect the large arterial trunks, given that values of carotid-femoral PWV were similar in individuals with or without trisomy 21. Considering that DS presents with chronic hypotension, it is reasonable to propose that the prolonged reduction of arterial distending pressure may contribute to the functional preservation of the arteries in patients with DS.

Keywords: Aging, Down syndrome, Pulse wave velocity, Arterial stiffness

Financial Support: FAPES and CDV-FACITEC
alterations in hypertensive animals. The doxycycline effects were associated with decreased MMPs activity. However, whether this inhibitor might also act as a ROS (reactive oxygen species) scavenger in hypertension is uncertain. Thus, we evaluated the effects of doxycycline in different doses on ROS formation and NADP(H) oxidase activity, besides MMPs inhibition, and if this may contribute to the amelioration of vascular alterations in two-kidney, one-clip (2K1C) hypertension.

Methods and Results:

2K1C hypertension was induced by clipping the left renal artery with a silver clip (0.2 mm). Sham operated or 2K1C hypertensive rats were treated with doxycycline 3, 10 e 30 mg/kg/day (or vehicle). Systolic blood pressure was assessed weekly throughout the experiment period by tail-cuff plethysmography. After four weeks of treatment morphometry of structural changes in the aortic wall were studied in orcein and picrosirius red sections. Aortic MMP activity was determined by in situ zymography. Superoxide production and aortic NADPH oxidase activity were evaluated. Procedures were approved by the local Ethical Committee (protocol number: 140/2009). All treatments attenuated the increases in SBP in hypertensive rats after the fourth week of the renal artery surgery (200.42 ± 12.8 mmHg versus 169.6 ± 9.6 mmHg, 165.8 ± 4.9 mmHg and 158.3 ± 7.9 mmHg in 2K1C and 2K1C+doxy 3, 2K1C+doxy 10 and 2K1C+doxy 30, respectively, all P

Conclusions:

Treatment with doxycycline ameliorates 2K-1C hypertension-induced vascular alterations in aortas in part by inhibiting oxidative stress generation and increased MMP activity.

Keywords: Doxycycline, metalloproteinases, renovascular hypertension, reactive oxygen species, oxidative stress

Financial Support: FAEPA, FAPESP, CNPq, CAPES

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Resumo:16-058

EXPRESSION OF ANGIOGENIC FACTORS IN THE HEART OF HYPERTENSIVE RATS: EFFECTS OF TREATMENT WITH ANTIHYPERTENSIVE DRUGS.

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Fundação Oswaldo Cruz, FIOCRUZ

Objectives:

Microvascular rarefaction is involved in the increase of blood pressure in hypertensive animals and humans. We previously demonstrated that antihypertensive treatment is able to reverse capillary rarefaction in the heart of spontaneously hypertensive rats (SHR) (J Cardiovascular Pharmacol. 51: 402, 2008). Growth factors such as vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF) promote survival of endothelial cells, inhibiting apoptosis and favoring the emergence of new vessels. Here we investigated the effects of chronic treatments with antihypertensive drugs on VEGF and PDGF expression in the heart of SHR.

Methods and Results:

We treated adult male (12-14 weeks) SHR and normotensive Wistar Kyoto rats (WKY) by gavage during 3 or 28 days with distilled water or antihypertensive drugs: atenolol (50mg/kg/day, ATE), enalapril (10mg/kg/day, ENA), losartan (10mg/kg/day, LOS) and nifedipine (20mg/kg/day, NIF). Blood pressure was determined by a computerized tail-cuff plethysmography system once a week and protein expression (VEGF and PDGF) was determined by western blotting. Compared to normotensive animals, SHR control group had lower PDGF (WKY 1.05±0.16 vs SHR 0.33±0.06 arbitrary units) and VEGF (WKY 1.07±0.07 vs SHR 0.41±0.04 arbitrary units) expression. After 3 days of treatment, even if blood pressure was still not reduced, there was an increase in PDGF in ATE (0.93±0.16), ENA (0.91±0.14) and LOS (0.90±0.19) groups and no effects on VEGF expression. After
28 days all treated groups had the systolic blood pressure decreased to the same extent, accompanied by an increase in VEGF expression in ATE (0.79±0.08), ENA (0.94±0.08) and LOS (1.03±0.06) groups and no effects on PDGF expression. Only nifedipine had no effect on PDGF and VEGF expression.

Conclusions:

It is concluded that PDGF and VEGF expression is reduced in the heart of SHR. The treatment with antihypertensive drugs acts differently on the expression of proteins involved in angiogenesis on the heart of hypertensive animals in acute and chronic treatment.

Keywords: angiogenesis, antihypertensive drugs, hypertension

Financial Support: FIOCRUZ, CNPq and FAPERJ.

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Resumo:16-059

TGF-BETA AND MATRIX METALLOPROTEINASES UPREGULATION CONtribute to Cardiac ALTERATIONS IN 2K1C HYpERTENSION VIA OXIDATIVE STRESS

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2 Morfología/Faculdade de odontologia de Ribeirao Preto, FORP
4 Patología/ FMRP-USP, FMRP-USP

Objectives:

Increased oxidative stress upregulates matrix metalloproteinases (MMPs) and transforming grow factor (TGF–&beta), which are involved in hypertensive cardiac remodeling. We tested the hypothesis that tempol (an antioxidant) could prevent these alterations in two-kidney, one-clip (2K1C) hypertension.

Methods and Results:

Sham-operated or hypertensive rats were treated with tempol (18 mg.kg-1day-1 or vehicle) for eight weeks. Systolic blood pressure was monitored weekly. At the end of the treatment, a catheter was inserted into the left carotid artery and into the left ventricle (LV) to assess arterial blood pressure and contractile function. Morphometry of the LV was carried out in hematoxylin/eosin sections and fibrosis was assessed in picrosirius red-stained sections. Cardiac TGF–&beta level was evaluated by immunofluorescence. Cardiac MMP-2 levels and activity were determined by gelatin zymography, in situ zymography, and immunofluorescence. Cardiac superoxide production was evaluated by dihydroethidium probe. Procedures were approved by the local Ethical Committee (protocol number: 122/2006). Tempol treatment attenuated 2K1C-induced hypertension and reversed the contractile dysfunction in 2K1C rats. Cardiac hypertrophy was ameliorated by antioxidant treatment. Hypertensive rats showed increased cardiac MMP-2 levels, however tempol did not decrease MMP-2 levels. Increased TGF–&beta level, total gelatinolytic activity and oxidative stress were found in untreated 2K1C rats. Tempol treatment decreased oxidative stress, TGF–&beta levels, and gelatinolytic activity in 2K1C rats to control levels.

Conclusions:

Tempol blunted the increases in TGF–&beta, the proteolytic imbalance, and the morphological and functional alterations found in 2K1C-induced
LOW-LEVEL LASER THERAPY REDUCES THE IL-6/IL-10 RATIO ON PLASMA AND SKELETAL MUSCLE IN RATS WITH HEART FAILURE.

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Objectives:

Heart failure (HF) is currently recognized as a multisystemic disorder that, besides the cardiovascular system, affects other systems, such as the musculoskeletal and immune systems. In HF the immune activation is followed by the imbalance between pro-inflammatory (e.g. IL-6) and anti-inflammatory (e.g. IL-10) cytokines in the skeletal muscle. Low level laser therapy (LLLT) has been used as an anti-inflammatory treatment in several disease conditions. Experimental studies show a decrease in the expression and synthesis of several inflammatory cytokines after LLLT in vitro and in vivo. However, the use of LLLT in skeletal muscle of rats with HF remains unclear. The present study aimed to evaluate the influence of LLLT on plasmatic and skeletal muscle IL-6/IL-10 ratio in rats with HF.

Methods and Results:

Male Wistar rats (n=49) with 200-230g (90 days) were assigned to one of six groups: sham rats (P-Sham; n=8), LLLT at a dose of 3J/cm2 sham rats (3J/cm2-Sham; n=8), LLLT at a dose of 21J/cm2 sham rats (21J/cm2-Sham; n=8), placebo HF rats (P-HF; n=9), LLLT at a dose of 3J/cm2 HF rats (3J/cm2-HF; n=8), LLLT at a dose of 21J/cm2 HF rats (21J/cm2-HF; n=8). Four weeks after induction of myocardial infarction or Sham surgery, animals were underwent to LLLT applied on the right gastrocnemius muscle for 10 consecutive days. Subsequently was performed the tissue collect for evaluation of inflammatory cytokines (IL-6 and IL-10) in plasma and in the right gastrocnemius muscle by multiplex bead array using MilliplexTM MAP rat cytokine kits (RCYTO-80K). For statistical analysis was applied two-way ANOVA test followed by post-hoc of Tukey (α=5%). Data are expressed in mean±SD. The plasmatic IL-6/IL-10 ratio in the P-HF (61.68±15.64) was higher compared to P-Sham (27.55±4.51) group (P)

Conclusions:

In conclusion, LLLT reduced the IL-6/IL-10 ratio on plasma and skeletal muscle in rats with HF. This results showed the systemic and peripheral anti-inflammatory effects of LLLT, which characterizes the LLLT as a new non-pharmacological possibility of the treatment for inflammation in HF.

Keywords: cytokines, inflammation, LLLT, myocardial infarction

Financial Support: CNPq; PIBIC-CNpq; PROAP; PIC-UFCSPA
EFFECT OF POLYUNSATURATED FATTY ACIDS ON MYOCARDIAL INFARCTION IN RATS

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Objectives:
The effect of dietary supplementation with omega-3 and 6 fatty acids on size of heart infarction was investigated in rats.

Methods and Results:
The heart infarction model used was the ligation of the left descending coronary artery. The infarct area was analyzed by echocardiography 24 hours after ligation. Inflammatory and metabolic parameters were also evaluated under these conditions. The source of omega-3 fish oil used was Hi-Omega™ containing approximately 50% of omega-3 fatty acid (42 and 17% EPA and DHA, respectively). The source of omega-6 was soybean oil (52% linoleic acid). The rats were supplemented by gavage for 20 days before induction of infarction. The area of infarction in the rats supplemented with fish oil (MI + FO) was 28% lower than in the rats infarcted without supplementation (MI) and 25% lower than in the rats infarcted supplemented with soybean oil (MI + SO). The activity of creatine kinase in plasma of the MI group was higher than in the non-infarcted group (759 ± 68 vs 394 ± 29,78 U/L). The supplementation with fish oil reduced the plasma activity of creatine kinase (409 ± 56) by 46% compared with MI (759 ± 68) and 37% compared with the MI + SO group (649 ± 52 U/L). Creatine kinase activity did not differ between the MI + SO and MI groups. The content of inflammatory cytokines (IL-1, IL-6, TNF and CINC) in the left ventricle of the MI group was higher than in the non-infarcted group. There was no modulatory effect of the fish oil on the content of inflammatory cytokines in infarcted ventricles compared with MI group. In the MI + SO group, an increase of 3 times in the concentration of these cytokines in ischemic ventricle relative to the MI group was found. Fish oil supplementation increased the concentration of glycogen content (2.7 times) and ATP (1.7 times) in non-infarcted left ventricle, relative to the control group. Supplementation with soybean oil did not alter the metabolic parameters.

Conclusions:
Supplementation with fish oil favorably affected energy metabolism possibly by increasing glycogen and ATP content in the left ventricle and protected it from ischemic injuries with no change in the inflammatory response.

Keywords: infarct size, fish oil, soybean oil, myocardial, infarction

Financial Support: CAPES, CNPq, FAPESP, NATURALIS and UNEMAT

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Resumo:16-062

ACTIVATION OF RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM BY ANABOLIC STEROID AND ITS DELETERIOUS EFFECTS ON THE EXERCISE-INDUCED CARDIOPROTECTION AGAINST ISCHEMIA/REPERFUSION INJURIES

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Objectives:

Coronary artery disease is the major cause of morbidity and mortality in the world. Exercise inactivity is one of the risk factors associated to this disease. Since the decade of 1970s, aerobic exercise had been purposed to prevent cardiovascular diseases. Studies in animal models have reported exercise-induced cardioprotection against ischemia/reperfusion injuries and its impairment by chronic use of anabolic androgenic steroids (AAS). This study aimed to investigate the role of the renin-angiotensin-aldosterone system on the impairment by AAS of the exercise-induced cardioprotection.

Methods and Results:

Fifty male Wistar rats were randomized in six experimental groups: Sedentary (S, n = 5), Trained (T, n = 10), AAS-treated Sedentary (AS, n = 5), AAS-treated Trained (AT, n = 10), AT plus spironolactone (ATS, n = 10) and AT plus losartan (ATL, n = 10). During 8 weeks, the trained rats were exercised on electrical treadmill (speed of 10-16 m/min, 15-60 min/day, 5 days/wk) and the treated rats received AAS (nandrolone decanoate; 10 mg/kg/week) spironolactone or losartan: 20 mg/kg/day. After, the isolated hearts were perfused with Krebs solution in a Langendorff apparatus, and subjected to a ischemia/reperfusion protocol (30 min of global ischemia and 60 min of reperfusion). Cardioprotection was assessed by evaluation of the postischemic recovery of left ventricular developed pressure (LVDP) and the infarct area. In the molecular study, RT-PCR was used to assess changes in the genic expression of angiotensinogen, angiotensin converting enzyme (ACE), subtype 1 of angiotensin II receptor (AT1-R), mineralocorticoid receptor (MR), and subunits (SUR 2a, Kir 6.1 and Kir 6.2) of ATP-sensitive potassium channels (KATP).

Results are expressed as mean ± SEM (p < 0.05 as significative). After global ischemia, hearts of T, ATS and ATL groups recovered about 67% of basal LVDP, whereas AT, S and AS animals recovered only about 20%. Infarct area was lower in the T, ATS and ATL groups compared to the others three groups (S, AS and AT). The relative mRNA content of angiotensinogen and ACE did not change, but AT1-R mRNA was increased significantly in AT compared to T, ATS and ATL groups. MR mRNA of AT group was increased relative T, ATS and ATL groups. The mRNA of the regulatory subunit of KATP (SUR2a) was reduced in AT group compared to T, ATS and ATL. The Kir6.1 mRNA was decreased in AT group compared to T and ATL, but not different of ATS group. ATL hearts had Kir6.1 mRNA increased compared to the others groups. In other hand, Kir6.2 mRNA was increased in AT hearts compared to T, ATS and ATL groups. In ATL hearts, the mRNA content of Kir6.2 was higher than that of T and ATS groups.

Conclusions:

The present results suggest that chronic treatment with AAS impairs the exercise-induced cardioprotection by changes of AT1-R, MR and KATP expression, and the block of MR and AT1 receptors by spironolactone and losartan, respectively, prevented the AAS-induced deleterious effects on cardioprotection.

Keywords: Anabolic Steroid, Cardioprotection, Ischemia/Reperfusion Injuries, Physical Exercise, Renin-Angiotensin-Aldosterone System

Financial Support: FAPERJ, CNPq and CAPES.

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Resumo:16-063

RIGHT VENTRICLE CONTRACTILITY 7 AND 60 DAYS AFTER MYOCARDIAL INFARCTION IN RATS WITH OR WITHOUT HEART FAILURE.

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Objectives:

Aim: The aim of this study was to assess right ventricle (RV) contractility and proteins involving in calcium transient early (1 week) and late phase (8 weeks) in rats with or without heart failure (HF) after myocardial infarction (MI).

Methods and Results:

Methods: MI was generated by ligating the left anterior descending artery. In sham-operated rats the ligation was placed but not tightened. One and 8 weeks after surgery, rats were anaesthetized for hemodynamic measurements. After that hearts were excised and strips from RV were removed and attached to an isometric transducer and superfused at 30ºC with Krebs solution, stimulated at 0.5 Hz and 80 mV. Infarction area was evaluated using transillumination. The planimetry was used to analysis of the infarction area in relation to the total area of the left ventricle. Only MI-rats with scar size between 20 to 35% of the LV were included for analysis. The experimental protocols were approved by the local animal ethics committee (CEUA-EMESCAM). Results: The Increment of LVEDP (left ventricle end diastolic pressure), lung weight and body weight ratio (LW/BW) and right ventricle to body weight ratio (RV/BW) was used to divide MI rats with (HF) and without (Inf) classical signal of HF. The scar size was not different between groups (1 week: Inf=32±3 and HF=33.7±2.2; 8 weeks: Inf=26.5±1.1 and HF=25±0.9%; n=9 in each group) neither was correlated to signals of HF. The scar size was not correlated with the HF signals: LVEDP, LW/BW and RV/BW neither with RV contractility. The RV inotropic response to Ca2+ and Isoproterenol were preserved in the HF group one week after MI and reduced at 8 weeks (P

Conclusions:

Conclusion: These results suggest that increased SERCA-2a expression contribute to maintain RV contractility in Inf group. The damage of RV in HF group at 8 weeks should involve the increment on PLB/PLBP ratio.

Keywords: contractility, heart failure, right ventricle, myocardial infarction

Financial Support: FAPES/FUNCITEC, CAPES, CNPq

Resumo:16-064

DEVELOPMENT OF CHAGASIC CARDIOMYOPATHY MODEL IN CHIMERIC MICE

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Objectives:

Chagas’ disease caused by Trypanosoma cruzi is one of the main causes of death due to heart failure in Latin American. About 30% of chagasic individuals develop a chronic chagasic cardiomyopathy. Although significant progress has been achieved, so far, there is no effective treatment. Animal models of cardiac dysfunction are still a great challenge to study cell based therapies. The aim of this study is to establish an experimental model of Chagas’ disease in chimeric mice for further studies related to bone marrow cells contribution to the damage heart.

Methods and Results:
Twenty two C57BL/6 mice, two-month-old male and female were used in the experiments. The animals underwent myeloablation and subsequent graft of 1x10^6 bone marrow mononuclear cells from animals that carry the gene for green fluorescent protein (GFP). Thirteen animals were infected with 3x10^4 trypomastigotes of Brazil strain by intraperitoneal route and had their parasitaemia monitored during the acute phase twice a week. The remaining animals were used as controls. The evaluation of cardiac performance was monitored by electrocardiography (ECG) and treadmill monthly. Electrocardiography was performed using the Bio Amp PowerLab System, recording the bipolar lead I. The ECG analysis included P-wave duration, PR interval, PR segment, QRS complex, QT interval, Bazett corrected QT (QTc) and heart rate. The exercise test was performed following the protocol of exhaustion where mice exercised at 5 different velocity (0.1, 0.2, 0.3, 0.4 e 0.5 m/s) with increasing velocity after 5 min of exercise. Throughout the study, sections of paraffin-embedded tissue from the animals that died were stained by standard hematoxylin-eosin (H&E) and picrosirius to observe inflammation and nests of amastigotes and fibrosis respectively, by optical microscopy. The peripheral blood leukocytes were examined in these chimeric animals by flow cytometry. A statistical analysis used was the test one-way ANOVA with Bonferroni’s post test and t-test student. The repopulation of bone marrow was analyzed by flow cytometry and only animals (n = 18) that presented 75% of engraftment were included in the study. Two months after infection, 77% of infected animals and 40% of noninfected animals died. The remaining three infected animals were sacrificed. Parasites were observed in peripheral blood of infected animals since fifth day post-infection with a parasitemia peak around day 27 of infection. ECG evaluation revealed no alteration although it was observed a first degree AV block two months after infection. A significant difference was observed in exercise testing one month after infection in infected animals compared to control mice at parameters of distance.

Conclusions:

In conclusion, C57BL/6 chimeric mice infected with Brazil strain parasites develop a significant inflammation in heart tissue sections and changes in cardiac function two months post infection, showing that these mice are susceptible to infection. However, this parasite load shows a rise of mortality in these animals when administrated by i.p route.

Keywords: Chagas’ disease, Chimeric mice, cardiac dysfunction

Financial Support: CNPq, Capes, FAPERJ, Ministério da Saúde

LIPID TRANSFERS TO HDL PREDICTS THE PRESENCE OF CORONARY HEART DISEASE IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Objective:

Systematic assessment of metabolic and functional aspects of HDL is important for the understanding of HDL anti-atherogenic role. Plasma lipoproteins exchange lipids constantly, a process facilitated by cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP). Lipid transfer between HDL and the other lipoproteins is a crucial step in HDL metabolism and reverse cholesterol transfer. The purpose of this study was investigate whether the susceptibility of patients with type 2 diabetes mellitus (DM2) to develop CAD is related with alterations in lipid transfers to HDL.

Methods and Results:

83 patients with DM2 and CAD (DM2-CAD) and 82 with DM2 (DM2 group) were studied. They were of both genders, aged 40-
80 yrs. Fasting plasma samples were incubated for 1h at 37°C with a donor artificial nanoemulsion labeled with 3H -cholesteryl-esters and 14C –phospholipids or 3H -triglycerides and 14C- free-cholesterol. Radioactive lipids transferred from the donor nanoemulsion to HDL were quantified in the supernatant after chemical precipitation of non-HDL fractions and nanoemulsion. HDL size was measured by laser light scattering, using a zeta potential analyzer. In DM2-CAD, LDL-cholesterol and triglycerides were higher than in DM2 patients, but HDL- cholesterol was lower. DM2-CAD showed diminished transfer to HDL of free cholesterol (DM2-CAD= 4.23±0.2426; DM2= 5.66±0.1384, p

Conclusions:

The reduction of free-cholesterol transfer to HDL may hinder cholesterol esterification and reverse cholesterol transport. Alterations in triglyceride and cholesteryl-ester transfer may affect lipoprotein stability and those disturbances in HDL metabolism may have facilitated the atherogenesis process in DM2-DAC.

Keywords: Diabetes mellitus, Doença arterial coronária, HDL, Transferência de lípides

Financial Support: CAPES, FAPESP

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Resumo:16-066

ANALYSIS OF THE MYOCARDIUM TISSUE ORGANIZATION AND ULTRASCTRUCTURE OF ANIMALS VACCINATED WITH RECOMBINANT PLASMIDS THAT EXPRESS PORTIONS OF THE MUSCARINIC RECEPTOR SUBTYPE M2.

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Objectives:

Dilated cardiomyopathy (DCM) has been associated with changes in the functioning of signaling pathways initiated by activation of protein coupled to G proteins and their membrane receptors, particularly the muscarinic receptor (M2AChR). Deteriorations in cardiac function of DCM have been currently related to defects in the cytoskeleton. Particularly, an altered distribution pattern of membrane cytoskeletal proteins (or costameric proteins related to focal adhesion regions of spreading cells) also found in cardiomyocytes, were reported in patients with DCM (Cell Tissue Res. 294:449-460, 1998, Heart disease 6th ed, Saunders. 503-33, 2001). These changes, characteristics of myocardial remodeling, parallels with the deterioration of the functional capacity of the heart in long term and the consequent appearance of signs and symptoms of heart failure and overall cardiac macroscopic remodeling.

Methods and Results:

In this work we submit groups of mice BALB / c to an immunization scheme with plasmids containing cDNA coding for the second extracellular loop (o2) and the third intracellular loop (i3) of muscarinic receptor subtype M2 and evaluated the presence of signs of cardiomyocyte alterations induced by autoantibodies through histological analyses. We also have a group vaccinated with an empty plasmid (pcDNA3) and one non-vaccinated. We examined the general appearance of these cells by transmission electron microscopy, to figure out if there is a displacement or structural change of mitochondria, nucleus, T tubules and specifically of the sarcomere Z disks morphology. The labeling pattern of proteins important in anchoring the sarcomere cytoskeleton to the membrane such as alpha-actinin, or of desmin which acts interconnecting the cytoskeletons from Z discs to the intercalated discs and also of caveolin, that arranges the distribution and positioning of membrane bound G coupled receptors; were analyzed by immunolocalization at the ultrastructural level (electron microscopy) and also by immunofluorescence (light microscopy) for tissue level analysis. The results showed the expected distribution pattern of these elements, with alpha-actinin and desmin localized in the Z line and caveolin in the cell membrane, a preliminary step towards understanding the results.
obtained in experimental animals. On electron microscopy, we could not detect changes in tissue organization and ultrastructure of the myocardium of animals immunized with recombinant plasmids that express portions of M2AChR, when compared to the control group.

Conclusions:

These qualitative analyses did not show the changes described in literature for DCM animal models or patients, which suggests that the DCM induced by this experimental vacination was not sufficient to generate changes at the structural level. New samples will be analyzed to confirm the absence of ultrastructural modification suggested in this preliminary examination. Additionally, new immunofluorescence experiments are underway in experimental animals in order to verify the tissue pattern of cytoskeletal organization and compare to the one identified in the negative controls (non-vaccinated) and also in dystrophic DCM animals.

Keywords: MUSCARINIC RECEPTOR, DILATED CARDIOMYOPATHY, ULTRASTRUCTURE, MYOCARDIUM TISSUE ORGANIZATION

Financial Support: FAPERJ, CNPQ

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Resumo:16-067

EFFECTS OF G-TYPE IMMUNOGLOBULIN’S FROM CHRONIC CHAGASIC PATIENTS IN A PHARMACOLOGICAL MODEL OF DRUG-ACQUIRED TYPE-2 LONG QT SYNDROME

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Objectives:

The presence of functional autoantibodies (Ab) able to activate β-adrenergic (Ab-β), M2 muscarinic (Ab-M) and both (Ab-Mβ) G protein-coupled receptor in chronic chagasic patients (CChP) has been described. They were also associated with cardiac electrical disorders modulating different cardiac ion currents. Previous works of our groups shown the arrhythmogenic effect of these autoantibodies in health isolated rabbit heart. However, since was described longer QT and QT interval in chronic chagasic patients, the objective of this study were: 1- to induce a pharmacological model of drug-acquired Type-2 long QT syndrome (LQTS2) in isolated rabbit heart, and 2- to evaluate whether the presence of Ab-M and Ab-Mβ increase the arrhythmic risk in this LQTS2 model.

Methods and Results:

Hearts from female and male rabbits were cannulated in a Langendorff apparatus and perfused with modified Tyrode solution (Tyr). The electrocardiogram in this preparation was recorded in different conditions. The I(Kr) blocker, E-4031 (5 μM), was used to elicit LQTS2 model. Sera from 10 CChP previously functionally characterized as Ab-M (n=2) or Ab-Mβ (n=8) were tested in a LQTS2 model in isolated rabbit hearts to study ventricular repolarization. The follow protocol was used: 30 min perfusion with Tyr, followed by 30 min perfusion with Tyr + E-4031 (5 μM); E-4031 + serum (1:100 v/v), and washed by 30 min with E-4031 perfusion. QT QTc and RR interval were measured. Statistical analyses were made using one-way ANOVA. The data are expressed as mean ± SEM. In the presence of E-4031 was observed a significant longer QT (Control: 208,3 ± 4,029 vs. E-4031: 314,0 ± 13,53 ms; P<0.05), QTc (E-4031: 384,1 ± 62.5 vs. E-4032/Ab-Mβ: 379,6 ± 34.4 ms; Washout: 370.5 ± 62.7 ms P>0.05) and RR (E-4031: 643,5 ± 95.9; E-4032/Ab-Mβ: 613.8 ± 86.6 ms; Washout: 652,1 ± 134.1 ms P>0.05) interval in this model. However, the preliminary data obtained with Ab-M (n=2) showed reversible increase of RR (E-4031: 519,5 ± 16.7; E-4032/Ab-Mβ: 683,4 ± 116.7 ms; Washout: 526.3,9 ± 14.5 ms), QT (E-4031: 350,5 ± 38.7; E-4032/Ab-Mβ: 479,9 ± 121.6 ms; Washout: 357,4 ± 43.6 ms) and QTc (E-4031: 486,2 ± 33.6; E-4032/Ab-Mβ: 570,2 ± 61.7 ms; Washout: 491,8 ± 58.5 ms) interval, in the LQTS2 model.
Conclusions:

In the present work was possible to induce a LQTS2 model in isolated rabbit heart. Also, was observed that, even in preliminary data, Ab-M reversibly increased ventricular repolarization duration reflected by longer QT and QTc interval in the same model. However, the Ab-Mβ did not modulate the electrocardiogram parameter in the LQTS2 model. In all experiment was not observed any arrhythmic event.

Keywords: electrophysiology, heart failure, autoimmune disease, Chagas disease, autoantibodies

Financial Support: FAPERJ, CNPq e CAPES.

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Resumo:16-068

ROLE OF THE RENIN-ANGIOTENSIN SYSTEM IN MODULATION OF THE MYOCARDIAL DYSFUNCTION INDUCED BY FEMALE SEXUAL HORMONES DEFICIENCY.

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Objectives:

It has been described that female sexual hormones present a protector role to the cardiovascular system. Studies demonstrate that young oophorectomized rats, 60 days after oophorectomy surgery, present a contractile myocardial dysfunction, although the mediator mechanisms remain still unknown. Thus, the goal of this study is to analyze the role of the Renin-Angiotensin System as a possible mediator of this contractile myocardial dysfunction.

Methods and Results:

Eight-week-year-old female Wistar rats have been split in 4 groups: SHAM (control treated with placebo,N=10); OVX (oophorectomized treated with placebo, N=8); OVX+LOS (oophorectomized treated with Losartan 15 mg/kg/day, i.m., N=8); and OVX+E2 (oophorectomized treated with Estrogen 1 mg/kg/week, s.c., N=8). After 60 days, under effect of the anesthesia with Tiopenthal (50 mg/kg,i.p.), experiments were conducted based on preparations of the left ventricle’s papillary muscles in isometric contraction. Functional and weight parameters of the myocardial contraction were measured by means of the following protocols: [1] variation of the extracellular Calcium (Ca2+) concentration (0.62, 1.25, 2.5 and 3.75 mM); and [2] β-adrenergic response on increasing concentrations of Isoprenaline (Iso 10-7 — 10-2 M). Biochemical parameters were also analyzed, by means of the Western Blot method, in order to quantitate the expression of SERCA2a, of Fosfolambam, of Phosphorylated Fosfolambam (PLB-Ser and PLB-Thr) and of Angiotensin II receptors subtypes AT1 and AT2. The activity of the plasmatic Angiotensin Converting Enzyme (ACE) and the plasmatic levels of Malondialdehyde (MDA) were also measured. The quantification of superoxide anion in situ (fluorescence) and the expression of the subunit p22phox from NADPH oxidase (immunohistochemistry) were realized, as well. Statistical analysis was performed with ANOVA one-way or two-way, post-hoc Bonferroni. Body weight was increased in the OVX group, compared to SHAM, and was normalized in OVX+LOS and OVX+E2 (SHAM=257±5; OVX=310±9*; OVX+LOS=277±3#; OVX+E2=251±10# g; *#p

Conclusions:

The myocardial dysfunction induced by oophorectomy was reversed by 60-day treatment with Losartan. It suggests that there is a role of the Renin-Angiotensin System in the modulation of the Estrogen’s cardiovascular effects, probably due to an Angiotensin II-induced overproduction of superoxide anion in oophorectomized animals, which could be one of the basic mechanisms leading
to myocardial dysfunction.

Keywords: Renin-Angiotensin System, Estrogen, Losartan, Myocardial Dysfunction, Heart Failure

Financial Support: CNPq; FAPES/FUNCITEC

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Resumo: 16-069

STUDY OF THE FUNCTION OF VOLTAGE-GATED CALCIUM CHANNEL ON HYPTERTENSIVE RATS VAS DEFERENS IN DIFFERENT AGES


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Objectives:

Previous studies of our laboratory indicated alterations of intracellular calcium ([Ca+2]i) homeostasis in smooth muscle and chromaffin cells of Spontaneously Hypertensive Rats (SHR). Several mechanisms are involved, including alteration of voltage-gated calcium channel (VCC). The high blood pressure comes in SHR between 12º and 16º week of life. However, it’s probable that occur changes in the activity of VCC even before appearance of phenotypic characteristic related to increase of blood pressure. In addition, this hypothesis was not considered by scientific literature. Therefore, we decided to study the role of VCC in vas deferens of young (3 – 4 week) and adult (16 – 20 week) SHR.

Methods and Results:

We used Normotensive Wistar Rats (NWR) and SRH of different ages (young and adults). The vas deferens were removed and mounted in isolated organ bath. We made four time-response curves for Noradrenaline (NA) (0.1 mM), KCl (80 mM) and ATP (1 mM) in the presence or absence of Nifedipine (0.3 to 3 µM) and Mibefradil (0.1 to 30 µM). The contractile response was measured and corrected by weight tissue. We observed that Nifedipine (type-L VCC blocker) was able to block the phasic (PR) and tonic response (RT) to NA and KCl, as well as ATP-induced contraction in young and adults NWR. However, the PR to NA in young SHR and TR to NA in adult SHR were not blocked by Nifedipine. Our data showed that 3 µM of Nifedipine block 60% of PR and TR to NA in young and adults NWR, without changes in the inhibition percentage to RF and RT to NA in young SHR. The same was observed in TR to NA in adult SHR. The PR to KCl was blocked more intensively by Nifedipine at concentration of 0.1 µM in vas deferens of young SHR when compared with adult SHR and young and adult NWR. We also found that TR was inhibited in the same way for Nifedipine, considering the tested concentrations. The ATP-induced contraction showed an increased sensibility in young animals when compared with adults, but the block effect of Nifedipine was the same between groups. Analyzing the effect of Mibefradil (type-T VCC blocker), we found absence of effect in the PR and TR for NA and KCl, as well as for ATP-induced contraction in vas deferens of young and adults NWR and SHR.

Conclusions:

From our data we suggest that physiological changes in VCC of the young SHR appear even before of the phenotypic characteristic related to hypertension. There are also differences on contractile response for NA, KCl and ATP between young and adult NWR as well as between young and adult SHR.

Keywords: Different ages, Spontaneously Hypertensive Rats, Vas deferens, Voltage-gated calcium channel, Voltage-gated calcium channel blockers
AEROBIC EXERCISE TRAINING IMPROVES PLASMATIC INFLAMMATORY PROFILE AND HEMODYNAMIC FUNCTION IN CHRONIC HEART FAILURE RATS.

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Objectives:

Chronic heart failure (CHF) is a syndrome characterized by a left ventricular (LV) dysfunction and marked physical capacity reduction. Hemodynamic dysfunction and high levels of plasmatic pro-inflammatory cytokines are associated with severity and progression of disease. Aerobic exercise training has been considered an important non pharmacological therapy in the treatment of CHF. The aim of the present study was to evaluate the effects of 8-week aerobic exercise training on hemodynamic function, cardiac hypertrophy and plasmatic levels of IL-6, TNF-α and IL-10 in CHF rats subsequent to myocardial infarction.

Methods and Results:

Male Wistar rats (220-270g) were submitted to myocardial infarction or sham surgery and assigned into four groups: CHF trained (n=8); CHF sedentary (n=8), Sham trained (n=8) and Sham sedentary (n=8). Four weeks after myocardial infarction or sham surgery, rats were submitted to 8-weeks of aerobic training in treadmill running (60 min/day, 5 times per week, 15m/min). At the end of exercise program, under anesthesia, the hemodynamic variables were recorded and the blood samples were collected to analysis of cytokines (IL-6, IL-10 e TNF-α) by ELISA. The cardiac hypertrophy was evaluated by left ventricular weight/body weight (LVW/BW) ratio. Statistical analysis was carried out by two-way ANOVA followed by Tukey test as post-hoc. Data are expressed as mean±SD. The CHF trained group demonstrate an important reduction in left ventricular end-diastolic pressure (LVEDP) compared to CHF sedentary group (21.1±8.7 mmHg vs 30.1±7.7 mmHg, P

Conclusions:

In conclusion the 8-week aerobic exercise training improves the inflammatory profile and cardiac function in CHF rats.

Keywords: Heart failure, Exercise, inflammatory

Financial Support: Capes and UFCSPA
Objectives:

It has been suggested that following a myocardial infarction, lymphocytes react with heart components and that this autoimmunity would be disadvantageous to heart healing. In the present work we investigated whether ingestion of heart proteins and the development of tolerance may modify the course of post-infarction myocardial repair.

Methods and Results:

Infarction-like myocardial lesions were induced in Wistar rats by injection of high doses of isoproterenol (140 &micro;mol/kg). The healing process was accompanied morphologically and functionally for 60 days. Cardiac function was evaluated using isolated and perfused heart (Langendorff) preparation. At day 14 after isoproterenol, lymphocytes collected from mediastinal lymph nodes proliferated when exposed in vitro to myocardial homogenate, indicating an autoimmunity response to cardiac components. In contrast, lymphocytes collected from rats turned tolerant to heart proteins, by means of oral exposure to cardiac antigens, did not proliferate. Furthermore, this group presented a milder inflammatory infiltrate, less collagen deposition, and improved cardiac performance when compared to animals that ingested saline before myocardial infarction.

Conclusions:

The present findings suggest that myocardial infarction induces the development of an autoimmune reactivity directed to cardiac components that is associated with a fibro-proliferative response and loss of function. If this event is modified by the establishment of immunological tolerance to heart proteins, myocardial healing proceeds with milder inflammation and fibrosis.

Keywords: Myocardial Infarction, Autoimmunity, Oral Tolerance, Cardiac antigens, Cardiac Remodelling

Financial Support: Capes, CNPQ, FAPESC

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Resumo:17-202

TOXICOPHARMACOLOGICAL EVALUATION OF FOSFORILHYDRAZONES COMPOUNDS

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² Depto de Química/ Instituto de Ciências Exatas, UFRRJ

Objectives:

To evaluate the pharmacological and acute toxicity of FH compounds through specific methods for the determination of safety for their use in veterinary medicine.

Methods and Results:

Antinociceptive activity was evaluated by the methods of accumulated writhing (a.w.) induced by acetic acid (J. Pharm. Exp Ther. 125, 237 and Fed.Proc., 18, 412, 1959, 1959) and toxicity was assessed by method of acetylcholinesterase activity (Biochem. Pharmacol. 7, 88, 1961). In the method of writhings, 1.2% acetic acid is administered intraperitoneally in mice and the
The number of contortions is counted as accumulated writhings during 30 minutes. In this method, we used the following groups: tap water, xantan gum 0.2%, indomethacin (10mg/kg) and test groups FH1, FH2, FH3 and FH4. Only the groups treated with FH3 and FH4 caused inhibition when compared with the water group (29.33 ± 2.64 a.w.). The FH3 group showed 36% inhibition (18.83 ± 4.22 a.w., P

Conclusions:

Therefore, our results show an antinociceptive action without inhibition of cholinesterase of the compounds FH3 and FH4, central or peripheral nature of which will be evaluated in later trials. The compounds FH1 and FH2 showed no antinociceptive activity, but FH1 was capable of causing cholinesterase inhibition, which directs us to conduct future trials with this drug to see how to use the same insecticide.

Keywords: fosforilhydrazones compounds, pain, toxicopharmacological evaluation

Financial Support: FAPERJ, DCF/IB/UFRRJ

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Resumo:17-203

INHIBITIVE EFFECTS OF HYDROGEN SULFIDE AND INOS ON OXIDATIVE STRESS IN ALLERGIC MICE LUNGS

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2 Universidade São Francisco, USF

Objectives:

Oxidative stress plays an important role in the pathogenesis of asthma due to an increase in reactive oxygen species (ROS). It has been suggested that H2S, proposed as an antioxidant, and the selective inhibitor of iNOS (1400W) have an important anti-inflammatory role in lung diseases. Our preliminary results showed a reduction in eosinophil infiltration in the bronchoalveolar lavage (BAL) of allergic mice treated with 1400W or H2S donor (NaHS) at 48h and 144h after OVA challenge. We, herein, investigated the effect of H2S and 1400W on oxidative stress in airway inflammation.

Methods and Results:

Methods: Balb/c mice were sensitized subcutaneously at day 0 and day 7 with 400 µl of a suspension of 100 mg ovalbumin bound to 4mg of aluminum hydroxide. Seven days after the second sensitization, the animals were briefly anesthetized with halothane and challenged intranasally with 10 µg of OVA in 50 µl of sterile saline. These OVA were performed twice a day for 4 consecutive days. Groups of animals were treated with 1400W (1.0 mg/kg in 300 µl of sterile saline) intraperitoneally (i.p.) 2 hours before each OVA challenge. In other groups of mice the NaHS (14 µmol/kg) was given i.p. 30 min before each OVA challenge. The control group received only sterile saline i.p. At 24, 48, 96, 120 and 144 hours after OVA challenge, mice were sacrificed and lungs were removed, flash frozen in liquid nitrogen and stored at -80°C. Lung tissue was homogenized in buffer containing a protease inhibitor cocktail and then centrifuged at 800 x g for 10 min at 4°C. The supernatant was used to analyze aconitase activity, a tricarboxylic acid (TCA) cycle enzyme easily inactivated by O2•-, and thiobarbituric acid reactive substances (TBARS). All experiments were approved by the animal ethics committee of USF (protocol 0021108). Results. No changes were detected in TBARS or aconitase activity in the lungs of allergic mice treated with NaHS or 1400W at 24h and 96h after OVA-challenge. NaHS treatment produced an increase of 132% and 49% in aconitase activity at 48h and 144h, respectively. The analysis showed that TBARS was decreased by about 50% in the lungs of NaHS or 1400W-treated mice at 48h and 144h after OVA-challenge, as compared to control mice.
Conclusions:

Treatments with NO synthesis inhibitors or an H2S donor produced similar effects on TBARS and on aconitase activity in the lungs of allergic mice. The increase in aconitase activity and decrease in TBARS levels suggest that both treatments decreased oxidative stress, which could account for the reduction in eosinophil lung infiltration previously observed in 1400W and NaHS-treated mice at 48h and 144h after OVA-challenge.

Keywords: hydrogen sulfide, 1400W, Inflammation, Oxidative Stress

Financial Support: FAPESP and CNPq

**SIMVASTATIN: TOPICAL ANTI-INFLAMMATORY ACTIVITY IN ANIMAL MODELS**

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Objectives:

The 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase inhibitors or statins represent a class of drugs that effectively lowers cholesterol levels in serum (Weitz-Schmidt, 2002; Arnaud et al., 2005). Besides his activity for lowering cholesterol, statins have pleiotropic effects, including promotion of vasculogenesis, prevention of bone loss, and immunomodulating and anti-inflammatory effects (Weitz-Schmidt, 2002). The use of topical or systemic statins may be useful in treating inflammatory skin diseases, especially those characterized by migration of activated leukocytes in the skin, such as psoriasis (Namaz, 2004). Otuki et al. (2006) demonstrated that topical application of simvastatin has an anti-inflammatory action in acute irritant contact dermatitis induced by croton oil. Otuki et al. (2006) demonstrated that topical application of simvastatin has an anti-inflammatory action in acute irritant contact dermatitis induced by croton oil. Once your anti-inflammatory activity was evaluated in the skin of mice (Otuki et al., 2006), there is a need for a more thorough assessment of some statins.

Methods and Results:

Female Swiss mice (25-30 g, N = 5-8) were used and ear edema was induced by topical application of 12-O-tetradecanoylforbol acetate (TPA). Edema was measured by the increasing of ear thickness, measured 6 h after of TPA (2.5 μg/ear) application in the acute model. The chronic inflammatory process was induced by multiple applications of TPA (2.0 μg/ear) for 9 days on alternate days. Topical treatment with ointment of simvastatin or dexamethasone started on the 5th day of experiment. Samples of ear tissue (6 mm of diameter) from acute and chronic model were collected, weighed and analyzed using the following parameters: histology, myeloperoxidase (MPO) enzymatic activity and ear weigh. Procedures have been approved by Institutional Ethics Committee under the number 390/UFPR. Results: Simvastatin in the acute model was able to reduce edema in concentration-dependent manner with an ID50 of 24 (11 to 56) mM and an inhibition of 70 ± 4% at a concentration of 119 mM, although the activity of MPO was reduced by simvastatin at all concentrations tested with an Imax 53 ± 4%. In the chronic model simvastatin was able to reduce edema formation in 16 ± 3% and weight of the ear in the 11 ± 2% after 7th day of induction of inflammation when used at a concentration of 1 %. Histological analysis showed the reduction of swelling and decrease in leukocyte infiltration which reaffirms the anti-inflammatory.

Conclusions:

The results confirm the anti-inflammatory activity of simvastatin when applied topically in both acute and chronic processes that
favor a possible clinical use of statins topically in diseases such as psoriasis.

Keywords: Topical Inflammation, skin, Simvastatin

Financial Support: CAPES, CNPq and REUNI.

THE INVOLVEMENT OF CENTRAL OPIOID MECHANISMS IN THE PROTECTIVE EFFECT OF ENDOGENOUS TESTOSTERONE ON TEMPOROMANDIBULAR JOINT NOCICEPTION DEVELOPMENT IN RATS.

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Objectives:

We have recently demonstrated that endogenous testosterone protects males from developing temporomandibular joint (TMJ) nociception. To investigate some of the mechanisms underlying this protective effect of testosterone, we asked whether it is mediated by the activation of central opioid receptors.

Methods and Results:

Intact, gonadectomized and sham gonadectomized male Wistar rats (230-300g) were used and all experimental procedures were approved by the Ethics Committee in Animal Research at the UNICAMP. TMJ injection of 0.5% formalin was used as nociceptive stimulus. The nociceptive behavior was quantified for 45 minutes and used as a quantitative nociceptive behavior measure that was defined as the cumulative total number of seconds that the animal spent rubbing the orofacial region asymmetrically with the ipsilateral fore or hind paw plus the number of head flinches counted during the observation period. (Pain, 94: 185, 2001). Administration of the opioid receptor antagonist naloxone or the combination of the mu-opioid receptor antagonists CTOP (30 microgramas/10 microlitros) plus the delta-opioid receptor antagonist Naltrindole (90 microgramas/10 microlitros) or of nor-binaltorphimine (90 microgramas/10 microlitros) plus Naltrindole (90 microgramas/10 microlitros) did not affect TMJ 0.5% formalin-induced nociception in intact, sham gonadectomized and gonadectomized rats.

Conclusions:

These findings suggest that the protective effect of endogenous testosterone on TMJ pain development depends on the release of endogenous opioids and on the subsequent activation of mu and kappa opioid receptors in the central nervous system. Selective activation of individual receptor subtypes is insufficient, the co-activation of mu - and k-opioid receptors is necessary to mediate the protective effect of endogenous testosterone.

Keywords: Opioid Receptors, Testosterone, TMJ pain

Financial Support: CNPQ

ANTIPRURITIC EFFECT OF PETROLEUM ETHER FRACTION AND OF TRITERPENES MIXTURE ISOLATED
FROM LECYTHIS PISONIS CAMB. LEAVES

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Objectives:

To examine the antipruritic activity of petroleum ether fraction (EF) and of triterpenes mixture (oleanolic and ursolic acids) (TM) from Lecythis pisonis leaves in compound 48/80 (C-48/80)-induced scratching behavior in mice, to verify the role of endogenous opioids and their effects on C-48/80-induced degranulation of rat peritoneal mast cells.

Methods and Results:

Male Swiss mice (25-30g, n=8) fasted 24h, were treated with control (0.9% saline, 10 mL/kg, po), EF (50, 100, 200 mg/kg, po), TM (12.5, 25 and 50 mg/kg, po), cyproheptadine (CYPRO,10 mg/kg, ip) or morphine (MOR, 5 mg/kg, ip) and after 60 min or 30 min the C-48/80 (100μg/100μL) was injected subcutaneously into rostral part of the back (50μL). Control mice received a similar quantity of normal saline injection instead. The scratching behavior was observed for 20 min. Only scratching of nose by fore- or hind paws and injections site by hind paws was counted (Kuraishi, Y., Eur J. Pharmacol. v.275 p.229, 1995). In order to verify the possible role of endogenous opioids in the suppressive effect of EF and TM mice were pretreated with saline, naloxone (NLX, 2 mg/kg ip), MOR (5 mg/kg, ip), EF (200 mg/kg po) or TM (50 mg/kg po) alone or in their combinations with NLX prior to the injection of C-48/80 (100μg/100mL). While FE or TM was administered 1h before, NLX and MOR were given 30 min prior to pruritogen. Wistar rats (male, 180-200g, n=7) were treated orally with vehicle (saline 0.9%), EF (200 mg/kg), TM (100 mg/kg) or ketotifen (1 mg/kg). 60 min later, the animals were killed and pieces of mesenteric vascular plexus were collected from respective groups into each for the glass tubes containing Ringer fluid (10 mL). Mast cell degranulation was induced by incubation of tubes containing C-48/80 (0.4μg/mL). The same volume of distilled water was added to tubes containing mesenteric tissue obtained from normal control rats that received only the vehicle. After 30 min the mesenteric tissue was mounted on glass slide and stained with toluidine blue (0.1%) for the observation of mast cells by light microscopy. The number of total mast cells presented and percentage of cells degranulated were noted. Mice that received EF (100 and 200 mg/kg) or TM (25 and 50 mg/kg) demonstrated potent inhibition of C-48/80-induced scratching (30.50±2.32; 10.17±1.70; 37.80±3.77 and 24.0±4.51, respectively) when compared to the control group (58.92±3.42). CYPRO (10 mg/kg) also caused marked inhibition of scratching response (8.33±2.65). MOR (5 mg/kg), EF (200 mg/kg) and TM (50 mg/kg) pretreatments resulted in significant suppression of scratching behavior mice (3.22±1.64; 10.17±1.70 and 24.0±4.50, respectively). Although NLX alone showed no significant influence, it could completely reverse the MOR effect (55.0±6.38). The suppressive effect of EF and TM was partially antagonized by NLX (42.80a±2.80 and 45.33±3.10), when compared to control group (53.0±3.16). Treatment with EF (200 mg/kg), TM (100 mg/kg) or ketotifen (1 mg/kg) significantly reduced the compound 48/80 induced degranulation by extent of 41.82±4.18; 36.33±1.40 and 16.1±1.31%, respectively, when compared to control group (82.2±4.40%). The percent numbers of saline treated normal rats was in the order of 7.3 ± 3.43%.

Conclusions:

The results clearly indicate the antipruritic effect of EF and TM and suggest that this effect may be related to possible involvement of the opioid system and mast cells a stabilizing action on mast cell membrane.

Keywords: Antipruritic activity, Lecythis pisonis , Compound 48/80

Financial Support: UFPI/CAPES

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Resumo:17-207
LYMPHOCYTES TREATED WITH LIPOPOLYSACCHARIDE INHIBIT PLATELET AGGREGATION BY CYCLIC
GMP-INDEPENDENT MECHANISMS. CARDELLI, N.J.A, ANJOS, D.J., ANTUNES, E., MARCONDES, S.
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Dep.Farmacologia, UNICAMP

Objectives:

Lipopolysaccharide (LPS) is currently used as experimental sepsis model since mimics various signals observed in sepsis such as increase of reactive oxygen (ROS) and nitrogen species (RNS) generation by different cells. Excessive production of nitric oxide (NO) and ROS in sepsis modulate different cell function. Some works show that the sepsis severity is associated with the degree of platelet activation. Since leukocytes may interact and cross-talk with platelets in many settings including inflammation, we decided to investigate the role of NO and ROS released by LPS-treated lymphocytes on platelet aggregation.

Methods and Results:

Methods: The present study was approved by the Human Ethics Committee of State University of Campinas (UNICAMP) no CEAA1711-1. Blood from abdominal aorta of male Wistar rats (250-320g) was collected in 3.8% sodium citrate. Two different experimental protocols have been carried out, as follows. In the first protocol, blood was centrifuged at 200g for 15 min to obtain platelet-rich plasma (PRP). Platelet suspension was incubated with saline or LPS (100 µg/ml) for 6 h. Next, PRP was centrifuged (200 g, 15 min), the number of platelets was adjusted to 2x10^8 platelets/ml with Krebs solution and aggregation assays were carried out using ADP (5 µM)-activated platelets. In the second experimental protocol, blood was centrifuged at 50g for 15 min to obtain leukocytes. The leukocyte layer was separated, and the remaining blood was centrifuged at 200 g for 15 min to obtain PRP. The differential leukocyte counts indicated a cell population of 100% lymphocytes. Next, the lymphocytes suspension (500 µl of 5x10^6 lymphocytes/ml) was added to the PRP (2.5 ml of 5-6x10^8 platelets/ml), and incubated with saline or LPS (100 µg/ml) for 6 h. The cell suspension was centrifuged again at 200 g for 15 min, and the supernatant (PRP containing 2x10^8 platelets/ml) was used to aggregation assays. Results: Incubation of platelets with LPS (100 µg/ml) for 6h did not affect ADP (5 µM)-induced platelet aggregation, but the incubation with lymphocytes (5x10^6 lymphocytes/ml) for 6h significantly reduced the aggregation (56±4 % and 25±3% of aggregation in absence and in presence of lymphocytes, respectively, n=6). The inhibitory effect of lymphocytes was increased in presence of LPS (reduction of aggregation by 53% and 80%, in absence and in presence of LPS, respectively). Incubation of NO synthase inhibitors L-NAME (300 µM) or 1400W (100 µM) prevented the inhibitory effect of lymphocytes in presence of LPS. However, the incubation of the cellular suspension in presence of LPS with the guanylyl cyclase inhibitor ODQ (10 µM) did not affect the inhibitory effect on platelet aggregation. Incubation of superoxide anion or peroxynitrite scavenger PEG-SOD (100 U/ml) and epigallocatechin gallate (10 µM), respectively, abolished the inhibitory effect of lymphocytes in presence of LPS on platelet aggregation.

Conclusions:

Conclusion: Our results show that the lymphocytes inhibit platelet aggregation and that effect is potentiated in presence of LPS. The inhibitory effect of lymphocytes in presence of LPS is mediated by NO through cGMP-independent mechanisms, involving peroxynitrite generation.

Keywords: Platelete, Sepsis, LYMPHOCYTES

Financial Support: CNPq

QuebraPagina

Resumo:17-208

EVALUATION OF PERIRADICULAR LESIONS IN RATS WITH DOXORUBICIN-INDUCED
CARDIOMYOPATHY: EFFECT OF ANTIOXIDANT THERAPY

Financial Support: CNPq
Objectives:

Cardiovascular diseases have been recently associated with an increased risk of tooth extraction and complications following dental procedures (Am J Epidemiol. 170; 615, 2009). This study aimed at evaluating whether periapical lesions develops in rats submitted to the doxorubicin model of cardiomyopathy. Furthermore, we have also assessed the therapeutic potential of systemic treatment with the antioxidant agent tempol on apical periodontitis establishment, in either control or doxorubicin-treated rats.

Methods and Results:

Male Wistar rats (150-200 g; N= 5 per group) were used. All the experimental protocols were approved by the local animal ethics committee (CEUA/09/00132). For cardiomyopathy induction, the animals received doxorubicin (1 mg/kg/day), by i.p. route, from day 0 to day 10, in a total dose of 10 mg/kg. Control animals received saline solution (0.9 % NaCl). Tempol (50 mg/kg) or saline solution (10 ml/kg) was orally administered from day 3 to day 10 after initiating doxorubicin treatment. The animals were checked daily to register body weight variations and doxorubicin-related toxicity signs. To induce periapical lesions, the pulps of the mandibular first molars were surgically exposed with a ¼ size round steel bur in high-speed rotation, under constant irrigation, and they were left open to oral cavity for 21 days. The extent of lesions was measured radiographically and compared among the different experimental groups. The rats in the cardiomyopathy groups displayed a progressive weight loss, associated to some visible signs of toxicity such reduced motor activity and piloerection, which were generally diminished in tempol-treated animals. Radiographic data demonstrates that doxorubicin-induced cardiomyopathy resulted in a significant increase in the extension of periapical inflammatory lesions, in comparison to the control group (107 ± 24 %). The treatment with tempol was able to significantly inhibit the apical periodontitis development, in either the control or doxorubicin groups (36 ± 23 % and 38 ± 13 %, respectively).

Conclusions:

Our results provide new evidence showing that doxorubicin-induced cardiomyopathy might be associated to serious complications of infection-related periapical lesions in rats, an effect that can be prevented by the implementation of anti-oxidant therapy.

Keywords: Doxorubicin, Cardiomyopathy, Apical Periodontitis, Antioxidant Therapy, Rats

Financial Support: BPA/PUCRS
Objectives:

Introduction: The ionotropic receptor TRPA1 belongs to the superfamily of TRP channels, is expressed mainly in nociceptive C fibers and has received increased attention due to its central role in inflammatory nociceptive mechanisms. Under inflammatory conditions, peripheral nociceptors are sensitized and a hyperalgesic state develops. Prostaglandins, especially those of the E2 series (PGE2), are considered the main inducers of this process that is mediated by formation of cAMP and the downstream activation of protein kinase A (pAkA) and the epsilon isoform of protein kinase C (pKCe). In this study, we asked if TRPA1 mediates PGE2–induced mechanical hyperalgesia and investigated the mechanism involved.

Methods and Results:

Methods: Male wistar rats (200-280g) were used. PGE2 (100ng), its downstream mediators db-AMPc (100µg), pAkA catalytic subunit (6, 9 and 24U), and pKCe (9 µg) or their vehicle (0.9%NaCl) were injected in the plantar surface of the rat’s hindpaw. The pharmacological blockade of TRPA1 receptors was induced by the co-administration of its specific antagonist (HC030031, 300 and 1200 µg). The TRPA1 gene silencing (confirmed by western blot) was induced by lumbar (L5-L6) subarachnoid administration of ODN antisense for TRPA1 during four days. The mechanical threshold was measured using an electronic von Frey algometer, 3, 6 and 24 hours after injection of PGE2 or its downstream mediators. A two-way repeated-measure or a one way ANOVA followed by Tukey test was used to determine if there were significant (p ≤ 0.05) differences in the hyperalgesic response among the groups. Data are present as mean + E.P.M. Results: PGE2 (33,0528 + 1,7919), db-AMPc (31,8583 + 1,4965), pAkA (28,5098 + 1,7642) and pKCe (26,0933 + 1,0286) induced similar hyperalgesia (showed as decrease in grams of mechanical nociceptive threshold) that peaked at 3 hours. For this reason, the involvement of TRPA1 in their hyperalgesic effect was analyzed 3 hours after their injection. The pharmacological blockade of TRPA1 receptor by co-administration of HC030031 significantly decreased PGE2 (15,8000 + 1,8716), db-AMPc (25,5333 + 0,6554), pAkA (20,0130 + 0,6213) and pKCe (22,5907 + 1,3639) induced hyperalgesia. Similarly ODN antisense for TRPA1 receptors significantly decreased PGE2 (4,7904 + 0,3280, pAkA (16,4500 + 1,7963) and pKCe (12,5750 + 2,2712) induced hyperalgesia.

Conclusions:

Conclusion: These findings indicate that PGE2 activates TRPA1 to induce hyperalgesia and suggest that this activation is dependent of pAkA and/or pKCe.

Keywords: TRPA1 receptor, Prostaglandin E2, Hyperalgesia

Financial Support: FAPESP

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**ANTI-INFLAMMATORY EFFECTS OF METHYL GALLATE ISOLATED OF PLANTS FROM ANACARDIACEAE FAMILY**

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Objectives:

Derivatives of phenolic acids, such as Methyl Gallate (MG) are suggested as substances with biological activity in plants from Anacardiaceae family. Previous results obtained in our laboratory showed that pre-treatment with MG (100µg/ml) inhibited mast cells degranulation and, consequently, histamine release in vitro (Int. Immunopharmacol., 8: 1552, 2008). In the present work, we
investigated the \textit{in vitro} and \textit{in vivo} anti-inflammatory effect of the Methyl Gallate.

Methods and Results:

The performed in vitro studies were MTT cytotoxicity (range concentrations from 0.01 to 100\(\mu\)g/ml), ELISA and macrophage chemotaxis using Boyden chamber assay. MG (100\(\mu\)g/mL) seems to inhibit IL-6 production (2.73 ± 0.2 for LPS vs 1.82 ± 0.18 for MG) from mice peritoneal macrophages in the presence of LPS (30ng/ml) for 6h. Bone marrow macrophages chemotaxis stimulated by KC (10\(\mu\)M) was inhibited in 60\% by MG in the concentration of 1\(\mu\)g\(\mu\)g/ml. To perform \textit{in vivo} studies, male Swiss mice were submitted to an oral pre-treatment with MG (1, 10 or 100 mg/ml) and pleurisy was induced by an intrathoracic injection (i.t.) of zymosan (100 \(\mu\)g/cavity). Control animals received an equal volume of sterile saline. After 4 hours the thoracic cavities were washed with PBS containing EDTA 10 mM and the pleural washes were used to analyze cell migration, protein extravasation and the production of inflammatory mediators. The i.t. injection of zymosan induced a significant increase of protein extravasation and leukocyte influx, 4h after stimulation. The 10 and 100 mg/kg concentrations of MG were able to inhibit the neutrophil influx and protein extravasation into the thoracic cavity.

Conclusions:

Our results suggest an anti-inflammatory effect for the Methyl Gallate \textit{in vitro} and \textit{in vivo} and also a possible therapeutic tool to the treatment of inflammatory diseases.

Keywords: Anacardiaceae family, Anti-inflammatory, IL-6, METHYL GALLATE

Financial Support: FAPERJ and FIOTEC/FIOCRUZ

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QuebraPagina

Resumo:17-211

\textbf{EFFECT OF OLIGOPEPTIDASES B FROM TRYPANOSOMA CRUZI AND TRYPANOSOMA BRUCEI IN AN EXPERIMENTAL MODEL OF PAIN IN MICE}

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\(^{2}\) Instituto Sírio - Libânes de Ensino e Pesquisa, ISLEP
\(^{3}\) Laboratorio de Fisiopatologia / Instituto Butantã, Instituto Butantã

Objectives:

Oligopeptidases B from \textit{T.brucei} (OPTb) and \textit{T.cruzi} (OPTc) are endo-oligopeptidases of the class of serine peptidases, similar to trypsin (Barrett et al. Br. J. Pharmacol, 152: 1155-1171, 2007), implicated in infectious processes of medical importance, marked by intense inflammatory reactions (Gorrão et al. Peptides. 28: 2146, 2007). Both OPTc and OPTb play an important role in the pathogenesis of trypanosomiasis of Chagas disease and sleeping sickness, respectively (Gorrão et al. Peptides. 28: 2146, 2007). OPTc is related to the process of invasion of \textit{T. cruzi} in the host cell and the activity of OPTb is involved in the disorderly degradation of peptides and hormones in the blood of patients with sleeping sickness (Gorrão et al. Peptides. 28: 2146, 2007). It has been proposed that peptidases may be important mediators in the pathogenesis of trypanosomiasis once it is demonstrated that the deletion of these enzymes from the parasites induces attenuation of virulence of trypanosomes (Caler et al. Embo J. 17:4957, 1998). The aim of this work was to evaluate the effect of purified forms of OPTc and OPTb in a murine model of inflammatory pain evaluation.

Methods and Results:

Clones of oligopeptidase B from \textit{T. cruzi} and \textit{T. brucei} were expressed as described (Burleigh et al. J. Cell Biol. 136(3):609, 1997; Morty et al. J. Biol Chem. 274(37): 26149, 1999). Male Swiss mice (18 to 22g) received intraperitoneal injections (i.p.) of
different concentrations of OPTc or OPTb (150µg, 50µg and 16.6µg) and after 1h were evaluated at the writhing test (KOSTER et al. Fed. Proc.18:412, 1959). OPTb decreased the number of abdominal contortions in all evaluated concentrations while OPTc decreased the was effective only at the concentrations of 150µg and 50µg. Inactivation of the enzymes by temperature reversed the inhibitory activity of both enzymes while the inhibition with TLCK, a specific serine peptidase inhibitor, was not able to interfere with the antinociceptive effect. Peritoneal exudates of mice previously treated with OPTc or OPTb and injected i.p. maintained the decreased in the number of abdominal contortions.

Conclusions:

Data obtained herein demonstrate that both oligopeptidases from T.cruzi and T.brucel are endowed of antinociceptive activity that depends on the intact structure of the enzymes to occur.

Keywords: Antinociception, Oligopeptidase B, Trypanosoma brucei, Trypanosoma cruzi

Financial Support: CNPQ and Instituto Sírio-Libanês de Ensino e Pesquisa

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THE ROLE OF TNF AND MELATONIN IN THE PRIMING OF ENDOTHELIAL CELLS CULTURES

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Objectives:

We have recently shown that melatonin primes endothelial cells. In fact, plasma melatonin levels are inversely correlated with cultured endothelial cells reactivity (PlosOne. 5:1, 2010). On the other hand, during an inflammatory response the nocturnal melatonin production by pineal gland is impaired by inflammatory mediators, such as TNF (J. Pineal Res. 41:344, 2006). In this way, we proposed the existence of an immune-pineal axis composed by a bidirectional communication between pineal gland and the immune system (Neuroimmunomodulation. 14:126, 2007). This work aimed to verify the relation of TNF production induced by LPS in vivo with the cultured endothelial cells reactivity and to correlate with plasma melatonin level.

Methods and Results:

Male adult rats were injected with LPS (0.5 mg/kg, i.v.) or LPS + melatonin (3.0 mg/kg, i.v.) and killed during the day or the night after two hours of treatment at the middle of the light or dark phases. Animals treated with vehicle (saline 0.9% + ethanol 5%) or not (naïve) were considered as control. Plasma was collected for melatonin and TNF quantification by ELISA and endothelial cells cultures were obtained from cremaster muscle and used after confluence (± 18 days). The expression of adhesion molecules (PECAM-1 and ICAM-1) and inducible nitric oxide synthase (iNOS) was determined by immunofluorescence. The results show the correlation of the plasma concentration of TNF versus the expression of adhesion molecules, iNOS or melatonin, for each rat. The slopes of the linear regressions obtained by plotting the plasma TNF levels versus the expression of PECAM-1 (0.28 ± 0.08), ICAM-1 (0.26 ± 0.12) and iNOS (0.14 ± 0.05), were significantly different from zero, according to analyzes of variance for linear regressions. In addition, plasma TNF was inversely correlated with nocturnal plasma melatonin level (Pearson’s r = -0.50; P<0.05).

Conclusions:

Our results suggest that LPS treatment could be impairing nocturnal melatonin production, enhancing endothelial cells reactivity. These results reinforce the concept of immune-pineal axis and show the same pattern observed in humans for the correlation of TNF and melatonin concentrations (J. Pineal Res. 41:136, 2006). Since melatonin primes endothelial cells diminishing the expression of adhesion molecules and iNOS, our data suggest that TNF could be also priming endothelial cells during an
inflammatory response in the opposite direction. Therefore, the control of endothelial cells by modulators of the inflammatory response is an important aspect in the control of inflammatory process. As a matter of fact, inflammatory patterns, known to be epigenetically modulated (Biochem. Pharmac. 72:1114, 2006), can be hereditarily transmitted. Therefore, it is possible that melatonin controls epigenetically modulation of inflammatory genes.

Keywords: Cellular memory, Endothelial cells, LPS, Melatonin, TNF

Financial Support: FAPESP, CNPq, CAPES

QuebraPagina

Resumo:17-213

(-)-CARVONE ACTIVATES TRPV1 CHANNELS IN DRG NEURONS AND TRPV1-EXPRESSING HEK CELLS

Goncalves, J. C. R. 1,3; Souza, H. D. N. D. 2; Nery, A. A. 2; Ulrich, A. H. 2; Prado, V. F. 3; Prado, M. A. M. 3, Araújo, D. A. M. D. 1

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2 Instituto de Química, IQ/USP
3 Robarts Research Institute, UWO

Objectives:

(-)-Carvone ((-)-Cv) is a monoterpenic ketone widely found in the essential oil of various aromatic plants such as Mentha sp.. Recently our group observed that ((-))-carvone-induced analgesic effect is associated with decreased peripheral nerve excitability as also by increasing the intracellular calcium levels ([Ca2+]) in dorsal root ganglia (DRG) neurons. The transient receptor potential vanilloid 1 (TRPV1) is fully expressed in DRG neurons and well-known implicated in pain transmission. Paradoxically, some studies attributed to TRPV1 activation and its latter desensitization as the analgesic mechanism of other monoterpenes like camphor. Therefore, we investigate whether ((-))-Cv might also act via TRPV1 in DRG neurons and in human embryonic kidney (HEK) cells expressing TRPV1 by calcium imaging assays

Methods and Results:

For all experiments ((-))-Cv was diluted to a final concentration of 1mM dissolved in a vehicle composed by 0.1% DMSO. Freshly isolated DRGs from young-adult Wistar Rats (~100g) were digested with 0.1% papain and 0.25% collagenase and neurons were mechanically dissociated with fire-polished glass pipettes. Fluorescence quantification was performed in small DRG neurons (15–30µm) loaded with 5µM Fluo-3/AM and imaged on a fluorescence microscope (Nikon, Japan) coupled to a CCD camera (Phitomecs, USA). Neurons (~10x2, n=3) were individually analyzed and the mean fluorescence intensity (F515/F488) normalized from basal levels. Transfection of purified TRPV1-cDNA (1µg/plate) was carried out in HEK 293 cells using the calcium phosphate method. Quantification of [Ca2+]i was performed by loading the transfected cells with 5µM Fura-2/AM and imaged on a fluorescence microscope (Leica, Canada) coupled to a CCD camera (Hamamatsu, Japan). Transfected cells (~15, n=3) were identified by mCherry fluorescent protein (Ex587/Em610) and the [Ca2+]i was determined by the formula: [Ca2+]i=Kd*(R-Rmin)/(Rmax-R)*[Fmax380/Fmin380], were Kd is the Fura-2 dissociation constant, R the 340/380nm ratio, RMin and RMax the ratios in the absence and in a saturating concentration of calcium respectively; Fmax380 and Fmin380 the maximal and minimal fluorescence achieved at 380nm. Ca2+ calibration was performed by EGTA 20mM and ionomycin 5µM. All cells were kept at 37ºC/5% CO2 atmosphere in DMEM (DRG neurons) or MEM (HEK cells) both supplemented with 10% FBS and 1% pen/strep. Capsaicin 5µM was used as positive control for TRPV1 activation and ruthenium-red (Rth-R) or capsazepine (CPZ) as its respective blockers. Significance was considered when p

Conclusions:

We demonstrated that ((-))-carvone increases intracellular calcium levels of small diameter DRG neurons via TRPV1 activation, an
evidence further confirmed in TRPV1-expressing HEK cells

Keywords: (-)-Carvone, TRPV1 channels, DRG neurons

Financial Support: CAPES and CNPq

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QuebraPagina

Resumo:17-214

BAP1-INDUCED PGE2 PRODUCTION IN B TYPE SYNOVIOCYTES IS DEPENDENT ON NF-κB PATHWAY

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1 Instituto Butantan, IBu
2 Instituto Clodomiro Picado, ICP

Objectives:

Snake venom metalloproteinases present homology with matrix metalloproteinases (MMPs), which are increased in inflamed articular joints during arthritis. Recently, we demonstrated that the metalloproteinase BaP1 is able to induce inflammatory events in the rat articular joints, including the release of PGE2 and TNF-α, both major mediators of pain in inflamed joints. However, the cell sources of these mediators were not identified. During inflammatory processes in joints, the synovial fibroblasts (B Type) are central cells for production and release of inflammatory mediators. In this study we evaluated the action of BaP1 in B type synoviocytes, focusing: 1) release of PGE2; 2) protein expression of ciclooxigenases-1 and -2 (COX-1 and -2), and 3) the role of NF-κB in the release of PGE2 and expression of COX-2.

Methods and Results:

B type synoviocytes were isolated from synovial membranes of male Wistar rats (Butantan Institute Ethical Committee ref. 576/09) and cultured in culture flasks with complete RPMI medium at 37°C and 5% CO2. Presence of the membrane protein Thy-1 was evaluated by immunocytochemistry as a marker of B type synoviocytes. These cells were incubated with BaP1 (12.5µg/mL) or RPMI (control) for 30 minutes, 1, 3 or 6 h, followed by evaluation of PGE2 concentration by enzyme immunoassay and protein expression of COX-1 and -2 by Western blotting. Participation of NF-κB in the release of PGE2 and in the protein expression of COX-2 was evaluated by treating synoviocytes with the specific inhibitor SN50 (50 µg/mL) 1 hour before stimulation with BaP1 (12.5µg/mL) for 3 h. Results showed that BaP1 induced release of PGE2 from synoviocytes after 1 up to 6 h incubation (362.26±45.77) when compared with control (114.36±7.749). In addition, BaP1 increased COX-2 protein expression at 1 and 3 h (0.4335±0.06970; basal: 0.2303±0.02292), but did not affect COX-1 expression. Treatment of cells with SN50 inhibited BaP1-induced PGE2 release (98%) and protein expression of COX-2 (43%).

Conclusions:

In conclusion, BaP1 is able to directly stimulate B type synoviocytes to produce PGE2 and expression of COX-2, which may be the primary mechanism for production of this mediator. Both effects of BaP1 are dependent on NF-κB pathway. Therefore, besides being targets for BaP1, B type synoviocytes are relevant sources for production of inflammatory mediators during joint inflammation induced by this metalloproteinase.

Keywords: metalloproteinase, synoviocytes, prostaglandin, arthritis, COX-2

Financial Support: FAPESP, CNPq
EVALUATION OF THE ANTINOCICEPTIVE AND ANTI-INFLAMMATORY EFFECTS OF HYDROALCOHOLIC AND BIFLAVONES RICH FRACTION OBTAINED FROM LEAVES OF CENOSTIGMA MACROPHYLLUM TUL. VAR. ACUMINATA TELES FREIRE (LEGUMINOSAE)

Cavalcanti, M. F.; Pereira, S. S.; Nunes, B. L. M.; Mendes, R. M. B.; Sousa, L. Q.; Piauilino, C. A.; Marques, R. B.; Costa, C. L. S.; Chaves, M. H.; Almeida, F. R. C.
Núcleo de Pesquisas em Plantas Medicinais/CCS, UFPI

Objectives:
Cenostigma macrophyllum Tul. var. acuminata Teles Freire (Leguminosae) (caneleiro) is used in folk medicine to treat stomach disorders and abdominal pain. The present study was designed to investigate the antinociceptive and anti-inflammatory effects of hydroalcoholic (FHA) and biflavones rich fraction (FRB) obtained from the leaves of this species in animal models.

Methods and Results:
Were used male Swiss mice (25-30 g) and female Wistar rats (150-210 g). In the formalin test (2%, 20 &muL i.pl.), FHA (200 and 400 mg/kg p.o.) inhibited only the second phase (p<0.001).

Conclusions:
Our data suggest that both fractions have a potential antinociceptive effect but not an antiarthritic or antiedematogenic effect (FHA) on the doses and routes of administration employed. The FRB showed a greater antinociceptive effect than FHA. Our data also suggest that opioid and nitrergic systems and KATP channels may have a role on the antinociceptive effect of FRB in mice glutamate test.

Keywords: Cenostigma macrophyllum, Antinociceptive, Anti-inflammatory effect, Hydroalcoholic fraction, Biflavones rich fraction

Financial Support: CAPES

EVALUATION OF THE ANTINOCICEPTIVE EFFECT OF HYDROALCOHOLIC EXTRACT LEAVES OF HYPTIS FRUTICOSA

Departamento de Fisiologia/ Universidade Federal de Sergipe, UFS

Objectives:
Evaluate the antinociceptive effect of the hydroethanolic extract (EE) of Hyptis fruticosa in formalin, capsaicin and glutamate induced orofacial nociception on mice.

Methods and Results:

Male Swiss mice (25-30 g) were used throughout this study. Mice (n = 6/group) were pretreated with EE (80 mg/mL in saline) at doses of 50, 100 and 200 mg/kg (v.o.) 60 min before each test. Nociception was quantified by measuring the time (s) the animals spent rubbing the face injected area with their fore or hind paws. Experimental protocols were approved by the Ethics Committee on Animal Research of the Federal University of Sergipe (CEPA: 65/09). Data were analyzed by ANOVA followed by Dunnet's post hoc test. Differences were considered significant when p < 0.05. The EE significantly reduced orofacial nociception in the first (37.7%, 38.1% and 69.6%; p < 0.001, respectively, at doses of 50, 100 and 200 mg/kg) and second (61.9%, 74.3% and 74.8%; p < 0.001, respectively, at doses of 50, 100 and 200 mg/kg) phases of the formalin test. In glutamate-induced orofacial nociception, EE produced a significant (p < 0.001) reduction in the orofacial nociceptive behavior (43.6%, 55.4% and 84.6%, respectively, at doses of 50, 100 and 200 mg/kg). The EE significantly (p < 0.001) reduced the painful response when capsaicin was used as nociceptive agent (44.4% and 61.2%) respectively, at doses of 100 and 200 mg/kg.

Conclusions:

Our results suggest that Hyptis fruticosa might be an important alternative for treating orofacial pain. Support: CNPq

Keywords: Hyptis fruticosa, orofacial pain, hydroethanolic extract

Financial Support: CNPq

QuebraPagina

IMPAIRED INFLAMMATORY AND PAIN RESPONSES INDUCED BY ETHANOLIC EXTRACT OF THE CAPSICUM CHINENSE (BIQUINHO PEPPER).

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Mestrado em Biotecnologia/Universidade Católica Dom Bosco, UCDB

Objectives:

Aim: Red peppers are the pungent fruits of various species of the genus Capsicum that are grown as condiments, spices, and pharmaceutical preparations. The peppers contain a great substances variety, mainly the capsaicinoids compounds. Capsaicin is the major alkaloid responsible for the mucosal irritant properties of plant species from the Capsicum family and has important pharmacological effects such as analgesic and anti-inflammatory activities. Thus topical capsaicin has been used in clinical practice for the treatment of a variety of painful conditions. But others compounds with the pharmacological effects are found in this family. However, little is known about Capsicum chinense (biquinho pepper variety), and this constitution as well as these pharmacological properties remains unclear. In this study, we attempted to further characterize the biological anti-inflammatory and analgesic properties of ethanolic extract from Capsicum chinense in mice.

Methods and Results:

Metodology: The methods were approved by CEUA-UCDB, which is the protocol number 001/11. The ethanolic extract was obtained from pepper fruit through Soxhlet extractor, using ethanol. After, the solvent was removed by rotaevaporation (40 °C). The extract was storage in -4°C. The anti-inflammatory and analgesic activity was carried out in Swiss mice, which were pre-treated with saline or ethanolic pepper extract (1, 5 or 15 mg/ s.c). In order to evaluate the anti-inflammatory activity
mice were pretreated with saline or ethanolic extract of the C. chinense, and 15 min later, they were injected i.p. with thioglicolate (3%) and the neutrophil migration into the peritoneal cavities performed 6 h after. The analgesic activity was determined through a model of visceral pain. Mice were injected with 0.8% acetic acid (i.p.) to evoke abdominal writhing, and received treatment (s.c.) 15 minutes before this stimulus. The number of writhes that occurred during 30 min after acetic acid was determined. Results: Our results demonstrated that pre-treatment of mice with 15mg of ethanolic extract from C. chinense was able to inhibit the neutrophil recruitment to the inflammatory site. The same profile was observed when the analgesic effect was evaluated, were the ethanolic extract of the C. chinense was able to inhibit the number of writhes in mice.

Conclusions:

Conclusions: These data suggest that ethanolic extract of the C. chinense exhibit a potential anti-inflammatory and antinociceptive profiles and the studies about these peppers compounds may be relevant to new drugs development.

Keywords: acid acetic, Capsicum chinense, inflammation, neutrophil migration, pain

Financial Support: Capes

QuebraPagina

Resumo:17-218

EXOGENOUS LEPTIN DOWN-REGULATES LUNG INFLAMMATION INDUCED BY LPS, REDUCING THE ENDOTHELIAL ICAM-1 EXPRESSION AND INSULIN LEVELS, IN MICE.

Landgraf, M. A. 1; Silva, R. C. 2; Vieira, P. M. M. M. 1; Pacheco-silva, A. 3; Camara, N. O. S. 13; Landgraf, R. G. 4

1 Imunologia, ICB/USP
2 Medicina, Unifesp
3 Nefrologia, Unifesp
4 Ciências Biológicas, Unifesp

Objectives:

Leptin is an adipocyte-derived hormone that influence a multitude of physiological systems including immunity, inflammation and hematopoiesis (JACI,116;1228,2005). However, the role of leptin in pulmonary inflammatory response is still unclear. Lipopolysaccharide (LPS) is an important factor in acute lung injury and airway exposure to LPS in mice induces acute pulmonary inflammation with recruitment and activation of neutrophils, vascular leakage and bronchopulmonary hyperreactivity (Am. J. Respir. Cell Mol. Biol. 24; 345, 2001). Considering these informations, the objective of the present study was to investigate the role of exogenous leptin in acute lung inflammation induced by LPS.

Methods and Results:

All procedures used in this study were approved and performed in accordance with guidelines established by the ethics committee of the ICB/USP (CEEA–83/2009). 5-6 male C57BL/6 mice at 8-9 wk of age were used for each group. Control group was given saline intranasally (i.n.,20uL). Experimental groups were given leptin (1ug/g/20uL), LPS (1.5ug/g/20uL) or leptin (1ug/g/20uL) and LPS (1.5ug/g/20uL) i.n. 24 h after instillation of LPS and/or leptin, the bronchoalveolar lavage fluid (BALF) was collected to evaluate cellular infiltration in lung. Blood was collected to measure serum insulin concentration. Lungs were harvested for measurement of the mRNA expression of keratinocyte chemoattractant (KC) by real-time PCR and to evaluate the intercellular adhesion molecule-1 (ICAM-1) expression. The i.n. administration of leptin into C57BL/6 did not alter any of the parameters evaluated, when compared to the control group; on the other hand, the i.n. administration of LPS increased all of them. The intranasal administration of leptin 30 minutes prior to LPS administration reduced inflammatory cell infiltration into airways (50%-total cells and 25%-neutrophils), levels of KC mRNA expression (32%), serum insulin levels (44.8%), and ICAM-1
expression (48.6%), when compared to the group that received LPS alone.

Conclusions:

These results indicate that exogenous leptin can attenuate the LPS-induced acute lung inflammation in mice by down regulation of insulin levels, in serum, and reduction of the KC and ICAM-1 expression, in lung tissue.

Keywords: lung, inflammation, LPS, Leptin, ICAM-1

Financial Support: FAPESP, CNPq and INCT Complex Fluids.

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**Objectives:**

Multiple sclerosis (MS) is the most common progressive, inflammatory and demyelinating disease of the central nervous system. Despite the great progress observed in the last decades on the mechanisms underlying the control of multiple sclerosis, no effective and safe therapies have emerged so far. Kinins are increased in MS patients, but the underlying mechanisms by which kinin receptor regulates MS development have not been elucidated. Here we investigated the role played by kinin receptors in the modulation of experimental autoimmune encephalomyelitis (EAE), analyzing the preventive and therapeutic effects of the selective blockade of kinin receptors in conjunct with gene deletion for both kinin B1 and B2 receptor in the different phases of EAE development. We also assessed the underlying mechanisms of kinin receptor in this experimental model.

**Methods and Results:**

Experiments were conducted using female C57BL/6, kinin B1R and B2R knockout mice (6-10 weeks old) (CEUA/UFSC23080038266/2008-43). EAE was induced by immunization with MOG35-55 peptide plus Mycobacterium tuberculosis extract H37Ra in incomplete Freund’s adjuvant oil. The animals received pertussis toxin i.p. (day 0 and day 2) post-immunization (p.i.). Mice were observed daily for clinical signs of EAE, locomotor activity, mechanical and thermal hypernociception. After 25 days p.i., cytokines production and proliferative response were evaluated in lymph node and spleen cells. The percentage of CD4+CD69+ and CD8+CD69+ T cells was investigated by flow cytometry assay. The inflammatory cell infiltrate, demyelination index, astrocytes, T lymphocyte and transcription factor CREB were evaluated in lumbar spinal cord at day 25 p.i. The real-time PCR and primary astrocytes culture were used to assess the autoimmune inflammation of the CNS. Here, we report that blockade of B1R in the induction phase, markedly suppressed the EAE progression, by interfering with the onset of immune response. Furthermore, B1R antagonist suppressed the production/expression of antigen-specific TH1 and TH17 cytokines and transcription factors, both in the periphery and in the CNS. The blockade of B1R in EAE chronic phase consistently impaired the clinical progression of EAE. Moreover, the B1R antagonist reduced IFN-γ induced up regulation of TNF-α, CXCL1/KC and IL-6 levels, as well as COX-2 and NOS2 expression in primary astrocytes cultures. Of note, administration of B1R agonist at EAE acute phase suppressed disease progression as well as inhibited the increase of permeability of the blood-brain barrier (BBB) and ongoing CNS inflammation. In contrast, blocking B2R had only moderate impact in all studied parameters of EAE progression.

**Conclusions:**

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**Resumo:**

**KININ B1 RECEPTOR PLAYS A DUAL ROLE IN PREVENTING THE NEUROINFLAMMATION AND THE CLINICAL SEVERITY DURING EXPERIMENTAL MODEL OF MULTIPLE SCLEROSIS.**

Dutra, R. C.; Leite, D. F. P.; Bento, A. F.; Manjavachi, M. N.; Patrício, E. S.; Figueiredo, C. P.; Pesquero, J. B.; Calixto, J. B.

1 Departamento de Farmacologia - FMC, UFSC
2 Departamento de Biofísica, UNIFESP

**Objectives:**

Multiple sclerosis (MS) is the most common progressive, inflammatory and demyelinating disease of the central nervous system. Despite the great progress observed in the last decades on the mechanisms underlying the control of multiple sclerosis, no effective and safe therapies have emerged so far. Kinins are increased in MS patients, but the underlying mechanisms by which kinin receptor regulates MS development have not been elucidated. Here we investigated the role played by kinin receptors in the modulation of experimental autoimmune encephalomyelitis (EAE), analyzing the preventive and therapeutic effects of the selective blockade of kinin receptors in conjunct with gene deletion for both kinin B1 and B2 receptor in the different phases of EAE development. We also assessed the underlying mechanisms of kinin receptor in this experimental model.

**Methods and Results:**

Experiments were conducted using female C57BL/6, kinin B1R and B2R knockout mice (6-10 weeks old) (CEUA/UFSC23080038266/2008-43). EAE was induced by immunization with MOG35-55 peptide plus Mycobacterium tuberculosis extract H37Ra in incomplete Freund’s adjuvant oil. The animals received pertussis toxin i.p. (day 0 and day 2) post-immunization (p.i.). Mice were observed daily for clinical signs of EAE, locomotor activity, mechanical and thermal hypernociception. After 25 days p.i., cytokines production and proliferative response were evaluated in lymph node and spleen cells. The percentage of CD4+CD69+ and CD8+CD69+ T cells was investigated by flow cytometry assay. The inflammatory cell infiltrate, demyelination index, astrocytes, T lymphocyte and transcription factor CREB were evaluated in lumbar spinal cord at day 25 p.i. The real-time PCR and primary astrocytes culture were used to assess the autoimmune inflammation of the CNS. Here, we report that blockade of B1R in the induction phase, markedly suppressed the EAE progression, by interfering with the onset of immune response. Furthermore, B1R antagonist suppressed the production/expression of antigen-specific TH1 and TH17 cytokines and transcription factors, both in the periphery and in the CNS. The blockade of B1R in EAE chronic phase consistently impaired the clinical progression of EAE. Moreover, the B1R antagonist reduced IFN-γ induced up regulation of TNF-α, CXCL1/KC and IL-6 levels, as well as COX-2 and NOS2 expression in primary astrocytes cultures. Of note, administration of B1R agonist at EAE acute phase suppressed disease progression as well as inhibited the increase of permeability of the blood-brain barrier (BBB) and ongoing CNS inflammation. In contrast, blocking B2R had only moderate impact in all studied parameters of EAE progression.

**Conclusions:**
These results demonstrate that kinin receptors, mainly the B1R subtype, play a dual role in EAE progression depending on the phase of treatment through the lymphocytes and glial cell-dependent pathways.

Keywords: Autoimmunity, Bradykinin receptor, Demyelination, Multiple sclerosis, Neuroinflammation

Financial Support: CNPq; CAPES; PRONEX; FAPESC.

QuebraPagina

Resumo:17-220

EUPHOL ATTENUATED THE AUTOIMMUNE DISEASE OF THE CENTRAL NERVOUS SYSTEM IN EXPERIMENTAL MODEL OF MULTIPLE SCLEROSIS: A POTENTIAL THERAPEUTIC ROLE.

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Dep. Farmacologia/ CCB, UFSC

Objectives:

Multiple sclerosis (MS) is the most common inflammatory demyelinating disease of the central nervous system (CNS) that cause neurological disability in young adults and still remains without an effective therapy. The hallmarks of MS include neuronal loss, axonal injury and atrophy of the CNS, due to a progressive inflammatory reaction involving both the adaptive and the innate immune systems. Adhesion molecules are specialized cell-cell adhesion structures and critical components of the blood-brain barrier (BBB) that have previously been shown to be abnormally distributed in MS tissue. Medicinal plants are largely used worldwide by the population and have proved to be a rich source of new active compounds, especially to treat pain and inflammatory processes. The tetracyclic triterpene euphol is the main constituent found in the sap of Euphorbia tirucalli, a plant belonging to the family Euphorbiaceae, known in Brazilian folk medicine as avelóz. Aim: In the present study, we used pharmacological and molecular approaches to investigate the role played by euphol in the modulation of experimental autoimmune encephalomyelitis (EAE) model. We also assessed the underlying mechanisms of euphol in this experimental model.

Methods and Results:

Experiments were conducted using female C57BL/6 (6-10 weeks old) and were approved by (CEUA/UFSC23080.030926/2010-62). EAE was induced by immunization with MOG35-55 peptide plus Mycobacterium tuberculosis extract H37Ra in incomplete Freund’s adjuvant oil. The animals received pertussis toxin i.p. (day 0 and day 2) post-immunization (p.i.). Mice were observed daily for clinical signs of EAE, locomotor activity, mechanical and thermal hypernociception. After 25 days p.i., mRNA of pro-inflammatory mediators and transcription factors were evaluated in spinal cord by real-time PCR. Animals were orally treated by gavage with 1, 3, or 10 mg/kg of euphol once a day from day 0 to day 25 p.i. (preventive treatment) or with 10 mg/kg from day 15 to day 25 p.i. (therapeutic treatment). All-vehicle solutions were used for the respective control animal treatments. Euphol oral treatment (10 mg/kg, p.o.), either before or at the early onset of EAE, ameliorate neurological signs of EAE-mice, such as clinical score and locomotor activity, without affecting the hyperalgesia induced by EAE. Moreover, euphol treatment was accompanied by a remarkable reduction of neuroinflammation by modulation of TH17 cells activation. These beneficial effects of euphol seem to be associated with a reduction of mRNA adhesion molecules, such as lymphocyte function-associated antigen 1 (LFA-1) at the inflamed spinal cord tissue, thereby limiting TH17 cell transmigration.

Conclusions:

Our findings show that the oral administration of the natural triterpene euphol reduces and limits the severity and development of EAE through the modulation of the adhesion molecules. Therefore, euphol might be a small molecule of clinical interest for human MS and other TH17 and TH1 cell-mediated inflammatory diseases.
LACTOBACILLUS ACIDOPHILUS PREVENTS THE INFLAMMATORY RESPONSE IN INTESTINAL MUCOSITIS INDUCED BY 5-FLUOROURACIL IN MICE

Departamento de Fisiologia e Farmacologia, UFC

Objectives:
Intestinal mucositis is a frequent side-effect associated to 5-fluorouracil (5-FU) clinical use and results in inflammatory events. It is characterized by epithelial ulcerations in the mucosa and clinical manifestations of abdominal pain, nauseas and diarrhea. Lactobacillus acidophilus (LA) is a probiotic which has been shown to protect the gastrointestinal microflora from disequilibrium and from associated gastrointestinal disorders. Objective was to evaluate effect of Lactobacillus acidophilus in inflammatory aspects of intestinal mucositis induced by 5-FU in mice.

Methods and Results:
Swiss male mice (30-35g) were treated with 5-FU (450 mg/Kg, i.p., only dose) or saline (control). One group, mice was treated with Lactobacillus acidophilus (LA; 0,2x10^8 UFC) daily for 3 days (3D). On the third day after administration of 5-FU or LA+5-FU, mice were sacrificed and samples of duodenum (D), jejunum (J) and ileum (I) were collected for assessment of ponderal analysis, MPO activity and GSH concentration for spectrophotometry, measuring villus crypt. All animal treatments and surgical procedures were approved by the local ethics committee (protocol 34/10). Significance statistics (tests ANOVA and Bonferroni), values considers with p

Conclusions:
Our results suggest that the treatment with Lactobacillus acidophilus reverted the inflammatory events on the intestinal mucositis induced by 5-FU in mice.

Keywords: INTESTINAL MUCOSITIS, Lactobacillus acidophilus, Probiotic, 5-FLUOROURACIL

Financial Support: CNPq

A PHOSPHOLIPASE A2 HOMOLOGUE (MT-II) ISOLATED FROM SNAKE VENOM UP-REGULATES ADRP EXPRESSION IN MACROPHAGES

Objectives:

ADRP (adipocyte differentiation-related protein) is a member of the PAT protein family that is involved in the transport and storage of neutral lipids in multiple cell types. This protein is highly expressed in macrophages differentiated into foam cells, where it is found on the lipid bodies surface. Lipid bodies (LBs) are important organelles for lipid metabolism and generation of inflammatory mediators such as eicosanoids. The accumulation of these organelles is associated with metabolic disorders and inflammatory diseases. MT-II, a Lys49 phospholipase A2 (PLA2) isolated from Bothrops asper snake venom, induces inflammation and activates several macrophages functions including release of eicosanoids (Toxicon. 45: 335, 2005). In this study, the ability of MT-II to induce protein expression of ADRP in macrophages was evaluated and correlated with this toxin-induced lipid body formation.

Methods and Results:

Thioglycolate-elicited macrophages from male Swiss mice (Butantan Institute Ethical Committee ref. 760/10) were incubated with MT-II (0.4 µM) or culture medium (control) from 1 up to 12 hours and protein expression of ADRP was evaluated by Western blotting. Lipid bodies were quantified by staining cells with osmium tetroxide (1%), followed by analysis under phase contrast microscopy. Our results showed that incubation of macrophages with MT-II significantly increased ADRP protein expression by 149% in comparison with controls AU=0,279, n=3) from 1 up to 3 hours. Moreover, MT-II induced a significant increase in numbers of LBs (average of 559%) from 1 up 48h of stimulation in comparison with controls (average: 1.09 ± 0.166 LB/cell, n=5), with a maximum at 48h stimulation.

Conclusions:

These data indicate that MT-II is able to up-regulate ADRP protein expression and induce lipid body formation in macrophages. Considering the time course of both cell phenomena, protein expression of ADRP may be relevant for the late increase of LB formation induced by the venom PLA2.

Keywords: venom phospholipase, macrophage, inflammatory response

Financial Support: CAPES, INCTTox, CNPq

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Resumo:17-223

EVIDENCE FOR THE EXISTENCE OF A STRESS-INDUCED ANALGESIC ENDOGENOUS SYSTEM IN THE FISH LEPORINUS MACROCEPHALUS

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Objectives:

Pain perception in basal vertebrates, such as fish, is a controversial issue. Recent studies have shown that fish have a nervous system capable to perceive noxious stimuli, central opioid receptors and analgesia in response to administration of morphine. This study examined the behavioral response of juvenile piaçu (Leporinus macrocephalus) to subcutaneous injection of formalin
3% in the region of the adipose fin (20μl) and the influence of restraint stress in modulating this response.

Methods and Results:

For this purpose, 32 fish were divided into four experimental groups (n=8, per group): (1) subcutaneous injection of saline (SAL), (2) subcutaneous injection of formalin (FOR), (3) stress + subcutaneous injection of saline (STRESS+SAL), (4) stress + formalin subcutaneous injection (STRESS+FOR). Before the treatments the behavior was analyzed for 5 minutes (baseline). After that, the fish were submitted to the restraint stress for 5 minutes, immediately before the injections. After the injections the behavior was analyzed for 5 minutes. The results are presented as the difference between the post treatment data and the baseline data, and were analyzed using analysis of variance one-way (One Way ANOVA), followed by test and post-hoc test (P

Conclusions:

These results indicate that the application of noxious stimulation promotes behavioral changes, mainly related to motor activity, and provide evidence for the existence of an endogenous analgesic system activated by stress in basal vertebrates.

Keywords: Comparative Physiology, Fish, Pain, Stress

Financial Support: CNPq

QuebraPagina

Resumo:17-224

INVolVEMENT OF FPR1 AND FPR2 RECEPTORS ON THE INHIBITORY EFFECT OF ANNEXIN-1 DERIVED PEPTIDE AC2-26 ON MICE PULMONARY FIBROBLASTS

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Objectives:

Silicosis is a chronic disease caused by inhalation of free crystalline silica particles, characterized by intense inflammation and fibrosis (granuloma formation). Fibroblasts are considered important cells in the context of fibrosis. There are several endogenous mediators able to regulate negatively some inflammatory responses, in order to guarantee the control of such processes. Glucocorticoids are considered as important agents based on their antiinflammatory activity, which has been shown to be, at least partially, dependent on the generation of an intermediate protein named annexin-1. In this study we investigated the participation of FPR1 and FPR2 receptors in the inhibitory effect of annexin-1 N-terminal derived peptide Ac2-26 on the reactivity of pulmonary fibroblasts.

Methods and Results:

Fibroblasts were obtained from lungs of normal wild type (C57 Bl6), FPR1 (FPR1−/−) and FPR2 (FPR2−/−) knockout mice by means of enzymatic dissociation with collagenase type 1. Cells were cultivated in DEMEN medium supplemented with SBF 20% until the 3rd passage. The analyses included real time-PCR for receptors FPR1 and FPR2, chemokine (MCP-1) generation (ELISA), collagen secretion (Sircol technique) and cell proliferation (3H-tymidine). Fibroblasts were stimulated with profibrotic cytokines such as IL-13 (20 - 80 ng/mL) and TGFb (5 - 20 ng/mL). In another set of experiments, the cells were incubated with Ac2-26 peptide (15 and 30 µM) 1 h before stimulation with IL-13 (40 ng/ml) and TGFb (10 ng/mL). All the analyses were performed 24 h post-provocation. All experimental procedures were performed in accordance with the guidelines of the Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation (L-034/09). In vitro stimulation of lung fibroblasts from wild type mice with IL-13 and TGFb led to cell proliferation and activation (collagen and MCP-1 production), in a
concentration-dependent manner, being only the former sensitive to Ac2-26 peptide. Lung fibroblasts exhibited basal expression of mRNA for both FPR1 and FPR2 receptors, a response increased after stimulation with IL-13. No effect was noted after TGFβ provocation. Interestingly, the inhibitory effect of Ac2-26 on the production of collagen and MCP-1 was abolished under conditions of FPR1−/− fibroblast stimulation with IL-13 and TGFβ. In the case of FPR2−/− cells, only collagen production was refractory to Ac2-26 peptide.

Conclusions:

Our results show that annexin-1-derived peptide Ac2-26 directly inhibited lung fibroblast activation caused by IL-13 and TGFβ, a phenomenon dependent on binding to FPR1 and FPR2. These findings also suggest that Ac2-26 peptide may possibly constitute a promising therapeutic approach for the case of inflammatory fibrotic diseases such as silicosis.

Keywords: Silicosis, Annexin-1, Ac2-26 peptide, lung Fibroblast

Financial Support: FIOCRUZ, CNPq, FAPERJ, CAPES

QuebraPagina

Resumo:17-225

ROLE OF GAMMA DELTA T LYMPHOCYTES IN PULMONARY INFLAMMATION DURING EXPERIMENTAL SEPSIS

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Objectives:

To investigate the involvement of gamma delta T lymphocytes in lung inflammation caused by experimental murine sepsis induced by CLP, characterizing the kinetics of gamma delta T cell accumulation in lung tissue and the involvement of chemokines in this phenomenon.

Methods and Results:

Severe sepsis was induced by cecal ligation and puncture (CLP; 9 punctures with 21 gauges needle and antibiotics treatment for 3 days) in male C57BL/6 mice previously anesthetized [ketamine (112.5 mg / kg) and xylazine (7.5 mg / kg), ip], as described (Benjamin et al., 2003). Mice were euthanized 1, 3 and 10 days after CLP and lymphocytes were recovered from lung tissue for flow cytometric analysis (FACScalibur, BD). One day after CLP, the numbers of gamma delta T lymphocytes were increased in lung tissue (SHAM 0.3 ± 0.0 vs CLP 4.0 ± 0.4 x104 cells/lung), due to the accumulation of Vgamma4+ (SHAM 0.3 ± 0.0 vs CLP 1.3 ± 0.2 x104 cells/lung), Vdelta4+ (SHAM 0.2 ± 0.0 vs CLP 1.6 Â± 0.1 x104 cells/lung) and Vdelta6.3+ subtypes (SHAM 0.2 ± 0.0 vs CLP 0.9 ± 0.2 x104 cells/lung). At day 3, Vdelta4+ and Vdelta6.3+ cell numbers were decreased; however Vgamma4+ T lymphocyte numbers remained elevated. Gamma delta T cell numbers remain elevated until 10 days post-CLP. Intracellular cytokine staining revealed that 10 days after CLP, gamma delta T lymphocytes produced IL-17 (SHAM 11.1 Â± 1.0 vs CLP 35.4 Â± 2.1 % of positive cells/lung), but not IL-4, IL-10, IL-12, IFN-gamma and TNF-alpha. Levels of CCL2, which is known to recruit gamma delta T lymphocytes in different models of inflammation, were evaluated in cell-free extracts lung by ELISA (enzyme-linked immunosorbent assay, R&D) and revealed increased levels of such cytokine in the lungs of CLP mice when compared to sham mice, within 10 days (SHAM 165.4 Â± 38.5 vs CLP 752.1 Â± 168.1 pg/lung). In order to investigate if CCL2 triggers gamma delta T lymphocyte migration into the lungs during sepsis, an in vitro chemotaxis was performed using cell free lung extracts as stimulus and CCL2 as positive control. The cells that migrated were collected and submitted for marking of CD3 and TRC gamma delta to be analyzed in flow cytometer. We observed that CCL2 neutralization in CLP lung extracts with anti-CCL2 mAb failed to inhibit gamma delta T lymphocyte migration, suggesting such chemokine is not crucial for the recruitment of this cell population during CLP-triggered lung inflammation (SHAM lung extracts 1.9 Â± 0.2 vs...
CLP lung extracts 1.9 ± 0.3 vs anti-CCL2 mAB-treated CLP lung extract 1.9 ± 0.3 x 10^3 cells/well). All values of P≤0.05 were considered statistically significant. All experimental procedures were performed according to The Committee on Ethical Use of Laboratory Animals of FIOCRUZ(Licence#004/08).

Conclusions:
Experimental sepsis triggers an important inflammatory response in the lungs, marked by an marked accumulation of gamma delta T lymphocytes which are an important source of IL-17. These results suggest a role for these cells during lung inflammation. Moreover, CCL2 seems not to be crucial for gamma delta T cell migration into the lungs in septic mice.

Keywords: Inflammation, Sepsis, Lymphocyte migration, Cytokines

Financial Support: PIBIC/CNPQ/FIOCRUZ/FAPERJ

QuebraPagina

Resumo:17-226

GENDER DIFFERENCES IN THE CUTANEOUS SENSITIVITY TO ELECTRICAL NERVE STIMULATION (TENS) IN HEALTHY YOUNG ADULTS

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2 Universidade Federal do Espirito Santo, UFES

Objectives:
In recent years, the sensory neural system (SNS) has been the target of numerous investigations in different biomedical areas. In study the objective was to investigate the gender differences in neuronal sensory threshold (NS) for transcutaneous electrical nerve stimulation (TENS) among young adults, and the probable effects of cutaneous thermotherapy.

Methods and Results:
The study was conducted according to national and international standards for research on humans (Resolution 196/96-CNS and CIOMS/OMS), and approved by the Institutional Review Board of the Integrated Healthcare Center - CEP/CIAS-UNIMED(under opinion 84/2006). 30 young healthy students (15 men and 15 women) with 22±2 years old were divided by gender. TENS was applied in both right and left knees of subjects with a frequency of 20 Hz and pulse duration of 230s. The amplitude of the electric current (mA) was gradually increased to record the perception threshold (PT) and tolerance threshold (TT), before/after thermotherapy. The warm knee-D was performed by infrared light (250W)a 70cm perpendicular, and cooling knee-E by icepack, both carried out for 15 minutes. The tissue temperature was recorded by digital thermometry. The data were analyzed and differences established at P

Conclusions:
The cutaneous sensitivity for TENS is gender-term dependent in healthy young adults.

Keywords: TENS, Hyperthermia induced, Cryotherapy, Sensory threshold, Skin

Financial Support: CDV-FACITEC and CNPq.
THE BRADYKININ RECEPTOR 1 DEFICIENCY EXACERBATES EXPERIMENTAL COLITIS INDUCED BY DEXTRAN SULFATE SODIUM IN MICE

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Objectives:

Inflammatory bowel disease are idiopathic inflammatory bowel disorders that leads to long term and sometimes irreversible impairment of gastrointestinal structure and function, as well as extensive and unbalanced activation of the mucosal immune system driven by the commensal flora. The two major forms of IBDs are Crohn’s disease and ulcerative colitis which exhibit marked diarrhea and bloody stools. Recent study have reported the involvement of bradykinin receptor 1 (B1R) in TNBS-induced experimental colitis. However, the role of kinins on experimental colitis still needs of further studies. The purpose of this study is to investigate some of the mechanisms underlying the effect of kinin B1R in the development of dextran sulphate sodium (DSS)-induced colitis in mice.

Methods and Results:

The procedures were approved by ethics committee with number 23080.016329/2010-25. We used in this study male C57BL/6 mice (6 to 8 per group) wild type (WT) or with genetic disruption of B1R (B1R−/−), which were provided with a solution of filtered water containing 3% DSS ad libitum over a 5-day period. At the end of this 5-day period, DSS was replaced by normal drinking water for 2 days. Control mice (WT or B1R−/−) received only normal drinking water. All animals were examined once a day and the disease activity index (DAI) and change body weight were assessed. On seventh day, the animals were euthanized and their colons were removed and evaluated the macroscopic scores and slices were stained with hematoxylin-eosin. The colons were processed to analyze the levels of cytokines, and myeloperoxidase (MPO) activity. We also examined the intestinal endothelial integrity by transmission electron microscopy, and expression of tight junctions, like occludin and claudins by quantitative real-time (PCR). The role played by intracellular pathway of mitogen-activated protein kinase (MAPK) were investigated by immunohistochemistry. Results: Interestingly, the disruption of B1 receptor greatly exacerbated the inflammatory response induced by DSS, observed by DAI. Hematoxylin and eosin staining of the colonic tissues as well the MPO activity of B1R−/− mice with DSS revealed a marked increase in the number of lamina propria polymorphonuclear leukocytes. This effect was associated with release of interleukin-1β and interferon-γ. PCR showed that B1R−/− mice in both, DSS or water treated animals down-regulated the mRNA transcription of occludin, which is important component of tight junctions and its down-regulation may have a key role on polymorphonuclear transmigration and cell integrity. Most notably, claudin-4 expression was significantly increased. Transmission electron microscopy shows that B1R−/− with DSS leads to microvillus effacement of mouse colonocytes. The immunohistochemistry microscopy showed that B1R−/− treated with DSS resulted significantly increase of phospho p38-MAPK which seems be involved on inflammatory exacerbation.

Conclusions:

Our data indicate that Kinin B1R seems to have a critical role in modulating the immunoinflammatory response observed in DSS-induced colitis, and suggest that altered transcription of tight junctions proteins and MAPK pathway has a role in the increase in permeability and integrity of epithelial cells.

Keywords: Colitis, Kinin, Inflammation, Kinin receptor 1, Occludin

Financial Support: CNPq, FAPESC and CAPES
ANTI-INFLAMMATORY EFFECT OF LOW-LEVEL LASER (LLL) AND LIGHT-EMITTING DIODE (LED) IN ZYMOSAN-INDUCED ARTHRITIS

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2 Laboratório de Fisiologia e Farmacologia, Univap

Objectives:

It has been suggested that LLL and LED irradiation can modulate inflammatory processes. In a previous work we showed that laser treatment significantly inhibited edema formation, vascular permeability, and hyperalgesia in zymosan-induced arthritis (Photomed. Laser Surg. 28; 2, 2010). The aim of this work was to investigate the effect of LLL and LED on leukocyte migration and release of cytokines IL-1 and IL-6 in zymosan-induced arthritis.

Methods and Results:

Arthritis was induced in male Wistar rats (250–280 g) by intra-articular injection of zymosan (1mg in 50 mL of a sterile saline solution) into one rear knee joint. Animals were irradiated immediately, 1 h, and 2 h after zymosan administration with a semiconductor laser (685nm and 830 nm) and an LED at 628 nm, with the same dose (2.5 J/cm2) for laser and LED. In the positive control group, animals were injected with the anti-inflammatory drug dexamethasone 1 h prior to the zymosan administration. Leukocytes were harvested 6 h after zymosan injection by washing cavities with 2 ml of saline. Aliquots of the washes were used to determine total cell counts in Neubauer Chamber. Differential cell counts were performed on stained Hema3 cell smears. IL-1 and IL-6 concentrations in sinovial exudates were quantified 3h after zymosan injection by using an antibody-capture enzyme immunoassay (EIA) technique. Irradiation with LLL and LED significantly (p< 0.05) inhibited leukocyte migration 6 h after zymosan injection by 79%, 58% and 71% for 685nm and 830nm laser and 639nm LED irradiation, respectively. Laser irradiation at two wavelengths, reduced the release of IL-1 by 62 and 44% by 685nm and 830nm laser, respectively and reduced IL-6 by 65% for the 680nm and 58% for 839nm laser. Treatment with LED (628 nm), with the same fluence as the laser, had no effect in the release of inflammatory cytokines.Ethics Committee A027/2006/CEP.

Conclusions:

LLL reduces inflammatory signs more effectively than LED irradiation with similar irradiation times (100 sec), average outputs (20 mW), and energy doses (2 J) in an animal model of zymosan-induced arthritis. The anti-inflammatory effects of LLL appear to be a class effect, which is not wavelength specific in the red and infrared parts of the optical spectrum.

Keywords: laser, LED, ARTHRITIS

Financial Support: FVE-UNIVAP; FAPESP

EFFECTS OF THE PHARMACOLOGICAL BLOCKADE OF MITOGEN-ACTIVATED PROTEIN KINASES (MAPKS) AND NFKB ON NOCICEPTIVE THRESHOLD IN A MICE MODEL OF INCISIONAL PAIN

Lima, 1,2; Nogueira, 1; Soares, 1,3; Villarreal, 1,4
Objectives:

The aim of the present study was to investigate whether the activation of mitogen-activated protein kinases (p38, Jnk, Erk) and transcription factor NFκB mediates mechanical hypernociception induced by plantar incision in mice.

Methods and Results:

Experiments were performed using male Swiss mice (25-30 g, n=5-6). We evaluated the effects of the pretreatment (30 minutes before / intraperitoneal route) with PDTC (NFκB activation inhibitor; 25-100 mg/kg), SB203580 (P38 activation inhibitor; 2.5-10 mg/kg), SB600125 (JNK activation inhibitor; 30 mg/kg) and PD98059 (ERK activation inhibitor; 3 mg/kg) against incision-induced pain behaviors in a mouse model of post-incisional pain. Mechanical threshold was assessed using von Frey filaments up-down method, before and up to 6 days following plantar incision. The control group was treated with vehicle. Institutional Animal Care and Use Committee FIOCRUZ 26/2009-1. The plantar incision decreased the mechanical nociceptive threshold of the paw 4, 6, 24, 48, 72 and 96 hours after incision. Pretreatment of mice with PDTC (100 mg/kg) significantly reduced the hypernociceptive response 4 (4.59±0.79 g), 6 (3.86±0.67 g), 24 (5.3±0.68 g), 48 (6.93±0.35 g), 72 (8.2±0.65 g) and 96 (8.4±0.54 g) hours after the plantar incision, when compared with control (0.61±0.2 g; 0.47±0.06 g; 0.6±0.11 g; 0.99±0.3 g; 2.45±0.8 g; 4.17±0.8 g, respectively). Additionally, intraperitoneal injection of SB203580 (10 mg/kg) significantly reduced the hypernociception 4 (3.43±0.8 g), 6 (4.88±0.33 g), 24 (7.3±0.9 g), 48 (6.6±0.4 g) and 72 (7.4±0.3 g) hours after incision, relative to control group (0.82±0.2 g; 1.33±0.3 g; 1.35±0.31 g; 1.77±0.3 g; 2.95±0.5 g respectively). However, pretreatment with JNK or ERK inhibitors had no effect on incision-induced pain-related behavior.

Conclusions:

Plantar incision-induced mechanical hypernociception is prevented by pharmacological inhibition of p38 and NFκB, suggesting a contribution of these signaling pathways in the experimental incisional pain.

Keywords: incisional pain, MAPKS, NFkB, pain

Financial Support: This work was supported by CNPq, MCT, FAPESB, and CAPES.
The intraplantar administration of venoms isolated from snakes of the *Micrurus* genus induces severe edema without hyperalgesia in rats, suggesting the presence of an antinociceptive constituent (CASAIS-E-SILVA, L. L. 2001. 208f. PhD in Physiology, Institute of Biosciences, University of São Paulo, São Paulo). Here we investigated the antinociceptive activity of *Micrurus lemniscatus* venom in experimental models of pain and evaluated its possible mechanisms of action.

Methods and Results:

The antinociceptive activity of *M. lemniscatus* venom was evaluated using the acetic acid-writhing assay (0.8% 0.1 mL/10 g i.p.) and tail flick test (water bath 48°C ± 0.5°C) in Swiss male mice (18-22 g, n=6). In the acetic acid-writhing assay was noted the number of writhings performed by the animal and in the tail flick test, the nociceptive threshold was evaluated before (baseline) and at different times after treatment. Mice motor performance was evaluated in open field (number of squares crossed in 3 minutes) and rota rod (length in the cylinder) tests. Differences between groups were detected by one-way ANOVA followed by Tukey post-hoc test or by two-way ANOVA followed by Bonferroni post-hoc test using GraphPad Prism version 5.0. The results were represented as mean + SEM of 6 mice per group and *p* ≤ 0.05 was considered significant.

Animal care and handling procedures were in accordance with International Association for the Study of Pain guidelines for the use of animals in pain research and Institutional Animal Care and Use Committee FIOCRUZ 012/2009. Oral administration of *M. lemniscatus* venom (59-1600 µg/kg) produced inhibition of acetic acid-induced writhing (10.83 + 5.06; *p* Conclusions:

The *M. lemniscatus* venom has potent antinociceptive activity in experimental models of pain. This antinociceptive effect is, at least in part, mediated by the opioid system.

Keywords: Antinocicepção, Dor, *Micrurus lemniscatus*, Sistema opióide, Veneno

Financial Support: This work was supported by FIOCRUZ and FAPESB.

QuebraPagina

Resumo:17-231

EVALUATION OF ANTI-NOCICEPTIVE AND ANTI-INFLAMMATORY ACTIVITIES OF N-MORPHOLINE ISATINS

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Objectives:

Isatin (1H-indole-2,3-dione) is an endogenous compound that is widely distributed in mammalian tissues and body fluid. It synthetic flexibility permits the synthesis of a great variety of derivatives. Isatin and derivatives present several biological activities, including anti-inflammatory and anti-nociceptive effects. The objective of this work was to evaluate the anti-inflammatory and anti-nociceptive properties of the derivatives of isatin, the N-morpholine isatins.

Methods and Results:

Male swiss mice (20-25g, n=4-5) were used in the acetic acid (2%, ip) induced writhing model, in the licking response induced by formalin (2.5%, intraplantar) and hot plate model. Animals received oral administration of ISA3 or ISA4 (0.1 and 1.0 mg/kg), 1h before acetic acid or formalin injections or 30 min before the beginning of hot plate model. Statistical analyses were performed by ANOVA and Bonferroni’s test (*p

Conclusions:
The new N-morpholine isatins presented anti-inflammatory and anti-nociceptive effects in experimental animal models and suggest that these substances could be new candidates to prototypes of new drugs.

Keywords: Anti-inflammatory, Anti-nociceptive, N-morpholine isatins, Formalin model, Acetic acid induced writhing model

Financial Support: CAPES, CNPq and FAPERJ

**QuebraPagina**

**Resumo:**

MORPHOLOGICAL AND EXTRACELLULAR MATRIX ALTERATIONS IN THE BRAIN DURING LPS INDUCED FEVER IS MEDIATED BY SP/ NK1 RECEPTORS.

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**Objectives:**

We demonstrated, recently, that SP participates in the febrile response induced by LPS through activation of NK1 receptors (Brain Research, 384, 161-169, 2011). Systemic administration of LPS can induce some morphological alterations on liver, kidney and lung. However, that is a lack of studies demonstrating if there is or not brain morphological and extracellular matrix alterations during febrile response induced by this agent. And if it does, what would be Substance P participation on these alterations.

Based on that, we investigated the effect of intracerebroventricular administration of selective NK1 receptor antagonist, SR140333B, on the febrile response and morphological and extracellular matrix alterations, induced by lipopolyssacharide (LPS).

**Methods and Results:**

Male Wistar rats (200 g) were implanted with guide cannula in lateral ventricle and with data loggers for measurement of body temperature in the peritoneal cavity one week before the experiment under the same anesthesia. Body temperature (°C) was registered every 15 min. The room temperature was kept at 28°C. The animals were pre-treated intracerebroventricular with SR140333 (3.0 µg/2µl, icv) or vehicle (2 µl) and after 30 min were also injected intraperitoneal with LPS (50 µg/kg) or saline. In the third hour, animals were deeply anesthetized with ketamine (50 mg/kg) and xylazine (8 mg/kg) and perfused transcardially with PBS pH=7,4 followed by ALFAC. The brain were removed, postfixed for 16 hs in ALFAC and then stored in Alcohol 70%. Our analysis were restricted in areas localized near hypothalamus and OVLT (Organum Vasculosum of Laminae Terminalis). And then, some histological stains were used such as: Hematoxilin and Eosin, Masson, Periodic Schiff Reagente (PAS), Alcian Blue pH=2,5 and Picrosirius. All the methods were previously approved by Institutional Ethics Committee under protocol no. 384. The results showed that SR140333B significantly reduced fever (about 85%) and morphological alterations induced, by peripheral injection of LPS, in rat brains, such as: edema, hydropic degeneration, spongiosis and lymphoplasmocytic and neutrophil infiltration. The extracellular matrix analysis to all groups were positive for PAS and Alcian Blue in all groups . Systemic administration of LPS induced synthesis of thick collagen fibers type I and III, but pre-treatment with NK1 antagonist receptor only presented thin collagen fibers type I.

**Conclusions:**

These data suggest that systemic inflammation using LPS can induce fever and alterations on brain morphology and extracellular matrix metabolism. Apparently, Substance P, through NK1 receptor, is involved in all those actions.

Keywords: extracellular matrix, NK1 receptors, LPS, fever
EMPATHY IN MICE: EFFECT OF HIPERNOCICEPTIVE THE COHABITATION WITH MICE SUBMITTED THE SCIATIC NERVE

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2 Departamento de Psicologia, UFSCar

Objectives:

It has been shown that just as in humans, animals may exhibit behaviors related to their cagemates. Evidence of this relationship has been identified in studies involving rodents. Some studies have shown that pain-related areas and that are activated in the array (where the process of pain occurs) are also activated in those who are just observing potentially painful situations (Science, 1967; 1970, 2006). Thus, when the mice observe other mice receiving footshock, observers showed activation of structures linked to the limbic system, which modulate behaviors related to empathy (Nat. Neurosci., 13; 482, 2010). In this sense, the objective of this study was to determine whether mice that lived in pairs, with animals subjected to constriction of the sciatic nerve, may have increased nociception assessed by the writhing test.

Methods and Results:

Swiss-albino male mice (35-45g) (n= 9-10/group). After weaning animals were housed in pairs and after 14 days living together subjects were randomly assigned to 3 groups based on surgical procedure: CONSTRICTION (n= 10), one animal from each pair was submitted to surgical constriction of the sciatic nerve; SHAM (n= 9), one animal from each pair was underwent surgery without constriction; CONTROL (n= 9), no surgical procedure was made. After surgery, animals returned to their home cage. On the test day, after 31 days living together, each mouse considered observer received an intraperitoneal injection of 0.6% acetic acid (0.1 ml/10g weight; nociceptive stimulus). The test started after beginning of contortions and was held for a period of 5 minutes. The number of writhes was recorded during this period. One-way ANOVA showed significant effects for type of lived factor (F(2.25) = 12.00, P < 0.05). Posterior comparisons (Duncan’s test) revealed that subjects that lived with mice with constriction of the sciatic nerve showed highest number of writhes when compared to the observers animals of the CONTROL and SHAM groups [CONTROL (13.11 ± 1.33), SHAM (11.44 ± 1.13), CONSTRICTION (19.3 ± 1.41), P <0.05].

Conclusions:

Ours results corroborate previous studies showing that occurrence of social modulation of nociception as evidence for empathy in mice (Science, 1967; 1970, 2006). In the present study the accommodation in pairs for 31 days is able to produced empathy, and increase the nociceptive response in mice that lived with animal submitted to the neuropathic pain model.

Keywords: Empathy, Mice, Hipernociceptive

Financial Support: UFSCar, CAPES, CNPQ
NOVEL ORALLY ACTIVE PDE4 INHIBITORS OF THE CLASS OF N-METHYL-N-ACYLHYDRAZONES INHIBIT ALLERGEN-INDUCED AIRWAY INFLAMMATION, REMODELING AND HYPERREACTIVITY IN MICE.

Cardozo, S. V. S. 1; Anjos-vallota, E. A. 1; Coutinho, D. D. S. 1; Kümmerle, A. E. 2; Silva, P. M. R. 1; Fraga, C. A. M. 2; Barreiro, E. J. L. 2; Martins, M. A. 1

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2 LASSBio/ Faculty of Pharmacy, LASSBio/UFRJ

Objectives:

Aim: Phosphodiesterase 4 (PDE4), the main enzyme responsible for the hydrolysis and subsequent inactivation of cyclic AMP, is an important therapeutic drug-target in lung inflammatory diseases such as asthma. In this study we have evaluated in a murine model of asthma a novel series of PDE4 inhibitors (6 compounds), of the chemical class of N -methyl- N -acylhydrazones (NAH), using rolipram (standard PDE4 inhibitor) as reference compound.

Methods and Results:

Methods: Male mice of the strain A were actively sensitized with a mixture of ovalbumin (OVA) and Al(OH)3 at the days 0 and 7 and, one week (wk) later, subjected to a sequence of intranasal instillations of OVA (50 µg, i.n.), once a wk during 4 wks. Oral treatments (100 µmol/kg) were performed curatively 1 h before the third and fourth allergen provocation. Airway hyperreactivity (AHR) to methacholine was assessed using invasive barometric plethysmography. Sub-epithelial airway fibrosis, mucus production and peribronchial eosinophil infiltration were measured by histomorphometry in formalin-fixed and paraffin-embedded lung sections, stained with trichrome gomori, periodic acid-schiff (PAS) or sirius red (pH 10.2). Cytokine and chemokine lung levels were quantified with ELISA. All analyses were done 24 h after the last OVA provocation. FIOCRUZ Ethics Committee approved all procedures concerning animals (CEUA-FIOCRUZ # 0213-4). Results: AHR and inflammation, subepithelial airway fibrosis and mucus secretion were clearly present in the OVA group as compared with the sham challenged group. All treatments, including rolipram (50 µmol/kg, dose which is at the limit of toxicity), inhibited AHR, concerning both increased lung resistance and elastance. OVA-challenged mice developed a 165-fold increase in the number of eosinophils in the lung tissue compared with the sham-group (from 1,2 ± 0,1 x103 to 192,3 ± 0,6 x 104 cells/mm2, P < 0.01, n=8). Except rolipram, all substances inhibited tissue eosinophil infiltrate (~ 50-65%, P

Conclusions:

Conclusion: Our findings show that the oral treatment with either LASSBio-1406 or LASSBio-1407 inhibits crucial features of severe asthma, including lung eosinophilic infiltration, ARH and tissue remodeling. Such effects are accompanied by a marked reduction in the levels of TH2 cytokines, suggesting a possible action of these compounds upon TH2 cells. Taken together, our results provide evidence that these N -methyl- N -acylhydrazone derivatives should be further investigated as templates in drug discovery for asthma therapy.

Keywords: PDE4 INHIBITORS, ASTHMA, HIPERREACTIVITY, INFLAMMATION

Financial Support: CNPq, FAPERJ, PRONEX and INCT-INOFAR

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Resumo:17-235

PPAR-&GAMMA; AND –&BETA; RECEPTORS ARE INVOLVEMENT IN LIPID BODY FORMATION INDUCED BY A PHOSPHOLIPASE A2 ISOLATED FROM BOTHROPS SNAKE VENOM

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Objectives:

LBs are lipid inclusions formed by neutral lipids involved in both lipid metabolism and inflammation process. The nuclear receptors known as peroxisome proliferator-activated receptors (PPAR-γ and -β) are lipid-activated transcription factors that have emerged as key regulators of lipid metabolism and inflammation. Recently we demonstrated that MT-III, a secreted phospholipase A2 (sPLA2) isolated from Bothrops asper snake venom, induces formation of lipid bodies (LBs) in macrophages (J. Leukoc. Biol. 2011 Apr 8). However, the involvement of PPARs in this MT-III-induced effect was not investigated. In this study participation of both PPAR-γ and PPAR-β in LB formation and protein expression of these receptors induced by MT-III were evaluated in macrophages.

Methods and Results:

Thioglycolate-elicited macrophages from male Swiss mice (Butantan Institute Ethical Committee ref. 744/10) were incubated with MT-III (0.4 μM) or culture medium (control) from 1 up to 24 hours and protein expression of PPAR-γ and –β were determined by Western blotting. Participation of PPAR-γ and –β in LBs formation was evaluated by treating macrophages with the specific inhibitors GW9662 and GSK660 (10 μM), respectively, 1h before stimulation with MT-III (0.4 μM) for 12 h. LBs were quantified by staining cells with osmium tetroxide (1%), followed by analysis under phase contrast microscopy. Incubation of macrophages with MT-III significantly increased PPAR-γ protein expression by 45 % and PPAR-β protein expression by 74% from 1 h up to 24 h in comparison with control cells (average AU: 0.373± 0.06, n=4), with no statistical difference among time periods evaluated. Incubation of macrophages with MT-III induced a marked increase in LB numbers (7.62±0.3 LBs/cell; basal: 2.2±0.21, n=4). Pretreatment of cells with either GW9662 or GSK660 compounds reduced MT-III-induced LBs formation by 74,44 % and 92,96 %, respectively.

Conclusions:

In conclusion, these results demonstrate that LB formation induced by a secreted PLA2 (MT-III) is dependent on PPAR receptors (γ and β). Up regulation of expression of both PPAR subtypes may be an important mechanism for LB formation induced by this sPLA2.

Keywords: Lipid body, macrophage, Phospholipase A2, PPARs, Bothrops snake venom

Financial Support: FAPESP, CAPES, CNPq, INCTTOX

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Resumo:17-236

P38 MAP KINASE IS INVOLVED IN RESISTANCE TO STEROID THERAPY IN A MURINE MODEL OF CHRONIC ASTHMA


Objectives:

Although glucocorticoids (GCs) are highly effective anti-inflammatory agents, a small subset of patients shows persistent tissue
inflammation and AHR despite treatment with high doses of GC. p38 MAPK activity is increased in severe asthma and it has been proposed that increased expression of IL-2 and IL-4 in the airways of patients with glucocorticoid-resistant asthma reduces GR function by mechanism mediated via phosphorylation of GR by p38 MAPK. We have investigated here the effectiveness of the GC therapy on Th2 cytokine generation and p38 MAP kinase activity in a murine model of chronic asthma, marked by resistance to steroid therapy according to previous findings.

Methods and Results:

Mice of strain A/J were sensitized with ovalbumin (OVA) in Al(OH)3 suspension, and challenged (OVA, 50 μg/25 μl, intranasal) once a week (wk) for 4 or 9 wks, starting after wk-2 post-sensitization. Dexamethasone (DEX, 3 mg/kg, oral), or vehicle, was administered 1 h before OVA challenge and the analyses were done 24 h after the last provocation. Cytokine and chemokine generations were quantified in the lung tissue by ELISA. Western blotting was used to investigate p38 MAP Kinase activity. Airway hyperresponsiveness (AHR) was measured using invasive and noninvasive barometric plethysmografi. Sub-epithelial airway fibrosis and mucus were quantified using histomorphometric techniques in formalin-fixed and paraffin-embedded lung sections, which were stained with Trichrome Gomori and PAS, respectively. License CEUA # LW-23/10. We found that A/J mice reacted to the sequence of 4 weekly intranasal allergen provocations with marked AHR, fibrogenesis and mucus production. These changes were clearly intensified in those animals subjected to the 9-wk regime as compared with those of the 4-wk regime of allergen provocations. DEX clearly inhibited allergen-evoked AHR, peribronchial fibrosis and mucus production noted in the 4-wk regime, but failed to alter these changes in the 9-wk regime. Similarly, increased generation of IL-4, IL-5, eotaxin-1 and eotaxin-2 also appeared sensitive to DEX in case of the short-term but not in the long-term regime of allergen provocations. Interestingly, DEX reduced the levels of the anti-inflammatory cytokine IL-10 in animals of the long-term but not in those of the short-term regime. In contrast, DEX not inhibited the levels of p38 MAPK phosphorylation following the 9-wk regime OVA provocations.

Conclusions:

These findings show that A/J mice develop asthma-like pathological changes that are progressively exacerbated by the successive allergen provocations, becoming resistant to the steroid treatment as the magnitude of the allergic response increased. Since, in parallel, the levels of p 38 phosphorylated in the lung also increases and are less sensitive to DEX, under conditions where IL-10 levels are down-regulated, it is not unlikely that activation of the p38 MAPK pathway and reduction in IL-10 production may contribute to the state of GC refractoriness in asthma conditions.

Keywords: Steroid resistance in severe asthma, p38 MAP Kinase, Th2 cytokine

Financial Support: FAPERJ and CNPq

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Resumo:17-237

STUDIES ON THE METABOLISM OF LASSBIO-998, A PROTOTYPE OF ANTI-INFLAMMATORY DRUG.

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2 ICB/Programa PG Farmacologia e Química Medicinal, UFRJ
3 IQ/Programa PG Química, UFRJ

Objectives:

LASSBio-998 is a prototype of an anti-inflammatory drug planned as an inhibitor of p38 MAPK. Its ability to inhibit the activation of p38 MAPK with a potency of 14 μM and the anti-inflammatory activity evaluated in a murine model of acute lung inflammation through a preventive regimen per os were described recently (Pharmacol Reports, 2011, in press). In the present study we describe the plasma and hepatic metabolism of LASSBio-998, the determination of plasmatic and microsomes half-lives
Methods and Results:

The plasma metabolism was studied in vitro employing pooled rat plasma incubated in the presence of LASSBio-998 (100 µM) at 37 oC in a water bath, followed by steps of deproteinization and chromatographic analysis by HPLC-PDA at different times 0, 30, 90, 150 and 210 min. The half-life (t1/2) was calculated using the expression t1/2 = 0.693/a, were a is the slope of the natural logarithm (ln) of sample concentration vs. time incubation curve. The hepatic microsomal metabolism was carried out in vitro by incubating the samples with the liver microsomal fraction of male Wistar rats. The incubation was performed in the presence and absence of cofactors [i.e. MgCl2; NADP+; glucose-6-phosphate and glucose-6-phosphate dehydrogenase in phosphate buffer]. After incubation at 37 oC samples were collected at 0, 60 and 120 min and subjected to deproteinization, analysed by HPLC and the half-life (t1/2) calculate as described above. The data obtained allowed us to determine the plasma half-life of LASSBio-998 showing a value of 76.8 min. The study of hepatic microsomal metabolism of LASSBio-998 showed that the metabolic process occurs regardless of the addition of cofactors (t1/2 cofactors (-) = 66 min; t1/2 cofactors (+) = 75.3), suggesting that its metabolism does not depend on the involvement of oxidative enzymes such as CYP450 and FMOs. So the results indicated the involvement of a hydrolytic metabolism, possibly catalyzed by carboxylesterases (CEs) present in plasma and in the rat microsomal fraction. This hypothesis was confirmed by co-incubation with the inhibitor of CEs (bis-p-nitrophenylphosphate) that inhibited 100% the metabolism of LASSBio-998 at a concentration of 2 µM. The main metabolite detected by HPLC-PDA was characterized by LC/MS/MS and it is the carboxylic acid derivative obtained by hydrolysis of ethyl ester group present in LASSBio-998.

Conclusions:

We described the in vitro plasmatic and hepatic metabolism of a new prototype of anti-inflammatory drug, LASSBio-998 and the characterization of the main metabolite. In drug development, predictive studies of pharmacokinetics and metabolism are of great importance and contribute to our understanding of the pharmacological effects produced by the new compound enabling the optimization of the molecule and its effects.

Keywords: LASSBio-998, MAPK p38, hepatic microsomal metabolism, plasma metabolism

Financial Support: CNPq, CAPES, FAPERJ, INCT-Inofar

Resumo:17-238

ETHANOL INCREASES THE IMMUNOEXPRESSION OF CYSTATHIONINE GAMMA LYASE IN THE GASTRIC MUCOSA OF MICE.

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Objectives:

To better understand the role of hydrogen sulphide (H2S) and detailed localization of your production in normal gastric epithelium and ethanol-induced gastropathy we studied the cell-specific expression of cystathionine-γ-lyase (CSE) isoform in mice.

Methods and Results:

Mice were treated with saline or with DL-propargylglycine (PAG, an inhibitor of H2S synthesis) for testing purposes. After 30
min, 50% ethanol was administrated by gavage. After 1 h, mice were sacrificed, and gastric damage was evaluated by microscopic analyses (data shown are medians with minimal and maximal and specimens were assessed according to the criteria of Laine et al., 1988). A sample of the corpus region of each stomach was fixed in 10% formalin for subsequent histopathological assessment. Hereafter, the sections (5 µm thickness) obtained from gastric mucosal were immunostained by avidin–biotin–immunoperoxidase technique, by using antibody against CSE isoform. All animal treatments and surgical procedures were approved by the local ethics committee (protocol No 63/07). Ethanol administration induced microscopic gastric damage in mice and disruption of the gastric gland superficial region, with epithelial cell loss (3, 2-3), oedema (2.5, 2-3) and intense hemorrhage (3.5, 3-4). However, the administration of PAG increases mainly the ethanol-induced oedema (4, 3-4). Additionally, we demonstrated that ethanol increase the CSE expression and occurs almost exclusively in the parietal cell.

Conclusions:

These results showed that the gastric epithelium therefore could be one source of H2S in the gastric wall. Furthermore, the coexpression of CSE in gastric mucosal might be an indication that it’s involved in the production of H2S that’s important to gastroprotection.

Keywords: CYSTATIONINE GAMMA LYASE, ETHANOL, GASTROPATHY, GASTROPROTECTION, IMMUNOEXPRESSION

Financial Support: CAPES, CNPq

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**Resumo:**

**TOLL LIKE RECEPTOR 9 DEFICIENCY IN NEUTROPHILS IMPROVES SEPSIS OUTCOME PREVENTING DOWN-REGULATION OF CXCR2**

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² Biochemistry and Immunology, FMRP-USP
³ Biochemistry and Immunology, UFMG

**Objectives:**

The understanding of the physiopathology of sepsis is imperative to design new therapies. TLR9 is an important pattern recognition receptor whose activation was implicated previously with impaired sepsis survival, but the mechanisms that govern this process still unclear.

**Methods and Results:**

Adult females C57BL/6 (wild type-WT) and TLR9/-/- mice at 6 to 8 week-old were used in this study. Sepsis was induced by cecal ligation and puncture (CLP) model. TLR9/-/- mice submitted to CLP had approximately 40% of survival while all WT mice died (n=5, Chi-Square: p=0.0243). TLR9/-/- mice presented higher neutrophil migration to focus of infection (9,81X10⁶ ±1,83 neutrophils/cavity) than WT mice (1,66X10⁶ ±0,41 neutrophils/cavity), lower bacterial load (4,33±0,17 log CFU/mL blood and 6,28±0,15 log CFU/mL peritoneal lavage) than WT mice(5,46±0,11 log CFU/mL blood and 7,55±0,11 log CFU/mL peritoneal lavage), lower circulating levels of TNF-alpha (27,52±14,2 pg/mL), CXCL2 (536,88±147,6 pg/mL) and IL-1beta (41,51±1,04 pg/mL) than WT mice (95,48±16,4 pg/mL, 908,16±11,82 pg/mL; 77±8,23pg/mL, respectively) (n=5, t test: p

**Conclusions:**
The poor outcome of severe sepsis was associated with TLR9 activation in neutrophils, triggering GRK2 expression and CXCR2 down-regulation. These events account to the reduction of the migration of the neutrophils to focus of infection, with consequent widespread of the bacteria, onset of the systemic inflammatory response and drop of the survival.

Keywords: SIRS, Infection, Chemotaxis, Failure of neutrophil migration, GRK2

Financial Support: FAPESP, CNPq and CAPES

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Resumo:17-240

PARTICIPATION OF P2X7 PURINERGIC RECEPTORS IN THE NOCICEPTIVE RESPONSES ASSOCIATED TO HEMORRHAGIC CYSTITIS INDUCED BY CYCLOPHOSPHAMIDE IN MICE

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Objectives:

ATP is released in response to cellular damage, and the P2X7 receptors have an essential role in the onset and maintenance of pathological changes (Pain, 114, 386, 2005). The hemorrhagic cystitis (HC) is a well known adverse effect of therapy with cyclophosphamide (CYP) used for the treatment of many solid tumors (Indian J Urol., 26, 2, 2010). These urotoxic effects are attributed to the toxic metabolic of the CYP, named acrolein, which can be partially prevented by sodium 2-mercaptoetanosulfonate (Mesna) (J Cancer Res. Clin. Oncol., 121, 128, 1995). The present study aimed to determine the role of P2X7 receptors in the nociceptive changes associated to hemorrhagic cystitis induced by CYP in mice.

Methods and Results:

Male Swiss, C57/BL6 mice and P2X7 receptor knockout mice (n= 4; 25-30 g) were used. HC was induced by a single administration of CYP (300 mg/kg, i.p.). Immediately after, mice were housed in individual plastic cages to observe the spontaneous behavior for 4 h, for 2 min every half-hour. Three behavioral parameters were considered: (i) activity; (ii) immobility and (iii) indicatives of visceral pain behavior (‘crises’). In addition, the spontaneous behavior of mice was also scored according to the scale described before (Eur. J. Pain., 3, 141, 1999). We have also measured the expression of c-Fos, a known biochemical marker of nociception, in the lumbar spinal cords and the brains at 6 h. To confirm HC establishment, we performed bladders gross evaluation (J. Urol., 136, 497, 1986). Swiss mice were treated with the selective P2X7 receptor antagonist A-438079 (50, 100 and 200 μmol/kg) (Neuroscience., 146, 1817, 2007), given 30 min before and 4 h after the CYP. Control animals received saline at the same intervals of time. All the experimental procedures were approved by the Local Ethics Committee (08/00074, CEUA, PUCRS). The pre-treatment with the selective P2X7 receptor antagonist A-438079 or genic ablation of P2X7 receptors inhibited the nociceptive behaviour score induced by CYP. The inhibition percentages using A-438079 at the doses of 50, 100 and 200 μmol/kg were 46 ± 9 %, 47 ± 5 and 48 ± 5 % respectively, whereas in knockout animals a reduction of 17 ± 3 % was observed. The A-438079 treatment also significantly decreased the positive staining for c-Fos in the lumbar spinal cord (45 ± 13%) and brain cortical areas (86 ± 5%).

Conclusions:
In the recent years, the interest in the therapeutic potential of purinergic receptors has dramatically increased (Pharmacol. Rev., 58, 58, 2006). Our study revealed the importance of P2X7 receptors in the nociceptive responses allied to HC induced by CYP. These results confirm and extend previous evidence suggesting that pharmacological inhibition of P2X7 receptors might represent a new therapeutic approach for treating painful conditions.

Keywords: A-438079, cyclophosphamide, genic ablation, haemorrhagic cystitis, P2X7 receptor

Financial Support: CNPq, PROBOLSAS-PUCRS.

RESISTANCE EXERCISE-INDUCED CENTRAL ANTINOCICEPTION WITH THE PARTICIPATION OF THE NITRIC OXIDE/CGMP/KATP PATHWAY IN RATS.

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Objectives:
The aim of this study was to investigate the efficacy of resistance exercise in producing antinociception, with a possible central involvement of the NO/CGMP/KATP pathway.

Methods and Results:
Wistar rats weighing 180-200 g were performed exercise weight-lifting exercise model. The nociceptive threshold was measured by mechanical nociceptive test (paw-withdrawal test). To investigate the involvement of the NO/CGMP/KATP pathway the following nitric oxide synthase (NOS) unspecific and specific inhibitors were used: N-nitro-L-arginine (NOArg), Aminoguanidine, N5-(1-Iminooethyl)-L-ornithine dihydrochloride (L-NIO), Nu-Propyl-L-arginine (L-NP); guanylyl cyclase inhibitor, 1H-[1,2,4]oxidiazolo[4,3-a]quinoxalin-1-one (ODQ); and KATP channel blocker, Glybenclamide; all were administered intrathecally and intracerebroventricularly before exercise started. Plasma and cerebrospinal fluid (CSF) nitrite levels were determined by spectrophotometry. Resistance exercise produced a significant increase (P < 0.05) of nociceptive threshold, measured by the paw-withdrawal test. The central involvement of the NO/CGMP/KATP pathway was demonstrated since nitric oxide synthase (NOS) unspecific and specific inhibitors, guanylyl cyclase inhibitor and KATP channel blocker reversed this effect (P < 0.05).

Conclusions:
In conclusion, our work demonstrated that acute resistance exercise produced antinociception in rats, with the central participation of the endogenous NO/CGMP/KATP pathway. This finding is important to clarify endogenous analgesic mechanisms related to exercise.

Keywords: Antinociception, Nitric Oxide, Pain, Resistance Exercise

Financial Support: FAPEMIG, CNPQ
OMEGA-3 FATTY ACID-DERIVED MEDIATORS 17(R)-HYDROXY DOCOSAHEXAENOIC ACID, ASPIRIN-TRIGGERED RESOLVIN D1 AND RESOLVIN D2 PREVENT EXPERIMENTAL COLITIS IN MICE

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Objectives:
The ω3-derivate mediator Docosahexaenoic acid (DHA) can originate the Resolvins of the D series, which are believed to exert a beneficial effect in inflammatory bowel disease (IBD). Herein, we have investigated the anti-inflammatory effects of the aspirin-triggered resolvin D1 (AT-RvD1), its precursor (17R-HDHA) and resolvin D2 (RvD2) in dextran sulfate sodium (DSS)- or trinitrobenzene sulfonic acid (TNBS)-induced colitis in mice.

Methods and Results:
In DSS-induced colitis, male BALB/c mice (25-30 g) received 3% of DSS solution in drink water during 7 days and were monitored for body weight change, stool consistence and bleeding, parameters that result in the disease activity index (DAI). On day 7 colonic tissues were scored for macro- and microscopic damage and analyzed for myeloperoxidase (MPO) activity, cytokines levels by ELISA assay and adhesion molecules mRNA expression by real-time PCR. In addition, colons were analyzed for NFκB expression by immunohistochemistry and real-time PCR. In TNBS-induced colitis, mice received an intrarectal injection of TNBS (1 mg/animal) and during 3 days were monitored for survival and body weight change. On day 3 colons were analyzed for macro- and microscopic damage and MPO activity. Additionally, we used a lipoxin A4 receptor (ALX) selective antagonist (BOC-1) in vitro (bone marrow-derived macrophage culture) and in vivo (DSS-induced colitis) to demonstrate the possible involvement between ALX and resolvins. Our results showed that the systemic treatment with AT-RvD1, RvD2 or 17R-HDHA (0.1-1 µg/animal, once a day, e.v.) significantly (p

Conclusions:
Our findings showed for the first time that the AT-RvD1, RvD2 and 17R-HDHA are effective in preventing colitis in two different models of intestinal inflammation. Furthermore, we have established the first experimental evidence that the epimer of RvD1, AT-RvD1, exerts its positive effects in an ALX-dependent way. These results suggest these lipid mediators as a possible therapy for the treatment of IBD.

Keywords: Docosahexaenoic Acid, Inflammatory Bowel Disease , AT-Resolvin D1, Resolvin D2 , Lipoxin A4 receptor

Financial Support: CNPQ, CAPES, FINEP and FAPESC

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Resumo:17-243

PHARMACOLOGICAL CHARACTERIZATION OF THE OEDEMA AND HYPERALGESIA INDUCED BY MOOZINCIN: A METALLOPROTEASE FROM BOTHROPS MOOJENI SNAKE VENOM

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Objectives:

In this work, we study the oedematogenic and hyperalgesic responses induced by a metalloprotease, Moozincin, isolated from Bothrops moojeni snake venom.

Methods and Results:

To investigate the dose of Moozincin able to induce oedema and hyperalgesia were used on the test previous curves with different doses (5, 25 and 50 µg in sterile saline 0.1mL). Group of rats (n=4) were pretreated with different classes of drugs in appropriate times intervals simultaneously or before injection of Moozincin (50 µg/paw). To investigate the involvement of arachidonic acid metabolites on hyperalgesia and oedema induced by Moozincin, different groups of rats were treated with dexamethasone (2.5 mg/kg, i.p., 60 min before), indomethacin (8 mg/kg, i.p., 30 min before) and nordihydroguaiaretic acid (100 mg/kg, i.p., 30 min before). To evaluate the contribution of histamine the animals received promethazine (15 mg/kg, i.p., 30 min before). To assess the participation the amines biogenics rats was injected with yohimbine (2.5 mg/kg i.p., 30 min before). The contribution of nitric oxide (NO) was also studied by treatment with L-NMMA (100 µg/paw, i.pl. injected simultaneously with the Moozincin).

Moozincin is a metalloprotease recently isolated from Bothrops moojeni venom. Intraplantar injection of Moozincin (5, 25 and 50 µg/paw) caused a dose and time-dependent hyperalgesia and edematogenic responses. The maximal oedematogenic and hyperalgesic effects were observed 3 and 4 hours after toxin injection, respectively. Dexamethasone and Indomethacin reduced significantly the oedema and hyperalgesic activity. Promethazine and nordiydroguaiaretic acid (100 mg/kg) reduced significantly the oedema but did not reduce hyperalgesic activity. L-NMMA reduced only the hyperalgesic effect. The result suggests that arachidonic acid metabolites are involving on pain and oedema mechanisms induced by Moozincin. Furthermore, histamine is specifically related with oedema and nitric oxide production with hyperalgesia induced by Moozincin.

Conclusions:

Moozincin, a metalloprotease isolated from B. moojeni venom is able to induce oedema formation and has hyperalgesic effect, in similar time-course. The oedema and hyperalgesic events are mediated by arachidonic acid metabolism. COX and LOX pathways seem to interact in the oedema formation, whereas COX appears to be involved in hyperalgesia. Interestingly, our results showed that histamine inhibits only the oedematogenic response, while NO participates only in the phenomena of hyperalgesia induced by Moozincin. These results suggest that Moozincin play a significant role in local inflammatory effects resulting from B. moojeni envenomation.

Keywords: Bothrops moojeni, Hyperalgesia, Metalloprotease, Oedema

Financial Support: FAPEMIG, CNPq, CAPES, MCT

QuebraPagina

Resumo:

ANTI-INFLAMMATORY ACTIVITY OF 4'-HYDROXY-7,8-(2''''2''''-DIMETHYLPYRAN)FLAVAN (BAS1), A FLAVONOID OBTAINED FROM BROSIUM ACUTIFOLIUM.

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Brosimum acutifolium (BA) is a plant distributed in the forest of Pará State, it is used in folk medicine by Amazon community as an anti-inflammatory and anti-rheumatic agent. In this study we analyzed a flavan isolated from BA in order to determinate their anti-inflammatory effect and to ratify the use of the vegetal as phytoterapic against inflammatory process. The aim of this study was to evaluate the anti-inflammatory properties of 4'-hydroxy-7,8-(2''2''-dimethylpyran)flavan (BAS1), a flavan isolated from BA in two recognized models of rat inflammation in vivo.

Methods and Results:

Adult male Wistar rats, weighting 170-200 g, temperature-controlled environment (23 ± 2 °C) with a 12:12-h light/dark cycle with free access to rat chow and tap water. To evaluate BAS1 effects, we performed the established models of paw edema and air pouch utilizing carrageenan as the flogistic agent. The inflammatory parameters utilized in the present works were the paw edema volumes, determination of exudate, cellular migration and nitrergic activation measured by Griess method. We also evaluated the possible toxicological effect of BAS1 in the liver and kidney functions by biochemical determination of albumin levels, plasma and urinary creatinine, alkaline phosphatase, total protein and plasmatic urea. The results shown that BAS1 early administered into the paw evoked an intense reduction in edema volume, 61 ± 4.2 % in the first hour when compared with the control (100%). Similar effect was observed in the group treated with indomethacin. In the BAS1 treated group the volume of exudate harvested from the air pouch was significantly decreased in comparison with the control. The maximum reduction was about 62 ± 5.73 % in the animals treated with 1.2 mg/kg of BAS1. Similar results were observed with the dexamethasone (0.6 mg/kg) treated group about 68 ± 1.94 %. In the present work we also evaluated the effect of BAS1 treatment in the cellular number present in the air pouches after carrageenan treatment, in the groups treated with 0.012 mg/Kg, 0.12 mg/Kg and 1.2 mg/Kg of BAS1 were observed a dose dependent decrease in the number of inflammatory cells about 20 ± 1.46 %, 38 ± 1.38 % and 72 ± 4.06 % respectively. The administration of dexamethasone also evoked an intense reduction about 98 ± 5.89 % in the cellular migration. Our results demonstrated that BAS1 treatment induced a significant decrease in the nitrite concentration in rats treated with carrageenan, nitrite levels in the control was about 6.5 µM and in the animals treated with 0.012mg/Kg, 0.12 mg/Kg and 1.2 mg/Kg of BAS1 were about 35 ± 6, 41 ± 6.6 and 44 ± 6.5 % respectively. Dexamethasone treatment also promoted a high decrease, about 92 ± 3.33 % in the nitrite concentration in the animals treated with carrageenan. The treatment with BAS1 did not induce significant alterations in the biochemical parameters when compared with the control group.

Conclusions:

Our results demonstrated that BAS1 treatment evoked a significant decrease in the volume of exudates, paw edema, cellular migration and nitrite levels. No disturbance was observed in liver or kidney of rats after the treatment with the higher dose of BAS1. The BAS1 represents an active chemical component of BA plant that exhibits anti-inflammatory effect in rats, as well as, its utilization do not induce dysfunction on the liver or kidney qualifying its possible utilization as a biopharmaco.

Keywords: Air pouch, Brosimum acutifolium, Flavonoids, Inflammation, Paw edema

Financial Support: EGPA, UFPA, CNPq.
The purpose of this study was to investigate the effect of intragengival infusion of Poly(vinyl pyrroldone) (PVP)/GSNO formulations, on experimental periodontal disease (EPD) in rats, evaluating the inflammatory parameters and antioxidants, as well like his effect of the alveolar bone.

Methods and Results:

EPD was induced by a nylon thread ligature surgically placed around the cervix of the second left maxillary molars of female Wistar rats (180-200g). Animals were treated with 50ìL GSNO (0.5, 2 or 10 mmol L⁻¹), PVP or saline subgingivally 30 minutes before periodontitis induction and daily until sacrifice on 11th day. The parameters analysed were alveolar bone loss (ABL), bone alkaline phosphatase, myeloperoxidase (MPO), cytokines levels (IL-1α and TNF-α), malondialdehyde (MDA), reduced glutathione (GSH) content, nitrite/nitrate levels, and immunohistochemistry for metalloproteinase (MMP-1/8), inducible nitric oxide synthase (iNOS) and nuclear factor-κB (NFκB). The GSNO in the concentrations of 0.5 and 2 mmol L⁻¹ reduced ABL (1.76±0.2 and 0.69±0.12 mm², respectively), MPO (6.96±0.9 and 6.49±0.9 U of MPO/mg, respectively), inflammatory cytokines; IL-1α (305.3±70.1 and 292.0±35.4 pg/ml, respectively) and TNF-α (371.5±28.9 and 308.4±30.6 pg/ml, respectively), nitrite/nitrate (60.8±3.9 and 35.7±1.5 µM) when compared with saline (3.87±0.36 mm² of ABL; 16.0±1.5 U of MPO/mg; 675.3±33.3 pg/ml of IL-1α; 572.3±81.7 pg/ml of TNF-α; 96.9±5.8 µM of NOx) or PVP groups (3.68±0.35 mm² of ABL; 14.7±2.2 U of MPO/mg; 666.3±61.5 pg/ml of IL-1α; 579.8±51.2 pg/ml of TNF-α; 87.0±7.6 µM of NOx). GSNO 0.5 and 2 mmol L⁻¹ also decreased the demarcation to MMP-1/8, NOSi and NFκB. However, just GSNO (2 mmol/L-1) reduced MDA.

Conclusions:

These results show that GSNO has a protective effect on the experimental periodontal disease by reducing inflammation and oxidative stress.

Keywords: Periodontal disease, S-Nitrosoglutathione (GSNO), Nitric oxid

Financial Support: CNPq

QuebraPagina

Resumo:17-246

PHARMACOLOGICAL CHARACTERIZATION OF EDEMATOGENIC AND HYPERALGESIC ACTIVITIES AND HISTOPATHOLOGICAL ANALYSIS OF TISSUE DAMAGE INDUCED BY A METALLOPROTEASE FROM BOTHROPS JARARACUSSU SNAKE VENOM

Fachinelli, T. C. ²; Gomes Filho, S. A. ¹; Mamede, C. C. N. ¹,4; Sousa, B. B. ³; Fonseca, K. C. ¹,4; Silva, T. K. A. ²; Pereira, D. F. C. ²; Stanziola, L. ³,4; Canabrava, H. A. N. ³,4; Oliveira, F. ¹,4

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³ Instituto de Ciências Biomédicas, UFU
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Objectives:

The aim of this study was pharmacological characterize the edematogenic and hyperalgesic responses and histopathological analyze the tissue damage induced by a metalloprotease from B. jararacussu snake venom.

Methods and Results:

To analyze the tissue lesion, 50 µg of the metalloprotease was intraperitoneally or intraplantarly injected in male Wistar rats groups, sterile saline was injected in control group by similar conditions. After 24 hours of the injection, the organs was removed
and fixed in formaldehyde 10%. The material was dehydrated and included on paraffin. Histological sections with five micrometers thickness were obtained and stained on Haematoxylin-Eosin (HE) technique and analyzed in light microscope. For the kinetic characterization of the edematogenic and hyperalgesic responses, 50 µg of the toxin was injected intraplantarly in rats. The evolution of the edema was measured by the volume variation (by plethysmometer) and the pain threshold as measured by paw pressure test (Arch.int.Pharmaco. 113:233, 1957) before and in several times after the metalloprotease injection (1, 2, 3, 4, 5 and 24 hours). The characterization of the chemical mediation of the inflammatory responses was made by pharmacological treatments: Promethazine (5 mg/kg; i.p.; 30min before), Indometacin (4 mg/kg; i.p. 30 min before) and HOE-140 (5 µg/paw; simultaneously). The toxin caused morphological changes in different tissues 24 hours after application. In the liver was observed necrosis, evidenced by morphological disorganization, inflammatory infiltrate and blood extravasation. The skeletal muscle shown necrosis and leukocyte infiltration after toxin injection. Lung, kidney and heart don’t shown relevant morphological alterations, compared with control group. The isolated metalloprotease led to an increase of 15% in the rats paw, and 19 % of decrease of nociceptive threshold in the first hour and average of 12% in the following hours. Although they are discrete, these effects are statistically significant (ANOVA, P

Conclusions:

The metalloprotease from B. jararacussu was able to cause morphological alterations and inflammatory response in the liver and skeletal muscle, edema formation and hyperalgesia in experimental animals. The pharmacological characterization suggest that the mechanism of edema formation of this metalloprotease is associated with several inflammation pathways, involving since lipid metabolites until release of vasoactive amines and kinins, but it has no relation with the hyperalgesic response. Others treatments, doses and tests are being investigated to confirm these mechanisms.

Keywords: Snake Toxin, Hyperalgesia, Edema, Histopathology, Metalloprotease

Financial Support: FAPEMIG/CNPq/MCT

QuebraPagina

Resumo:17-247

EFFECT OF INFlixIMAB ON CHRONIC INFLAMMATION CAUSED BY SILICA PARTICLES IN MICE.

Ciambarella, B. T. ; Arantes, A. C. ; Ferreira, T. P. T. ; Jurgilas, P. B. ; Sant’anna, E. S. ; Pires, A. L. A. ; Cordeiro, R. S. B. ; Martins, M. A. ; Silva, P. M. R.

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Objectives:

Long-lasting inhalation of silica particles leads to silicosis, a disease characterized by leukocyte infiltration, collagen deposition and an extensive granuloma formation in the lung parenchima. TNF-α has been described as a proinflammatory/profibrotic mediator known to induce leukocyte recruitment and NF-κB activation. We previously demonstrated the involvement of TNF-α in the airways hyperreactivity and granuloma formation in silicotic mice, indicating that it may be a potential therapeutic target in the disease. Thus, this study was carried out to investigate the effect of a TNF-α neutralizing antibody infliximab (Remicade®) on the experimental silicosis in mice.

Methods and Results:

Male Swiss-Webster mice (18 – 20 g; n=5/group) were instilled with 10 mg/50uL of silica and with a similar volume of saline (controls). Treatment consisted of intraperitoneal administration of infliximab (1.25 and 2.5 mg/kg) into mice once a day on days 7, 14 and 21 post-silica provocation. The parameters were analyzed on day 28 and included i) lung function (resistance and elastance) and airways hyperreactivity to increasing concentrations of methacholine (Finepoint, Buxco System); ii) lung tissue morphology and morphometry; iii) collagen deposition (Sircol technique); iv) chemokine/cytokine (ELISA) and metalloproteases (MMP) 2 and 9 (zymography) quantification. All experimental procedures were performed in accordance with the guidelines of
the Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation (L-034/09). Results: Our data showed that silicotic mice had an increase in the basal levels of lung resistance and elastance together with hyperreactivity to aerosolized methacholine. A marked granuloma formation, mediator generation (MIP-1α, MIP-2, INF-γ and TNF-α) and MMP-2 and -9 release were also noted in the lung tissue of silicotic mice as compared to controls. Treatment with infliximab, at the dose of 1.25 mg/kg, inhibited lung function compromise and airways hyperreactivity. The fibrotic response including granuloma formation, cytokine and chemokine generation as well as MMP-9 release were also markedly reduced by infliximab. The compound failed to alter the MMP-2 release in the silicotic mice.

Conclusions:

Altogether our findings show that treatment of silicotic mice with the TNF-α neutralizing antibody was effective to inhibit important features of the disease including lung function and tissue compromise. They also reinforce the proposition that TNF-α is an important therapeutic target in silicosis and indicated that treatment with infliximab seems to constitute a promising pharmacological tool for the treatment of chronic fibrotic diseases such as silicosis.

Keywords: Silicosis, Infliximab, Chronic Inflammation, Lung Function

Financial Support: FIOCRUZ/CNPq/FAPERJ

QuebraPagina

Resumo:17-248

GASTRIC CYTOPROTECTIVE EFFECT OF CHRESTA MARTII (DC.) H. ROB. DEPENDS ON Á2-ADRENERGIC RECEPTOR ACTIVATION BUT NOT ON NITRIC OXIDE ACTIVITY

Val, D. R. 1; Silva, A. A. R. 1; Vieira, A. M. 1; Araujo, E. B. 1; Ribeiro, K. A. 2; Chaves, H. V. 1; Bezerra, M. M. 1; Pinto, V. P. T. 1; Filho, G. C. 1; Maia, M. B. S. 3

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2 Universidade Estadual vale do Acaraú, UVA
3 Universidade Federal de Pernambuco, UFPE

Objectives:

Chresta martii (Asteraceae) is found in the Xingó region (semi-arid area) in Northeast of Brazil, and recognized by local population as a traditional herb used to manage gastric-related complications. The aim of this study was to evaluate the role of α2-adrenergic receptors and nitric oxide on gastric cytoprotective effect of hydro-alcoholic extract (HAE) of the aerial parts (leaves and flowers) from C. martii.

Methods and Results:

The experimental protocols used in this study were approved by Committee on Animal Ethics (EAEC) from Federal University of Pernambuco (process nº 23076009313/2003-04), Recife, Brazil, in accordance with international guidelines (NIH publication No. 85-23, revised 1985). The ulcerogenic procedure was made by ethanol 99,9% (0.2 ml/animal, p.o.) and administrated in 24 h fasted mice (n=06 animals/group). After 30 min, mice were euthanatized and its stomachs were removed, opened and photographed to hemorrhagic area evaluation (ImageJ ® – National Institutes of Health – NIH). The involvement of nitric oxide was evaluated by L-NAME (20 mg/kg, i.p.) pretreatment 15 min before HAE (100 or 400 mg/kg, p.o.), L-arginine (600 mg/kg, i.p.), or saline (15mL/kg p.o.) administration. The involvement of α2-adrenoceptors was evaluated by Yohimbine (2 mg/kg, s.c.) pretreatment, 20 min before HAE (100 or 400 mg/kg, p.o.), clonidine (0.05 mg/kg, p.o.), or saline (15mL/kg p.o.) administration. After 30 or 60 min of the treatments (i.p. or p.o., respectively), each group received ethanol. A control group without L-NAME or Yohimbine was used to compare different treatments. HAE (100 or 400 mg/kg.) treated groups showed significantly (P
Conclusions:

The results suggest that the HAE of aerial parts of C. martii shows significant cytoprotective effect against ethanol-induced gastric lesions. This confirms its folk utilization. Previous administration of L-NAME did not alter the mucosal protective effect of HAE. This may signify that HAE effect is not linked to nitric oxide pathway. On the other hand, the loss of HAE protective effect in presence of Yohimbine shows that α2 adrenergic receptors are involved in its effect.

Keywords: Chresta martii, Cytoprotective, Ulcerogenic, Xingó, α2-adrenergic

Financial Support: Funcap and CNPq.

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Resumo: 17-249

TAUROLITHOCHOLIC ACID INDUCES PANCREATITIS WITH NEUTROPHIL PARTICIPATION AND PRODUCTION OF PROINFLAMMATORY CYTOKINES

Costa, J. V. G. ¹; Silva, L. M. N. ¹; Nogueira, A. F. ¹; Melo, L. F. M. ¹; Franco, A. X. ¹; Ribeiro, R. A. ¹; Souza, M. H. L. P. ¹; Criddle, D. N. ³; Soares, P. M. G. ²
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² Dept de Morfologia, UFC
³ Dept of Physiology, UL

Objectives:

Acute pancreatitis is an acute clinical disease, characterized by acute onset, rapid progression, systemic inflammatory response (SIR) and high mortality. Its pathogenesis has still not been fully elucidated till now and likewise no ideal therapy has been proposed. Our aim was to evaluate the inflammatory responses in a surgically induced mouse model of pancreatitis.

Methods and Results:

Swiss male mice (25-30g) were assigned for saline (S), tauroliothocholic (TC) or sham (Sh) groups. Thaurolithocolic acid (TLC-S), 50 ml 2%, or saline was retrogradely infused into the mouse pancreatic duct. The animals were killed 24 hours later and samples of pancreas (P) and lungs (L) were collected for histological analyses, immunohistochemistry and assessment of MPO activity. Plasma (Pl) samples were collected to determine amylase and cytokines concentrations (IL-10, CXCL1), by ELISA. All animal procedures were approved by the local ethics committee (protocol 34/10). Significance statistics (tests ANOVA and Bonferroni), values considers with p

Conclusions:

Retrograde infusion of thaurolithocholic acid elicits acute pancreatitis in animal models, with participation of neutrophils and production of proinflammatory cytokines.

Keywords: inflammation, neutrophil, pancreatitis, tauroliothocholic acid

Financial Support: CNPQ

QuebraPagina
EVALUATION OF OSTEOCLATOGENESIS AND MATRIX METALLOPROTEINASE-9 EXPRESSION IN APICAL HEALING FOLLOWING TREATMENT

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Objectives:

Aim: The purpose of this study was to evaluate the osteoclastogenesis and the expression of matrix metalloproteinase-9 (MMP-9) in teeth with apical periodontitis prior to and following root canal treatment.

Methods and Results:

Twelve dogs were selected for experimentation after approval by the Ethics Committee on Animal Use at University of Sao Paulo. Forty eight premolar teeth were assigned to 4 groups according with the treatment performed: single visit root treatment in teeth with experimentally induced apical periodontitis (Group 1); root canal treatment in teeth with experimentally induced apical periodontitis using calcium hydroxide as root canal dressing (Group 2); induction of apical periodontitis without root canal treatment (positive control) (Group 3); and healthy teeth without root canal treatment (negative control) (Group 4). One hundred and eighty days following treatment, the presence of inflammation was examined and tissues were stained to detect osteoclasts by means of a tartrate resistant alkaline phosphatase (TRAP) assay. To investigate the expression of MMP-9 in the tissues, a peroxidase-based immunohistochemistry assay and Western Blotting were used. Data were analyzed using one way ANOVA and Tukey post-test (alpha = 0.05). Teeth treated with hydroxide calcium as root canal dressing (Group 2) present a lower percentage of inflammatory cells (55% versus 70%; p < 0.05), lower expression of MMP-9 detected by Western blotting (p < 0.05) and lower percentage of cells positively stained for MMP-9 (80% versus 90%; p < 0.05) than teeth treated in single visit (Group 1). Regarding the amount of osteclastic cells, root canal treatment reduced osteoclastogenesis during periapical healing, regardless of the root canal treatment protocol used (5.0 +/- 3.0 for group 1, 4.0 +/- 2.0 for group 2, 15.0 +/- 5.0 for group 3).

Conclusions:

The expression of MMP-9 was lower in teeth treated with calcium hydroxide, suggesting that the MMP-9 is important for regulation of periapical inflammation. Root canal therapy modulated osteoclastogenesis in apical periodontitis whether a root canal dressing was used or not.

Keywords: apical periodontitis, matrix metalloproteinase-9, root canal treatment, calcium hydroxide, osteoclastogenesis

Financial Support: Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP 2006/51161-0.

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EVALUATION OF ANTINOCICEPTIVE ACTIVITY OF CARVACROL (5-ISOPROPYL-2-METHYLPHEONOL) IN MICE

Melo, F. H. C. ; Fernandes, M. L. ; Citó, M. D. C. O. ; Santos, L. K. X. D. ; Rios, E. R. V. ; Rocha, N. F. M. ; Sousa, F. C. F. D.
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Objectives:

Carvacrol (5-Isopropyl-2-methylphenol) is a monoterpenic phenol present in the essential oil of Labiatae. It is the major component of the essential oil fraction of oregano and thyme. Previous studies have demonstrated antinociceptive properties of CVC in mice when administered i.p. The present work was undertaken to evaluate the antinociceptive effect of carvacrol in oral route using behavioral models of pain, such as acetic acid-induced abdominal writhing, formalin, and hot-plate thermal tests, analyzing the involvement of nitric oxide/cyclic guanosine monophosphate pathway and opioid system.

Methods and Results:

Carvacrol (CVC) was administered orally, in male mice, at single doses of 50 and 100 mg/kg while morphine (7.5 mg/kg) and indomethacin (5 mg/kg) were used as standard drugs and naloxone (1 mg/kg) and L-arginine (150 mg/kg) were used to elucidate the possible antinociceptive mechanism of CVC on acetic acid-induced abdominal writhing and formalin tests. The results are presented as mean ± S.E.M. Data were analyzed by ANOVA followed by Student–Newman–Keuls’s post hoc test. Results were considered significant at *P* < 0.05.

Conclusions:

In conclusion, acute treatment with carvacrol at doses of 50 and 100 mg/kg seem to possess antinociceptive activity as demonstrated in the acetic acid-induced abdominal constrictions test and formalin test in mice. The central effects of CVC are not clear once naloxone and L-ARG failed in reverting the CVC action in the acid writhing test and formalin test.

Keywords: antinociceptive, carvacrol, nitric oxide, opioid

Financial Support: CNPq and CAPES

QuebraPagina

Resumo:17-252

INFLUENCE OF STATINS IN SEPSIS AND OXIDATIVE STRESS IN LIVER.

Stolf, A. M. ; Acco, A. ; Souza, C. E. A. D. ; Lívero, F. A. R. ; Dreifuss, A. A. S; Leite, A. P. B. ; Chicorski, R.

Universidade Federal do Paraná, UFPR

Objectives:

Statins are hypocholesterolemic drugs that also demonstrate antiinflammatory effects and has shown some benefits during sepsis. Some studies have demonstrated reduction of mortality in models of sepsis promoted by statins treatment. The major objective of this study is to determinate if the effects of statins in sepsis are related with reduction of hepatic oxidative stress under endotoxemia.

Methods and Results:

All the experimental protocols were approved by the Ethical Committee for Animal Research (CEEA) of Biological Sciences Sector of UFPR (certificate n. 475). Male Wistar rats were treated orally with simvastatin (1.17 mg/kg), atorvastatin (0.59 mg/kg) or vehicle (polysorbate 80 and distilled water) once a day. This doses were obtained after alometric extrapolation of the doses indicated for treatment of hypercholesterolemia in humans. After 30 days of treatment, sepsis was induced by CLP (Cecal Ligation and Puncture) on control, simvastatin and atorvastatin groups (n = 6), The sham group (n = 6), that received vehicle, was submitted only to laparotomy. After 24 hours of the CLP or laparotomy, the animals were anesthetized (ketamine hydrochloride + xylazine, intraperitoneally) for sample collection. The hepatic tissues were collected and immediately frozen at −70°C for
further analysis and the blood was drawn for plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) measurements. The quantifications of parameters that indicate oxidative stress (Superoxide Dismutase - SOD, Reduced Glutathione - GSH, Glutathione-S-Transferase - GST and Lipid Peroxidation - LPO) were performed. These obtained values were correlated with the amount of protein measured in each sample by Bradford method. The results were statistically analyzed by ANOVA and Newman-Keus as post-hoc test (p

Conclusions:

SOD and GSH are involved in redox balance and their reductions in CLP groups were probably caused by increase of reactive species production in sepsis. In the tested doses and period of treatment, neither atorvastatin nor simvastatin induced a protective effect in the liver in the CLP sepsis model, at least concerning to oxidative stress and plasma hepatic enzymes. Regarding these enzymes, AST seems to be more sensitive than ALT in the first 24 hours of endotoxia.

Keywords: CLP, Liver, Oxidative Stress, Sepsis, Statins

Financial Support: CAPES/REUNI

QuebraPagina

Resumo:17-253

ANTINOCICEPTIVE EFFECT OF ETHANOLIC EXTRACT OF POLYGALA SABULOSA IN A MODEL OF COMPLEX REGIONAL PAIN SYNDROME TYPE I

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CFS/UFSC, UFSC

Objectives:

Plants of genus Polygala are widely distributed in south of Brazil and have been employed to treat disorders of bowel and kidney, as a topical anaesthetic, expectorant and have antinociceptive properties (J Pharm Pharmacol. 2006 Jan;58(1):107-12; Basic Clin Pharmacol Toxicol. 2008 Jul;103(1):43-7). The aim of this study was evaluated the antinociceptive properties of Ethanolic Extract of Polygala sabulosa (EE) in a model of CRPS – type I (Complex Regional Pain Syndrome – Type I). Protocol approved by CEUA-UFSC under code: PP00503.

Methods and Results:

The model of CRPS – Type I, described for Millecamps, 2010, was generated following exposure to prolonged hind paw ischemic/ reperfusion injury. The animals (male mice – swiss) were divided in four groups (control and treated at doses of 30, 100 and 300 mg/kg – EE; n=6 to 7) anesthetized over a 3h period (cloral hidrate – 6%, i.p.). After induction of anesthesia, a ring was placed around the right ankle joint of mice for 3h. After a period of 7 days pos surgery, the animals was treated and submitted to a model of mechanical nociception (0.4g Von Frey filament - frequency response to ten presentations)In the 8th day after surgery the animals were submitted to a thermal model (double plate technique at temperature of 30°C and 10°C - 5 min) and in the 9th day after surgery the animals was submitted to a model of locomotor activity (Open Field). The animals were treated twice a day (12h interval) and mechanical analysis was realized for a period of 16 days. The EE of Polygala sabulosa reduced the nociceptive behavior in Von Frey model in many points of chronic treatment (16 days) at different doses (p0.05), when compared with the control group. The EE did not affect the number of crossings in a open field model.

Conclusions:

The ethanolic extract of Polygala sabulosa, was able to reduce the pain in a model of mechanic nociception (Von Frey), but not able to reduce the thermal nociception in a model of thermal preference (double plate). The EE not altered the number of
crossings in a open field model, indicating no deficits in locomotor activity.

Keywords: complex regional pain, Polygala sabulosa, Nociception

Financial Support: CNPQ - UFSC

POLYLAMININ MODULATES THE INFLAMMATORY PROCESS FOLLOWING SPINAL CORD INJURY.

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Objectives:
Polyaminin is a biomimetic laminin polymer capable of inducing a robust neuritogenesis in neurons cultivated in vitro. Polyaminin is also able to promote axonal growth and functional recovery after spinal cord injury in rats. The kinetics of the locomotor improvement as well as the analysis of the distribution of macrophages in the lesioned cord suggest a possible immunomodulatory role of the polymer. In the present study, we investigate this hypothesis, aiming at characterizing the mechanism of the immunomodulatory effect of polyaminin in spinal cord injury.

Methods and Results:
First, we observed that a functional improvement occurred as early as 24 hours after treatment, as measured by the BBB Score (0.6 + 0.5 to 2.7 + 0.7). In the same period, less neutrophil infiltration occurred at the lesion epicenter (29.1 + 5.1 cells/field to 11.0 + 2.0 cells/field). This decrease correlates with a statistically significant decrease in IL-1ß and TNF-á in the cord tissue (from 6.4+1.2 to 3.0+0.7; 8.7+0.8 to 2.8+0.3, respectively). Although these cytokines have not been detected in the serum, two other molecules related to the resolution of inflammatory processes had their serum levels altered by the polyaminin treatment, VEGF and L-selectin. Murine peritoneal macrophages cultivated for 24 hours on polyaminin showed a morphology that was compatible with that of inactivated cells, while macrophages on polyornithine, a substrate that promotes cell adhesion, showed a cell morphology compatible with the active state. Besides having distinct morphologies, macrophages on polyaminin were able to secrete factors that promoted neuritogenesis and increased cellularity in rat embryonic cortical cells.

Conclusions:
These data confirm the immunomodulatory properties of polyaminin and suggest that the polymer could directly signal to macrophages.

Keywords: cytokines, laminin, macrophages, spinal cord injury

Financial Support: CNPq, FAPERJ
EFFECTS OF TREATMENT WITH 1-(3-CHLOROPHENYL)PIPERAZINE (MCPP) ON IN VITRO NEUTROPHIL MIGRATION

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Objectives:

mCPP (1-(3-chlorophenyl)piperazine) is the main metabolite of antidepressants, like trazodone, and it is well-known and extensively used as a probe of serotonergic function in scientific researches. Recently, it has been seized in the recreative drug market instead of MDMA as an abuse drug. Nevertheless, there is few data concerning its actions in the immune system. The aim of this study was investigate the effects of mCPP on neutrophil migration in vitro and the mechanisms involved in this process.

Methods and Results:

Neutrophils were obtained from peritoneal cavity of male Wistar rats, 4h after injection of oyster glycogen (1%) and incubated in the presence or absence of 10ÌM, 100ÌM and 1000ÌM of mCPP (1–2h). Neutrophil migration was determined using fMLP as chemotactic agent in Boyden chamber and the expression of adhesion molecules L-selectin, ß2integrin and PECAM−1 was quantified by flow cytometry. Experiments were performed according to the Brazilian College of Animal Experimentation (COBEA; Protocol 233). mCPP treatment per se induced neutrophil migration (100ÌM = 46%) and increased the expression of L-selectin (100ÌM = 29%; 1000ÌM = 70%) but did not modify the expression of PECAM−1 and ß2integrin in cell membranes. On the other hand, mCPP 1000ÌM inhibited the migration induced by fMLP.

Conclusions:

Obtained data, although preliminary, suggest that mCPP changes neutrophil functions related to cell migration. Effects seem to be dependent, at least in part, on induction of adhesion molecules expressions, in neutrophils, involved in leukocyte-endothelial interactions and in the chemotactic activity. Thus, the results show that mCPP can modify the development of innate immune response, considering its neutrophillic actions in the early stages of the process.

Keywords: mCPP, migration, neutrophil

Financial Support: Capes

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UREASE OF HELICOBACTER PYLORI: ROLE IN INFLAMMATION

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Objectives:

Ureases (EC 3.5.1.5), nickel-dependent enzymes that hydrolyze urea into ammonia and CO2, are present in plants, fungi and bacteria. The spirochete Helicobacter pylori is the etiological agent of gastric ulcers and is possibly involved in the development
of gastric cancer. Urease produced by *H. pylori* (HPU) is considered a virulence factor since its ureolytic activity enables the bacterium to survive in the acidic medium of the stomach. Previous data of our group showed that HPU induces paw edema in a dose- and time-dependent manner. Here, purified HPU was evaluated for biological effects not related to its enzymatic activity. We investigated: 1) the induction of lipoxygenase expression by HPU-activated neutrophils; 2) the role of HPU on neutrophil apoptosis; 3) the chemotactic potential of HPU on human neutrophil migration; 4) the production of reactive oxygen species by HPU-stimulated human neutrophils.

Methods and Results:

Recombinant urease produced in *Escherichia coli* was purified by ion exchange and gel-filtration chromatographies and used to evaluate biological effects independent of its enzyme activity. Treatment of human neutrophils with HPU (100 nM) leads to a 2.4-fold increase in lipoxygenase levels, determined by immunoblotting, and a decrease (40.5% compared to control) in apoptosis. HPU is able to induce the expression of Bel-XL, an anti-apoptotic enzyme, and the degradation of Bad, a pro-apoptotic protein. HPU-induced neutrophil chemotaxis was 88% of that observed for fMLP (100 nM), a strong chemoattractant used as positive control. The anti-apoptotic and chemoattractant activities of HPU are abolished by AA861, a 5-LO inhibitor. HPU is also able to induce the production of reactive oxygen species by HPU-activated human neutrophils (approximately 2-fold as control).

Conclusions:

These newly described pharmacological properties indicate that HPU could play an important role in the pathogenesis of the gastrointestinal disease caused by *H. pylori*.

Keywords: Urease, Helicobacter pylori, inflammation, neutrophil, eicosanoids

Financial Support: CAPES, CNPq, FINEP, Fapergs.
Methods: All our experiments were previously approved for Ethical Committee of UNESC according to protocol (n.21/2011). Male Wistar rats were subjected to technique of cecal ligation and puncture (CLP) to induce sepsis. 3, 6 and 12 h after induction the animals were killed and the renal artery, serum and lung were removed. Renal artery and lung was performed immunocontent assessment of SOD1, SOD2 and SOD3 by immunoblotting and 3-nitrotyrosine. In the serum was measured SOD activity, immunocontent of SOD1 and SOD3 by immunoblotting and 3-nitrotyrosine as a parameter of oxidative damage. The data are expressed in mean ± standard error. In the nitrotirosine test, for Western blotting, the results are expressed as (Nitrotirosine - U/protein), while the results about SOD expression are expressed as (Arbitrarity Units).

Results: In the renal artery of rats with sepsis had decreased SOD1 after 6 h the induction in comparison to sham group (8043±362,8 vs. 11051.2±1023,3) and a decrease of SOD2 and after 6 and 12 h. While SOD3 increased after 12 h (8810,2±946,3 vs. 1672±276,3). The 3-nitrotyrosine levels decreased 6 h (0,14±0,01 vs. 0,16±0,001) and 12 h in sepsis (0,12±0,004 vs. 0,1623±0,003). In serum, the SOD3 decreased 6 h (2469±461,9 vs. 7837±431,7) and 12 h (2703,5±244,03 vs. 7156,2±870,8) after CLP, and no change was observed in SOD1. The levels of 3-nitrotyrosine is elevated in the septic 6 h (0,17±0,02 vs. 0,10±0,01), and SOD activity increased after 6 h (8,3±0,9 vs. 6,8±0,44) and 12 h (9,9±0,6 vs. 6,8±0,44). In the lung of septic rats was reduced SOD1, 3 h and 12 h after induction. The SOD2 increased 3 h after induction, while decreased 6 and 12 h, while SOD3 increased 6 h (9116,2±795,2 vs. 5383,5±785,4) and 12 h (11338,7±183,3 vs. 7546,7±1377,1) after induction of sepsis. The levels of 3-nitrotyrosine increased 3 h (0,21±0,02 vs. 0,15±0,004) and 12 h (0,20±0,01 vs. 0,16±0,001) after induction.

Conclusions: The decrease in SOD3 associated with increased serum levels of 3-nitrotyrosine indicates excessive formation of peroxynitrite radical. However, lung and especially in the renal artery increased the expression of SOD3 seems to protect the vessel of this oxidation process, indicating that SOD3 plays an important role in vessels protection against oxidative damage.

Keywords: Sepsis, extracellular superoxide dismutase, Inflammation, oxidative damage

Financial Support: UNESC, CAPES, FAPESC, CNPq and INCT

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Resumo:17-258

ANTINOCICEPTIVE EFFECTS OF FRACTIONS OF RHEEDIA LONGIFOLIA PLANCH & TRIANA METHANOLIC EXTRACT.

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Objectives:

In previous studies, fractions of methanolic extract from leaves of Rheedia longifolia demonstrated important antinociceptive effects, with different profile of action. Among these fractions, ethyl acetate fraction (RhFAcEt) was the most potent antagonist of purinergic receptor P2X7 in vitro and presented the best activity in murine models of inflammatory pain. On the other hand, butanolic fraction (RhFBuOH) prevented neurogenic pain acting through opioid receptors. The aim of this study was investigate the mechanisms of action of RhFAcEt and RhFBuOH and their effect on motor capacity.

Methods and Results:

Methods: Animals: Adult male Swiss Webster mice weighing 20 to 30 g (CEUA License 033/09). Formalin-induced nociceptive: mice were injected with formalin in the right hind paw (2.5%/20 μL/paw) one hour after the treatment with RhFAcEt or RhFBuOH (10 mg/kg, p.o.). The time licking spent was counted from 0 to 5 and 15 to 30 minutes. Acetic acid writhing test: mice received i. p. injection of 0.8% acetic acid solution. Animals were treated p.o. with fractions one hour before the stimulus and
writhing numbers was counted for 10 minutes. Paw inflammation induced by carragenin: mice were treated with RhFAcEt or RhFBuOH (1mg/kg, p.o.) 1h before carragenin injection (300µg/paw), the analgesic effect was evaluated by mechanical stimulus with Von Frey filaments and paw volume was evaluated by plethysmometer. Rota Rod Test: mice previously trained received RhFAcEt or RhFBuOH (10mg/kg, p.o.) 1h before assay on Rota Rod (9rpm). Open Field: one hour after the treatment, each animal was placed on the open-field arena and allowed to have free ambulation for 5 min. It was observed the number of floor units walked by the animal using all its limbs. Morphine (10 mg/kg, i.p.), diclofenac (50 mg/kg, p.o.), clonidine (0.01 mg/kg, i.p.) and phenobarbital (50 mg/kg, p.o.) were used as standard drugs while yohimbine (0.15 mg/kg, i.p.), efaroxan (1 mg/kg, i.p.) and idazoxan (3 mg/kg, i.p.) were used as antagonists. Results: RhFBuOH inhibited licking time in the both phases of formalin model, while RhFAcEt acted only on the inflammatory pain. When inflammation was induced with carragenin, RhFBuOH and RhFAcEt did not have antiedematogenic activity in spite of the antinociceptive action showed in Von Frey model. In the acetic acid writhing test, RhFAcEt and RhFBuOH decreased significantly the number of abdominal constrictions (40.8±5.5 and 23.3±4.5 respectively) in relation to the control group (70.9±5.6). Yohimbine did not affect analgesic activity of RhFAcEt (39.8±4.6) or RhFBuOH (30.2±4.1) suggesting that none of the fractions acts through α2 receptors. When mice were pretreated with efaroxan, RhFAcEt effect did not have its effect affected (52.5±5.3), while RhFBuOH had its action reversed (57.3±4.5), otherwise pretreatment with idazoxan did not change the effect of both fractions (44.4±7.9 and 23.3±4.5, respectively) suggesting that RhFBuOH acts through I1 but not I2 receptors. It was not observed loss of motor capacity in animals treated with or RhFAcEt or RhFBuOH in rota rod and open field tests. Data are summary of two experiments (mean±SEM, n>5).

Conclusions:

Our results confirm the species R. longifolia as a promising source of bioactive compounds for the treatment of different kinds of pain, with efficacy and safety necessary.

Keywords: Rheedia longifolia, antinociceptive, antiinflammatory, α2 receptor, imidazolinic receptor

Financial Support: IOC-Fiocruz and CNPq

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Resumo:17-259

SPINAL CORD Y1 RECEPTOR BLOCKADE REVERSES THE HYPONOCICEPTIVE EFFECT OF H1 AGONIST IN THE RAT KNEE-JOINT.

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Objectives:

Low dose injection of histamine, or a H1 agonist, into knee-joint decrease articular nociception induced by formalin in the rat. Such effect was reversed by H1 antagonists as cetirizine (SBFTE, 2010). It was reported that H1 receptors are expressed in a subtype of peptidergic afferent that contains neuropeptide Y (NPY), and it is known that NPY plays a hyponociceptive role in spinal cord. Thus, the aim of this study was to test the hypothesis that spinal cord NPY could be mediating the hyponociceptive effect of the knee-joint H1 receptor activation.

Methods and Results:

Articular incapacitation was measured by counting the paw elevation time (PET; s) during 1 min period of forced walk either each 5 min throughout 60 min after formalin knee-joint injection. Formalin 1.5% induced two phases (P1: 0-5 min, and P2: 10-60 min) of nocifensive behavior. The Y1 receptor agonist (Leu 31, Pro 34)-NPY (0.07; 0.7; 7 and 70 imol/i.t) or antagonist Y1 receptor BIBO 3304 (0.4; 4 and 13 imol/i.t) were administrered 20 min before formalin injection. The agonist H1 receptor 2-PEA (5 mmol/i.a) was co-injected with formalin. This work was approved by the local ethical committee for animal use (CEUA 23080.034306/2009-69). The (Leu 31, Pro 34)-NPY (0.7 and 7 imol/i.t. P
Conclusions:

These results suggest that the Y1 receptor in the spinal cord is involved with the hyponociceptive effect of intraarticular H1 receptor activation.

Keywords: Histamine, Neuropeptide Y, Formalin, Arthritis

Financial Support: Capes, Fapesc/Pronex/CNPq

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Resumo:17-260

FUCOIDIN, AN P AND L-SELECTIN BLOCKER, PREVENTS SEVERE ACUTE PANCREATITIS INDUCED BY CERULEIN AND LPS IN MICE.

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Objectives:

Acute pancreatitis, especially severe acute pancreatitis, is often followed by gastrointestinal dysmotility and systemic inflammatory response syndrome (SIRS). A new mouse model of severe acute pancreatitis, induced with cerulein and lipopolysaccharide (LPS), has recently been described, which is non-traumatic and possesses the same pathological characteristics as those of severe acute pancreatitis in humans (Am J Physiol Gast Liver Physiol. 297:G981, 2009). The aim of the present study was to assess the effects of the polysaccharide fucoidin, an P and L-selectin blocker, in severe acute pancreatitis in mice.

Methods and Results:

Methods: Severe acute pancreatitis was induced in Swiss mice by 12 i.p. injections of cerulein (50 µg/kg, hourly intervals), challenged by LPS (3 mg/kg i.v.) 12 hours after the first injection of cerulein. In another group of animals, fucoidin (25 mg/kg, i.v.) was administered 30 minutes before the first cerulein injection and immediately after LPS treatment. Twelve hours after the last cerulein injection, serum amylase, pancreas weight, lung and pancreas myeloperoxidase (MPO) activity, gastric emptying (GE) and gastrointestinal transit (GIT) were measured. In order to study gastrointestinal motility, mice were gavage-fed (300 µl) with the test meal (5% glucose solution with 0.05 g/mL phenol red) and sacrificed 20 mins later. Then, GE and GIT (using the geometric center method) were measured. Experimental protocols were approved by the Institutional Committee on Care and Use of Animals for Experimentation (No. 26/10). Statistical analysis was performed using an ANOVA test. Results: Cerulein with LPS caused a significant (p

Conclusions:

Fucoidin ameliorates the severity of acute pancreatitis induced by cerulein+LPS reducing the systemic inflammatory response syndrome, by decreasing neutrophil infiltration in pancreas and lung. Moreover, fucoidin improves disturbed motility in severe acute pancreatitis.

Keywords: PANCREATITIS, FUCOIDIN, CERULEIN
SWIMMING EXERCISE INHIBITS SPINAL PHOSPHOLIPASE C-Γ AND CAMP RESPONSIVE ELEMENT BINDING PROTEIN PHOSPHORYLATION IN A MOUSE MODEL OF NEUROPATHIC PAIN.

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Objectives:

Exercise-induced synaptic plasticity in the spinal cord, may help to recover motor and sensory function following nerve injury. However, the effect of physical exercise on cellular and molecular changes induced by pathological conditions remains poorly understood. Here, we investigated whether chronic forced swimming activity influences tactile stimulus-induced neuropathic pain hypersensitivity and the phosphorylation status of the transcription factor cAMP responsive element binding protein (CREB). Upstream regulators of CREB such as ERK1,2, p38, JNK, and phospholipase-Cγ (PLCγ) were also monitored.

Methods and Results:

Adult Balb/C male mice weighting 20-25 g were used. Neuropathic pain was induced by partial sciatic nerve ligation whereas sham operated mice were used as control. Chronic exercise activity was performed by swim training which lasted 40 min/day, 5 days/week for 6 weeks. Nerve injured and sham-operated animals swam for the same period of time whereas naïve mice swam for 30 seconds for 6 weeks. The development of tactile hypersensitivity following nerve lesion was monitored by von Frey filaments whereas the phosphorylation of CREB, ERK1,2, p38, JNK, and PLCγ was evaluated by immunohistochemistry and western blots. Seven days after nerve injury, animals showed a marked hypersensitivity to mechanical stimuli which lasted for at least 6 weeks (p < 0.01, Mann-Whitney test, n= 8). However, under moderate swim exercise training, injured mice showed a progressive recovery of the sensory abnormality from the second week of training (p < 0.01, Mann-Whitney test). By the end of six weeks of training no difference was observed in the mechanical threshold of injured and sham-operated animals (p > 0.05, Mann-Whitney test). No sensory abnormality was observed in non-lesioned mice submitted to chronic exercise training. Peripheral nerve injury also increased the phosphorylation status of CREB, ERK1,2, p38, JNK , and PLCγ in the spinal dorsal horn. Six weeks of forced swimming, marked reduced CREB phosphorylation after nerve injury. In addition, persistent exercise reduced PLCγ phosphorylation but not ERK1,2, p38 or JNK activity (p < 0.05, Student t-test, n= 8).

Conclusions:

Our data indicate that swimming exercise has a remarkable beneficial effect on tactile stimulus-induced neuropathic pain hypersensitivity which may be induced, at least partially, by reducing CREB activity via PLCγ phosphorylation. In addition, the results indicated that the exercise effect on chronic pain is reliant on a time-dependent plasticity in the sensory system.

Keywords: CREB, PLC, Neuropathic pain, Chronic pain, Physical exercise

Financial Support: The São Paulo State Research Foundation (FAPESP grant # 07/03757-4).
Objectives:

RNA-dependent protein kinase (PKR) is one of the best characterized of many proteins induced by type I interferon. PKR is stimulated by a wide range of different cell stresses such as cellular viral double-stranded RNA, proinflammatory mediators, growth factors and cytokines. However, PKR has not been identified in the nociceptive system and its intracellular signaling pathway is unknown under peripheral inflammatory condition. In the present study we combine genetic, molecular and cellular methods with pharmacological and behavioral paradigms to uncover the role played by PKR in the spinal cord dorsal horn during chronic inflammatory disease.

Methods and Results:

Male C57Bl/6 mice weighting 20-25g were used. Chronic inflammation was induced by subcutaneous injection of Complete Freund’s Adjuvant (CFA) (20uL/paw) in the dorsal aspect of the left paw whereas control animal was injected with mineral oil. The development of tactile hypersensitivity during chronic inflammation was monitored by von Frey filaments 3 days after CFA administration. PKR mRNA expression was investigated RT-PCR. Protein analysis was performed by immunohistochemistry and western blot. Under chronic inflammatory condition, PKR mRNA and the protein are up-regulated in the spinal cord dorsal horn ipsilateral to the inflamed paw (Student t-test, p < 0.05, n = 6/group). Intrathecal administration of PKR inhibitor completely reversed inflammation-induced thermal hypernociception in a concentration-dependent manner (two-way ANOVA, p < 0.05, n = 12/group) whereas PKR deficient mice (PKR+/−) showed a significantly reduced CFA-induced hypernociception (two-way ANOVA, p < 0.05, n = 12/group). Moreover, inhibition of PKR activity in the spinal cord marked inhibits p38 and JNK MAP kinase family members as well as IKK increased phosphorylation induced by peripheral inflammation (Student t-test, p < 0.05, n = 6–8/group). Conversely, PKR inhibition had no effect on Eif2-α and p42/44 MAP kinase activity.

Conclusions:

Here we show for the first time that PKR play a key role in chronic inflammatory pain processing as a pronociceptive molecule. The results strongly indicate that PKR acts through different signaling pathways in the spinal dorsal horn and therefore may participate in the regulatory mechanisms underlying pathological pain. Our results offer a completely new view to the mechanisms of action of PKR and should lead to a better understanding of the fundamental biology of chronic pain. Most important, this study revealed a novel target for the development of more effective analgesic drug.

Keywords: PKR, pain, inflammation

Financial Support: State of São Paulo Research Foundation (FAPESP grants # 08/04994-2)
Objectives:

Cyclophosphamide (CYP) is a chemotherapeutic drug widely used in the treatment of solid tumours, B-cell malignant diseases and some non-neoplastic conditions such as nephrotic syndrome and rheumatoid arthritis. Hemorrhagic cystitis (HC) is a common side effect observed in patients under chemotherapy with CYP. The urotoxic side effects of CYP are attributed to the metabolic compound acrolein and can be partially prevented by the uroprotector agent 2-mercaptoethene sulfate (Mesna). This study analyzed the anti-inflammatory and the antinociceptive effects of the selective CXCR2 and TRPV1 receptor antagonists - SB225002 and SB366791 - in the rat model of CYP-induced HC.

Methods and Results:

Female Wistar rats were used (150–200 g) and HC was induced by a single injection of CYP (200 mg/kg, ip). Some groups of animals were pretreated with Mesna (21.5 mg/kg, ip, 30 min before CYP injection), SB225002 (1 mg/kg, ip, 30 min before CYP injection) or SB366791 (500 μg/kg, ip, 30 min before CYP injection). Breathing rate, closing of the eyes, and specific posture were scored at different time points after cystitis induction as nociception indexes. The mechanical hypernociception was measured with Von Frey filaments in the bladder area and in the rat paw, before and after CYP-induced HC. The response of the rats to a noxious thermal hypernociception was determined by Hargreaves’ plantar test, in the bladder area and in the rat paw, before and after CYP-induced HC. As inflammatory parameters, hemorrhage presence, oedema formation, and bladder wet weight were determined macroscopically at 24 h after CYP administration. The neutrophil migration and inflammatory cytokines profile was assessed 4 h after cystitis induction by means of myeloperoxidase (MPO activity) and IL-1β, respectively. All the experimental procedures were approved by the Animal Ethics Committees of Universidade Federal de Santa Catarina (protocol number PP00607). As expected, Mesna treatment was able to reduce in a significant manner all the inflammatory and the nociceptive parameters induced by CYP. Of note, the pre-administration of SB225002 and SB366791 significantly attenuated the hemorrhage, the oedema formation, as well the increase in MPO activity and the elevation of IL-1β. Interestingly, the pretreatment with either SB225002 or SB366791 markedly reduced the bladder wet weight and the mechanical and thermal nociceptive responses.

Conclusions:

The present results indicate that CXCR2 and TRPV1 antagonists might represent important alternatives to prevent inflammation and nociception following chemotherapy with CYP.

Keywords: Hemorrhagic cystitis, Cyclophosphamide, CXCR2, TRPV1, nociception

Financial Support: Capes, CNPq

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Resumo:17-264

ANTI-INFLAMMATORY POTENTIAL OF A NATURAL PRODUCT LAPACHOL AND ITS DERIVATIVE LQB 118,A NEW PTEROCARPANQUINONE

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Objectives:
The aim of our investigation is to study the anti-inflammatory effect of the natural product extracted from Tabebuia, lapachol, and its synthetic derivative, a new pterocarpanquinone, LQB 118.

Methods and Results:

Using a well-established inhaled lipopolisacharide (LPS)-induced lung inflammation model, with C57J/BL6 mice (animal protocol approved by CEUA/UFRJ - reference number HU 008), our results demonstrated that intraperitoneal treatment with 1 mg/Kg of lapachol or LQB 118 reduced neutrophil influx to lungs in mice submitted to inhaled LPS (0,5 mg/mL). Additionally, LQB 118 reduced the concentration of the inflammatory mediators TNF-α and KC in the bronchoalveolar lavage fluid (BALF). These effects of LQB 118 are comparable to those of dexamethasone or aspirin, two anti-inflammatory drugs. The reduction of mediators concentration by LQB 118 is partially due to reduction of NF-κB activation in the cell lungs. In vitro, LQB 118 also inhibited TNF-α production in LPS-stimulated mononuclear cells of human peripheral blood (PBMC). The highest concentration tested (100 μM) was able to inhibit almost 100% of TNF-α liberation.

Conclusions:

These data confirm the anti-inflammatory action of lapachol observed in a paw edema model (J. Ethnopharmacol. 29:239, 1990.), and reveal the lapachol derivative, a new pterocarpanquinone LQB 118, as a potent modulator of inflammation, through the reduction of inflammatory mediators.

Keywords: Inflammation, Pterocarpanquinone, Lapachol

Financial Support: CAPES, CNPq, FAPERJ.
C57BL/6 female mice (25-30 g) were given saline (0.5 mL, ip) or infliximab (5, 10 mg/kg ip) 1h before saline or IFO (400 mg/kg) injection. Visceral nociception was performed, 12h after IFO injection, using a digital analgesymeter (von Frey). The animals were killed 12h after IFO injection and bladder wet weight (BWW), vascular permeability (VP), macroscopic and microscopic parameters, myeloperoxidase assay (MPO), in vitro bladder contractility to Carbachol and KCl were performed. The study was approved by Ethics Committee (Protocol 09/06) Results were expressed as mean ± S.E.M. and analyzed by ANOVA, considering significant when p value was < 0.05. IFO increased nociceptive threshold (10.05 ± 0.55 g) when compared to saline group (1.76 ± 0.87 g), and infliximab inhibited (4.83 ± 0.81 g) such effect. Furthermore, IFO increased BWW (49.77 ± 3.46 mg/20g), VP (9.33 ± 1.49 µg/mL) edema (2[1-2]), hemorrhage (2[2-2]), microscopic (2[2-2]) scores when compared to saline group (18.06 ± 1.579; 0.60 ± 0.30; 0[0-0]; 0[0-0]; 0[0-0], respectively). These effects were prevented with infliximab treatment (24.91 ± 2.41; 4.17 ± 0.33; 0[0-1]; 1[0-1]; 1[1-2], respectively). Conversely, infliximab was not able to prevent IFO-induced MPO increase (1.54 ± 0.45 U/mg; 2.84 ± 0.30 U/mg, respectively), and control was 0.11 ± 0.11 U/mg. Furthermore, IFO induced bladder hyporresponsiveness to carbachol (0.14 ± 0.03 g/mg) and KCl (0.13 ± 0.02 g/mg) when compared to control group (0.25 ± 0.04 and 0.21 ± 0.04, respectively), and infliximab did not prevent this effect (0.15 ± 0.02 and 0.10 ± 0.02, respectively).

Conclusions:

This study showed the efficacy of infliximab in controlling the edema, hemorrhage, and nociception in a mice model of IFO-induced HC. However, infliximab was not able in preventing bladder dysfunction and neutrophil infiltration.

Keywords: Hemorrhagic cystitis, Ifosfamide, Infliximab, TNF alpha

Financial Support: CNPq/CAPES/FUNCAP

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Resumo:17-266

WHITE ADIPOSE TISSUE INFLAMMATION IN WALKER 256 TUMOR-INDUCED CACHEXIA

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Objectives:

Aim: To investigate gene and protein expression of proinflammatory cytokines from different WAT (White Adipose Tissue) deposits (WMAT; White Mesenteric AT, WEAT;White Epididymal AT and WRAT;White Retroperitoneal AT) in rats with cachexia induced by Walker 256 tumor.

Methods and Results:

Methods and results: Male Wistar rats, (062/04/CEEA), 8 weeks old, were inoculated subcutaneously with a 1 mL preparation containing ~107 tumor cells. Samples of AT were collected at days 0, 4, 7 and 14 after Walker 256 tumor cells inoculation and stored at -80oC. The animals were divided in groups (5 animals per group). For gene expression, RT-PCR (Real time-PCR) was used to perform mRNA detection and quantification and ELISA was chosen for protein expression. The results showed that the proinflammatory genes, such as F4/80, CD 68 and MCP-1, have increased their expression level only in the 14th day after inoculation, respectively, WMAT (462, 1016 e 145%), WEAT (1430, 241 e 1920%) and WRAT (878, 629 e 725%). However, gene expression of haptoglobin has increased since the 7th day after inoculation in both WMAT and WEAT (867%), but not in WRAT (617%). TNF-α protein level has also increased since the 7th day after inoculation in all AT deposits (221, 205 e 167%).

Conclusions:
Conclusion: The results suggest that white AT is primarily affected by inflammatory systemic route and not by the autocrine one, because TNF-β expression increase occurs before alterations in cytokines gene expression. This sort of behavior reflects an increase of active and infiltrated macrophages in all white AT deposits.

Keywords: Adipose tissue, Cachexia, Inflammation

Financial Support: Sources of research support: FAPESP and 2008/54091-9 2007/52782-1

PARTICIPATION OF NONPEPTIDERGIC NOCICEPTORS IN INFLAMMATORY PAIN

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Objectives:

The small-diameter nociceptive fibers (C fibers) have an important role in detecting noxious stimuli, initiating the transmission of painful information, and are classified as nonpeptidergic and peptidergic C fibers. However, a possible functional difference between these two classes of C fibers in the genesis of acute nociception as well as inflammatory pain is still unclear. Thus, this study aims to elucidate the role of non-peptidergic fibers in acute nociception induced by mechanical, thermal and chemical stimuli as well as in inflammatory hypernociception.

Methods and Results:

In order to elucidate differences between these two classes of C fibers, a neurotoxin was used to selectively eliminate the non-peptidergic C fibers: a saporin conjugated to isolectina B4 (IB4). Nociceptive threshold was evaluated through thermal (Hargreaves) and mechanical (filaments and electronic von Frey) tests in C57BL/6 mice. This study was approved by Animal Ethics Committee of FMRP/USP (nº 101/2010). Firstly, it was observed that the intrathecal administration of IB4-saporin not changed the thermal and mechanical basal threshold of the mice when compared to saline and saporin-control groups. Moreover, we demonstrated that mechanical inflammatory hypernociception induced by carrageenan and prostaglandin in mice was reduced by the intrathecal administration of IB4-saporin. In agreement, the treatment with IB4-saporin inhibited the nociception caused by intraplantar injection of the capsaicin.

Conclusions:

These results suggest that the nonpeptidergic C fibers are important in driving the nociception induced by inflammation but not in basal state. Furthermore, we found that the nonpeptidergic C fibers appear to be important in models of acute nociception as induced by capsaicin.

Keywords: C fibers, IB4-saporin, inflammatory pain, nociception, nonpeptidergic

Financial Support: FAPESP, CNPq.
EVALUATION OF ANTINOCICEPTIVE AND ANTI-INFLAMMATORY ACTIVITIES OF NOVEL N-ACYLHYDRAZONES DERIVATIVES DESIGNED BY MOLECULAR SIMPLIFICATION AT PROTOTYPE LASSBIO-294

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2 Laboratório de Avaliação e Síntese de Substâncias Bioativas, UFRJ

Objectives:

This work aimed the pharmacological evaluation of novel N-acylhydrazones derivates planned by molecular simplification at the structure LASSBio-294, a cardioactive compound with antiinflamatory and analgesics activities.

Methods and Results:

Methods: Experiments were conducted using adult Swiss mice (20-30g), males or females, 6–8 weeks of age. All animals came from the breeding unit of the BIOCEN–UFAL and approved by the Ethics Committee –UFAL (number: 026681/2009-23) for animal handling. We performed functional models of nociception and inflammation in vivo: abdominal writhing induced by acetic acid (Brit. Jour. Pharm., 32; 285, 1968), nociception induced by formalin (Pain, 30; 103, 1987) and peritonitis induced by carrageenan (Infla. Res., 32, 2838, 1991). Derivates and standards drug were administrated 40 minutes before the tests, by oral route, at the dose of 100 µmol/kg. The results were analyzed by test t in the tutorial Prism® (*p

Conclusions:

The results suggest that the compounds present peripheral antinociceptive and anti-inflammatory activities. Further studies are needed to establish the comparative potency of derivatives.

Keywords: antinociceptive, anti-inflammatory, acylhydrazones

Financial Support: INCT-INOFAR/CNPq (573.564/2008-6), PROSUL (#490.600/07-7), CNPq, FAPEAL.

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Resumo: 17-269

EVALUATION OF THE ANTIINFLAMMATORY EFFECT OF NDP-MSH IN THE CARRAGENIN-INDUCED PERITONITIS.

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Objectives:

Neutrophil migration leading to infectious focus is critical to immunity. A rapid and regulated neutrophil migration from the vascular compartment to sites of infection is required for successful host responses during a bacterial invasion. However, excessive neutrophil recruitment is associated with deleterious organ-specific consequences. The Scholinergic anti-inflammatory pathway, a recent discovered parasympathetic anti-inflammatory neural route, reduces the neutrophil migration during an acute inflammatory response. Therapeutic approaches to activate this pathway may be utilized to control inflammatory diseases such as
is melanocortins. In the present study, we investigated the effect of NDP-MSH - a semi-synthetic melanocortin peptide - in the carrageenin-induced neutrophil migration, discussing the probable mechanisms involved in this effect.

Methods and Results:

The peritonitis was induced in male Swiss mice (22-26 g) by intraperitoneal carrageenin injection (500 ug/cavity). The animals were pretreated with different doses of NDP-MSH (100-1000 ug/kg, s.c.) or vehicle (saline), 30 min before carrageenin administration. After 4 h, the peritoneal cavity was washed with 2 mL of sterile saline containing EDTA. Total leukocyte counts were performed in a Neubauer chamber, while the differential leukocyte counts were performed in cytospin preparations. The cytokines were determined by ELISA. Moreover, mice neutrophils were treated with NDP-MSH (30-1000 nmol/l) or vehicle (RPMI with 2% DMSO) for 30 minutes, and then assayed for chemotaxis using a Boyden chamber. In order to investigate the role of the spleen, the NDP-MSH effect was investigated in splenectomized animals. The results were presented as mean ± S.E.M and analyzed statistically by one-way ANOVA, with the differences between groups assessed using the Bonferroni post-test (P < 0.05). The Pretreatment with NDP-MSH inhibited, in a dose dependent manner, the recruitment of neutrophils to the peritoneal cavity (100ug/kg = 6.18 ± 0.21x10^6 cells, 300ug/kg = 4.30 ± 0.73x10^6 cells and 1000ug/kg = 3.56 ± 0.44x10^6 cells) as compared to the carragenin group (8.66 ± 0.25x10^6 cells). This mechanism was dependent on the spleen but independent of cytokines production due to the fact that the levels of cytokines (TNF-alfa, IL-10, KC and IL-1beta) were not changed as compared to the control group. Moreover, this compound was not able to inhibit MIP-2-induced neutrophil chemotaxis suggesting no direct effect in this cellular function.

Conclusions:

These preliminary results showed that NDP-MSH shows a promising anti-inflammatory activity in this peritonitis model of inflammation. However, further studies are necessary in order to evaluate the mechanism of anti-inflammatory action.

Keywords: carrageenin, inflammation, cytokines, migration neutrophil

Financial Support: Fapesp, CNPQ, CAPES

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Resumo:17-270

ANTINOCICEPTIVE AND ANTIINFLAMMATORY ACTIVITIES OF OIL-RESIN FROM COPAIFERA GLYCARYCARPA (DUCKE)

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Objectives:

To study the pharmacological properties of oil-resin(OR)extracted from Copaifera glycybarpa(DUCKE)through preliminary evaluation of the antinociceptive and anti-inflammatory. To determine the 50% inhibitory dose(ID50)and to study the mechanisms involved in such activities.

Methods and Results:

Mice SW55, adult,males weighing between 20-35 grams (n = 6-8) were used here and methods of writhings, hot plate and ear oedema. In the Writhing method (Fed. Proc. 412:18,1959) we use the following experimental groups treated orally (po): water (negative control), tween 1% (negative control), indomethacin 10 mg/kg (positive control), the OR of C. glycybarpa at doses of 10 to 1000 mg/kg. One hour after the treatment was injected acetic acid 1.2% intraperitoneally (ip) and then were counted the
accumulated writhings (a.w.) for 30 minutes. Indomethacin inhibited in 63.89% (19.50 ±1.65 a.w., P < 0.001). The OR inhibited 54.32 ±3.87%(24.67;P < 0.01 a.w., P < 0.001); 61.11 ±2.70%(21.00 ±1.46 a.w., P < 0.001) and 70.99 ±2.42%(15.67 ±1.31 a.w., P < 0.001) at doses of 100, 300 and 1000 mg/kg, respectively, and allowed the determination of the ID50 of 145.24 mg/kg. In the Hotplate method (J Pharmacol Exp Ther. 300: 80-1944) Five experimental groups were treated: water (p.o), tween 1% (p.o), fentanyl 30 μg/kg (positive control, subcutaneous (sc)), OR (100 to 1000 mg/kg, p.o). In this model, the OR in the range of doses used was not able to increase the reaction time of animals in the hotplate. Fentanyl produced an increase of 221.96% at 30 minutes (29.17 ±0.83 seconds, P < 0.001) in the reaction time of animals compared to water (9.03 ±0.76 seconds). In the Ear edema method (Drug Res. 230: 32.1982) we used the groups: water (p.o), tween 1% (p.o), dexamethasone (positive control, sc), OR at doses of 1 to 1000 mg/kg (p.o). Dexamethasone inhibited the oedema in 66.67%(2.20 ±0.29 mg, P < 0.001). The OR at doses of 100, 300 and 1000 mg/kg inhibited the oedema formation in 26.57 ±4.22% (5.07 ±0.29 mg, P < 0.05), 45.65 ±4.89%(3.75 ±0.34 mg, P < 0.001), 79.95 ±3.33% (1.38 ±0.23 mg, P < 0.001) respectively compared to tween 1% (6.99 ±0.61 mg) and allowed the determination an ID50 of 321.32 mg/kg.

Conclusions:

Our results demonstrate that the OR C. glycycarpa has antinociceptive and anti-inflammatory based on the methods of writhings and edema, respectively. Moreover, the antinociception seems to be related to anti-inflammatory activity without the participation of morphine-like activity.

Keywords: antinociceptive activity, copaiba oil, diterpenes, kaurenoic acid

Financial Support: CAPES/FAPERJ/UFRRJ

QuebraPagina

Resumo:18-058

EVALUATION OF CEREBRAL MALARIA INFECTION IN MSG-OBESE MICE

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Objectives:

Obesity is a metabolic syndrome characterized by increased storage of adipose tissue, which affects various endocrine functions and also the immune system, and now is emerging as a problem in developing countries (Malaria Journal 2008, 7:81). Malaria is a Tropical disease that causes lots of deaths worldwide yearly (Korean J Parasitol. 2009 June; 47(2): 93–102). Due to the importance of these diseases separately and the complexity of the immune response of the obese subject, the aim of this work was to evaluate the immunity of MSG-obese mice during the course of cerebral malaria infection.

Methods and Results:

Neonatal C57Bl/6 mice were treated with monosodium glutamate (4 mg/g) subcutaneously for the first 5 days of life, whereas the control group was given equimolar saline solution at the same conditions. Both groups were infected with Plasmodium berghei-ANKA (5 x 106 iRBCs/mL) at 8 weeks of age, and their parasitemia were monitored until their day of death by collecting a bit of their tail’s blood daily. Obesity was proved by Lee Index. Although there was no difference at the day of death between the control (n=6) and the obese group (n=11), the MSG animals showed a significant lower parasitemia (SE = 4.19 ± 1.3) compared to the control group (SE = 8.885 ± 2.1) at the same day.

Conclusions:

The MSG-obese mice are characterized by an increased production of proinflammatory cytokines, such as leptin, IL-6 and TNF-
α, along with many others. In fact, the immunological profile in the MSG animals could be leading to a stronger immune response against P. berghei parasites, resulting in a lower peripheral parasitemia as observed, making us to think about two hypothesis: the first one is that, despite of the better parasitemia control, when the parasites reaches their brain, their stronger immunity lead to their death, or, on the other hand, as the animals were supposed to die before the controls, something is protecting them from brain lesions. Therefore, more studies are required to elucidate what could be happening with the immunity of MSG-obese mice.

Keywords: monosodium glutamate, mortality, obesity, parasitemia, proinflammatory cytokines

Financial Support: Fapemig

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THE ROLE OF SOCS-2 IN THE REGULATION OF IMMUNE RESPONSE DURING EXPERIMENTAL HERPETIC ENCEPHALITIS

Sousa, L. F. C. 1; Lima, G. K. 2; Miranda, A. S. 1; Vilela, M. C. 1; Lacerda-queiroz, N. 1; Saito, V. M. 1; Kroon, E. G. 2; Machado, F. S. 1; Rachid, M. A. 1; Teixeira, A. L. 1

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3 Centro de Pesquisas René Rachou, FIOCRUZ

Objectives:

The Herpes Simplex Virus-1 (HSV-1) is a pathogen responsible for sporadic encephalitis in humans. Lipoxins are eicosanoids mediators that have potent anti-inflammatory properties. Recent evidence indicates that these effects may be, at least in part, related with Suppressor of cytokine signaling (SOCS) (Nat. Med. 12(3): 330, 2006). We aim to evaluate the role of SOCS-2 in the inflammatory process in an experimental model of encephalitis caused by HSV-1.

Methods and Results:

Eight-to-10–week-old male C57BL/6J wild type (WT) and SOCS2−/− mice were intracranially infected with 10² PFU of neurotropic HSV-1. We evaluated myeloperoxidase activity (MPO) and immune cell populations using fluorescence-activated cell sorting (FACS). Data are expressed as mean ± SEM and statistical analysis was performed using one-away ANOVA or t-student test (p

Conclusions:

SOCS-2 may play a role in the regulation of neuroinflammation during HSV-1 encephalitis.

Keywords: Encephalitis, Herpes, HSV-1, SOCS-2

Financial Support: CNPq, CAPES

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Resumo:18-059

THE ROLE OF SOCS-2 IN THE REGULATION OF IMMUNE RESPONSE DURING EXPERIMENTAL HERPETIC ENCEPHALITIS

Sousa, L. F. C. 1; Lima, G. K. 2; Miranda, A. S. 1; Vilela, M. C. 1; Lacerda-queiroz, N. 1; Saito, V. M. 1; Kroon, E. G. 2; Machado, F. S. 1; Rachid, M. A. 1; Teixeira, A. L. 1

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Objectivos:

The Herpes Simplex Virus-1 (HSV-1) es un patógeno responsable de la encefalitis esporádica en humanos. Los lipoxinas son mediadores eicosanoïdes que tienen propiedades antiinflamatorias potentes. Recientemente, se ha indicado que estos efectos pueden, al menos en parte, estar relacionados con el Suppressor of cytokine signaling (SOCS) (Nat. Med. 12(3): 330, 2006). Se desea evaluar el papel de SOCS-2 en el proceso inflamatorio en un modelo experimental de encefalitis causada por HSV-1.

Métodos y Resultados:

Ocho a diez–semana de edad, machos C57BL/6J tipo salvaje (WT) y SOCS2−/− murciélagos fueron infectados intracranealmente con 10² PFU de HSV-1 neurotípico. Se evaluó la actividad del peroxidasa de mielo (MPO) y poblaciones de células inmunes utilizando flujo celular activado (FACS). Los datos se expresan como media ± SEM y la análisis estadístico se realizó usando ANOVA de un paso o t-student test (p

Conclusiones:

SOCS-2 puede desempeñar un papel en la regulación de la inflamación nerviosa durante HSV-1 encefalitis.

Palabras clave: Encefalitis, Herpes, HSV-1, SOCS-2

Apoyo financiero: CNPq, CAPES

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Resumo:18-060
EVALUATION OF THE IMMUNOLOGICAL STIMULUS DELIVERED BY WHOLE AND PURIFIED PEANUT PROTEIN EXTRACT

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Objectives:

Food allergies are currently a major health problem affecting 4-8% of human populations. Proteins derived from milk, eggs, fish, tree nuts and peanuts are considered the most important in the clinical setting. Our group has established a mouse model that simulates the natural history of food allergies. Thus our AIM was to perform a total protein extract from peanuts and then purify this extract and compare its immunogenicity.

Methods and Results:

Raw peanuts were ground in an electric coffee grinder and mixed in extraction buffer (10 mM borate buffer - pH 8.5) 1:10 w/v and incubated at room temperature for 2 h to obtain total Peanut Protein Extract (TPPE). The TPPE was then mixed in a saturated Ammonium sulfate (NH₄)₂SO₄ solution at the concentrations of 20, 30, 40 and 50% v/v under constant agitation for 20 minutes. After this, all preparations were centrifuged for 15 minutes and the precipitates were resuspended in physiological saline to obtain Purified Peanut Protein Extract (PPPE). SDS-PAGE was used to evaluate the differences in the protein profile at each Ammonium sulfate concentration. To determine the immunogenicity of these extracts, C57BL/6, male adult mice weighting from 20 to 25 g (n = 6/group), bred and maintained at the Núcleo de Animais de Laboratório (NAL/UFF) were housed in an air controlled environment, (22°C) in polypropylene cages. These received ad libitum acidified water (pH 2.5), and diet. Animals were immunized subcutaneously with 100µg of PPPE or TPPE + 1 mg Al(OH)₃ in the primary immunization and 21 days later all animals received a booster immunization without the adjuvant. ELISA was used to quantify the levels of total IgG anti-peanut and revealed that the immunogenicity of PPPE 10% (3.794 ± 0.310), PPPE 20% (3.544 ± 0.319) are similar to TPPE (3.9805 ± 0.017) and significantly higher (p

Conclusions:

Although the profile of proteins in PPPE differs from TPPE the immunogenicity of this protein preparation is maintained rendering it a good antigenic stimulus for studies in peanut food allergies and tolerance.

Keywords: Extract, Peanut, Tolerization

Financial Support: CNPq

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Resumo:18-061

ENVIRONMENTAL AND AGING INFLUENCES ON ANTIBODY-ENHANCED DENGUE DISEASE OUTCOMES IN AN IMMUNOCOMPETENT MURINE MODEL

Pinho, B. G. 1; Rego, C. M. D. 1; Ramos, J. P. 2; Magalhães, M. C. 1; Gomes, G. F. 1; Foro, C. A. R. 1; Turiel, M. C. P. 1; Diniz, D. G. 1; Sosthenes, M. C. K. 1; Perry, V. H. 5; Vasconcelos, P. F. D. C. 3; Diniz, J. A. P. 3; Casseb, S. M. M. 3; Picanço-diniz, C. W. 1; Demachki, S. 1; Cunningham, C. 4

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Objectives:

T-lymphocytes are proposed to promote clearance during primary dengue virus (DENV) infection but contribute to immunopathology during heterologous infections. Since an enriched environment enhances T-cell activity during viral infections, and active older adults show less functional decline in T- and B-cell mediated adaptive immunity, we hypothesized that enriched environment and aging would increase clinical symptoms and deaths after antibody-enhanced dengue disease model.

Methods and Results:

Nine and 19 months old female albino Swiss mice were housed from weaning either in impoverished or in enriched conditions. To mimic multiple infections as it may occur in human disease, we performed serial i.p. injections following one of two experimental protocols. In the 1st experiment series of every other day injections of 100µl of DENV3 (genotype III) infected brain homogenate were done. In the 2nd experiment, animals were serially inoculated every other day with anti-DENV2 hyperimmune serum containing anti-DENV2 antibodies (200µl, 1:5 dilution in sterile saline), followed 24 hours later by DENV3 infected brain homogenate, to simulate antibody-enhanced DENV replication and infection with two serotypes. Control subjects received equal volumes and dilution of anti-DENV2 hyperimmune serum followed 24h later by uninfected brain homogenate. All subjects were sacrificed when clinical signs (dyspnea, ruffled fur, hunched posture, preterminal paralysis or shock) became apparent or when reduced burrowing activity below 40%. Tissues were perfused with aldehyde fixatives and liver, lungs and brain were paraffin embedded, cut and stained with hematoxylin-eosin or immunolabeled with anti-CD3 and anti-CD20 antibodies and counterstained with eosin. We found that subjects with enriched environment and aging after antibody-enhanced dengue disease showed more intense clinical symptoms and deaths than subjects with impoverished environment (Kaplan-Meyer analysis of survival probability, Logrank test p= 0.0312) and these evidences were associated with T lymphocytic hyperplasia in both liver and lungs.

Conclusions:

The present results add evidence to the hypothesis of both T cell-mediated immunopathogenesis and antibody-enhanced dengue disease in immunocompetent murine model where expansion of these memory cells during acute DENV infection and serotype cross-reactive antibodies may facilitate DV infection of myeloid cells by promoting virus entry via Fcc receptors and these events correlate with disease severity.

Keywords: Dengue, Secondary Infection, Inflammation, Enriched Environment, Aging

Financial Support: CNPq, INCT-FHV/CNPq/CAPES/FAPESPA, FINEP/FADESP, PROPESP-UFPA-FADESP and IBNnet

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Resumo:18-062

ROLE OF ENDOGENOUS GLUCOCORTICOIDS HORMONES ON NEUTROPHIL MOBILIZATION FROM THE BONE MARROW INTO BLOOD

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2 CENTER OF BIOCHEMICAL PHARMACOLOGY-WILLIAM HARVEY INSTITUTE, WILLIAM HARVEY
Objectives:

Our group has demonstrated that absence of endogenous glucocorticoids (EG) cause neutrophilia (Br. J. Pharmacol. 152; 1292, 2007), and the mechanisms involved are not fully elucidated. Therefore, this work aimed to investigate the role of the glucocorticoid cytosolic receptor and the participation of the membrane receptors CXCR-4, and adhesion molecules integrins (CD18 and CD49d) and CD62L on this effect.

Methods and Results:

Balb/C male mice (n=12) were treated with vehicle or glucocorticoid antagonist receptor RU 38486 (mifepristone) (RU, 10 mg/Kg, i.p.). Four hours later, Peripheral blood (PB) and Bone Marrow (BM) were collected. Total and differential numbers of leukocytes were quantified by Neubauer chamber and May-Grunwald stained smears using optical microscope, respectively. Expressions of CXCR-4, CD62L, CD49 and CD18 were measured by flow cytometry. In the PB, RU treatment caused neutrophilia (p<0.01).

Conclusions:

Results here obtained show that blockade of glucocorticoid cytosolic receptor affect the differentiation phases of neutrophil maturation and cause neutrophilia. This latter effect may be dependent on EG actions on expressions of receptors on membrane of neutrophils in the BM or circulating compartments.

Keywords: adhesion molecules, bone marrow, CXCR4, endogenous glucocorticoids, neutrophil mobilization


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Resumo: 18-063

LEPTIN ACTIVATES LIPID BODY-DRIVEN LEUKOTRIENE C4 SYNTHESIZING MACHINERY WITHIN EOSINOPHILS.

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2 Instituto de Biofísica Carlos Chagas Filho, UFRJ
1 Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, IOC/FIOCRUZ

Objectives:

Aim: Leptin is an adipocytokine involved not only in the control of body weight but also in the neuro-immuno-endocrine modulation. Leptin activates immune system and enhance cell proliferation in different tissues. Eosinophils are potential sources of inflammatory mediators, including cysteinyl leukotrienes, which are pertinent to allergic inflammation, and is emerging as an important target for leptin effects. We decided to investigate the human and murine eosinophil activation induced by leptin using in vivo and in vitro approaches.

Methods and Results:

Methods and Results: We evaluated the means by which leptin act on human eosinophils to affect migration and capacity to release leukotriene C4 (LTC4). Within human eosinophils treated in vitro, leptin dose dependently (0.5-50 nM) elicited lipid body biogenesis and priming for enhanced calcium ionophore-activated synthesis of LTC4, but not of prostaglandin E2. Intracellular signaling involved in leptin-induced lipid body-driven LTC4 synthesis involves PI3K activation, since wortmanin blocked both lipid body biogenesis and LTC4 synthesis triggered by leptin. Moreover, pertussis toxin inhibited leptin-induced eosinophil activation, although leptin receptors are not G-protein coupled. Explaining pertussis toxin effect, leptin actions were
found to be mediated in an "autocrine fashion", not of eosinophil-derived platelet-activating factor, but rather of endogenous CCR3-acting eotaxin. The effects of leptin were blocked by neutralizing anti-eotaxin and anti-CCR3, but not by platelet-activating factor receptor antagonists (BN52021). Therefore, leptin activation of eosinophils elicits the extracellular release of pre-formed eotaxin, which then in an autocrine fashion act on plasma membrane CCR3 receptors to stimulate lipid body-driven enhanced LTC4 synthesis within eosinophils. It was also evaluated the leptin capacity to induce mouse eosinophil migration by in vivo experiments in C57Bl6 mice. The intraperitoneal injection of leptin induced a concentration dependent (0.5 to 2 mg/kg) eosinophil influx after 24 h. The eosinophil migration was inhibited in the TNFRI-/- mice, showing that TNFRI is involved on this eosinophil recruitment. Leptin induced eosinophil influx was PI3K&gamma dependent, showing that this pathway may be indirectly important for this effect, since leptin signaling pathway activates PI3K&alpha. Interestingly the eosinophil recruitment by leptin in the 5-LO-/- mice was not inhibited.

Conclusions:

Conclusion: We observed that leptin directly and indirectly activates human eosinophils, in vitro to form lipid bodies and produce LTC4. Moreover the in vivo experiments in mice show that leptin clearly induce eosinophil recruitment in a TNFRI and PI3K&gamma dependent and leukotriene independent manner. Our results establish eosinophil activation by leptin as an important player on the leptin modulation of immune system, a connection between obesity and allergy disorders.

Keywords: leptin, eosinophil, leukotriene, lipid body, allergy

Financial Support: CNPq, FAPERJ, CAPES, FIOCRUZ

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Resumo:18-064

IL-17A SUPPRESSES EOSINOPHILPOIESIS IN MURINE BONE-MARROW THROUGH A NOVEL INOS- AND CD95-DEPENDENT, LTC4-SENSITIVE MECHANISM

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3 IMPPG / CCS / Universidade Federal do Rio de Janeiro, UFRJ
4 Departamento de Farmacologia, Universidade de São Paulo, . USP

Objectives:

Interleukin(IL)-17A is a powerful modulator of inflammatory responses, with multiple, wide-ranging contributions to the pathogenesis of autoimmune and allergic diseases. For granulocytes, most of the IL-17A effects are stimulatory and selective for the neutrophil lineage. However, the possibility of separate regulatory effects on the eosinophil lineage remains unexplored. Because IL-17 was shown to transduce the stimulatory effects of systemic neutrophil turnover, occurring on bone-marrow neutropoiesis, we have examined its impact on murine bone-marrow eosinophil production and analysed its mechanisms and interactions with other immunomodulators.

Methods and Results:

Bone-marrow was cultured with the eosinophil differentiation factor, IL-5, alone or associated with IL-17A, from 12 female BALB/c mice, or mice 6 female deficient in inducible Nitric Oxide (NO) Synthase (iNOS -/- ), 6 female CD95 (lpr), 6 female IL-17RA, 6 female IFN-gammaRI, or 4 female IL-4, and their respective wild-type controls. Synergism between NO-dependent and -independent mechanisms mobilized by IL-17A, as well as antagonisms between IL-17A and the immunomodulatory agents, Leukotriene D4, IL-13, eotaxin and IL-4 were further explored. IL-17A had no stimulatory effect of its own on eosinopoiësis. In the presence of IL-5, however, it dose-dependently suppressed eosinophil differentiation over a 7 day-period, by acting at a very short window of opportunity (24 h). Its effect was prevented by terminal caspase inhibitor, zVAD-fmk. The effects of IL-17A (0.1-1 ng/mL) were abolished in the presence of iNOS-selective inhibitor Aminoguanidine, and
undetectable in iNOS (-/-) bone-marrow. In both conditions, IL-17A (10 ng/mL) retained significant suppressive effect. Synergism was detectable between concentrations of IL-17A and NO donor Sodium Nitroprusside that had no effect by itself. Suppression of apoptosis by IL-17A was undetectable in bone-marrow lacking IL-17RA and CD95, and counteracted by LTD4, IL-13 and eotaxin. Sensitivity to IL-17A was increased by an order of magnitude in bone-marrow lacking either IFN-gammaRI or IL-4; addition of IL-4 to the cultures, as predicted, counteracted this increased sensitivity in IL-4 deficient bone-marrow.

Conclusions:

IL-17 has a powerful suppressive effect on eosinophilopoiesis, which is partly mediated by a novel proapoptotic pathway requiring both iNOS and CD95. Its effects are effectively counteracted by mediators of allergic responses, including LTD4, IL-13 and eotaxin, as well as by the immunoregulatory cytokines, IL-4 and IFN-gamma.

Keywords: Interleukin 17, Eosinophil, Bone-Marrow, Nitric Oxide

Financial Support: CNPq, Capes, Faperj

QuebraPagina

Resumo:18-065

ROLE OF TLR2 AND PPARγ IN SCHISTOSOMA MANSONI INFECTION

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Objectives:

Mansonic schistosomiasis is a disease caused by parasitic trematode Schistosoma mansoni, endemic to tropical countries. There is great interest in understanding the mechanisms used by this parasite that causes a modulation of the immune system in order to improve efficiency in treating this disease and to reproduce such modulation in the treatment of autoimmune diseases. Several studies have shown the participation of Toll-like receptors (TLR) in the recognition of pathogen-associated molecules in the innate immune response and the roles of lipid-ligand activated nuclear receptors (PPARg and LXR) in regulating inflammatory and metabolic genes, but few studies have addressed the involvement of these receptors and characterized the pathogen-derived ligands in S. mansoni infection. In the present study we aimed to investigate the role of S. mansoni-derived lipid molecules to modulate the macrophage response. In addition we investigated the role of S. mansoni infection to regulate the expression of liver TLR and nuclear receptors.

Methods and Results:

Peritoneal macrophages extracted from five wild type or TLR2/-/- mice (C57BL/6, male, 2 months old) were stimulated in vitro with lipids extracted from adult worms of Schistosoma mansoni. After 24 hrs we quantified the induction of lipid droplets by osmium staining and eicosanoids levels by EIA. Pretreatment with the PPARg inhibitor, GW9662, were performed in selected groups. For gene expression analysis in the liver, a group of 15 mice (C57BL/6, male, 2 months old) were subjected to intraperitoneal infection with about 70 cercariae each one. After 5, 20 and 90 days, five mice were sacrificed and the liver was collected for analysis. The mRNA was purified and the expression of TLR2, PPARg and ADRP were analyzed by Real Time PCR. RESULTS: We observed that lipids extracted from S. mansoni, mostly lysophosphatidylcholine (LPC), are capable to promote an increase of the production of lipid droplets, PGE2 and LTB4 in macrophages in vitro after 24hrs. The effects of schistosomal lipids in macrophages were inhibited in the TLR2/-/- mice and by the treatment with GW9662. Our results showed an increase of TLR2 and ADRP expression, the lipid droplet specific protein, in the liver from infected mice after 20 days of infection. In addition, the expression of LXRb was increased after 90 days of infection.

Conclusions:
The results suggest that lipids of S. mansoni have immunomodulatory activity being capable to promote an increase of lipid droplet formation and eicosanoid production by a mechanism partially dependent of TLR2 and PPARg. In addition, S. mansoni infection modulates the expression of TLR2, ADRP and both nuclear receptors PPARg and LXRβ, and these receptors may have roles in the pathogenesis of schistosomiasis.

Keywords: Toll-like Receptor, Schistosoma mansoni, lysophosphatidylcholine

Financial Support: PIBIC/CNPq, FAPER, CNPq and IOC/FIOCRUZ

**QuebraPagina**

**Resumo:**

LACK VITAMIN A INCREASES SUSCEPTIBILITY TO INFECTION WITH LEISHMANIA AMAZONENSIS AND PREVENTS THE EFFECTIVENESS OF ORAL VACCINE LAAG

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**Objectives:**

Induction of oral tolerance has emerged as feasible strategy to prevent immunopathologies. We showed that oral immunization with LaAg (*Leishmania amazonensis*’s lysate) protected BALB/c mice against *L. amazonensis* infection (Vacc. 21; 3534, 2003). We observed that Jones–Motes (JM) hypersensitivity reaction and IL-4 was diminished whereas IFN-γ was increased in peripheral lymph nodes, indicating Th2 responses toleterization and expansion of Th1. Regulatory cytokines was increased in mesenteric lymph nodes (MLN). Role of T reg cells in restraining pathogenic responses during C57BL/6 infection with *L. amazonensis* and balance between Treg and Th1 effector cells in outcome of cutaneous leishmaniasis has been demonstrated (J. Immunol. 174; 7145, 2005). Retinoic acid, a Vitamin A (VitA) metabolite, is powerful inducer of FoxP3 expression and enhances commitment to T reg cell lineage (J. Exp. Med. 204(8); 1765, 2007). Then, we evaluated the oral LaAg vaccine ability in the CD4+Foxp3+ T regulatory cells expansion, their efficacy in VitA deficient BALB/c mice and outcome of infection with *L. amazonensis* in VitA deficient C57BL/6 and BALB/c mice.

**Methods and Results:**

BALB/c (n=9) and C57BL/6 (n=5) gravid females at 7-10 days of gestation received lacked VitA (VitA-) diet or normal (VitA+) diet. Pups were weaned at 3 weeks of age and maintained on the same diet. Serum retinol levels were analyzed by HPLC to confirming VitA deficiency. Moreover, a group was treated with Citral (CT), 14 days before vaccination until the day of second dose (300mg/Kg). VitA+, VitA- and CT BALB/c mice (n=8) received 2 doses of LaAg (100 µg) or PBS once a week, by gavage. MLN were collected 2 days after second dose. LaAg vaccine increased CD4+FoxP3+ expansion only in VitA+ (from 5.9% to 10.9%) but not in VitA- (from 4.5% to 4.8%) or CT (7.4% to 4.8%). After, VitA+, VitA- and BALB/c mice were immunized, as previously described, and 7 days after second dose, the mice were infected in footpad with 2x106 *L. amazonensis* promastigotes. JM reaction (0, 15, 18, 24 and 48 hours post infection) was measured and lesion growth was monitored until 10 weeks (vaccine efficacy). LaAg did not impair the capacity of infected VitA- and CT mice to mount a disease-associated hypersensitivity response. Based on lesion growth and parasite burden, VitA- mice were more susceptible to infection than VitA+. Oral LaAg was only effective in VitA+ mice, producing higher IFN-γ gamma and TGF-β and decreased IL-4 and IL-10 production in the infected footpads and peripheral lymph nodes. Moreover, VitA+ and VitA- C57BL/6 mice (n=9) were infected in footpad with *L. amazonensis*. Outcome of infection was evaluated until 12 weeks and we observed that VitA- mice were more susceptible to infection than VitA+, presenting higher parasite load and diminished IFN-γ and TGF-β production in peripheral lymph nodes and infection site. All experiments were reproduced at least twice and a representative experiment is shown. The statistical differences between the groups were determined by the Student’s t-testing the GraphPad Prism 4 software, and were considered significant when p≤0.05.

**Conclusions:**

...
Dietary Vitamin A is required for an effective response against cutaneous leishmaniasis caused by *L. amazonensis*, and for the effectiveness of oral LaAg vaccine in BALB/c mice. The requirement of Vitamin A may be associated with the expansion of CD4+Foxp3+ T regulatory cells in the gut-associated mucosa.

**Keywords:** leishmaniose, vitamina A, vacina oral, T reguladora

**Financial Support:** CNPq
CCL25/TECK INDUCES THE MIGRATION OF IL-17+ GAMMA DELTA T LYMPHOCYTES DURING ALLERGIC REACTION VIA ALPHA4BETA7-INTEGRIN

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Objectives:
Gamma delta T cells accumulate in the airways during allergic reaction and modulate the inflammatory response. CCL25 is described as a homeostatic chemokine restrictedly expressed in lymphoid organs and small intestine. CCR9 (CCL25 receptor) is mainly expressed on alpha4beta7-integrin+ T lymphocytes and dictates their homing to intestinal mucosa. Herein we investigated the role of CCL25/TECK and alpha4beta7-integrin on gamma delta T cell migration during allergic inflammation.

Methods and Results:
The intra-pleural (i.pl.) injection of CCL25 (200ng/cavity) triggered the accumulation of gamma delta T lymphocytes expressing the CCL25 receptor, CCR9, (SAL 1.4±0.1 X CCL25 2.7±0.4 x10^3 cells/cavity; p=0.001) and alpha4beta7-integrin (SAL 1.1±0.1 X CCL25 1.9±0.2 x10^3 cells/cavity; p=0.003) in C57BL/6 mouse pleura, but failed to attract alpha beta T lymphocytes. CCL25 i.pl. injection also attracted gamma delta T cells expressing the marker of IL-17-producing cells, CCR6, (SAL 1.8±0.3 X CCL25 3.2±0.4 x10^3 cells/cavity; p=0.008), and producing IL-17 (SAL 2.3±0.2 X CCL25 3.3±0.3 x10^3 cells/cavity; p=0.017), but not IFN-gamma or IL-4. In fact, CCL25 (100ng/ml) induced the chemo-taxis of IL-17+ gamma delta T lymphocytes in vitro (chemotatic index= RPMI 1.0±0.3 X CCL25 2.4±0.02; p=0.001), even though it failed to alter IL-17 production by gamma delta T cells after in vitro stimulation (RPMI 1.3±0.7 X CCL25 1.6±0.4 % IL17+ gamma delta T cells; p=0.091). In a allergic pleurisy mouse model, the antigenic challenge with ovalbumin (OVA) (12.5μg/cavity, i.pl.) into immunized mice [50μg OVA + 5mg AL(OH)3] triggered increased production of CCL25 (SAL 136.8±7.0 X OVA 301.7±33.4 ng/ml; p=0.002), followed by the accumulation of CCR9+ (SAL 0.5±0.03 X OVA 2.5±0.3 x10^3 cells/cavity; p=0.003), alpha4beta7+ (SAL 1.0±0.1 X OVA 4.0±0.3 x10^3 cells/cavity; p=0.003) in the mouse pleural cavity. During the allergic response, the in vivo neutralization of CCL25 (anti-CCL25 mAb 10μg/cavity, i.pl.) was able to inhibit alpha4beta7+ (OVA 4.0±0.3 X anti-CCL25 2.7±0.3 x10^3 cells/cavity; p=0.002) and CCR6+/IL-17+ gamma delta T cell (OVA 2.1±0.4 X anti-CCL25 1.0±0.3 x10^3 cells/cavity; p=0.012) migration into the pleural cavities of OVA-challenged mice. In addition, the in vitro blockade of alpha4beta7-integrin (anti-alpha4beta7 mAb 100μg/mouse, i.p.) also inhibited the migration of total gamma T lymphocytes (SAL 5.6±0.2 X OVA 16.8±1.2 X anti-alpha4beta7 12.9±1.2 x10^3 cells/cavity; p) gamma T lymphocytes (SAL 2.7±0.2 X OVA 6.4±0.2 X anti-alpha4beta7 4.8±0.5 x10^3 cells/cavity; p) alpha4beta7-integrin blockade as well as CCL25 neutralization failed to inhibit the accumulation of alpha beta T lymphocyte into mouse pleural cavities after OVA challenge, suggesting that CCL25,alpha4beta7-integrin pathway is selective for gamma delta T cells.

Conclusions:
Overall, our results reveal a particular in vivo migration pathway for IL-17+ gamma delta T lymphocytes, which requires CCL25/CCR9 axis and is mediated by alpha4beta7-integrin. Here we provide evidence that CCL25 plays a pivotal role for IL-17+ gamma delta T cell trafficking in allergic response; however, the relevance of this chemokine in Th17-mediated immune responses is yet to be defined.

Keywords: gamma delta T lymphocyte, chemokine, Integrin

Financial Support: CNPq, FAPERJ, FIOCRUZ.

QuebraPagina

Resumo:18-069

MDMA (ECSTASY) INDUCES IMMUNOSUPPRESSION AND ENHANCES SUSCEPTIBILITY TO LISTERIA

QuebraPagina
MONOCYTOGENES INFECTION

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Objectives:

We have previously shown that MDMA - Ecstasy decreases neutrophil activity and changes the leukocyte distribution in blood, spleen and bone marrow. The HPA axis activation and consequently the corticosterone released was shown to be responsible for these effects and the catecholamines seem not to be involved. It has been reported that Ecstasy users are often more susceptible to infectious diseases, and such alterations could be important in this issue. Therefore, the aim of this study was to search for the MDMA-resistance to infection in a model of infection by Listeria monocytogenes (LM).

Methods and Results:

Balb/C male mice (6 per group) were used and divided randomly in 2 groups: Saline (NaCl 0.9%) and MDMA (10 mg/kg), 60 min after i.p. treatment, we inoculated i.p. 5x10³ LM per animal. Bone marrow, spleen and blood samples were harvested after 24, 48 and 72 hours in order to evaluate the growth of granulocyte-macrophage progenitor cells (CFU-GM) and to investigate the colony-stimulating factors production (colony-stimulating activity - CSA). Previously to the LM inoculation, mice were subdivided randomly in 2 other groups: infected and not infected by LM. We observed that the treatment with MDMA per se was able to decrease the CFU-GM (%) on bone marrow (F(11,60)=8,408; p

Conclusions:

Taken together these data indicate that MDMA induced a persistent myelosuppression persisting for 72 hours caused by a lower reserve of myeloid progenitors in bone marrow and lack of increasing of colony-stimulating factors, which in turn enhanced mice susceptibility to LM infection.

Keywords: Ecstasy, immunosuppression, Listeria monocytogenes, MDMA, myelosuppression

Financial Support: FAPESP and CNPq

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Resumo:18-070

ROLE OF ENDOGENOUS GLUCOCORTICOIDS IN THE REGULATION OF EOSINOPOIESIS IN A MURINE MODEL FOR ALLERGIC ASTHMA

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Objectives:
Atopic asthma is a chronic inflammatory disease, characterized by hyperresponsiveness of airways, eosinophil-rich inflammatory infiltrates in the lungs and reversible obstruction of the air flow, which is becoming increasingly prevalent worldwide. Eosinophils are produced and stored in bone-marrow, in response to interleukin (IL)-5, and subsequently released in the circulation and recruited into peripheral tissues, under the influence of chemotactic factors such as eotaxin, which is released from inflammatory sites, including asthmatic lungs. Stress, defined as a state of threatened homeostasis, is increasingly regarded in the scientific literature as a predisposing factor for the development of allergic diseases, including asthma. The hypothalamus-pituitary-adrenal (HPA), the central neuroimmunoendocrine component of the physiological response to stress, is activated by inflammatory cytokines, including tumor necrosis factor-α (TNF-α). Glucocorticoids, in the therapeutic dose range, suppress the eosinophilia of blood and peripheral tissues, through inhibitory effects on production of cytokines, including IL-5, granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-3, and through induction of mature eosinophil apoptosis. However, in specific conditions, glucocorticoids are stimulatory, rather than inhibitory, for eosinopoiesis in bone-marrow. We aimed to evaluate the role of the adrenal glucocorticoid hormones in the immunological regulation of eosinopoiesis in sensitized mice, using an inhibitor of adrenal glucocorticoid synthesis (metyrapone), along with an antagonist of the glucocorticoid receptor (mifepristone, RU486).

Methods and Results:

Methods: BALB/c mice (n=3-9, female and 4-8 weeks old) sensitized to ovalbumin, were treated with metyrapone or RU486 before intranasal allergen challenge. The following aspects were evaluated: a) the presence of eosinophils in the recently harvested bone-marrow, and following bone-marrow culture in the presence of IL-5; b) the presence of eosinophils in peripheral blood. In wild-type mice of the C57BL/6 strain, as well as mice of the same genetic background, lacking the type I receptor for TNF, we evaluated the in vivo bone-marrow response to challenge. The comparisons used analysis of variance with the Tukey (HSD)correction (Systat forWindows 4; Systat Inc.,Evanston, IL) p≤0.05. Results: Treatment with RU486 before ovalbumin challenge abolished the increase in numbers of eosinophils in bone-marrow, both in vivo and ex vivo. Corticosterone levels were increased in plasma by challenge and this increase was prevented by metyrapone treatment. Bone-marrow responses to allergen challenge were not observed in mice lacking the type I receptor for TNF, but were present in wild-type controls.

Conclusions:

Together, these observations point to an important role of adrenal glucocorticoid secretion, induced by TNF, in the hematological response to airway challenge.

Keywords: Asthma, Eosinophilopoiesis, Glucocorticoid, Hematopoiesis, Stress

Financial Support: CNPq, FAPERJ.
reduces the severity of the disease preventing further bone erosion. Thus, we evaluated the function of NK cells in periodontal disease.

Methods and Results:

Eight week female Balb/c mice (n = 5 per group) were infected or not with Aggregatibacter actinomycetemcomitans (1x10^9) as control, and other two groups (infected or not) were treated with anti-NK for NK depletion. After 30 days of infection, the animals were subjected to euthanasia and the submandibular lymphnodes were removed for FACS analysis and the jaws were removed for bone resorption evaluation. Statistical analysis was performed using ANOVA followed by Bonferroni test. Infected-mice treated with anti-NK showed a 100% lower bone resorption compared to the group of infected animals in the presence of NK cells.

Conclusions:

The depletion of NK cells suggests an immunomodulatory effect on the recruitment of regulatory T cells and may be associated with the mechanisms of bone resorption generated by the immune response in periodontal disease.

Keywords: Bone resorption, Natural Killer Cell, Peridontal Disease

Financial Support: Financial support: FAPEMIG (PPM 097/09)

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Resumo:18-072

PRODUCTION OF MIP-1 β BY BRONCHIAL EPITHELIAL CELLS STIMULATED WITH CCL5/RANTES.

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Objectives:

The allergic inflammatory process of airways consists in the production and release of inflammatory mediators, like cytokines and chemokines. The airway epithelial cells have a great role in the process, since can be stimulated to release inflammatory mediators potentiating the process allergic. The objective of this study was to investigate the production MIP-1 β by airway epithelial cells stimulated with CCL5/RANTES (regulated upon activation, normal T cell expressed and secreted) and ovalbumin.

Methods and Results:

Cells were plated in a 24 wells plate. After reach the confluence, cells were stimulated with RANTES 1, 10 and 50 ng/mL or ovalbumin (OVA) 1, 10 and 100 µg/mL. The MIP-1 β production was evaluated 6, 24 and 48 hours after. The MIP-1 β production was analyzed by ELISA. Our results suggested that RANTES-stimulated bronchial epithelial cells increase the MIP-1 β production with 50 ng/mL in the period of 24 hours, but not with 1 and 10 ng/mL the same period. The RANTES stimulus of 6 hours not promoted changes in MIP-1 β release, however, observed that in 48 hours stimulus with 10 ng/mL, there was a tendency to decrease of chemokine production. The OVA-stimulus did not present changes in MIP-1 β concentrations at 6 and 24 hours. However at 48 hours, 10 µg/mL of OVA induced decrease of MIP-1 β production by bronchial epithelial cells. Statical analysis was performed using ANOVA with correction of Bonferroni where p< 0.05.hours.

Conclusions:

RANTES stimulation increased the production of MIP-1 β by bronchial epithelial cells. This result indicates that further studies
are needed to clarify the mechanism involved in the RANTES-stimulated bronchial epithelial cell.

Keywords: Airway inflammation, CCL5/RANTES, Epithelial cells, MIP-1 β, Ovalbumin

Financial Support: FAPESP and CAPES

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Resumo:18-073

PARTICIPATION OF NUCLEAR FACTOR KAPPA B (NFκB) IN LPS-INDUCED MELATONIN SYNTHESIS IN RAW MACROPHAGES.

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Objectives:
Activated human and rat macrophages are known to synthesize melatonin (MEL), which plays a role in the recovery phase of an innate immune response. Here we evaluated whether the synthesis of MEL by macrophages stimulated with LPS is a result of the activation of the NFκB transcription factor.

Methods and Results:
RAW macrophages (1x106 cells/well) cultured in RPMI were activated with LPS (1 µg/mL) for 3 or 6 h. MEL content in the supernatant was measured by ELISA kit, and the participation of NFκB in the induction of MEL synthesis was tested by inhibiting the translation of NFκB RelA and cRel subunits with small interference RNA (siRNA). Transfected cells (1x105/well) seeded in chamber-slides were activated or not with LPS (1 µg/mL, 1h). The expression of the key enzyme in melatonin synthesis aryl-alkyl-amine-N-acetyltransferase (AA-NAT) was determined immunocytochemical assay. The primary antibodies (1:400) were reacted with secondary antibodies stained with Texas Red (1:400). The slides were analyzed by confocal microscopy. The profile of NFκB subunits was determined by super-shift assay. In this case the cells were stimulated with LPS (1 µg/mL) for only 5 min. LPS induces an increase in melatonin production when compared to non-stimulated cells. The basal production of melatonin was 4.38 ± 1.59 pg/mL (n=3) and 4.41 ± 1.43 pg/mL (n=5) at 3 and 6 h, respectively. Stimulation with LPS increased MEL production by 63 times in 3 h and 3 times in 6 h. Inhibition of RelA or cRel translation, confirmed by super-shift with specific antibodies, reduces the LPS-induced expression of AA-NAT.

Conclusions:
Our data clearly show that LPS leads to the synthesis of melatonin by a mechanism dependent of the nuclear translocation of NFκB. Both the subunits RelA and cRel are relevant for the expression of the key enzyme in melatonin production. These are important data for understanding the Immune-Pineal Axis, as it shows that the same signaling pathway that induces the LPS-induced suppression of nocturnal pineal melatonin synthesis leads to the activation of macrophage synthesis. In other words, the NFκB transcription factor, which is a hallmark of inflammatory response, is responsible for the shuttle between pineal and extra-pineal production of melatonin induced by LPS.

Keywords: AA-NAT, LPS, macrophages, melatonin, nuclear factor kappa B (NFκB)

Financial Support: CAPES, CNPq and FAPESP (2007/07871-6)
EFFECTS OF GENISTEIN ON NITRIC OXIDE PRODUCTION BY RAT PERITONEAL MACROPHAGE:
COMPARATIVE STUDY BETWEEN MALE AND FEMALE

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Objectives:
The phytoestrogen genistein is an isoflavone found in abundance in soybeans. There is considerable interest in soy isoflavones as an alternative to endogenous estrogens, not only on hormonal changes (menopause), but also in inflammatory processes. Genistein has estrogenic activity and can bind to estrogen receptor (ER). ER has been reported in macrophages from mice, rat and human. In fact, the sexual dimorphism of the immune system is well established and also estrogens modulate macrophage function. However, there are few studies that elucidate the role of bioactive compounds such as genistein between the sexes. In the present study we have investigated the effects of genistein on nitric oxide production by rat peritoneal macrophage comparing males and females in different phases of the estrous cycle.

Methods and Results:
First of all we evaluated the cytotoxicity of genistein (10μM), quercetin in the same concentration (as antioxidant activity control) and 0,01μM of the 17β-estradiol (as estrogenic activity control) using the technique of reduction of MTT. It was observed that the compounds tested are not toxic. NO production was estimated from nitrite levels. In resume macrophages of different group (4 x 10⁵/well) were or not stimulated with LPS (10μg/mL) in the presence of genistein (10μM) or quercetin (10μM) or 17β-estradiol (0,01μM) for 24 h. After this period, 100 μL of culture supernatant was mixed with the same volume of Griess reagent and absorbance was determined at 550 nm with a microplate reade. Genistein decreased basal NO production (non-stimulate macrophages) in all group. Nitrite levels decreased on male (n=12), on female in diestrous (n=11), and on female in proestrous (n=14) respectively by 34% (±5,51), 40% (±8,62) and 17% (±4,91) compared to the control of the group.

Conclusions:
Our results suggest that genistein appears to be a more effective in the inhibition of NO production in conditions with low hormone concentration, as found in males and females in diestrous. In cells previously modulated by endogenous hormone (17β-estradiol), as shown by other researchers, the effect of genistein was less pronounced. In conclusion we can infer that the use of genistein should be taken into account sex, and therefore, the serum concentration of 17β-estradiol. However other studies must be carried out to elucidate the involved mechanisms.

Keywords: 17β-estradiol, genistein , macrophages, phytoestrogens, sexual dimorphism

Financial Support: CAPES

ACTIVATION OF CHOLINERGIC MECHANISMS DECREASES INFLAMMATION AND IMPROVE MDX MUSCLE REGENERATION

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Keywords: Cholinergic mechanisms, inflammation, MDX muscle regeneration

Financial Support: CAPES
Objectives:

Mdx mice, the animal model of Duchenne Muscular Dystrophy (DMD), develop inflammatory myopathy characterized by progressive degeneration of skeletal muscle fibers and connective tissue replacement. Recently, nicotinic acetylcholine receptor (nAChR) activation has been shown to regulate the production of pro-inflammatory cytokines. This work aimed to investigate whether muscle nAChR activation would interfere with TNFα production, activity of matrix metalloproteinases (MMP)-9 and -2, NFκB expression and regeneration on skeletal muscle of mdx mice.

Methods and Results:

Gastrocnemic muscles of male control C57 and mdx mice were used (n=5 for each experimental group) at weaning 2 weeks-old (w), myonecrosis prevalence (4w), regeneration (12w) and fibrosis (24w). Mdx mice at 3w received intraperitoneal injection of nicotine (400 mg/kg) or vehicle twice daily from day 21 until day 28 of life and sacrificed at end of treatment (day 29). Total surface and areas occupied by inflammatory infiltrate, regeneration and collagen deposition (syrius red) were assessed by histological staining and morphometric analysis. Western blot and RT-PCR were used for analysis of protein and mRNA of TNFα and nAChRα7, zymography to assess MMP activity, immunohistochemistry to demonstrate nAChRα7 co-localization on F4/80+ macrophages and NCAM+ regenerating myofibers. Mdx muscles presented characteristic pattern of muscle inflammation evident at 4w followed by dense inflammatory infiltrate at 12w (84 ± 30%, p < 0.05) and significant reduction at 24w (80 ± 15%, p < 0.01). At 12w mdx muscle showed a 3.5-fold increase (p < 0.001) of nAChRα7+ F4/80+ macrophages per mm2 in the inflammatory infiltrate compared with mdx muscles at 4w and 24w. In contrast, TNFα production within mdx muscle was higher at 4w than 2w (48 ± 9%, p < 0.05) but reduced at 12w (61 ± 3%, p < 0.001) and 24w. In comparison with mdx at 4w, MMP9 activity involved on proteolytic cleavage of TNFα decreased at 12w (87 ± 13%, p < 0.05), whereas active-MMP2 involved in tissue remodeling increased 8.3-fold (p < 0.01) and NCAM+ increased 3.2-fold (p < 0.01). Nicotine treatment for 8 days decreased MMP9 activity (38 ± 11%, p < 0.05), NFκB expression (35 ± 11%, p < 0.05), TNFα production (32 ± 5%, p < 0.01) and reduced inflammatory infiltrate areas (61 ± 18%, p < 0.05) in comparison with mdx muscles at 4w age with myonecrosis prevalence. In addition, treatment improved skeletal muscle regeneration through identification of fibers with strong basophilic sarcoplasm and centrally located nuclei (28 ± 9%, p < 0.05) beyond expression analysis of myogenin protein.

Conclusions:

Altogether, the results indicate that nAChR activation reduced the progressive degeneration of skeletal muscle fibers by decreasing local production of inflammatory mediators and promoting tissue remodeling and regeneration through a cholinergic mechanism.

Keywords: Acetilcholine Receptor, Cholinergic mechanisms, Inflammation, Duchenne Muscular Dystrophy, skeletal Muscle

Financial Support: CAPES; CNPq; FAPERJ

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Resumo:18-076

COMPARISON BETWEEN RESVERATROL EFFECTS ON NITRIC OXIDE PRODUCTION OF THE MALE AND FEMALE RAT PERITONEAL MACROPHAGES.

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Objectives:

The sexual dimorphism of the immune system is well established. In fact, sexual hormone, testosterone and estrogen, modulate immune responses. In addition, estrogen receptors (ER) have been reported in macrophages from mice, rat and humans. Resveratrol, which is a polyphenol present in red wines and vegetables included in human diets, exerts many biological effects, such as potent antioxidant activity and low estrogenic activity. However, there are few studies that elucidate the role of resveratrol between the sexes. The aim of the present study was to investigate its effects on oxide nitric production by male or female in diestrous or female in prestrous-isolated macrophages.

Methods and Results:

Before assay NO production, we evaluated if the compounds tested are well tolerated and safe to macrophages. The assessment of cytotoxic was performed using the MTT reduction assay. Resveratrol (10 μM), quercetin (10 μM) as antioxidant activity control, and 17β-estradiol (0.01 μM) as estrogenic activity control did not show cytotoxic effects after 24 hours. To determine the effects of resveratrol on nitric oxide (NO) production by macrophage, peritoneal lavage cells were cultured in the presence of these compounds. The cells were treated for 24 h. After this period, cell-free culture media were mixed with Griess reagent. The concentration of nitrite was determined spectrophotometrically. The treatment of peritoneal macrophages with 10 μM resveratrol decreased significantly the production of NO of all group tested (male n=12, female in proestrous n=11 and female in diestrous n=14). Basal nitrite levels (from non–stimulated macrophage) decreased on male, on female in diestrous, and on female in proestrous respectively by 47% (± 6,38), 41% (± 4,10) and 35% (± 4,38) compared to the control of the group (p

Conclusions:

Thus, resveratrol modifies the NO production macrophage, but this effect is not clear and perhaps it depends on previously modulated by endogenous hormone of the cells. Thus future experiments are needed, varying the experimental conditions in vitro and mainly in vivo to better evaluate the possible resveratrol effects between sexes. Taken together, these results indicate that the resveratrol modulates NO production with the same pattern of quercetin and, consequently, is likely to affect macrophage as antioxidant compounds. The small dose of resveratrol used is attainable with a diet including red wine and vegetables confirming its protective role against some pathological processes such as inflammation, coronary heart disease, and cancer.

Keywords: Antioxidants, 17β-estradiol, Macrophage, Resveratrol, Sexual Dimorphism

Financial Support: CAPES

QuebraPagina

Resumo:18-077

INFLAMMATORY RESPONSE OF THE OBESE RATS SUBMITTED TO SEPSIS BY CECA LIGATION AND PUNCTURE (CLP)

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Objectives:

Several studies in animal models suggest that obesity and overweight are associated with immunocompetence alterations. Considering that obesity may be seen as a chronic inflammatory state one can imagine that obese individual might respond to
Sepsis differently than normal individual. In the study, the obesity repercussions on inflammatory activity (nitric oxide (NO) production, IL12 and C-reactive protein) were analyzed in adult rats fed with a hiperlipidic diet over 20 weeks and subjected to sepsis by colon ligature and puncture (CLP).

Methods and Results:

The experimental groups were based on the diet offer after weaning. Control groups were fed with a rat chow diet. The obese group was fed with a high fat diet starting after weaning (22nd day). The high fat diet consists of foods that constitute the basic meals of cafeteria. All rats received these diets during experiment of the 20 weeks. On the 140th days of life, sepsis was induced by CLP. The body weight of the rats was checked on the 140th day of life. Abdominal fat was collected after the experimentation through an midline incision the abdomen wall. Blood was collected before and 4h and 24h after CLP. The total leukocyte’s counting and differential counting was done. C-Reactive Protein (PCR) levels were measured by immunotubidimetry. In alveolar macrophages (AMs) of bronchoalveolar lavage (BAL) IL-12 were determined by a double-ligand ELISA and NO release was measured indirectly using a quantitative, colorimetric assay based on the Griess reaction. Statistical analysis was performed using SPSS for windows 12.0. The results are expressed as mean ± SEM and p< 0.05 were considered statistically significant. The body weight, as well abdominal fat of rats that received high-fat diet, showed significant increase when compared to those fed with the standard diet. In this study, CLP induced sepsis resulted in a significant elevation white blood cell counts compared to sham CLP rats after four hours sepsis. A similar response occurred after 24h of surgery. In both times of induction of sepsis was also observed neutrophilia and lymphopenia. Comparing all groups no significant difference in levels of PCR after 4h and after 24h of sepsis. NO release by AMs from the obese septic group after 4h CLP surgery was higher compared to normal septic and to the sham operated. The NO analysis performed 24h after the surgery showed that AMs from obese rats CLP release greater amounts of NO than the sham operated obese and normal rats CLP. Found in AMs of BAL increased amount of IL-12 in normal sham-operated group compared to sham-operated obese (p = 0.03); but the sham-operated obese group showed lower production of IL-12 than the obese group CLP. The obese CLP group had higher production of IL-12 than the normal group CLP. When the assessment was conducted in the serum of rats obtained the following results: normal sham-operated rats showed lower basal IL-12 than the obese sham-operated. The sham-operated obese rats produced more IL-12 than normal sham-operated after 24h. However, when comparing obese and normal 24 hours CLP found no difference.

Conclusions:

Obesity does not appear to have influenced the levels of PCR in this model of sepsis. Alterations in the NO production in animals submitted to sepsis were observed only in obeses. The coordinated innate immune response during sepsis seems to protect normal rat within 24h of sepsis in order to preserve these important organs for survival different of the obeses.

Keywords: obesity, sepsis, inflammation

Financial Support: FAPESB, CAPES

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**QuebraPagina**

Resumo:21-158

**NEUROGENESIS IN THE HIPPOCAMPUS OF ADULT MALE SWISS MICE IS RESISTANT TO ENVIRONMENTAL ENRICHMENT.**

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Objectives:

Neurogenesis (birth of new neurons) persists in the dentate gyrus (DG) of hippocampus throughout mammalian lifespan. In adult rats and mice, environmental changes were reported to increase or decrease the rate of newborn neurons formation in DG. In fact, isolation rearing has been reported to decrease neurogenesis in the DG whereas enriched environment increased the number of
newborn neurons and their survival or maturation in the DG of some rodents. Data from our laboratory suggest that the neurogenesis in the DG of adult male Swiss mice is sensitive to isolation rearing but resistant to enriched environment. Indeed, it was observed that 4 or 8 weeks in enriched environment failed to increase the number of immature perikaria in the DG of adult Swiss mice. However, the degree of neuronal survival or maturation in these conditions was not evaluated. Therefore, we hypothesized that enriched environment could have favored the maturation of newborn neurons in DG of adult Swiss mice instead of increase the number of newborn neurons. The degree of maturation could be estimated by the number of dendrite-extension and bifurcations in the immature neurons.

Methods and Results:

Nine male adult-Swiss mice were kept under standard (SE, n=5) and enriched environment (EE, n=4) for 30 days before euthanasia (anesthesia followed by heart perfusion with PFA 4%). After perfusion the brains were removed, frozen and sliced. Sections (50 um) were submitted to immunohistochemistry for detection of doublecortin (DCX). Bilateral DG (at least 4/animal) were photographed (400 x magnification) with a digital camera adapted to a bright field microscope for posterior analysis. An examiner blind to treatment counted, throughout the dorsal or ventral blades of DG in the dorsal hippocampus, the number of soma and filaments-expressing DCX. Filaments were classified as primary, secondary or tertiary according to the presence of zero, one and two or more bifurcations, respectively. The total number filaments counted in the DG was (mean±SEM): primary (SE: 62.4 ±19.36; EE: 96.25 ± 53.61), secondary (SE: 26.2 ± 9.44; EE: 36± 17.94) and tertiary (SE: 13 ±SE 6.55, EE: 32.25± 21.67). No significant differences between treatments were detected (significance level p

Conclusions:

Present results further confirm that neurogenesis in the DG of adult male Swiss mice is resistant to environmental enrichment.

Keywords: enriched environment, Male mice, Neurogenesis


QuebraPagina

Resumo:21-159

MOTOR BEHAVIOR: CORRELATION BETWEEN MOTOR FUNCTIONAL CAPACITY AND QUALITY OF LIFE IN THE ELDERLY OF CAMPINA GRANDE-PB

Department of Physiotherapy, UEPB

Objectives:

The aging is a natural physiological process, dynamic and progressive where are involved several factors, which promote changes in body systems, as the decline in the motor abilities and cognitives functions. This study aimed to evaluate the motor functional capacity and its correlation with the quality of life of elderly in the city of Campina Grande - PB.

Methods and Results:

METHODS: The research´s participants were 169 elderly of both genders, linked to the Family Health Units of Campina Grande - PB. The instruments used were: Brazilian OARS Multidimensional Functional Assessment Questionnaire – BOMFAQ, to analyze the motor functional capacity, and Flanagan’s Quality of Life Scale, to evaluate the quality of life of the elderly. The data were analyzed using the software Graph Pad Prism 4.00, and the values were expressed in percentage, mean and standard deviation, considering significant values with p.
Conclusions:

It is possible suggest that there is a correlation between motor functional capacity and quality of life of the Campina Grande city’s elderly.

Keywords: Elderly, Functionality, Quality of life

Financial Support: PIBIC/UEPB.

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EFFECTS OF ANTIEPILEPTIC DRUGS ON SPREADING DEPRESSION IN THE CHICK RETINA: IMPLICATIONS FOR MIGRAINE PROPHYLAXIS

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Objectives:

Spreading depression (SD) is a physiological response of nervous tissue to a local stimulus, which might be mechanical, chemical or electrical in nature. In the cerebral cortex, SD is characterized by a strong depression of both spontaneous and evoked electrical activities, which initiates at the stimulated locus and propagates slowly (ca. 3 mm.min⁻¹) to adjacent regions. The SD eletrocorticographic period lasts 2-5 min, and normal activity is usually restored 15-20 min after SD onset. Propagation of SD waves respects neither the limits of vascular territories, nor cytoarchitectural or functional boundaries throughout the cerebral cortex. Moreover, SD can be elicited and propagated in avascular tissue, such as the chick retina, which constitutes an excellent model for pharmacological studies of this phenomenon. Given its pharmacological and dynamic properties, SD has been clinically related to epilepsy and, even more closely, to migraine. Here, we tested how several antiepileptic drugs, at clinical dosages, affect the physiological characteristics of SD in the chick retina model, using in vitro and ex vivo preparations.

Methods and Results:

1) Isolated retinas from 2 week-old chicks (white leghorn, Gallus domesticus) were prepared according to Martins-Ferreira & Oliveira-Castro (J.Neurophysiol., 29:715, 1966). Briefly, each retina was dissected and kept in a superfusion chamber under a flow of 1.2 ml/min of Ringer’s reference solution at 31°C, pH 7.4 and 5%CO2. Mechanical or chemical stimuli of graded intensities were applied every 15 minutes, as slow retinal potentials were registered using two glass microelectrodes inserted in the internal plexiform layer, connected to a WPI223 electrometer, and recorded on a Grass5D polygraph. SD waves were characterized by their speed (mm/min), amplitude (mV), deflagration threshold (DT, tested with KCl-stimulus) and absolute refractory period (ARP, sec) with and without the drugs under study. Five antiepileptic drugs with proven effect on the modulation of GABAergic transmission were tested: Topiramate, Valproate semisodium, Gabapentin, Lamotrigine and Levetiracetam. 2) Additional retinas were treated by the drugs for 15 min, then homogenized to measure the activity of the enzyme GABA-transaminase (GABA-T). 3) Finally, additional animals were treated systemically with the drugs for 15 days, before their retinas were removed and SD parameters were assessed as above. Analysis of variance was used to compare results from different experimental groups. All five tested drugs reduced the speed and amplitude of SD in a dose-dependent and reversible manner, in vitro as well as ex vivo. All drugs also reversibly increased the DT, as well as the ARP for SD. Topiramate was the most effective drug in modifying all of the above mentioned parameters. Levetiracetam, in spite of its sui generis mechanism of action, was considered the least effective drug. In our model, the enzyme GABA-T displayed only a slight decrease in activity in the presence of Topiramate, Valproate semisodium and Gabapentin.

Conclusions:
Antiepileptic drugs, and more especially Topiramate, can significantly affect both the threshold and the propagation of SD in a dose-dependant manner. Our results reinforce the notion that SD may underlie the physiopathology of migraine and that, although further investigations on cortical SD are needed, our model may be a useful tool for the test of new prophylactic drugs.

Keywords: Migraine, Spreading Depression, Antiepileptic drugs, Neuropharmacology, Retina

Financial Support: CNPq, FAPERJ

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**EFFECTS OF PHYSICAL TRAINING IN THE MEMORY, ANXIETY AND MOTOR BEHAVIOR IN MICE**

do Carmo Cunha, J. ; Shimizu, W. A. L. ; Rosa, D. ; Schultz, M.
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Objectives:
The physical exercise has a great capacity in promoting chemical and morphological changes on the mammals’ central nervous system (CNS), and it can activate molecules involved with learning and memory processes. The aim of this work was to analyze the effects of treadmill physical training on the parameters of memory, anxiety and motor behavior.

Methods and Results:
Male C57/Bi6 mice (3 weeks old, n=15) were randomly assigned in two groups: sedentary (n=7) and trained (n=8). The experimental protocol has been approved by Ethics Committee of Federal University of São Paulo (protocol #0355/10). The animals were submitted to an adaptation period of 5 days in the treadmill (Versatille, Imbramed; 8 m/min). The treadmill training program consisted in one daily session in a speed of 10 m/min, 5 days/week, with a gradual increase of 10 min per day (from 20min - 1st day - to 60min - 5th day). The behavior of the animals was assessed by: i) spontaneous locomotor activity evaluation by an animal activity cage (model 7430, Ugo Basile, Comerio, Italy); ii) the long term memory evaluation by means inhibitory avoidance equipment (Ugo Basile, Comerio, Italy; intensity: 0.5 mA with duration of 2 sec, maximum exposure of 180 sec); and iii) the anxiety analysis by elevated plus maze. The data were expressed as means ± S.E.M. and two-way ANOVA (repeated measures) and student t test were used to statistical analysis. The locomotor activity’s analyses showed difference in vertical movements in first minute of the test (p=0.014). The time of locomotion of the animals in the cage was different between the groups (F[4, 13] 6,139 p= 0,0004), and there was interaction between time of locomotion and training (F[4, 13] 2,125 p=0,0908). The training program exerted effect on the time of horizontal movements (F[4, 13] 3,771 p=0,0091). The long term memory test was applied before and after the training protocol and demonstrated a time effect in both groups (F[1, 13] 31,10 p=0.0001). The analysis of the effect of time on the obtained data showed an increase of 63.05% for the trained group and 37.65% for the sedentary group. In the elevate plus maze test, the trained group had a decrease in exploratory capacity in open arms test (37,84% faster) when compared to sedentary. Similar effect was observed in the exploration of the closed arms in the trained group (28,67% faster than sedentary group).

Conclusions:
In conclusion, the training on the treadmill for 5 days did not deflagrate alterations in long-term memory in animals, but decreased the level of anxiety and influenced the locomotor exploratory activity.

Keywords: physical training , memory, anxiety, motor behavior, neuroplasticity

Financial Support: FAPESP
Objectives:

This work had two aims: 1) To investigate how the sleep-wake cycle affects the phosphorylation levels of Ca(2+)/calmodulin-dependent protein kinase II (CaMKII), which plays a key role in memory consolidation and Zif268/Arc transcription; and 2) to investigate the relationship between spindle features and CaMKII phosphorylation levels.

Methods and Results:

Young adult male rats (n=24) were implanted with electrodes for chronic recordings of local field potentials in the dentate gyrus and cerebral cortex, to identify WK, SWS and REM (Neurosci 24(49): 11137-11147, 2004). Experimental animals were allowed to explore 4 novel objects introduced in the recording box for 10 minutes as previously described (PLoS Biol 2(1): E24, 2004), while controls were unexposed to novel stimuli. The animals were then kept awake for 3 hours, to avoid detecting CaMKII phosphorylation directly related to the exploration of novel objects. Animals were then allowed to sleep and were decapitated immediately after entering SWS (at least 10 minutes) or REM (at least 2 minutes). Animals in the WK group had an additional 10 minutes of sleep deprivation before killing. The brains were quickly removed and the left hemispheres were frozen for immunohistochemistry assays, while the right hemispheres were used for protein extraction and immunoblots. Global phosphorylation levels of CaMKII α (Thr286) assessed in immunoblots revealed a significant decrease in CaMKII activation during REM in rats exposed to novel objects, in comparison to WK. As expected, no statistically significant differences occurred in controls. Immunohistochemistry used to investigate CaMKII inactivation in three specific hippocampal regions (dentate gyrus, CA3 and CA1) revealed significant differences in animals exposed to novel objects: CamKII phosphorylation decreased from WK to SWS in the hippocampal cell layers analyzed, but there was a significant increase from SWS to REM. Controls displayed no significant differences. Novel experience was concomitant in our dataset with an increase in spindle density, which was strongly correlated with CAMKII phosphorylation levels in the REM group, but not in the WK or SWS groups.

Conclusions:

Our results suggest that experience-dependent changes in the density of spindles during SWS and/or intermediate sleep before REM determine subsequent levels of CAMKII phosphorylation in local hippocampal circuits, when assessed immediately after REM. A similar relationship relates spindle power and Zif-268 mRNA expression 30 minutes after REM (Frontiers in Neuroscience 1: 43-55, 2007), suggesting a causal chain linking spindles, CAMKII phosphorylation and zif-268 expression.

Keywords: Sleep, memory, hippocampus, kinases, spindles

Financial Support: AASDAP, FINEP, FAPERN, CNPq.
VARIATION IN BODY WEIGHT AND SUSTAINED HYPERPHAGIA AFTER CYCLES RESTRICTION/REFEEDING IN RATS IS ASSOCIATED WITH PERINATAL MALNUTRITION.

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Objectives:
In an experimental model of binge eating, analyze food intake and body weight of rats malnourished during the perinatal period.

Methods and Results:
We used female Wistar rats whose mothers were malnourished (8% casein diet) and nourished (17% casein diet) during pregnancy and lactation. At 52 days old, we started the protocol of binge eating with 10 rats in each group. This sequence consisted of three cycles of restriction/refeeding of four days each. The restriction was to supply 40% of daily intake of the animal before the protocol. In the first two days of the 1st and 2nd cycles the animals had access to two hours of palatable food (high fat) during refeeding. Body weight (g) and food intake (kcal/g body weight) were measured daily during all cycles. After three cycles, we performed the feeding test, where all the animals had 24 hours with access to palatable food. The intake was measured after 2, 4, 6 and 24 hours of food availability. Data are expressed as mean ± standard error. Malnourished rats (66.9 ± 0.5) had higher (p

Conclusions:
The occurrence of adverse events in early life promotes organic adapt and metabolic disease in adulthood. The findings in this animal model of binge eating, suggest that perinatal malnutrition may act as an important etiologic factor for the development of a hyperphagic behaviour after cycles of restriction/refeeding.

Keywords: binge eating, body weight, intake, perinatal malnutrition

Financial Support: CAPES/FACEPE

LOW CONCENTRATION OF ETHANOL INCREASES QUANTAL CONTENT AT THE FROG NEUROMUSCULAR JUNCTION.

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Objectives:
It has been shown, at the frog neuromuscular junction, that ethanol has both pre and postsynaptic effects, increasing the rate of spontaneous transmitter release as well as the postsynaptic response to iontophoretically applied acetylcholine. In order to separate the action of ethanol on evoked release of transmitter from its postsynaptic effect, we measured the quantal content of endplate potentials (EPPs), which reflects the mean number of quanta released per stimulus, as well as paired pulse facilitation of quantal content, in low calcium Ringer solution.

Methods and Results:

The experiments were performed using cutaneous pectoris or sartorius nerve-muscle preparations from Rana catesbeiana. We recorded endplate potentials with intracellular electrodes and measured quantal content using the method of failures. We studied paired pulse facilitation by comparing the difference in quantal content of EPPs evoked by two stimuli separated by a 15 ms interval. Our results show that 0.02% ethanol increased quantal content by 43±35% (mean±95% confidence limits, n=15). Lower and higher doses (0.004%, 0.1%, 0.5%) did not have a statistically significant effect. Paired pulse facilitation was not changed.

Conclusions:

We conclude ethanol increases quantal content within a narrow range of concentrations. This may reflect an interaction between both excitatory and inhibitory effects, although we never observed inhibition, even at the highest dose tested. The lack of effect of ethanol on paired pulse facilitation suggests that it did not affect vesicle release probability.

Keywords: Neurotransmission, Ethanol, Acetylcholine, Neuromuscular junction, Quantal content

Financial Support: FAPEMIG, CNPq

QuebraPagina

Resumo:21-165

EVALUATION OF THE INFLUENCE OF OESTROUS CYCLE STAGE ON DEFENSIVE BEHAVIORS EVOKED BY ELECTRICAL STIMULATION OF THE PERIAQUEDUCTAL GRAY (PAG) OF THE RAT

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Objectives:

Panic disorder affects adults aged between 25 and 44 years, and its incidence is 2.2 times higher in females, especially during their fertile age. However, it is unknown what is the exactly influence of women hormonal fluctuations on this prevalence. Symptoms of spontaneous panic attacks are similar to the defensive behaviors evoked by intracranial electrical stimulation (EI) of PAG, which is involved with the control of aversive emotional state of animals. Then, this study aimed to investigate the effect of the stage of oestrous cycle on PAG-evoked defensive behaviors, a model of panic attack, in female rats.

Methods and Results:

Female Wistar rats, weighting between 270-300gr, were housed in individual glass walled cages (25cm×15 cm×30 cm) with food and water ad libitum. The cages were kept in a temperature-controlled room (20–25 oC) and 12-h light/dark cycle (lights on at 6:00 am). Rats were surgically implanted with electrodes in the dorsal PAG, and five days after surgery, electrical stimulations of dPAG and behavioral evaluations were begun. Rats were stimulated in a plexiglass cylindrical open-field apparatus (60cm wall height and diameter), and stimuli consisted of 20 s trains of sine-wave pulses (0–70μA, 60 Hz, a.c.) applied at 20s intervals or at 4 min intervals when animals presented any defensive behavior. The intensity was increased in steps of 5μA up to the production
of flight behaviors (galloping and/or jumping) or the cutoff intensity of 70μA. Defensive behaviors evaluated was Exophthalmus, Immobility, Trotting, Galloping, Jumping, Defecation and Micturition. Vaginal smears were taken daily for oestrous cycle evaluation, starting five days before surgery, and it was always conducted immediately before the electrical stimulations, to ensure that each animal would be stimulated in all stages of oestrous cycle. Response probability threshold curves were obtained by logistic fitting of accumulated response frequencies in function of the logarithm of the stimulation intensity. Curve comparison was performed by likelihood-ratio coincidence tests, with Bonferroni post-hoc (PP < 0.007), there was not influence of the stage of oestrous cycle on all the other defensive responses

Conclusions:

These results do not show clear evidences for the influence of oestrous cycle stage on the defensive responses evoked by electrical stimulation of the dorsal PAG. The most markedly altered response (i.e. micturation) may have been influenced by progesterone levels, since this hormone presents anti-mineralocorticoid properties. Anyway, hormonal measurements are necessary for pairing hormonal levels with the observed behaviors, what will be useful to better understand the effects of oestrous cycle stage on the defensive behaviors elicited by dPAG stimulation.

Keywords: periaqueductal gray, oestrous cycle, electrical stimulation, defensive behaviors

QuebraPagina

Resumo:21-166

NITRIC OXIDE AND CARBON MONOXIDE: INTERPLAY IN THE RAT LOCUS COERULEUS DURING FEVER.

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Objectives:

Locus coeruleus (LC) has been reported to take part in lipopolysaccharide (LPS)-induced fever in freely moving rats exposed to an isothermal, subneutral environment, 23°C. Interestingly, despite carbon monoxide and nitric oxide activate the same intracellular target, soluble guanylyl cyclase, LC carbon monoxide has been documented to be an antipyretic molecule whereas LC nitric oxide is a propyretic one. Therefore, aiming at further exploring the mechanisms underlying the anti- or propyretic role of these gaseous molecules in the LC we sought to investigate the putative interplay between these neuromodulators.

Methods and Results:

To attain this goal, male Wistar rats (250-300 g) were implanted with a guide cannula toward the fourth ventricle to perform microinjection intracerebroventricular. The animals were microinjected with a non-selective inhibitor of the nitric oxide synthase (L-NMMA; 4 μmol in 2 μl) or an inhibitor of the heme oxygenase (ZnDPBG; 75 nmol in 2 μl) and injected with LPS (100 μg/kg; intraperitoneal). Two and a half hours later, they were decapitated, and the brains frozen and cut in a cryostat. LC punches (500-μm thick; 500-μm diameter) were excised and processed to assess LC nitrite/nitrate (NOx) and bilirubin levels. Microinjection of ZnDPBG reduced LC bilirubin (75±2 vs. 23±1 μg of bilirubin/mg of protein; n=5) and increased LC NOx (44±5 vs. 71±3 nmol of NOx/mg of protein; n=5), whereas L-NMMA diminished LC NOx (42±4 vs. 21±5 nmol of NOx/mg of protein; n=5) and augmented LC bilirubin (73±3 vs. 96±2 μg of bilirubin/mg of protein; n=5).

Conclusions:

These findings are consistent with the notion that in the LC during LPS fever the nitric oxide synthase/nitric oxide pathway is
down-modulated by the heme oxygenase/carbon monoxide pathway and also that the former up-modulates the latter.

Keywords: Body temperature, Lipopolysaccharide, Brainstem, Nitrite/nitrate, Bilirubin

Financial Support: FAPESP

CHARACTERIZATION OF THE MUSCULOSKELETAL SYSTEM ENABLING HEAD MOVEMENTS IN THE BARN OWL (TYTO ALBA): I. MUSCLE FUNCTIONAL ANATOMY AND MORPHOMETRY.

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Objectives:
The static and dynamic control of the head relies on the complex orchestration of muscles enclosing the cervical spine. Here, we provide a thorough anatomical description of these muscles in the barn owl (Tyto alba), a bird species that exhibits a rich repertoire of head/neck movements. The paramount importance of such movements for owl gaze relocation can be better appreciated considering the lack of expressive eye movements in these birds.

Methods and Results:
Anatomical data are based on the dissection of four owls terminally anaesthetized with tiopental sodico (50 mg/kg). Each neck muscle was carefully characterized with respect to its origin, insertion, weight and pennation angle. Some of these measurements served to calculate the physiological cross-section area (PCSA), which reflects the maximum force capacity generable by a given muscle. To investigate the functional contribution of each muscle, kinematical and electromyographic (EMG) data were collected while two birds were cued to rotate their head in different axes. An electromagnetic 3D position tracker system (3D guidance trakSTAR™, Ascension Technology Corporation) was used to measure head positional changes. The GRASS QP511 Quad AC Amplifier (band-pass filtering: 30-3000 Hz; sampling frequency: 10kHz) was used to record EMG activity. Conventional signal processing procedures were employed to analyze EMG data. All experimental and animal care procedures used in this study were approved by the University Ethics Committee for Animal Experimentation. The origin and insertion of primary head-movement muscles were well correlated with cervical column mobility and PCSA measurements. Specific muscular groups were coupled with the upper, medium and low cervical bones. The m. rectus capitis lateralis was seen to originate from the second and third vertebrae and to attach to the mastoid process medially to the m. complexus, which was superficially located on the dorsal part of the skull. Underneath the m. complex insertion, the m. splenius capitis insertion covers a large part of the posterior skull. These muscles originate from the axis spinous process (C2). The pair of m. biventer attachments on the medial part of the posterior skull was seen to have its first insertion about C2 and its second insertion onto C16 and C17, where m. longuissimus dorsi attachment was also observed. Confirming the above anatomical data, m. splenius and m. rectus capitis displayed high EMG response amplitude during rotation and lateral flexion of the owl head. The same pattern of EMG response was recorded from the m. biventer and m. longuissimus dorsi during head extension.

Conclusions:
Our study reveals that the basic design of the barn owl’s neck musculature is surprisingly similar to that of mammals. Nevertheless, important differences were noted, especially with respect to the sites of origin and insertion of the primary muscles involved in head/neck movements. Such differences are likely to result in significant kinematical modifications in the owl.
Biomechanical modelling would be a valuable approach to address this issue.

Keywords: avian kinematics, avian anatomy, avian morphometry, electromiography, neck head movement

Financial Support: Brazilian agencies CAPES and FAPEMIG

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Resumo:21-168

LOCOMOTOR ACTIVITY OF SWISS MICE IN THE OPEN FIELD IS AFFECTED BY BLOCKAGE OF NMDA RECEPTORS BUT NOT BY ACTIVATION OF GABAA RECEPTORS DURING THE BRAIN GROWTH SPURT

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Objectives:

In rodents, both the blocking of NMDA glutamate receptors and the activation of GABAA receptors during the brain growth spurt (a period equivalent to the third trimester of human gestation) trigger widespread apoptotic neurodegeneration. Considering that neuronal loss plays a major role in the lifelong neurobehavioral disturbances resulting from developmental exposure to several neurotoxicants and that there are marked differences between NMDA and GABAA receptors regarding function and spatial distribution in the brain, here, we evaluated the effects of exposure to MK-801 (non-competitive antagonist of NMDA) or to muscimol (GABAA agonist) during the third trimester equivalent period of human gestation on locomotor activity of prepuberal Swiss mice males.

Methods and Results:

From postnatal day (P) 2 to P8, 48 males from 14 litters were assigned to received, every other day, one single intraperitoneal injection of one of the following substances: saline (SAL, n = 13), MK-801 (MK) 0.1mg/kg (n = 9), MK 0.3mg/kg (n = 7), MK 0.5mg/kg (n = 9), muscimol (MU) 0.1mg/kg (n = 7), MU 0.3mg/kg (n = 8) or MU 0.5mg/kg (n = 8). At P25, the distance travelled was automatically assessed under red dim light in a square chamber (50x48x50 cm) for 15 min. An univariate ANOVA was performed using the average of values from mice of the same litter. Neonatal treatment was used as the between-subject factor and post-hoc pairwise comparisons by Fisher¡’s Protected Least Significant Difference (FPLSD). The treatment with MK promoted locomotor hyperactivity in a dose-dependent way [F (6,34) = 2.8, P<0.05 in all pairwise comparisons). No differences were observed between SAL, MU 0.1mg/kg (28.9±1.6 m), MU 0.3mg/kg (26.7±2.7 m) and MU 0.5mg/kg (30.6±2.5 m) (P>0.05 in all pairwise comparisons).

Conclusions:

Our data suggest that, during the brain growth spurt, the blockade of NMDA receptors but not the GABAA activation promotes a long-lasting locomotor hyperactivity in mice. These data open up interesting possibilities for the development of therapeutic interventions that aim to ameliorate neurotoxicant-induced hyperactivity.

Keywords: Development, GABAA agonist, Locomotor Hyperactivity, Neurotoxicity, NMDA antagonist

Financial Support: FAPERJ, SR2 /UERJ

QuebraPagina

Resumo:21-169
MALNUTRITION DURING LACTATION ALTERS NICOTINE EFFECTS ON OPEN FIELD TEST IN ADOLESCENCE MICE.

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Objectives:

AIM: Several studies have demonstrated that insults during critical periods of development increase the susceptibility to drug use/abuse during adolescence and adulthood. In this regard, a recent study demonstrated a positive association between gestational exposure to famine during the Dutch hunger winter of 1944–45 and addiction later in life. Nicotine is one of the most frequently used/abused drugs and most smokers begin their habit during adolescence. Despite that, to our knowledge, there are no experimental studies that investigate the effects of malnutrition during development on nicotine susceptibility during adolescence. Thus, this study investigated if early malnutrition programs for increased susceptibility to nicotine effects in the open field arena test during adolescence in mice.

Methods and Results:

METHODS: At postnatal day 2 (P2), lactating female mice and their pups were divided into 3 groups: Control Group (CT) – fed a standard diet (23% protein), Protein-Restricted Group (PR), fed a diet containing 8% protein; and Caloric-Restricted Group (CR) – fed a reduced amount of standard diet (similar average amount of consumption of the PR group in the previous day). After weaning (P21), all pups had free access to standard diet. At postnatal day 30, the offspring either received a nicotine i.p injection (0.05 mg/kg (NIC) or saline (SAL) immediately before being tested in the open field arena. Accordingly, there were 6 treatment groups: CT/SAL (n=12), standard diet and saline; CT/NIC (n=16), standard diet and nicotine; PR/SAL (n=8), hypoproteic diet and saline; PR/NIC (n=10), hypoproteic diet and nicotine; CR/SAL (n=12), caloric-restricted and saline; CR/NIC (n=14), caloric-restricted and nicotine. The open field arena is a rectangular box, which each animal explores freely for 5 minutes. We quantified the time spent in the center of arena (anxiety indicator) and total ambulation (total traveled distance – activity indicator). Results were evaluated by univariate analyses of variance (uANOVAs) followed by post-hoc analyses using Fisher's Protected Least Significant Difference test (FPLSD). Results: In mice that received standard diet, subsequent nicotine exposure elicited an increase in activity (CT/NIC: 269±35cm vs. CT/SAL: 94±24cm, P

Conclusions:

Conclusion: Caloric restriction during lactation programmed for hyperactivity later during adolescence. This result corroborates the hypothesis that malnutrition during lactation elicits long-term behavioral alterations. In addition, in caloric-restricted mice, nicotine exposure during adolescence elicited an anxiolytic effect, even though in this group, nicotine failed to affect activity levels. These results indicate that nicotine effects on locomotor activity and anxiety are independent. Finally, the anxiolytic effect in the Caloric-Restricted group subsequently exposed to nicotine confirms the influence of nutritional insults during development on drug susceptibility later in life.

Keywords: anxiety, nicotine, protein restriction, caloric restriction, hyperactivity

Financial Support: Fellowships from CNPq, CAPES, FAPERJ

QuebraPagina

Resumo:21-170

HIGH FAT DIET MATERNAL AFFECTS THEIR ADULT RAT OFFSPRING IN EXPERIMENTAL MODELS OF MOOD DISORDERS.
Objectives:
Maternal exposure to high fat diets appears permanently alter structure and function brain their offspring, possibly affecting the neurotransmitter serotonergic system. According to experimental and clinical studies the serotonergic system is involved in the pathophysiology of mood disorders or neuropsychiatric. Therefore, this study had aim to investigate the performance of adult offspring suckled by dams submitted to high fat diet in experimental models of depression and aggressiveness.

Methods and Results:

Twenty-six were used (approximately three litters; 8 offspring/dams/litter) of Wistar newborn rats weighing 6-7 g, later they were divided into two experimental groups: 1) Control Diet (CD) and 2) high fat diet (HFD). The animals were breastfed during the 21 days postnatal (P) by mothers subjected to CD (commercial diet) or HFD (high saturated fat). Were used the following experimental models of mood disorders: forced swim test (FST, for depression) and the foot-shock test (aggressive defensive behavior analysis). Data are expressed as means ± SEM. An unpaired Student’s t-test was used for statistical analysis of data.

HFD animals showed a deficit of body weight during the suckling period and gain in adulthood compared to controls (HFD, P7 = 13.51 ± 0.70 g; P14 = 27.18 ± 1.86 g; P21 = 43.05 ± 3.08 g; P71 = 325.94 ± 28.17 g; P94 = 433.81 ± 30.44 g, n=17, versus CD, P7 = 16.98 ± 4.11 g; P14 = 31.40 ± 3.58 g; P21 = 49.87 ± 8.10 g; P71 = 298.75 ± 8.88 g; P94 = 351.88 ± 19.07 g, n=9, p = 0.001). In the FST the swimming time was lower in HFD animals compared to controls (HFD, 2.71 ± 0.18 s, n=17, versus CD, 13.50 ± 4.11 s, n=9, p = 0.034). In addition, the latency of aggressive responses was higher in the HFD animals compared to controls (HFD, 86.63 ± 32.07 s, n=16, versus CD, 6.25 ± 0.56 s, n=8, p=0.024).

Conclusions:
It seems that the high fat diet maternal during suckling has affected the offspring, promoting undernutrition in childhood and overweight in adulthood. Furthermore, HFD animals also showed behavioral changes, such as depressive and aggressive behaviors increased.

Keywords: HIGH FAT DIET MATERNAL, OFFSPRING RAT, DEPRESSION, AGGRESSIVENESS, MOOD DISORDERS

Financial Support: Scholarship and Master Scholarship from CNPq-PIBIC/CAPES-REUNI-Brazil (1 and 5)
mammals, which used to evaluate the behavioral specificity of feeding-modulating stimuli. Detailed descriptions of the BSS and its regulation have not been examined in birds; here, the post-prandial behaviors of pigeons after fasting- and hedonically-evoked feeding are described in detail, and a protocol to evoke and study BSS in this bird is depicted and tested.

Methods and Results:

Sixteen pigeons (both sexes, adult, 380-420 g bw, individually caged) were acclimatized to lab conditions (12:12 light-dark cycle, 24 ± 1 °C) and regular chow (RC) for 2 weeks, and then continuously video-recorded (3 h, from 15-18 h in their own home-cages, n=8) in 1) baseline conditions, 2) after palatable food (PF, a 3-seeds mix), 3) refeeding with RC and PF after a 24 h fasting (RF24), 4) refeeding with RC and PF after a 4h-period (RF4) or 5) after a 2-h-food withdrawal (RF2). Duration and frequency of feeding (F), drinking (D), preening (P), sleep (S), locomotor (L), alert immobility (AI) and exploratory (E) behaviors were recorded in 4-min bins. Similar experiments were carried out in 8 naïve pigeons transferred to a plastic experimental cage (EC) especially constructed to allow for controlled food/water delivery. To test for response stability, the RF2 protocol was repeated 4 times in the EC (7-day inter-test interval). All protocols (either in the home cage or in the EC) reliably evoked intense feeding bouts (RF24=RF4>PF=RF2>baseline) followed by drinking, then preening and then by increased sleep incidence. This BSS can be evoked by the mere exposure to a palatable food in the absence of food deprivation (PF protocol). Minimum square distance fitting of 4-min bin data helped to describe this sequential pattern as a consistent succession of waves whose morphological features (amplitudes, inter-wave intervals) can be quantitatively compared across experiments. Refeeding with RC and PF after a 4 h-period of food withdrawal (RF4, in the EC, after a 10-day habituation period) evoked a reliable, statistically-confirmed stable BSS (completed within 60-80 min from re-exposure to food/water), in absence of stressful, extensive food/water deprivation.

Conclusions:
Similar to mammals, pigeons exhibit a robust and consistent BSS, which can be evoked in low stress protocols, suggesting that its presence is a conserved functional attribute in amniotes. This type of behavioral analysis can be helpful to discriminate between drugs or feeding-modulating manipulations that preserve or disrupt the BSS in birds, and may represent a powerful tool in comparative studies on the physiology and neurochemistry of ingestive and sleep behaviors.

Keywords: feeding, drinking, sleep, satiety, avian

Financial Support: Supported by CNPq, Capes and FAPESC.

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Resumo:21-172

CORTICOCORTICAL CONNECTIVITY OF HAND AND FOOT REPRESENTATIONS IN PRIMATE PARietAL CORTEX

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Objectives:
The parietal cortex is involved in different roles, such as somatosensory processing, spatial perception, object manipulation, and tool use. It is subdivided into anterior parietal cortex (APC), responsible for processing somatosensory information; posterior parietal cortex (PPC), a node of multisensory integration; and lateral parietal cortex (LPC). In humans and some primates APC comprises areas 3a, 3b, 1 and 2, each one containing a separate and complete somatosensory representation of the body. A large part of the somatotopic maps in APC is devoted to the representation of hand and mouth. In PPC, area 5 is almost entirely dedicated to the representation of the hand and forelimb. Whereas the function of these parietal areas is determined by their connectivity, we raised the question of whether the connectivity of the lesser magnified foot/hindlimb representation follows the same pattern as the hand/forelimb representation. To answer this question we injected retrograde anatomical tracers in the representation of the foot in different areas of the parietal cortex in order reveal the ipsilateral cortical projections to this region.
Methods and Results:

Initially, hindlimb representations in parietal cortical areas were identified by electrophysiological mapping in one monkey of the species *Cebus apella*. In the same experimental session, fluorescent anatomical tracers were injected in the foot representation of areas 5 and 1. After a survival of 14 days, the animal was perfused. Resulting parasagittal brain slices were subjected to histological processing. Distribution of retrogradely labeled cell bodies was digitized using a fluorescent microscope equipped with the Neurolucida system (MBF, Inc). Architectonic identification of cortical areas containing labeled neurons was performed on adjacent Nissl-stained sections. We then compared the distribution of labeled neurons obtained in this experiment with that obtained in other previous experiments in which tracer injections was performed in the hand/forelimb representation of these same areas. The foot representation in area 1 received strong projections from all areas of APC (areas 3a, 3b, 1 and 2) and a weaker projection from cingulate cortex. Different from what had been demonstrated for the representation of the hand in area 1, no projections from cortical areas of PPC, LPC, and motor cortex were detected in our case. In area 5, the foot representation received projections from area 2, motor cortex, and cingulate cortex. This pattern of connections was more restricted than the one found for the hand representation in area 5, characterized by retrograde labeled neurons found in all areas of APC, area 7, motor areas (F1, F4 and F5), cingulate cortex, insular cortex, claustrum, and LPC (areas PV and S2).

Conclusions:

In conclusion, the connectivity pattern of the representation of foot/hindlimb differs from that of the representation hand/forelimb, both in areas 1 and area 5. The foot/hindlimb representation displays a more restricted pattern of distribution. This indicates that circuits processing information from forelimb and from hindlimb are distinct, despite the fact that they might be part of a same cortical area. Connectivity differences like these probably reflects functional differences in the way fore- and hindlimbs are used during ordinary behavior.

Keywords: Cebus monkey, corticocortical connectivity, foot representation, parietal cortex

Financial Support: FAPERJ

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Resumo:21-173

N5-STZ DIABETIC-INDUCED MODEL DEVELOPS ALTERATIONS IN SCIATIC NERVE AND DORSAL ROOT GANGLIA NEURONS OF WISTAR RATS.


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Objectives:

Diabetes is a disease that affects 246 millions humans around the world and in Brazil there is an estimative of 10 million with this disease. One of the most common complications of diabetes is the neuropathies and several animal models were developed to study its complications. Therefore this work has the objective to study the electrophysiological alterations produced by a diabetes-induced model in sciatic nerve (SN) and dorsal root ganglia (DRG) of rats.

Methods and Results:

For diabetes-induction we used a single injection of streptozotocin, (STZ, 120 mg/kg via i.p.) in the 5th day of life of Wistar rats. Body mass and glicemic level were monitored weekly and to confirm the induction of diabetes, we realized a glucose tolerance test (75 mg/100 g of weight) in 11th week of life. The animals were sacrificed in the 12th week by cerebral concussion for dissection of SN and DRG, which were immediately placed in Locke’s solution. To measure electrophysiological parameters we
used extracellular, intracellular and patch clamp recording techniques and this work was submitted to local ethical committee (CEUA-UECE, protocol number 06379067-0). The male STZ rats showed mean mass value inferior to control group, while this effect was not observed in female STZ group. Glycemic concentration in control group was 112.8 ± 2.6 mg/dL (n=8) whilst in STZ animals we divided in two other groups: one called hyperglycemic in which the mean glicemic level was 359.8 ± 24.4 mg/dL (n=4) and the other, called near-normoglycemic whose mean glicemic level was 135.4 ± 13.6 mg/dL (n=7). We performed a glucose tolerance test in near-normoglycemic group and they showed intolerance to glucose when compared to control, with glicemic values of 181.6 ± 28.0 mg/dL 2h after test onset (108.5 ± 7.7 dg/mL, for control group in the same time). SN chronaxie and reobase were altered in hyperglycemic group when compared to control but this was not the case for near-normoglycemic group. Furthermore hyperglycemic rats showed modifications in conduction velocity. In DRG, we separated the neurons in two groups: without inflexion in the falling phase of action potential (named here N0) and with inflexion (Ninf). Values of limia current of both cellular groups were not altered in hyperglycemic and near-normoglycemic animals when compared to control. Regarding action potential electrophysiological parameters, N0 and Ninf cells in near-normoglycemic group were similar. There was depolarization of resting membrane potential in all neurons of hyperglycemic animals when compared to control (4 and 9 mV to N0 and Ninf, respectively) and a reduction in action potential amplitude. For near-normoglycemic group there were no modifications in these parameters. Concerning patch clamp experiments, there were no changes in Na + currents in both diabetic animal groups compared to control.

Conclusions:

In conclusion, n5-STZ diabetic model develops alterations in SN and in DRG, but the alteration of excitability cannot be attributable to alterations in sodium current. This kind of model is useful to study peripheral neuropathy and its complications in SN and DRG but one restriction is that animals must presents high glycemic level to better distinguish those alteration.

Keywords: DIABETIC NEUROPATHY, n5-STZ DIABETIC-INDUCED MODEL, SCIATIC NERVE, DORSAL ROOT GANGLIA, SODIUM CURRENT

Financial Support: CNPq, Funcap

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Resumo:21-174

MEASURING THE DISTRIBUTION OF THETA-LOCKED SPIKING ACTIVITY IN HIPPOCAMPAL NEURONAL ENSEMBLES OF RATS BEFORE AND AFTER SPATIAL EXPLORATION

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Objectives:

In the rodent hippocampus, theta oscillations are associated with exploratory activity and REM sleep. Recent evidence has pointed to an involvement of theta-locked neuronal activity in novelty-induced synaptic plasticity, short-term memory storage, and the encoding of new memories. In humans, successful memory formation is predicted by the phase-locking of hippocampal and amygdalar neurons to theta oscillations (Nature. 464: 963, 2010). Furthermore, a phase shift of spiking activity with respect to the LFP was reported in rats during post-novelty REM sleep (Brain Res. 855:176, 2000). To understand how exploratory activity induces changes in phase-locking patterns during post-exploratory sleep, we measured the spike coupling to theta oscillations in rat hippocampal neurons before and after a spatial exploration task.

Methods and Results:

We used chronically implanted microelectrode arrays to record single neuron activity and LFP from the CA1 region of the
hippocampus in two freely behaving Wistar rats. Recordings were performed during sleep sessions (30-45 minutes) before and after a spatial exploratory task. To understand the coordination of spike timing to the ongoing theta oscillation we first selected LFP segments with increased theta power (theta/delta ratio > 6, as in PNAS. 98: 9386, 2001). We then measured the phase of spikes with respect to theta oscillations, and statistically compared the resulting distribution with an independent uniform distribution. Finally, we selected the significantly coupled neurons and measured (1) the strength of theta-locked spiking activity using the mean vector length and (2) the preferential theta phase for the discharge of individual neurons. We sampled 485 hippocampal neurons in 14 recording sessions. We found that 63.6% of neurons were statistically coupled to theta oscillations (Rayleigh test p

Conclusions:

The amounts of theta-locked neurons were not different before and after a spatial exploration task. Almost half of the neuronal population remained permanently coupled before and after the task, and approximately a quarter changed their coupling status. Interestingly, the mean vector length analysis revealed that 58.7% of the neurons coupled in both situations reduced their strength of coupling after task execution. Our findings suggest that spatial exploration reduces the strength of theta-coupling of hippocampal neurons during subsequent sleep.

Keywords: changes, phase-locking, spatial exploration task, theta oscillations.

Financial Support: AASDAP, FINEP, CAPES, CNPq.

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Resumo:21-175

STUDY OF BRAIN PLASTICITY INDUCED BY PHYSICAL EXERCISE DURING THE POSTNATAL BRAIN DEVELOPMENT OF RATS SUBMITTED TO MULTIPLE STATUS EPILEPTICUS IN EARLY LIFE

Novaes; Gomes da Silva; Toscano-silva; Almeida; Scorza; Cavalheiro; Arida
Departamento de Fisiologia, UNIFESP

Objectives:

There are several evidences showing the capacity of environmental factors to compensate the neural function after insult of the central nervous system. Studies in adult animals have demonstrated a beneficial effect of physical exercise on epileptic insult. Thus, it has been observed that the physical exercise increases threshold of seizures and confers a neuroprotective effect. Although the effects of physical exercise in the mature brain are well documented, its influence in the developing brain has been little explored. The purpose of our study was to investigate whether a physical exercise program undertaken during the postnatal brain development could influence cerebral plasticity of rats submitted to multiple status epilepticus (SE) using the pilocarpine model.

Methods and Results:

Male Wistar rats aged 7 postnatal days old (P7) were randomly divided into four groups: exercise group, control group, SE group and SE exercise group. Animals from SE and SE exercise groups were treated with a single intraperitoneal injection of pilocarpine at 380 mg/kg for three consecutive days (P7 to P9). Control rats were injected with 0.9% saline under the same conditions. After weaning, animals from exercise and SE exercise groups were submitted to daily exercise program in a treadmill (between P21 and P60). At P60, animals of all groups were assigned to histological analyses. Results. Nissl and Neu-Nissl stained sections did not reveal the presence gross anatomic changes among the studied groups, such as gliosis and neuronal loss in brain areas involved to seizure-related injury or mossy fiber sprouting in the supragranular layer. In immunohistochemical analysis, the neurofilament protein parvalbumin expression was significantly enhanced in the hippocampal formation of rats from exercise groups (exercise and SE exercise) when compared to rats from control groups (control and SE). A significant reduction in brain-derived neurotrophic factor (BDNF) immunoreactivity was also observed in SE group when compared to control group. Nevertheless, no significant difference in the BDNF immunoreactivity was detected between the exercise and SE

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Resumo:21-175

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exercise groups.

Conclusions:

These findings indicate that physical exercise program during the development promotes neuroplastic changes in brain of animals submitted to multiple SE in early life.

Keywords: exercise, development, brain plasticity, seizure, epilepsy

Financial Support: FAPESP, CNPq, CAPES, ClnAPCe e INNT.

QuebraPagina

Resumo:21-176

NEUROCHEMICAL ALTERATIONS IN THE BRAIN OF ISCHEMIC RATS SUBMITTED TO THE PILOCARPINE-INDUCED CONVULSIONS MODEL

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4 Laboratório de Neurofisiologia , UNIFESP-EPM

Objectives:

Pilocarpine-induced convulsions model is known to significantly decrease levels of dopamine and its metabolites in the rat striatum. Our objective in the present work is to determine striatal levels of dopamine and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in rats after brain ischemia followed or not by the treatment with a high dose of pilocarpine.

Methods and Results:

Male Wistar rats (250-300g) were submitted to a transient brain ischemia by the occlusion of both common carotid arteries for 60 min, followed by reperfusion. For that, the animals were anesthetized with sodium tiopental (50 mg/kg, i.p.) and submitted to a medial incision for exposure, isolation and clamping of the carotid arteries from both sides. The sham-operated group (Sham) was submitted to all the surgical procedure, except for the carotid clamping. One ischemic group was treated with pilocarpine at the dose of 300 mg/kg, i.p. (Ischemia+Pilo300). Another group without ischemia was also treated with pilocarpine at the same dose (Pilo300). While one sham-operated group was untreated (Sham) the other one received pilocarpine (Sham+Pilo300). After treatments, the animals were sacrificed by decapitation and their brain dissected on ice. Striatal homogenates were used for determinations of DA) and its metabolites (DOPAC and HVA) by HPLC. The data (ng/g wet tissue) were expressed as means ± SEM and analyzed by ANOVA and Student-Newman-Keuls (post hoc test) and considered significant at p

Conclusions:

Brain ischemia significantly decreased DA levels and values were not different in ischemic rats after the pilocarpine treatment. However, DOPAC levels were altered in all groups, ischemic or not, after the treatment with pilocarpine. Reduced levels of HVA were also observed after brain ischemia in the absence or presence of pilocarpine. In conclusion we showed that ischemia leads to striatal monoamine alterations indicating the participation of dopamine neurotransmission in this type of brain injury.
PURINERGIC MECHANISMS REDUCE PILOCARPINE-INDUCED SALIVATION IN RATS

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Objectives:
Adenosine-5′-triphosphate (ATP) is considered an important neurotransmitter in the peripheral and central nervous system (CNS) and the purinergic receptors are present in the CNS and in the salivary glands. The intraperitoneal (ip) injection of pilocarpine (PILO, cholinergic agonist) induces salivation by acting in the salivary glands and in the CNS. Previous studies demonstrated that the activation of purinergic receptors in isolated salivary glands induces salivation, however, this same activation reduces the salivation induced by carbachol (another cholinergic agonist) in isolated submandibular glands of rats. Thus, in the present study we investigated the effects of ip and intracerebroventricular (icv) injections of ATP on the PILO-induced salivation in rats.

Methods and Results:
Male Holtzman rats (280-320 g, n=10-17/group), part of them with stainless steel cannulas implanted into the lateral ventricle (LV), were used. To test the salivation, rats were anesthetized with ketamine (100 mg/kg of body weight) ip. The saliva induced by PILO (1 mg/kg of body weight) injected ip was collected using small cottons balls previously weighed inserted into the oral cavity of the rat. Previous ip injection of ATP (50, 200 and 400 mg/kg of body weight) reduced the salivation induced by PILO ip (225 ± 37, 341 ± 68 and 138 ± 36 mg/7 min, respectively, vs. saline: 491 ± 48 mg/7 min). The icv injection of ATP (300 nmol/1 μL) also reduced the salivation induced by PILO ip (494 ± 26 mg/7 min, vs. saline: 579 ± 38 mg/7 min).

Conclusions:
The results suggest that central or peripheral purinergic receptor activation inhibits salivation produced by peripheral administration of pilocarpine.

Keywords: ATP, pilocarpine, purinergic, salivation

Financial Support: FAPESP and CNPq
Objectives:

The adult olfactory epithelium (OE) is able to replace olfactory receptor neurons (OSNs) lost as the result of an intrinsic turnover or injury, throughout life. However, in contrast to the peripheral components of the auditory and visual systems, olfactory receptor neurons are true bipolar neurons directly exposed to the external environment. The dendritic processes of these olfactory receptor neurons project into the nasal lumen optimizing olfactory transduction, but rendering these neurons vulnerable to injury from infectious, mechanical and chemical agents. In consequence, an innate inflammatory response is triggered upon lesion in order to eliminate such agents. The endogenous glucocorticoids (GCs) act as a negative feedback on inflammatory responses, preventing them an exacerbated and damaging effect. Accordingly, GC receptor agonists have been used faithfully in the treatment of inflammatory processes, but little is known about their effect on OE regeneration. This work aimed to verify if administration of a strong GR agonist, dexametasone (DEX), is detrimental or beneficial to OSNs replacement subsequent to OE lesion.

Methods and Results:

OE lesion was induced in C57Bl/6 adult mice by means of methimazole administration (50g/kg, i.p.). Twenty-four hours after complete mature OSNs loss, the animals were submitted to intranasal (i.n.) infusion and assigned to three groups: Control, saline intranasal infusion (3 days); DEX-1, a 200 ng single-dose of DEX (saline infusion the next 2 days); DEX-3, three successive 200 ng doses (one dose/day) of DEX. Upon OE recovery, the mice were perfused with saline 0.9% followed by 4% formaldehyde 2 weeks after the first i.n. infusion. The noses were dissected, decalcified in EDTA, cryoprotected for 24h in 30% sucrose PBS solution and embedded with Tissue-Tek OCT compound. Coronal 14 µM sequential sections were obtained using a cryostat and collected onto adhesive microscope slides. The sections were processed for immunofluorescence using anti-OMP (Olfactory Marker Protein) to verify the reappearance of mature OSNs. The florescence signal was measured on digital photomicrographs using Image J software. Animals treated with DEX three consecutive days presented a significant reduction (~ 50%) of neuronal recovery, as judged by OMP labeling, compared to saline and DEX-1 groups (p

Conclusions:

Continuous use of DEX, differently from a single-dose procedure, causes impairment of the expected OE regeneration. Our results corroborate the studies that point towards a negative effect of excessive antiinflammatory use on neuronal recovery and might help explain as well, the failure of glucocorticoid-based therapy to promote olfactory recover in chronic upper respiratory inflammatory disease.

Keywords: neuroregenetation, inflammation, olfactory, glucocorticoids

Financial Support: FAPESP

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Resumo:21-179

PERFORMANCE OF A CAPUCHIN MONKEY (CEBUS APELLA) IN A MANUAL TASK EXECUTED IN DIFFERENT SENSORY CONDITIONS

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Objectives:
Dexterity is one of the most spectacular evolutionary achievements of primates. Capuchin monkeys inhabit Central and South Americas, sharing a common ancestor with humans that lived circa 35 million years ago. They are endowed with a semi-opposable thumb and are able to handle basic tools in captivity and in the wild, thus offering an excellent model for the study of evolution of manual behavior. Such feature relies on a sensory-motor circuitry that processes haptic and visual information simultaneously. The aim of this work is to investigate the performance of capuchin monkeys in a simple manual reaching and grasping task in the presence and absence of visual inputs.

Methods and Results:

All experimental protocols were conducted following the NIH guidelines for animal research. One adult male (*Cebus apella*) weighting 3.5 kg was trained to stay positioned in front of a testing device with both hands on two levers as the initial position, and to wait for a go signal in order to retrieve a food reward from one of five randomly indicated well positions located in a fixed platform in front of the animal. The device with this platform was illuminated and each well was equipped with a photocell that signaled the presence or absence of the treat. The testing device was connected to a computer through a LabVIEW program interface (National Instruments). This apparatus recorded the reaction time (T1) concerning the period between the go signal and the removal of the monkey’s hand from the lever. It also recorded the motor execution time (T2) between movement onset and reward withdrawal. This experiment was conducted in two different conditions. In the visual condition (VC) the animal saw the position of the reward before and while retrieving it by looking throughout a transparent window. In the non-visual condition (NVC) the go signal was given only after the sight of the platform was hidden from the monkey by an opaque field. In this condition the go signal corresponded to the lifting of the opaque screen, while in the VC the go signal consisted of a verbal command combined with the withdrawal of a stick from the top of the well. The animal performed one session of 50 trials per day during 8 days. A trial was considered invalid when T1 or T2 was greater than 2 sec. A total of 278 VC trials and 121 NVC trials were recorded computing an amount of 283 valid trials out of 399. The following parameters were quantified, comparing VC with NVC: (1) Mean time spent to retrieve the reward; (2) Mean reward retrieval velocity; (3) Manual preference during task execution. Reaction and motor execution time – In VC: T1 = 931.2 ms (SD=61.9); T2 = 801.9 ms; (SD=96.2); Vel. (T2) = 15.8 cm/s (SD=3.3). In NVC: T1 = 762.5 ms (SD=66.2); T2 = 924.6 ms (SD=102.9); Vel. (T2) = 13.4 cm/s (SD=2.6). Manual preference: In VC, the left hand was used in 98% of the trials (N=218), while in NVC the right hand was preferred in 96% of the trials (N=71).

Conclusions:

Hand preference changed according to the visual condition of the task. Additionally, in the visual condition, there was an increase in the velocity of retrieval, as expected. Surprisingly, the reaction time (T1) is longer when visual feedback is present. This may reflect an increase in the time required to plan the movement, due to additional information provided by the visual system. Alternatively, it might be a result of the difference in go signals of each condition.

Keywords: Capuchin Monkey, *Cebus apella*, manual abilities, posterior parietal cortex, multisensory integration

Financial Support: CNPq, FAPERJ, PRONEX.

QuebraPagina

Resumo:21-180

**EVENT RELATED SYNCHRONIZATION/DESYNCHRONIZATION FOR IMAGINATION OF FLEXION AND EXTENSION MOVEMENT OF THE INDEX FINGER**

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2 Programa de Pós-Graduação em Neurociências / ICB, UFMG

Objectives:
The motor imagery (MI) can be defined as a dynamic state where the representation of a specific motor action is activated inside a neural network without any motor response and is directed by the principles of the central motor control. The present study aims to identify the pattern cortical activation of the MI and movement execution (ME) of the flexion and extension index finger, by using the event related desynchronization and synchronization (ERD/ERS) of cerebral rhythms on electroencephalogram signals (EEG).

Methods and Results:

Six healthy subjects participated of this study. EEG signals during MI (85 trials) and ME (85 trials) of the flexion and extension of dominant index finger were collected using the 10-20 international system (BrainNet BNT - 36, bandpass 1-100Hz and sampling rate of 600Hz). First, the event related potential (ERP) was estimated by coherent mean of 40 trials (free of artifacts) selected randomly at C3, C4, Cz, P3, P4 and Pz electrodes. Furthermore, the reactive frequency bands to use in ERD/ERS were determined by comparing (paired Wilcoxon test with significance level of 5%) short-time power spectra of 1s reference window without motor task and one during preparation of task and another one after task. Besides, the ERD/ERS was processed in the frequency intervals identified previously. All experimental care procedures used in this study were approved for the Local Ethics Committee. The subjects presented different reactive frequencies in different electrodes; however, all of them showed significant frequencies that belonged to the alpha rhythm during the preparation motor task and to beta rhythm after the motor task. During the preparation of MI and ME, five subjects showed an ERD in the alpha frequency band in central and parietal electrodes. In the post-MI, an ERS in the beta frequency band was found in four subjects in central electrodes. On the other hand, in the post-ME, this ERS was identified in five subjects but at parietal electrodes.

Conclusions:

Results confirm a cortical activity pattern in somatomotor cortical areas. An ERD in the alpha frequency band shows an increase of mental activity, whereas an ERS post-MI and post-ME in beta frequency band indicates a decreased of the mental activity. In this way, ERD in alpha can be related with the motor planning while the ERS in beta shows the end of the motor task. This suggests a similar pattern for the MI and ME of the index finger in alpha and beta rhythms. This type of study would be a valuable approach for the development of brain computer interfaces and for rehabilitation applications.

Keywords: ERS/ERD, motor imagery, EEG

Financial Support: FAPEMIG, CAPES, CNPq

Resumo: 21-181

ULTRASTRUCTURE OF THE CEREBRAL GANGLIA OF THE SNAIL MEGALOBULIMUS ABBREVIATUS SUBMITTED TO EXPERIMENTAL ANOXIA

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Objectives:

The land snail *M. abbreviatus* is an anoxia-tolerant species, when it stays burrowed in the ground. The natural tolerance to these periods must involve mechanisms to protect the nervous system and other organs of the reduced availability of O2 or reoxygenation phases. The aim of this study is analyze the effects of anoxia exposure and reoxygenation on the structure of neurons and glial cells in the cerebral ganglia of *M. abbreviatus*.

Methods and Results:

Thirty-five (35) snails *Megabulimus abbreviatus* were divided in 5 experimental groups that are submitted to different periods
of anoxia, followed or not by reoxygenation period: control (CT), 3h anoxia (ax); 12h ax; 3h ax + 15 h of recovery (reox); 3h ax + 14 days (d) reox; 12h ax + 15h reox; and 12h ax and 14 d reox. An area of the mesocerebrum (containing the giant neuron) was analyzed. The tissue samples were processed for electron microscopy using standard procedures (Acta Zool. Stockholm 86;135, 2005). 3h ax: The neurons showed a nuclear swelling and chromatin condensation, whereas nuclear membrane (NM) remained intact. The rough endoplasmic reticulum (RER) showed electron-lucid cisternae and few ribosomes; the Golgi apparatus (G) has scanty vesicles; few mitochondria (MT) was found; and vacuoli smooth endoplasmic reticulum (SER) adjacent the plasma membrane (PM). 3h ax + 15h reox: the neurons presented a decrease of the nuclear swelling, an increase of vesicles between the G cisternae, compared to 3h, MT with increased density of the matrix; and the vacuoles adjacent of the MP have persisted. 3h ax + 14 d reox: the neurons were similar to the CT cells, the chromatin was homogenously distributed throughout the nucleus, numerous ribosomes were visualized, G displayed dilated cisternae and numerous vesicles associated; M were similar to the 3h ax + 15h reox. 12h ax: the chromatin was condensed adjacent to the NM. The NM integrity was maintained. There were G with few cisternae and vesicles, and the MT were very small. 12h ax + 15h reox: the neurons displayed the nuclei with very condensed chromatin, and NM indentations were observed, but maintaining the membrane integrity. Thin RER cisterns with scanty ribosomes were also observed. The G cisternae were decreased with few vesicles; MT seemed swelled when compared to neurons exposed to 12 h ax. 12h ax + 14 d reox: the neurons showed similar features to the control neurons, with numerous free ribosomes, well-developed G, and the MT were similar to those observed in the other groups submitted to reoxygenation. The PM recovered the control aspect, without adjacent vacuoles. No remarkable differences were observed in the glial cells of the mesocerebrum. No signs of necrosis or apoptosis were observed in the neural cells of the mesocerebrum in any experimental group.

Conclusions:

The absence of necrosis or apoptosis phenomena in cerebral neurons of *M. abbreviatus* submitted to anoxia, followed or not by reoxygenation, point out the existence of anoxia tolerance mechanisms in this species. The neurons at anoxia showed altered cytological aspects, which are reversible. We are facing an anoxia-tolerant animal model which deserves further investigation.

Keywords: ANOXIA, CEREBRAL GANGLIA, MEGALOBULIMUS, REOXYGENATION, ULTRASTRUCTURE

Financial Support: CAPES
maceration process (tenebrionid beetles), the bones were cleaned with hydrogen peroxide 3% (CRQ Ltda, Brazil). A digital caliper (100µm resolution) was used to measure the dimensions of each vertebra. All experimental and animal care procedures used in this study were approved by the University Ethics Committee for Animal Experimentation. Thirteen cervical vertebrae were identified. Based on previous avian studies, these vertebrae were divided into three cervical groups. The first one comprised the superior cervical vertebrae C1 (axis) and C2 (atlas). These bones have a paramount importance for head extension, rotation and anterior-lateral flexion. Compared to many other birds, the owl dorsal spinous and transversus processes of C2 were found to have a more prominent muscle attachment area. Cervical lordosis is essentially due to the second vertebral group (between C3 and C7), whose zygapophysis format also permits anterior-posterior and, to a lesser degree, lateral movements. A relatively similar osteological arrangement was found for the vertebrae belonging to the third group (C8-C13), except that the face of zygapophysis processes had a more pronounced lateral extension, thereby reducing lateral flexions and axial rotations.

Conclusions:
The present study reports original osteo-articular data on the cervical vertebrae of barn owls. To provide more realistic information for future biomechanical modeling of head movements in this species, we intend to estimate quantitatively the ranges of movement and axis of rotation for single vertebrae during flexion, extension, side rotation and lateral bending.

Keywords: avian, cervical vertebrae, head/neck movements, osteology

Financial Support: Brazilian agencies CAPES and FAPEMIG

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Resumo:21-183

EFFECTS OF CENTRAL CHOLINERGIC ACTIVATION ON SODIUM DEPLETION-INDUCED SODIUM INTAKE.

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Objectives:
Central cholinergic activation in fluid replete, particularly with carbachol, stimulates water intake and natriuresis, but no hypertonic NaCl intake. In the present study, we investigated if intracerebroventricular (i.c.v.) injection of carbachol could change sodium depletion-induced 0.3 M NaCl intake.

Methods and Results:
Male Wistar rats (n=9) with a guide cannula implanted in the lateral ventricle (L.V.) had free access to water, food and 0.3 M NaCl for at least 5 days before starting the test. At the moment of the test, sodium depleted rats (treated with the diuretic furosemide at the dose of 20 mg/kg of body weight followed by sodium deficient diet for 24 h) or fluid replete rats received injection of carbachol (7 nmol/1 µl) or saline i.c.v. Immediately after the i.c.v. injections rats had free access to water and 0.3 M NaCl for 2 hours. Injection of carbachol i.c.v. reduced 0.3 M NaCl intake by sodium depleted rats (2.5 ± 1.3, vs. saline: 7.0 ± 1.3 ml/2 h), without changing 0.3 M NaCl intake by fluid replete rats (0.8 ± 0.3, vs. saline: 0.7 ± 0.2 ml/2 h). Water intake after i.c.v. injection of carbachol was similar in sodium depleted and fluid repletes rats (7.3 ± 0.9 and 7.5 ± 2.3 ml/2 h, respectively).

Conclusions:
The present results show that i.c.v. injection of carbachol reduces sodium depletion-induced 0.3 M NaCl intake, suggesting that central cholinergic activation inhibits sodium depletion-induced sodium intake.
SHORT-TERM EFFECTS OF LOW FREQUENCY STIMULATION IN THE SPONTANEOUS LOCAL FIELD POTENTIAL OF ANESTHETIZED RATS.

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Objectives:

Previous studies have shown that neural stimulation may induce synaptic plasticity in a frequency-dependent manner (Neuron, 44:5, 2004). However, the effect of synaptic plasticity on the spontaneous oscillations is unknown. Here, we investigated the effects of low frequency stimulation (LFS) applied to the angular bundle on the generation of local field potentials (LFP) in the hippocampus of anesthetized rats.

Methods and Results:

Adult male rats were deeply anesthetized and placed in a stereotaxic apparatus. A pair of insulated stainless steel electrodes (100µm diameter) were placed in the angular bundle and were used to deliver monophasic square pulses (100µs pulse duration) at different frequencies. Spontaneous and evoked LFP were recorded from the dentate gyrus of the dorsal hippocampus using a bundle of insulated stainless steel electrodes (2-6 electrodes with 50 µm diameter each). After constructing stimulus intensity response curves and recording 20 min of basal activity (stimulation frequency: 0.1Hz), LFS (1Hz) was applied for 15min (stimulation intensity: 80% of the intensity needed to evoke maximum response). To evaluate synaptic depression, evoked responses were normalized for the basal condition. Duration of the synaptic plasticity was accessed for 40 min after termination of the LFS protocol. Evoked amplitudes of the extracellular postsynaptic potential (fEPSP) and population spike (PS) were analyzed and compared to the spectrum of the spontaneous activity observed in the 1 sec preceding application of the stimuli. After the experiments, animals were transcardiically perfused and histological analysis were performed to confirm electrodes location. All experiments were approved by the local ethical committee (# 07/2010). Evoked fEPSP and PS were correlated with the power of spontaneous activity in different frequency bands. Analysis of variance was used to assess statistical differences (P

Conclusions:

These results suggest that LFS can modulate spontaneous activity in the hippocampus of the anesthetized rat, possibly by reducing local excitability. Modulation of spontaneous oscillation in the hippocampus by direct stimulation might be relevant for the development of neuromodulation protocols in disorders of rhythmogenesis, as occurs in temporal lobe epilepsy.

Keywords: Local Field Potential, Neuromodulation, Rats, Synaptic Plasticity

Financial Support: CNPq (Processos: 134260/2010-4 and 474900/2010-0), CAPES.
FEAR MEMORY IMPAIRMENT IN A HYPERTENSION ANIMAL MODEL

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Objectives:
The increase in sympathetic activity is a mechanism for both initiating and sustaining the blood pressure elevation. Sympathetic nervous activation also confers specific cardiovascular risk. Additionally, hypertension is associated with mild decrements in cognition. Here we investigate the performance of Mas knockout mice (MasKo), which did not express the Ang-(1-7) endogenous receptor, in fear memory tasks. Based on the fact that MasKo mice present high blood pressure and elevated sympathetic tone, our hypothesis is that fear memory is impaired in these mice.

Methods and Results:
MasKo adult mice on the FVB/N background and its wild-types littermates were used. We analyzed fear memory using three different protocols: inhibitory avoidance, contextual and cued fear conditioning. Animals are submitted to a classical fear condition (FC) protocol and inhibitory avoidance task (IA). At FC animal was putted in an acrylic chamber with a metallic grid floor for 120s and after a 80dB 1KHz tone for 30s, that preceded a scrambled foot 0.7mA shock for 2s. The pairing by sound and foot shock was repeated tree times. 24h after animal was putted in another chamber and tested by freezings episodes when the same tone of day before was presented. We submitted the mice (10 per genotipe) for five consecutive days for the same sound test, to evaluated extinction memory. To test the context compound, another group of mice (8 per genotype) were allowed freely exploration in a neutral chamber for 150s and received a scrambled foot 0.7mA shock for 2s followed an 90s interval, in a total of 5 shocks per session. 24h the animals were tested by freezing behavior when putted again in the same chamber (context). At IA task mice (12 MasKo, 17 WT) were putted in an acrylic platform in the extremity of a chamber with a metallic grid floor. When animal putted the four paws on the grid floor a 0.3mA 2s shock was delivered and animal were immediately removed. 1.5h after the animal were putted in the platform again to test short term memory and 24 after to evaluate long term memory, using the latency to down the metallic grid we tested the aversive episode learning. We tested for five consecutive days to evaluate extinction of IA memory.

Results. At FC, when we tested tone, we found that MasKo can learn, but presented litter performance compared WT animals (n=8/group. Tone x 3rd pairing WT P=0.05; Test x Day5: WT P=0.05). When we tested the context, we found a deficit in the two groups to learn the task (Unpaired T Test - 5th pairing x Test: WT P=0.2647, MasKo P=0.7138). At IA while WT animals learned the task (Trial x Test- Dunn´s test: n=12, STM P=0.0002 and LTM P=0.003) and showed litter latencies long the days (Test x Day 1 P=0.05), MasKo mice (Trial x Test- Dunn´s test: n=17, STM P=0.05).

Conclusions:
MasKo mice exhibited deficits to learn cued and contextual fear conditioning task, but at the second, it’s probably due a strain-specific deficit observed in other FVBn behavior studies. We found too clear deficits in extinction memory at FC and IA tasks. We can conclude that MasKo mice, a hypertension model have deficits in fear related memories.

Keywords: FEAR MEMORY, HYPERTENSION, MAS RECEPTOR, ANGIOTENSIN(-1-7)

Financial Support: Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq

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Resumo:21-186

STROKE: EVALUATION OF THE FUNCTIONAL CAPACITY AND LIFE QUALITY OF THE BASIC ATTENTION USERS IN THE CITY OF CAMPINA GRANDE – PB

Paixão, L. M. ; Barbosa, K. A. Q. ; Araújo, M. L. D. B. ; Franco, C. I. F. ; Silva, M. D. S. B. E.
Objectives:

The stroke is defined as a neurological deficit that as a sudden start caused by a vascular disturb leading to a stereotyped unilateral motors disturbs associated with sensitive, mental, perceptive and/or language sequels, depending on the side of the brain that was affected. The functional capacity is presented as one of the most important sequels, that have great impact in life quality of the hemiparetic individuals. So, this study has the aim to evaluate the functional capacity and life quality of the basic attention users in the city of Campina Grande – PB affected with the stroke.

Methods and Results:

The sample is composed by 32 patients of both genders, with the maximum of 5 years since the stroke and linked with the Family Health Basic Units (FHBUs) in the city of Campina Grande – PB. As evaluation instruments it was used: Research Formulary to characterize the socio-demographic and clinical data, the Motor Evaluation Scale (MES) to evaluate the functional abilities, and the Stroke Specific Quality of Life Scale (SS-QOL), to verify the stroke impact over the life quality. The collected data were analyzed using the GrandPad Prism 4.00 program, and the exposed values were expressed in frequency, percentage, mean and standard deviation, considering the significant values with p

Conclusions:

Based on the results it was possible to suggest that the functional capacity is presented lightly affected with impact in the life quality, once the self-care activity, the language and the mobility were the most affected aspects in the users of basic attention in the city of Campina Grande – PB.

Keywords: FUNCTIONAL CAPACITY, LIFE QUALITY, STROKE

Financial Support: PIBIC/UEPB

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Resumo: 21-187

EFFECTS OF ETHANOL ON HIPPOCAMPAL NEUROGENESIS DEPENDS ON THE CONDITIONED REWARDING RESPONSE.

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Objectives:

Neurogenesis in the subgranular layer of the dentate gyrus (DG) has been suggested to underlie some forms of associative learning, including the conditioned place preference (CPP) produced following repeated exposure to some drugs of abuse. The present study was undertaken to determine whether there is also a role of neurogenesis in the ethanol induced CPP.

Methods and Results:
Mice were conditioned with five injections of ethanol (EtOH- 2.0 g/kg) in one compartment of an unbiased place preference chamber and saline in the other compartment. This procedure produced three groups of mice; some mice developed a conditioned preference (EtOH_Cpp), others developed a conditioned aversion (EtOH_Cpa) and others demonstrated indifference to the context previously paired with ethanol (EtOH_Ind). In order to verify whether: (1) there was neurogenesis in these animals; (2) these cells were proliferating and (3) they became adult neurons, immunohistochemistry analyses were conducted on tissue from 6 randomly chosen mice from each group. Tissue from the same mice were used for all analyses [doublecortin (DCX), BrdU and double-labeled BrdU+DCX] – those were perfused 24 h post-conditioning phase, except for the double-labeled BrdU+NeuN that was conducted on tissue from mice perfused 28 days after the post-conditioning phase. When measured 24 h following the CPP test, there was no effect of EtOH on doublecortin expression (F(3,20)=0.50;P>0.05). We found a significant decrease in EtOH_Cpa and EtOH_Ind groups BrdU+ cells number, but not in the, EtOH_Cpp group. The BrdU+ cells number of EtOH_Cpp was higher than the EtOH_Ind and EtOH_Cpa (F(3,26)=5.92; P

Conclusions:

These results suggest that positive associative learning is important to maintain normal levels of neurogenesis in DG to counteract ethanol-produced decreased cell proliferation/survival rate.

Keywords: NEUROGENESIS, conditioned place preference, Hippocampus, Ethanol, Drug addiction

Financial Support: FAPESP, CNPq

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Resumo:21-188

NOS BLOCKADE-INDUCED PLASTICITY INVOLVES ADENOSINE AND AMPA RECEPTOR MODULATION.

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Objectives:

The rat retinotectal pathway develops from initially diffused projections to discrete and precise connections within the critical period. Nitric oxide (NO) is a well documented retrograde messenger for retinotectal refinement, and it was shown that a short-term blockade during the second postnatal (PN) week induces sprouting of retinotectal arbors upon superior colliculus (CS). Important regulators of synaptic plasticity that are modulated by NO are AMPA receptor subunits. Furthermore, adenosine receptors system is present in the same temporal-window than NO in the SC, and extensive data show that adenosine levels and receptors are modulated by NO. Herein we investigate whether the systemic blockade of NO synthase (NOS) modulates the expression of AMPA subunits and purinergic receptors in the superior colliculus.

Methods and Results:

For this purpose, pigmented rats were daily treated with intraperitoneal L-narg (50mg/kg) from PND 9 through PND12, and SC western blot samples or immunohistochemistry fixed-tissue sections were obtained at PND13. Our results show that L-narg treatment induces an increase in GluR1 AMPA receptor subunit expression on SC neuropil, whereas reduces GluR2 levels. Concomitantly, NOS blockade induces adenosine A1 receptor overexpression, and doesn’t change A2a receptor expression.

Conclusions:

In conclusion, we suggest that the sprouting behavior induced by NOS inhibition might be generated by decrease in GluR2 expression and GluR1 increase. This modulation may induce mature synaptic depression and reactive growth to new targets, resulting in new synapses, since GluR1 is the first AMPA receptor subunit recruited to previous silent synapses. Additionally, we
suggest that the A1 receptor increase found here destabilize pre-formed connections, leading to the sprouting of retinotectal axons.

Keywords: adenosine receptors, AMPA receptors, nitric oxide, retinotectal

Financial Support: CNPq, PROPPi-UFF, FAPERJ, CAPES, UFF-PROAP, PRONEX-MCT

ANALYSIS OF THE EXPRESSION OF THE GLUR2 RECEPTORS ON MICE SUBMITTED TO LOCOMOTOR SENSITIZATION INDUCED BY ETHANOL.

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Objectives:
The abusive use of drugs leads to a number of neuroadaptations responsible for dependence. There we analyzed the expression of GluR2 (an important subunit of AMPA receptors) on animals submitted to the protocol of locomotor sensitization induced by ethanol.

Methods and Results:
Adult male Swiss mice were used. For the sensitization, ethanol 15% was administered (2g/Kg, i.p.) for 21 consecutive days. Afterwards, the animals were in their cage for 4 days, without any manipulation, and then challenged with just one dose of ethanol 15% (1.4 g/Kg, i.p.). The locomotor activity was evaluated for 15 minutes in an automated box (Insight). This procedure was done one day before the beginning of treatment with ethanol (basal activity), after the 1st, 7th, 14th and 21st administration of the drug (acquisition phase) and after the challenge with ethanol (expression phase). According to the expression, the animals were classified as sensitized (Et_Sens) and non-sensitized (Et_NSens). One day after the challenge, the animals were perfused and an immunohistochemistry for GluR2 was made. The animals that received saline were the control group. For the statistical analysis, one-way or repeated measurements ANOVA were made followed by Turkey test a posteriori. There was no difference between the groups at the basal locomotor activity [F(2,21)=2.11; P=0.16]. The Et_Sens group showed a greater locomotor activity after the challenge with ethanol when compared with the other groups [F(2,21)=57.93; P

Conclusions:
We suggest that the specific increase of GluR2, might be associated to the resistance to develop a locomotor sensitization induced by ethanol.

Keywords: ethanol, GluR2, Sensitization

Financial Support: CNPq and Fapesp

INFORMATION-THEORETIC ANALYSIS OF WHISKER-RESPONSIVE TRIGEMINAL GANGLION NEURONS
TO WHITE NOISE STIMULATION

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Objectives:

Rodents acquire tactile information using whiskers, whereby tactile perception is constrained by the information transmitted through (at least) two classes (slowly and rapid adapting, SA & RA) of primary trigeminal ganglion afferents that innervate the whisker follicles (J. of neuros. 26, 7933-41, 2006). We employed extracellular single unit recordings from the trigeminal ganglion of anesthetized animals in response to band-limited white noise stimulation of the corresponding whisker (lower cut-off frequency of 100 Hz), to investigate if differential responses would be elicited depending on which neuron class was stimulated.

Methods and Results:

Four female (12-18 weeks) Sprague-Dawley rats (240-300g), were used in this study. The animals were deeply anesthetized and head-fixed. A single platinum-tungsten sharp electrode was lowered into the trigeminal ganglion and neuronal responses were elicited through automatic whisker stimulation. Recorded spike trains were analyzed using standard information theoretic measures (Nat. neuros. 2, 947-57, 1999; J. of vision 6, 484-507, 2006; J. of neuros. 17, 4809-19, 1997). Yielding the total information carried under no assumptions of what and how information is represented by the afferents (‘direct method’), as well as the channel capacity (upper bound), and stimulus reconstruction methods (lower bound). Results obtained from 20 trigeminal ganglion cells show that primary afferents respond to whisker deflections in a highly reliable manner and that they are able to convey information about the stimuli at high transmission rates (~200 bits/s), although SA cells have, in general, higher information transmission rates (Mann-Whitney: MedianSA=375bits/s; MedianRA=145 bits/s; p=0,0049). Comparison of channel capacity quality confirms that the two classes of primary afferents reliably encode whisker frequencies related to textures between 50-100 Hz. Information coding was fairly linear in this frequency range. In contrast, frequencies typically related to whisker movement (5-10 Hz) were found to be less well encoded and rely more on non-linear encoding. When the stimuli were reconstructed from the spike trains using a linear filter, SA cells showed better reconstruction capability then RA cells as shown by the correlation measures between estimated and actual stimulus (Mann-Whitney: MedianSA=0,86; MedianRA=0,66; p=0,0018).

Conclusions:

Our results show that, as shown for the primary afferents of other animals, the rat primary afferents are capable of linear coding and high information transmission, with little noise corrupting the system. Moreover the differences observed between RA and SA cells are in disagreement with the literature, where no differences between the responses of the cell classes were found (J. of neurophys 92, 665-8 2004, Science 304, 1986-9 2004).

Keywords: Information theory, Primary afferents, stimulus reconstruction, White noise

Financial Support: Centre of Integrative neurosciences – Tübingen Universität.
Objectives:

Sleep disorders in university students lead to decreased alertness and concentration on the development of academic activities. Those students try to reach a good level of professional qualification and experience requirements for high outcome and time required in studies. Although an individual needs to sleep a few hours a day, the determination of this function is the sleep quality obtained, not the quantity of time sleeping. The aim of this study was to draw a sample profile of sleep quality and daytime sleepiness of physical therapy students, allowing to identify the prevalence of sleep disorders in the sample analyzed.

Methods and Results:

It was a cross-sectional study, approved by the Ethics and Research of Faculdade Integrada do Recife (FIR-Estacio). Data were collected at the School Clinica of Estacio-FIR. We applied the Pittsburgh Sleep Quality Index (PSQI) and Epworth Sleepiness Scale (ESS), which were previously translated and validated to Portuguese. The sample comprised 260 students, 42 males (16.15%) and 218 females (83.85%) between 18 and 30 years and mean age of 22.5 ± 1.3 years. Our results showed that 48% (n=124) of the respondents had scores of PSQI ≥ 5, indicating poor quality of sleep and only 20% (n=52) reported perception of this condition. Regarding to sleep duration, our findings presented that 31% (n=82) slept more than seven hours per night and the average was 6.97 ± 1.37 hours per night. We pointed out that 64.6% (n=168) of the students had trouble sleeping because they have bad dreams or nightmares, 30% (n=78) reported difficulties in sleeping because they feel too cold or too hot less than once a week and 46.9% (n=122) described sleep impairment because of pain. Approximately 32% (n=84) of the students showed high levels of discomfort during the day. ESS application presented that 28% (n=74) students analyzed rated daytime sleepiness divided into three levels: mild somnolence (n=66), moderate sleepiness (n=7) and severe sleepiness (n=1).

Conclusions:

According to our results, we conclude that the students analyzed underestimate their sleep quality, as they do not consider the negative influences to which they are exposed. Considering the role of sleep in the individual life and their possible harmful effects in its absence, an appropriate level of sleep does not directly influence the commitments required from the academic life, affecting the quality of life. For this reason, it is essential to identify the mechanisms responsible for alterations in the normal function, leading to awareness of sleep hygiene.

Keywords: DAYTIME SLEEPINESS, PHYSICAL THERAPY, SLEEP QUALITY, STUDENTS
Over the last few years, the mobile communication system has increased significantly. In 2011, there are more than 210 million habituated mobile phones in Brazil. Some researches about the radiation emitted by mobile phones have been trying to verify possible modifications on gland functions. The hypothalamus-pituitary axis acts orchestrating the functionality of the endocrine systems. ERK1, ERK2 and PKCδ enzymes are of great importance for controlling synthesis and secretion of pituitary and hypothalamic hormones. Thus, the aim of this work was to evaluate the effect of the radiation emitted by mobile phones into the expression of ERK1, ERK2 and PKCδ and also into the activity of ERK1 and ERK2 in pituitary cells of Wistar rats.

Methods and Results:

Wistar male rats, 60 days old, were exposed to the radiation emitted by mobile phone (1800 MHz) during 1 or 3 hours uninterrupted. After that, the pituitary gland of the animals of the exposed and control group were removed in order to analyze the expression of ERK1, ERK2 and PKCδ and the activity of ERK1 and ERK2, by Western Blot. The activity of ERK1 and ERK2 can be measured by their phosphorylation. We evaluated that phosphorylation using specific antibodies against these phosphorylated enzymes. After 1 hour of exposure, it was not observed any significant modification in the expression of ERK1 (control: 73.30 ± 0.4289, n= 6; treated: 73.33 ± 0.4519, n= 6), ERK2 (control: 39.12 ± 4.386, n= 6; treated: 37.92 ± 3.664, n= 6) and PKCδ (control: 41.67 ± 1.592, n= 3; treated: 42.20 ± 1.906, n= 5). The activity of ERK1 and ERK2 was shown to be reduced in the exposed animals (ERK1/ control: 22.21 ± 2.351, n= 5; treated: 8.669 ± 2.491, n= 5; ERK2/ control: 24.21 ± 1.902, n= 5; treated: 9.129 ± 2.056, n= 5, p<0.05). The activity of these enzymes and the expression of PKCδ was reduced in the exposed animals (ERK1/ control: 48.74 ± 2.755, n= 4; treated: 40.25 ± 1.551, n= 6; ERK2/ control: 50.15 ± 2.488, n= 4; treated: 40.65 ± 1.238, n= 6; PKCδ/ control: 39.20 ± 0.6493, n= 4; treated: 36.01 ± 0.2714, n= 4, p<0.05).

Conclusions:

Our results show reduced activity of ERK1 and ERK2 in pituitary cells of exposed rats in both experiments (1 and 3 hours). The expression of PKCδ was modified only after 3 hours of exposition. The expression of ERK1 and ERK2 was not modified on both experiments. According to these data, the exposure to this kind of radiation may alter the signaling pathways in which ERKs and PKCδ are involved, and, consequently, the normal physiology of pituitary cells.

Keywords: RADIATION, MOBILE PHONES, PITUITARY CELLS, WISTAR RATS

Financial Support: CNPq and FAPEMIG.

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Resumo:21-193

ENDOGENOUS CENTRAL AND PREOPTIC HYDROGEN SULFIDE POTENTIATES THE HYPOXIA-INDUCED HYPOTHERMIA.

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Objectives:

Hypoxia leads to an array of physiological responses, including a fall in deep body temperature (Tb) called regulated hypothermia. Although the involvement of gaseous messengers such as nitric oxide has been studied in the hypoxia-induced hypothermia, no information exists as to the putative role of hydrogen sulfide (H2S) in this response—a gaseous neuromodulator synthesized by cystathionine-α-synthase (CBS) in the brain and shown to be capable of inducing a suspended animation-like state. We therefore aimed at investigating whether H2S modulates the hypoxia-induced hypothermia.
Methods and Results:

Male Wistar rats (270-300g) were implanted with a guide cannula toward the third ventricle (microinjection intracerebroventricular, icv) or the preoptic area (POA) (microinjection into the POA) and with a temperature datalogger capsule in the peritoneal cavity (to record Tb). Hypothalamus samples were homogenized in potassium phosphate buffer (100 mM; pH 7.4) using a microprocessor. Each sample (50% w/v; 100 μl) contained L-cysteine (10 mM; 20 μl), pyridoxal 5′-phosphate (2 mM; 20 μl) and PBS (30 μl). The reaction was performed in paraffilmed eppendorf tubes and initiated by transferring the tubes from ice to bath at 37° degree C. After incubation for 2 h, zinc acetate (1% w/v; 100 μl) was added to trap evolved H2S followed by tricloroacetic acid (10% w/v; 50 μl) to precipitate proteins and thus stop the reaction. After centrifugation, N,N-dimethyl-p-phenylenediamine sulphate (20 mM; 50 μl) in HCl 7.2 M followed by FeCl3 (30 mM; 50 μl) in HCl 1.2 M was then added to 50 μl of the supernatant, and optical density was measured at 670 nm. The calibration curve of absorbance was obtained using Na2S solutions (0.1 - 100 μl/mL). Animals were microinjected icv or intra-POA with a CBS inhibitor (AOAA 200 pmol/2 μl) or saline (2 μl) and exposed to normoxia or hypoxia (7%, for 1 hour). Under normoxia, AOAA given icv or intra-POA had no effect on Tb: AOAA icv (n = 7) 2.22 ± 7.38 vs. saline icv (n = 7) 4.68 ± 10.22; AOAA intra-POA (n = 7) 11.07 ± 8.25 vs. saline intra-POA (n = 7) 4.69 ± 10.23° C.min. Under hypoxia, microinjection of AOAA (icv or intra-POA) significantly attenuated hypoxia-induced hypothermia: AOAA icv (n = 7) -37.16 ± 11.16 vs. saline icv (n = 7) -63.60 ± 8.83, P < 0.05; AOAA intra-POA (n = 7) -37.16 ± 11.17 vs. saline intra-POA (n = 7) -77.92 ± 8.26° C.min, P < 0.05. Additionally, animals were icv microinjected with AOAA (200 pmol) or saline (2 μl) and exposed to normoxia or hypoxia for 60 minutes. Hypoxia caused an increase in hypothalamic production of H2S (hypoxia (n = 8) 0.74 ± 0.05 vs. normoxia (n = 8) 0.48 ± 0.05 μg/mg protein/h, P < 0.05).

Conclusions:

These findings are consistent with the view that endogenous central and preoptic H2S favors the hypoxia-induced hypothermia, hypothermic response which is accompanied by an increase in the hypothalamic production of H2S.

Keywords: Gaseous neuromodulator, AOAA, Body temperature, H2S, Hypothalamus

Financial Support: CAPES, FAPESP, and CNPQ.

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Resumo:21-194

BEHAVIORAL SATIETY SEQUENCE (BSS) IN FREE-FEEDING PIGEONS (COLUMBA LIVIA) AFTER CENTRAL INJECTIONS OF SEROTONIN (5-HT) OR METERGOLINE.

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Objectives:

In mammals, increased central 5-HT levels inhibit food intake and accelerate the BSS (the postprandial sequence of drinking, grooming and resting) without disrupting its integrity, while ICV or intra-amgydaloid injections of metergoline (MET, a nonspecific 5-HT1/2 receptor antagonist) increase feeding and sleep in rodents. Mammals and birds share numerous neurochemical, pharmacological and anatomical attributes of 5-HT circuits, and central injections of 5-HT was shown to evoke hypoglic, hyperdipsic and hypnogenic effects in pigeons. Here we examine the effects of hyporexigenic (5-HT) or hyperorexigenic (MET) doses of these drugs in the whole BSS sequence of pigeons.

Methods and Results:

Thirty-six pigeons (both sexes, 8-12 month old, 380-450 g bw, free-feeding/drinking, 12:12 light-dark cycle, 24 ± 1°C RT) were continuously video-recorded (1 h, in their own home-cages) in 1) baseline conditions (N=8), 2) during refeeding after a 24 h...
fasting (RF24, N=8), 3) after intracerebroventricular (ICV) injections of 5-HT (150 nmol, N=8), 4) of metergoline (MET, 150 nmol, N=6) or of 5) vehicle (ascorbic acid, 5% in saline, N=6) in free-drinking/feeding pigeons (FF/FD). Total 1-h food/water intake, as well as duration and frequency of feeding (F), drinking (D), preening (P), sleep (S), locomotor (L), alert immobility (AI) and exploratory (E) behaviors (recorded in 4-min bins) were carried out. The RF24 protocol evoked a sequentially organized increase (as compared to baseline profile) in feeding, then drinking, then preening, which was followed by increased sleep behavior (the BSS). ICV injections of 5-HT in FF/FD pigeons inhibited food intake and accelerate the BSS, producing a behavioral sequence of magnitude and profile similar to that observed after refeeding in RF24 animals: intense and abrupt increase in D, followed by augmented P and then S. MET injections evoked increased feeding, and a complete, but delayed postprandial sequence, with inter-peak intervals between D, P and S curves higher than those observed in RF24 animals.

Conclusions:

Similar to observed in rodents, the BSS is accelerated by 5-HT and delayed by 5-HT1/2 receptor blockade, but its sequential components and structure are preserved after both treatments, which is taken as indicative of their association with physiological mechanisms of satiety expression. Moreover, these data indicates that the crucial role of 5-HT circuits in this post-consummatory sequence may represent a functional attribute phylogenetically conserved in the ammniote brain.

Keywords: Behavioral Satiety Sequence, Intracerebroventricular, Pigeon, Serotonergic receptors 5-HT1/2, Serotonin

Financial Support: Supported by CNPq, Capes and FAPESC.
Conclusions:

These findings confirm the hypothesis that PRL act as a cytoprotective agent in primary glial culture exposed to LPS.

Keywords: cytoprotection, glia, lipopolysaccharide, prolactin

Financial Support: CNPq and UFPA

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Resumo: 21-196

MATERNAL UNDERNUTRITION: EFFECTS ON DIENCEPHALIC EXPRESSION OF ANGIOTENSIN AND GLUCOSTEROID SIGNALING PROTEINS

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Objectives:

The current study investigates whether gestational undernutrition alters the cerebral expression and localization of Angiotensin II receptors (AT1R and AT2R), Arginine vasopressin (AVP), Pro-opiomelanocortin (POMC), Adrenocorticotropic Hormone (ACTH), Mineralocorticoid receptor (MR) and the Glucocorticoid receptor (GR).

Methods and Results:

Virgin female Wistar rats were fed during pregnancy with normal-protein (NP 17% casein, n=6) or protein-restricted diets (LP 6% casein, n=6). The male pups were followed and maintained with normal chow until adulthood. Cerebral tissues were obtained from male Wistar offspring of time-mated rats at 16 week-old. Protein expression of AngII receptors (AT1R and AT2R) and AVP, POMC, ACTH, MR and GR was measured by immunoblotting and immunohistochemistry. Data obtained over time were analyzed using appropriate ANOVA. Post hoc comparisons between selected means were made by Bonferroni’s contrast test.

Results: The arterial blood pressure increased significantly in LP rats, from 116.2±6.5 mmHg to 137.9±6.9 mmHg, P<0.05. The data obtained in the present study show by immuno stained technique a striking immuno expression of AT2R, POMC and ACTH associated with reduction of AT1R, AVP, MR and GR in hipothalamic-hypophisis axys of 16 wk-old LP rats.

Conclusions:

The current study showed a pronounced increase of hypothalamic-pituitary POMC and ACTH expression associated with downregulation of MR and GR immunomarker in LP suggesting a lack of control of Hypothalamus-Hypophysis-Adrenal system with presumible high corticosterone plasma levels in undernutrition adulthood progeny. In LP group, these findings are accompanied by reduced diencephalic AT1R and antidiuretic hormone (ADH) probably resulting in decreased kidney water/salt reabsorption and fall of blood pressure as counterregulation phenomem.

Keywords: Angiotensin, maternal undernutrition, Fetal programming, Diencephalic, Arterial pressure

Financial Support: CNPq and FAPESP supported this work

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EVALUATION OF BEHAVIOR, WORKING MEMORY AND LONG-TERM MEMORY IN AN OBESITY MODEL INDUCED BY HYPOTHALAMIC LESION IN WISTAR RATS.

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Objectives:

The administration of L-monosodium glutamate (MSG) in neonatal rats causes lesions in the arcuate nucleus of the hypothalamus affecting neuroendocrine function in adulthood in these animals. The arcuate nucleus is rich in NMDA type glutamate receptors, which when excessively stimulated creates a big calcium influx, which is toxic to cells. Because of the many neuronal connections between this region with important regulating centers of behavior and memory, and through empirical observations the aim of this study was to evaluate the behavioral patterns that these animals show, as well as determine the possibly memory type affected.

Methods and Results:

In the first 5 days of life, male Wistar rats received subcutaneous doses of MSG (4 mg /g body weight) in the neck area. Control animals received saline equimolar. At 90 days of life was initiated behavioral experiments in an open field arena regarding: number of episodes of grooming, freezing, number of traversed squares and the time spent in the center of the arena at 5-minutes experiment. After the adjustment period of one day was conducted the tests of memory by the object recognition test. This test evaluated working memory - 30 seconds - and long-term memory - 24 hours - for a period of 5 minutes. The animals were killed with a lethal dose of thiopental sodium (60mg/kg). We calculated the Lee Index (LI) (body weight (g) 1 / 3 / naso-anal length (cm) x 1000), to estimate obesity, and the perigonadal fat pad was removed and weighed to check the hypertrophy of adipose tissue. The MSG group showed a significant increase in the number of episodes of grooming (Control = 6.1 ± 0.7 n = 23 MSG = 3.5 ± 0.4 n = 26, p = 0.0019) and freezing (Control = 2.5 ± 0.4 n = 23, MSG = 7.9 ± 0.7 n = 26, p < 0.0001) and decrease in the number of squares traversed (Control = 48.7 ± 2.5 n = 25, MSG = 31.1 ± 1.3 n = 26, p < 0.0001) and time in the arena (Control 18.4 ± 2.5 n = 23, MSG = 7.8 ± 1.1 n = 26, p = 0.0002). In the 24 hours memory test 24 MSG animals showed a reduced discrimination index (control = 0.6 ± 0.03 n = 9, MSG = 0.5 ± 0.05 n = 9, p = 0.0214), the 30 seconds test showed that there was no significant difference between the groups. The MSG group had a bigger LI than in the control group (Control = 281.2 ± 1.07 n = 40, MSG = 300.7 ± 1.6 n = 40, p < 0.05) and perigonadal fat pad mass was 67% higher.

Conclusions:

MSG rats showed a behavior pattern that indicates a heightened state of stress and anxiety, as evidenced by the time spent in the center of the arena as well as a lower locomotion activity. The working memory was not affected, but the long-term memory showed impaired, this may be due to the numerous neural connections that exist between the hypothalamus and hippocampus.

Keywords: MSG, Behavior, Memory, Obesity, Hypothalamus

Financial Support: CNPq and UFJF

STRUUCTURAL STUDIES WITH THE CEREBRAL DOPAMINE NEUROTROPHIC FACTOR (CDNF) AND ITS NEUROPROTECTIVE EFFECTS AGAINST THE TOXICITY OF ALPHA-SYNUCLEIN AGGREGATES.
Objectives:

Parkinson’s disease is characterized by the loss of dopaminergic neurons in the substantia nigra of the brain. There is no available therapy to treat this disease, which is the second most prevalent neurodegenerative disorder worldwide. Neurotrophic factors promote survival, differentiation and maintenance of neurons in developing and adult vertebrate nervous system. A potent neurotrophic factor for dopaminergic neurons recently described is the cerebral dopamine neurotrophic factor (CDNF). Little is known about its structure and mechanism of action. The main goal of the present study is to solve the atomic structure and the dynamics of CDNF in solution by NMR. We also aim to evaluate its neuroprotective effects against synuclein aggregates, which are toxic to cells in culture.

Methods and Results:

Initially, CDNF (18kDa) was cloned, expressed and purified in a pure and soluble state as well as with 2H, 13C and 15N. We observed that the purified protein undergoes spontaneous proteolysis releasing a fragment of 6kDa from its C-terminus; thus we used a fragment of 12kDa for structural determination. We have already assigned all residues of CDNF by using triple resonance NMR experiments and this structure will be presented. With the pure protein in hands, we also evaluated its thermodynamic stability by the use of chemical and physical tools taking advantage of the presence of a single tryptophan residue. In the presence of 5M urea, the tryptophan emission shifts to 4nm to the red indicating denaturation of CDNF. By circular dichroism, the protein presents a negative peak at 208 and 222nm, suggesting the presence of alpha-helices. In the presence of urea and upon heating, the a-helix signal decreases suggesting disruption of the secondary structure of the protein. Moreover we observed a massive aggregation of the protein after 50ºC by light scattering. Finally, we are investigating whether CDNF could alleviate the toxic effects of alpha-synuclein aggregates added to SHSY-5Y cells in culture. Our data show an important protection of dopaminergic neurons by CDNF pre-treatment. Cells previously treated with 10 µM of CDNF and then incubated with 140 µM of alpha-synuclein aggregates (48h-old) showed a survival of approximately 80%. On the other hand, the cells treated with aggregates only presented 50% survival.

Conclusions:

Our data suggest that CDNF is a well-folded protein with promising protective activity against alpha-synuclein aggregates. Further studies are on the way to unravel the precise mechanism of action of CDNF, what might contribute to the elucidation and alternative treatments for Parkinson's disease.

Keywords: Fator Neurotrófico, Ressonância Magnética Nuclear, Estabilidade Estrutural, Sinucleína, Citotoxicidade

Financial Support: CNPq, Faperj, Capes

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Resumo:21-199

INTRACRANIAL PRESSURE: NEW APPLICATION’SCENARIOS WITH MINIMALLY INVASIVE AND NON-INVASIVE METHODS.

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Objectives:

The intracranial pressure (ICP) is a critical parameter for the intensive care of neurological patients. All existing monitoring methods in hospitals use invasive sensors, which are inserted into the central nervous system and can cause problems as infection, bleeding, blood clots and swelling. Another problem is the high cost of these monitoring systems, the Brazilian public health system does not pay this costs, denying the poorest people access to this important medical parameter. Our group works in the developing of new equipments, minimally invasive (small incision in the patient's skin) and non-invasive to monitor the ICP, showing sensitivity, safety, reproducibility of results and low cost.

Methods and Results:

The minimally invasive system for monitoring intracranial pressure consists to glue externally a strain sensor on the cranial bone, through a small incision in the skin and removal of the periosteum. Variations of internal pressure lead to changes in cranial volume (micrometric variations), as linear effects without hysteresis. The method was called minimally invasive in comparison with the invasive currently used systems. This method has been applied in several animal models, such as hydrocephalus, epilepsy, tumor simulations, depth of anesthesia and future tests will be performed with animal models of stroke. Besides the animal studies, 9 human patients received our system. In all tests we observed the efficiency observing the rising and decrease of ICP as expected and as described in the literature. The invasive method shows the results in millimeters of mercury, our system monitors the patient in millivolts. The variation of 10 mmHg in invasive system corresponds to a variation of approximately 1.6 mV in our equipment. This equipment is in ANVISA registration phase and will soon be available for use in hospitals. The non-invasive method arose as an enhancement of the minimally invasive method, and initially consisted in the use of a stereotaxic apparatus that allowed the patient fixation and a touch sensor (without shaving or surgical incision) on the parietal region of the patient. The physical characteristics of this equipment resulted the name BRAIN HELMET. Several tests and maneuvers were performed with this equipment, among which we highlight the compression of the jugular veins, which takes to increased ICP and exercises, leading to increased arterial blood pressure and consequent rise in intracranial pressure. Once again the results were consistent with expectations, showing that the non-invasive monitoring of ICP is valid. The brain helmet had another further development, a strap that surrounds the head region above the temporal muscle of the patient. This system is already showing good results, which will be described elsewhere.

Conclusions:

The monitoring of ICP with our new methods is possible, minimizing the risks to the patient, allowing the emergence of new fields of research and important clinical parameters with extensive applications of social importance in view of its simplicity and cost-effectiveness.

Keywords: ICP, monitors, intracranial pressure

Financial Support: FAPESP, MCT CNPq, Ministry of health of Brazil through the Pan American Health.

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Resumo:21-200

STATUS EPILEPTICUS INDUCED BY LITHIUM-PILOCARPINE AND ITS EFFECTS ON ANIMAL MODELS OF ANXIETY AND DEPRESSION

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Objectives:
The incidence of mood and anxiety disorders amongst patients suffering from temporal lobe epilepsy (TLE) is greater than in other types of epilepsy as well as in the general population. Behavioral analysis of animal models of TLE submitted to tests representing anxiety-like and depression-like behaviors could bring new perspectives on the study and comprehension of these comorbidities.

Methods and Results:
Male Wistar rats weighing 200-280g were submitted to induction of Status epilepticus (SE) by LiCl (127mg/kg, i.p.) and Pilocarpine (30mg/kg, s.c., 18-20h after LiCl treatment). Sustained seizures were ceased after 2 hours with injection of anticonvulsants (diazepam 5mg/kg, i.p. or thiopental 40mg/kg, i.p.). The control group were submitted to injection of vehicle (NaCl 0,9%) instead of LiCL and Pilocarpine. During the next 15 days animals went through a rehabilitation period with spontaneous recurrent seizures video monitoring. On the 15th day, rats were tested in the open field (OF) test for 12 minutes for anxiety-like and exploratory behavior observation. On the 16th day rats were submitted to 15 minutes of forced swimming, where only the first 5 minutes were recorded for immobility quantification. 24 hours later they were tested in the forced swimming test (FST) for 5 minutes. On the 19th day, they were submitted to learned helplessness (LH) induction protocol (40 inescapable shocks, 10 secs, 0,6mA) in the shuttle box apparatus. 24 hours later they were tested in the shuttle box test (30 escapable shocks, 10 secs, 0,4mA) for quantification of failures to escape shocks. Statistical analysis was performed by using the t-test and Mann-Whitney rank sum test as complementary. Total distance cursed on OF (SE (n=14) 1992,200±381,735 cm; Control (n=19) 324,469±60,746 cm; P
Conclusions:
Exploratory and ambulatory behavior of rats are affected after 15 days of Status epilepticus induction, but depression-like behavior in the forced swim test and learned helplessness test was not affected when compared to control group.

Keywords: temporal lobe epilepsy, depression, Li-PILO model, learned helplessness, forced swimming test

Financial Support: CNPq, FAPESP, CAPES, CInAPCe, FAEPA

QuebraPagina

Resumo:21-201

COMPARATIVE ANALYSIS OF NR1 AND NR2 NMDA SUBUNITS EXPRESSION RECEPTORS IN ANIMAL MODELS OF ANXIETY DISORDERS

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Objectives:
Although anxiety consists of a typical human phenomenon, it is possible to correlate this kind of emotion to animal response defenses in front of threaten stimuli. Thus, several studies define fear and anxiety as a motivational system related to defense reaction to adverse situations. The hippocampus has been identified as one main component of this system, since hippocampal ablation reduces anxiety behavior in many animal models. Recently, two Wistar rats’ lineages were genetically selected to
present high (CHF) or low (CLF) defense reactions to aversive conditioned stimuli, representing important animal models of anxiety. Certainly, there are different biological systems involved in this emotional status. NMDA receptors are highly involved in cognition and emotionality, and several works have shown that its antagonism can reduce anxiety both in humans and animal models. In the present work, we investigated NR1 and NR2b NMDA receptor subunits expression in the hippocampus of CHF, CLF and matched-control Wistar rats (CTL) in relation to tubulin expression as housekeeping protein.

Methods and Results:

For this purpose, we conducted Western Blot analysis of the hippocampal formation of CHF (n=4), CTL (n=4) or CLF (n=4). Our results show that CHF presents high expression of NR1 (Mean of 0.845 ± 0.105 SD) and NR2b (Mean of 0.756 ± 0.102 SD), whereas CLF presents low expression of NR1 (Mean of 0.615 ± 0.241 SD) and NR2b (Mean of 0.383 ± 0.201 SD). CTL animals, in turn, present intermediate levels of NR1 (Mean of 0.696 ± 0.367 SD) and NR2b (Mean of 0.701 ± 0.228 SD) protein expression.

Conclusions:

It seems that anxiety profile is correlated to NMDA activity in the hippocampus, particularly NR2b-containing receptors.

Keywords: anxiety, NMDA receptor, rats

Financial Support: CNPq, PROPPi-UFF, FAPERJ, CAPES, UFF-PROAP, PRONEX-MCT

QuebraPagina

Resumo: 21-202

MELANIN CONCENTRATING HORMONE (MCH) DECREASES PRESUMED SEROTONERGIC NEURONAL ACTIVITY IN THE DORSAL RAPHE NUCLEUS OF THE RAT.

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Objectives:

Hypothalamic neurons that utilize melanin-concentrating hormone (MCH) as a neuromodulator, exert a positive control in the generation and/or maintenance of sleep. MCHergic neurons project to several regions of the central nervous system and particularly, the dorsal raphe nucleus (DR) have dense innervation of MCHergic fibers. This nucleus has a high density of serotonergic neurons and is involved in the control of wakefulness and rapid-eye movements (REM) sleep. In addition, MCH-labeled tanycytes are present in the DR. These cells are specialized in transport substances from the CSF to the neuronal parenchyma; this fact suggests that MCH could be absorbed from the CSF in order to produce a tonic effect onto DR neurons. Indeed, we have quantified the levels of MCH in CSF from several mammals, included rats. Recently, we determined that MCH microinjections into the DR produced a significant increment of REM sleep while immunoneutralization of MCH produced the opposite effect. In addition, we also have shown that microinjections of MCH into this region produce a depressive-like effect that is reverted by pretreatment with fluoxetine, a known antidepressant drug of the selective serotonin reuptake inhibitor class. The blockade of MCH within the DR exerts an antidepressive effect. Due to the fact that tanycytes at DR are MCH-labeled, that MCH is present at CSF of the rat, we asked if MCH could have effects on DR neurons. Thus, the aim of the present study was to analyze the effects of microinjections of MCH into the lateral ventricle on the neuronal activity of DR neurons.

Methods and Results:

Adult Wistar rats of both sexes (250-310 g, n=13) anesthetized with urethane (1.5 g/kg, i.p.) were prepared for standard extracellular recording of DR neurons. Once the neurons were recognized, MCH (5 mcg/min) or saline were microinjected by a guide cannula implanted into the left lateral ventricle and its effects evaluated on electrophysiological properties of the neurons.
In one animal, we obtained several units to record. At the end of the experiments, the recording site was determined by histological procedures. We observed that in about 70% of the neurons recorded (n=16), MCH decrease its firing rate from an average of 3.33 Hz (±2.88) to 1.74 (± 1.57) with a latency of about 1.5 minutes and a duration of 3 to 5 minutes, compared with saline microinjections (n=7). Most of these units were presumably serotonergic according to their electrophysiological characteristics such as broad action potentials (aprox.2 ms), low discharge rate (0.1 to 4 Hz), regular firing, and inhibition of their discharge by a 5HT-1A agonist (8-OH-DPAT, 200 mg, i.p.). On the contrary, neurons with short duration of their action potentials and high firing rate do not respond, or increased their firing rate in response to MCH, in comparison with saline.

Conclusions:

We conclude that MCH regulate the DR neuronal activity and part of this effect could be mediated by volume conduction through the CSF.

Keywords: serotonin, MELANIN CONCENTRATING HORMONE, DORSAL RAPHE NUCLEUS

Financial Support: PDT-Salud 76/36 and CSIC, Uruguay.

QuebraPagina

Resumo:21-203

BRAIN STRAP: A NEW NON-INVASIVE SYSTEM FOR MONITORING INTRACRANIAL PRESSURE.

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2 Department of Physiological Sciences, UFSCAR

Objectives:

The methods currently used to monitor intracranial pressure (ICP) are invasive and can cause problems for patients (infections, the emergence of blood clots, swelling and bleeding). The way that this current sensor is inserted into the patient's skull does not allow the use of this important medical parameter to all patients, as well restricts the ICP field of study. Our group works with the development of alternative methods to monitor intracranial pressure, several prototypes have been developed and this work will describe the brain strap, the latest model of equipment developed by our team (patent required).

Methods and Results:

The skull strap consists of an elastic band equipped with a strain sensor that detects the volumetric changes of the skull. This sensor touches the surface of the patient's head over the parietal region, without the need for trichotomy or surgical incision. The sensor is connected to the acquisition system that digitizes the signal and sends it to a computer, the information is displayed in real time and stored for further analysis. The variation in intracranial pressure, as opposed to what is proposed by the Monro-Kellie doctrine, leads to changes in the skull volume, small variations, that our system is able to capture and which we showed in previous works, are proportional to variations of the ICP. The operation of this system was proved by postural maneuvers, as described in the literature and shown in our earlier work, lifting the body upper region leads to a decrease in intracranial pressure, and elevation of the body lower body region leads to increased ICP. Postural maneuvers were performed in 10 young adults, raising and lowering their heads in succession with an interval of 5 minutes between each move and with 3 repetitions for each person, data were stored and analyzed. The results shows that the sensor was able to capture changes in intracranial pressure, as well as to record cardiological and respiratory signals of volunteers. The ICP variation with postural maneuver shows a standard deviation of 1% in each individual, showing the reproducibility of the method. All results correspond to expectations, demonstrating the efficiency of this new method.

Conclusions:
This new non-invasive equipment to monitor the intracranial pressure was able to monitor the intracranial pressure of human volunteers, and it is thus a new simple, cost-effective system to investigate the intracranial pressure in normal and pathological conditions and circumstances opening new frontiers in the physiology of ICP, not accessible before.

Keywords: ICP, Intracranial pressure, Non-invasive monitor

Financial Support: FAPESP, MCT,CNPq, Ministry of Health of Brazil through the Pan American Health

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INVOLVEMENT OF KININ B2 RECEPTORS IN THE DEVELOPMENT OF THE PILOCARPINE-INDUCED EPILEPSY IN FEMALE RATS

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Objectives:

Kinins, a class of polypeptides represented by bradykinin, kallidin and their metabolites, acting via B1 and B2 receptors, have been related to inflammation, cytokines action, glutamate release and prostaglandin production. Several studies indicate that in the central nervous system and peripheral tissues, estrogen regulates the expression of B2 receptor and reduces cytokine production and inflammatory responses. Accordingly, the present work aimed to investigate the expression of kinin B2 receptor in non-castrated and castrated female rats, submitted to the pilocarpine model epilepsy

Methods and Results:

The animals were divided in four groups: OVX + SE ovariectomized female rats, which presented status epilepticus (SE); SE: intact female rats that presented SE; OVX ovariectomized female rats that received saline instead pilocarpine; SAL intact female rats that received saline instead pilocarpine. The results showed a decrease of immunoreactivity of kinin B2 receptor in the hippocampal formation during the acute and silent but not in chronic periods of this model in SE group when compared to SAL group. In contrast, the immunoreactivity in OVX + SE group was increased during the acute and silent periods when compared with OVX group. The Western Blotting showed an increased expression of kinin B2 receptor in OVX +SE group during the acute (2872.±236.82) (p=0.0034) and silent periods (2391.32±188.18) (p=0.0277), when compared with its proper controls (OVX) (1739.08±425.16). In addition, a decreased expression in SE group during the acute (1171.35±259.73) (p=0.0039) and silent periods (1661.87 ±345.55) (p=0.0121) were found, when compared with its proper control (SAL, 2470.92± 509.58).

Conclusions:

This study showed that the expression of kinin B2 receptor is modified in female rats during epileptogenesis and modulated by steroid hormones.

Keywords: Kinin, Pilocarpina, Rats

Financial Support: CNP, FAPESP
Objectives:
Considering the role of immune cytokines in hippocampal development and functions, as well as in regulating neurogenesis, we evaluated its involvement as mediators of the hippocampal changes induced by early environmental variations.

Methods and Results:
Wistar female rats from the institution’s central colony were kept on our setorial animal room, under a 12-12 hours dark-light cycle with food and water ad libitum. Pregnant females were observed daily to determine the day of delivery which was designated as day 0. Pups were handled one minute a day from day one to five and then have their hippocampus surgically removed after decapitation. The structures were homogenized in buffer containing Tris-HCl 10mM pH 7.4, EDTA 1mM, PMSF 0.001mM, pepstatine 1ug/ml and Triton X-100 1% for protein extraction. In the supernatant we dosage interleukin 1 beta by ELISA and interleukin 6 by Cytometric bead array. Handled puppies showed significantly increased levels of hippocampal interleukin (IL)-1beta (97.6pg/mg of tissue protein ± 8.89; N=10) in comparison to non-handled controls (62.08pg/mg of tissue protein ± 7.5; N=12 p=0.006; t=3.074; df=20) . The handling procedure had also effect on hippocampal IL-6 levels decreasing it in handled pups (8.046pg/mg of tissue protein ± 0.9648; N=8) in comparison to non-handled controls (11.91pg/mg of tissue protein ± 1.101 N=12; p=0.02; t=2.468; df=18).

Conclusions:
The increased IL-1beta and decreased IL-6 levels in the hippocampus of handled pups suggests a cytokinergic profile that supports cellular proliferation and alters morphogenesis of hippocampus, considering the role of IL-1beta in cellular migration, proliferative activity and differentiation. It also support the data showing a facilitate learning of hippocampus dependent tasks found in handled animals, since IL-1beta acts like a gliotrophic factor for astrocytes during development, being the astrocyte derived IL-1beta essential to hippocampal long-term potentiation and learning in adults. The increased feedback to glucocorticoids found in handled animals can also be related to IL-1beta since it can up-regulate the glucocorticoid receptor transcription, by acting on its promoter.

Keywords: Interleukin 1, Interleukin 6, Early Environmental Variations, Hippocampus

Financial Support: CNPq; CAPES; FAPERGS
Objectives:

Cerebellar primary cultures have provided a reliable model for studying the toxicity of methylmercury (MeHg), a well-known neurotoxicant contaminating the environment. In the present study we investigate the potential neuroprotective effects of 17β-estradiol (E2) on the neurotoxicity induced by MeHg in mixed and semipure cerebellar primary cultures.

Methods and Results:

We evaluated the acute toxicity associated with 24 h exposure to doses of MeHg ranging from 1-10 μM (at day 8 in culture) in mixed and semipure cerebellar cultures of cerebellum obtained from neonatal Wistar rats (P5-10) by using the MTT colorimetric assay (Mosmann, J Immunol Methods, v.65, p.55-63, 1983). Also, in some experiments we added E2 (10μM on day 7) to our cultures, associated or not with MeHg. As expected, MeHg exposure resulted in significant cell loss dependent both on concentration and exposure time. Mixed cultures exposed to MeHg for 24 h showed significant reduction in cell viability from the concentration of 2 μM to 10 μM (values ranging from 85.3 ±1.6% to 21.7 ±7.1%). Exposure of semipure cultures to the same conditions, on the other hand, resulted in deleterious effects at all concentrations (values ranging from 89.0 ±8.1% to 16.7 ±2.2%). Treatment of both mixed and semipure cultures to E2 resulted in neuroprotective effects, although more prominent in mixed cultures (from 56.8 ±3.6% to 91.8 ± 4.4% and from 21.7 ±7.1% to 101.0 ± 10.5%, respectively) than in semipure cultures (from 21.0 ±1.5% to 31.6 ±11.2% and from 16.7 ±2.2% to 33.4 ± 11.6%, respectively).

Conclusions:

Semipure cultures showed to be more sensitive to the neurotoxic effects of MeHg when compared to mixed ones. Accordingly, neuroprotective effects of E2 also seems to be more prevalent in mixed cultures, suggesting an important role for the glial component in the effects associated to mercuric compound in the nervous system.

Keywords: mercúrio, cerebelo, estrogenos, in vitro, neuroproteção

Financial Support: CNPq

QuebraPagina

Resumo:21-207

CHRONIC TREATMENT WITH D-CHIRO-INOSITOL PREVENTS SOMATIC NEUROPATHY IN STZ-DIABETIC MICE.

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Objectives:

Diabetic neuropathy is a dysfunction that affects the autonomic and somatic nervous system in diabetes type 1 and 2. The diabetic neuropathy can result from various causes, like the microvascular deficiency and the oxidative stress. The D-chiro-inositol (DCI) is a component of the inositol family, frequently found in many medicinal plants, that acts as a putative mediator of the intracellular insulin action. It was previously described that DCI prevents endothelial dysfunction in diabetic rats and rabbits, being that effect associated with decrease of oxidative stress, inhibition of the hexosamine pathway, inhibition of the advanced glycoside end products and decrease of the protein cinase C activity. This study evaluated the potential preventive effect of DCI.
Methods and Results:

Male Swiss mice, weighting between 30-35g, were randomly divided in three groups: the control group treated with saline solution (CTL) (NaCl 0.9%; 0.1 mL/10g/12h), and two diabetic groups induced by streptozotocin (175 mg/kg, indicar a via): the diabetic group treated with saline solution (DSS) and the diabetic group treated with DCI (DDCI) (20 mg/kg/12h). The treatment was made by orogastric gavage during 60 days. The somatic neuropathy was evaluated by comparing the parameters of evoked compound action potential (CAP) recorded in response to electric stimulation of isolated sciatic nerve between the studied groups. The induction of diabetes promoted a significant (p

Conclusions:

The results show neuroprotective effect of DCI in the experimental diabetic neuropathy.

Keywords: Diabetes, Neuropatia, D-Chiro-Inositol, Potencial de Ação Composto, Sistema Nervoso Periférico

Financial Support: FUNCAP e CNPq
The anorexia of Walker 256-tumor bearing rats may result of several factors including the participation of microglial cells, oxidative stress and neuropetides.

Keywords: tumor walker-256, anorexy, oxidative stress

Financial Support: fapespa

QuebraPagina

Resumo:21-209

ANALYSIS OF THE DYNAMIC OF LIQUORIC MELATONIN

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Objectives:
The hormone melatonin is produced by the pineal gland of mammalian in a circadian way and its secretion is higher at night. The melatonin's plasmatic concentration follows an ontogenetic pattern, with decreased values in aged animals. Melatonin is released in the cerebral ventricular system though the pineal recess of the III ventricle. We know that the concentration of melatonin is higher in the cerebral ventricles than in plasma and lumbar cerebrospinal fluid. Our aim was to evaluate the dynamics of liquoric melatonin in youth, aged and pinealectomized rats.

Methods and Results:
To evaluate the dynamic of melatonin in the cerebrospinal fluid (CSF) in Wistar rats, we collected, throughout microdialisys, samples of cerebrospinal fluid in the III ventricle of three different animals: one aged (12 months), one youth (2 months) and one youth pinealectomized rat (2 months old). To estimate the ontogenetic decrease in melatonin production and release we compare the concentrations of CSF melatonin between the young and the aged animal. We observed similar amounts of melatonin which corresponded to approximately 3 ng/mL in the cerebrospinal fluid of all specimens investigated.

Conclusions:
We did not observe an expressive decrease in melatonin's concentration in the aged rat as expected, but this may be one particular case, since we are considering just one individual. Once the amount of melatonin is similar in control and pinealectomized rats we consider that melatonin in the cerebrospinal fluid is mainly derived from the deep pineal gland that is not removed by the pinealectomy.

Keywords: melatonin, pineal gland, microdialysis, cerebrospinal fluid, aging

Financial Support: Capes, CNPq

QuebraPagina

Resumo:21-210

ENDOTHELIN-1 MICROINJECTION AS MODEL FOR ACUTE SPINAL CORD INJURY

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Objectives:
To establish ET-1 microinjections aimed directly into the corticospinal tract as a model for acute spinal cord injury.

Methods and Results:
Eight adult male Wistar rats (250-300g) were anesthetized with an intramuscular injection of 0.1ml Rompun (9mg/kg) and 0.7ml ketamine (72mg/kg). A partial laminectomy was performed at the C4 level to expose the dorsal columns. We injected 10 pMol of ET-1 (Brain Res. 1200:78-88, 2008) close to the medial dorsal artery at a depth of 1 mm from the pial surface and ipsilateral to the rat's dominant forepaw. Five minutes after the injection the micropipette was gently withdrawn, the animal was sutured and then returned to its home cage. To evaluate the effect of the lesion on the symmetry of forepaw use, we applied the vermicelli handling test (J. Neurosci. Methods. 170:229-244, 2008) at post-lesion days 1, 3, 7 and 14. We observed a clear asymmetry of skilled forepaw movements after the ET-1 injection that did not show any improvement over time.

Conclusions:
ET-1 spinal cord microinjections are a reliable method for focal lesions of the spinal cord that can be used to evaluate the efficacy of experimental treatment regimes.

Keywords: Corticospinal tract, Endothelin-1, Spinal Cord Injury, White matter

Financial Support: CNPq

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QuebraPagina

Resumo:21-211

HGF PROTEIN PROMOTES FUNCTIONAL RECOVERY AFTER SPINAL CORD INJURY IN RATS

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3 Faculdade de Farmácia UFRJ, FF-UFRJ

Objectives:
Spinal cord injury induces a complex cascade of tissue reactions which includes immediate loss of nerve fibers and damage to blood vessels. Revascularization of the damaged area can be a crucial precondition for induction of tissue repair. Hepatocyte growth factor (HGF) presents potent angiogenic and neuritogenic activities, mediating neurotrophic functions during repair of the central nervous system (CNS). In this work we investigated the effect of HGF administration in functional recovery after experimentally induced spinal cord injury.

Methods and Results:
Twelve Wistar-Albino adult female rats weighing 200-250 g were included in this study. Treatment with 10 µl HGF protein (0.1 ng/µl) was performed immediately after the spinal cord compression, whereas the control group received only vehicle (PBS buffer). Locomotor activity was weekly evaluated in an open field, by using the BBB scale. After 8 weeks the rats were perfused transcardially, the spinal cords were removed and submitted to serial cryosection (20 µm). Blood vessels were visualized and quantified after immunostaining with the rat endothelial cell marker, RECA-1 antibody. Acute HGF treatment promoted a significant increase in BBB scores as compared with the control group. The final BBB scores were 17.5 ± 0.81 and 11.6 ± 2.02 for treated and control groups, respectively, a difference that corresponds to p

Conclusions:

Our results indicate that the injection of HGF into the lesion site after spinal cord compression improves recovery of motor function by inducing angiogenesis and probably by contributing to improve cell survival.

Keywords: angiogenese, spinal cord injury, HGF, blood vessel

Financial Support: Faperj, Cnpq, CAPES

Resumo:22-048

SUBACUTE RESPONSE OF GLIAL CELLS AND HEMOCYTES AFTER LESION BY TRANSECTION OF THE PROTOCEREBRAL TRACT OF THE CRAB UCIDES CORDATUS.

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Objectives:

In a previous paper of our laboratory, the protocerebral tract (PCT) of the crab Ucides cordatus was analyzed after extirpation of the eyestalk (Brain Behaviour Evol. 66; 145, 2005). One result of this paper showed that among axons with morphologic aspect of axoplasmic degeneration, granular cells resembling hemocytes (blood cells) were seen. Based on this observation, we classified these cells and their participation in an acute degenerative process, 24 hours after lesion (Cell Tissue Res. 342; 179, 2010). In addition, it was showed that granular hemocytes may be activated and can interact with glial cells after migration to the injured site. In this work, our objective was to study the relationship between glial cells and hemocytes 48 hours after lesion (subacute response) and to observe the morphological features of the PCT.

Methods and Results:

The PCT of five adult male crabs (carapace length: 6 -8 cm) were processed by immunohistochemistry in order to identify GFAP (glial fibrillary acidic protein-present in glial cells), iNOS (inducible nitric oxide-present in hemocytes) and histamine (present in hemocytes). In addition, we used histochemistry for isolectin B4 (to identify macrophages/microglia) and transmission electron microscopy in order to observe subcellular characteristics. The distal stump, 48 hours after lesion, showed a high quantity of iNOS in the injured site, associated with a region of increased GFAP and IB4 positive cells. We also observed that these cells could migrate further into the damaged tract when compared to the acute response (24 hours) of the tract (Cell Tissue Res. 342; 179, 2010). It is interesting to note that some of these cells expressed histamine. Electron micrographs of the PCT exhibited changes in the nuclear aspect of the cells containing granules that infiltrated into the lesioned tract.

Conclusions:

Our results suggest that the presence of iNO and histamine in the lesion site may be considered important factors involved in the activation of hemocytes and/or glial cells attracted to the injury, in crabs Ucides cordatus. In addition, we believe that besides
phagocitizing neural debris, a function that is shared with the local glial cells, hemocytes may have been attracted to the lesion in order to produce important factors necessary for glial proliferation and activation.

Keywords: Neurodegeneration, Hemocytes, Glial cells, Decapode crustecean

Financial Support: CNPq, CAPES, FAPERJ

QuebraPagina

INDOMETHACIN INHIBITS MICROGLIAL ACTIVATION AND ENHANCES NEUROBLAST MIGRATION TO THE RAT STRIATUM FOLLOWING MIDDLE CEREBRAL ARTERY OCCLUSION.

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Objectives:

To investigate whether the modulation of inflammation using the non-steroidal anti-inflammatory drug indomethacin influences neuroblast migration to the striatum following middle cerebral artery occlusion (MCAO) in adult rats.

Methods and Results:

Sixteen male adult Wistar rats weighing 280 to 320 g were used in the study. All procedures were approved by the Ethics Committee in Animal Experimental Research of the Federal University of Pará and they were conforming of the National Institute of Health and were performed in agreement the Society for Neuroscience guidelines. Animals were submitted endothelin-1 induced-MCAO and treated (i.p) with indomethacin (2,5mg/kg) (n=4) or sterile saline (n=4) starting at 1 day postinjury, with subsequent doses (twice a day) for 7 days. Animals were perfused at 8 and 14 days postinjury. Gross histopathology was assessed by cresyl violet staining. Immunohistochemistry was performed to assess Neuronal loss (anti-NeuN), microglia in general (Iba1), microglial activation (ED1) and migrating neuroblasts (anti-double cortin) in all investigated survival times. The numbers of NeuN, ED1 and DCX positive cells were counted in the ischemic striatum. Only DCX also was counted in the subventricular zone. Indomethacin treatment reduced microglial activation in general and the number of ED1+ cells at both 8 and 14 days postinjury, compared to control, but it was significant only in 14 days (p0.05, ANOVA-Bonferroni).

Conclusions:

Indomethacin treatment induces inhibition of microglial activation concomitant with increased neuroblast migration following MCAO. This is a promising outcome, considering that indomethacin is already used in non-neural human diseases and that adult neurogenesis may underlie functional recovery following stroke.

Keywords: INDOMETHACIN, INFLAMMATION, MIDDLE CEREBRAL ARTERY OCCLUSION, MICROGLIA, NEUROGENESIS

Financial Support: Fundação de Amparo e Desenvolvimento à Pesquisa do Estado do Pará

QuebraPagina

Resumo:22-050
EVIDENCE FOR COMPENSATORY PATHWAYS IN HUMAN CALLOSAL DYSGENESIS

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Objectives:

Callosal dysgenesis (CD) is characterized by a developmental failure of formation of the major commissural fiber bundle in the human brain, together with absence of a typical disconnection syndrome. So far, direct evidence for possible compensatory interhemispheric connections through other commissures is lacking. Since diffusion tensor imaging (DTI) can quantify the integrity and connectivity of major white matter bundles in vivo, we posed the objective of investigating, by DTI, the existence of possible compensatory pathways through the other cerebral commissures in patients with CD.

Methods and Results:

Volumetric anatomical (3DT1) and DTI (2.5mm isotropic voxel) images were acquired (3T, Achieva Philips) in six patients (2 with callosal agenesis (total CD), 2 with callosal hypoplasia and 2 with partial CD) and seven healthy volunteers (HVs). Anatomical analyses were carefully performed, with a special attention to the thalamic adherence, the anterior commissure (AC) and posterior commissure (PC). Regions-of-interest (ROIs) were placed in AC and PC on 3DT1 images in the sagittal plane. The area, mean fractional anisotropy (FA), and mean diffusivity (MD) were quantified based on AC and PC ROIs. Comparisons between groups were done using a non-parametric test (Mann-Whitney). Probabilistic tractography of AC and PC was also performed in patients and HVs. Anatomical images showed a similar aspect of AC and PC in patients and controls, in size and general topography. The thalamic adherence was present in 100% of the controls, but only in 50% of patients. There was no difference in AC or PC values of FA or MD between groups. FA maps, however, showed increased volume of PC in patients, compared to controls. In addition, probabilistic tractography of PC revealed a peculiar pattern of connectivity in patients, with fibers coursing through the internal capsule up into the cerebral hemispheres, suggesting a possible cortical connection.

Conclusions:

Our preliminary results indicate the absence of structural changes in the AC and the thalamus in CD, suggesting that these interhemispheric pathways should not display any compensatory role in these patients. DTI maps and tractography, however, suggested an abnormal PC connectivity in these patients. Although additional functional reorganization can be present, these results are compatible with a possible compensatory reorganization through the PC in CD.

Keywords: callosal dysgenesis, DTI, commissures

Financial Support: FAPERJ, CNPq and Capes

Resumo:22-051

A SEMI-AUTOMATED MACHINE FOR ISOTROPIC FRACTIONATION AND SUBSEQUENT CALCULATION OF CELL NUMBERS IN LARGE BRAINS

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Objectives:

Isotropic fractionation is a recently developed method that allows a precise quantification of the absolute number of cells in the brain or its dissectable regions. Originally, it was developed as a manual procedure that works fast for small brains, but becomes very laborious when used to process large brains. To solve this problem, we developed a semi-automated machine that optimizes the fractionation process, and tested its efficiency, consistency, and reliability, as compared with the manual procedure.

Methods and Results:

The machine consists of a set of electronically-controlled rotation and translation motors coupled to 6 glass potters, which grind tissue pieces semi-automatically, transforming them into homogeneous nuclear suspensions. Speed and torque of both movements can be independently regulated by electronic control circuits, according to the volume of tissue being processed, and to its mechanical resistance to fractionation (white matter > gray matter, for instance). Eight male adult Wistar rats were sacrificed with ether at 120-150 days of age. They were perfused with saline followed by 4% paraformaldehyde. Their brains were removed from the skull and divided into two groups: the semi-automatically processed group (n=6) and the manually processed group (n=2). Both groups followed the original protocol of the method: DAPI and NeuN staining, then observation of nuclei morphology and counting with a Neubauer chamber at the fluorescence microscope. We compared both groups according to nuclei morphology and degree of clustering, as well as the counts obtained after the manual and the semi-automatic procedures. The machine was faster than the manual processing, showing healthy nuclei and very few clusters. Numbers obtained after semi-automated fractionation were 346.6 million average total brain cells, 60% of them being neurons. These results were statistically similar to those obtained by manual processing, and to those previously reported in the literature.

Conclusions:

The machine proved more efficient because it utilizes six potters simultaneously, consistently produces high quality material for counting, and is quantitatively reliable as compared to manual processing.

Keywords: Isotrop Fractionator Machine, Absolute number of neural cells, Large brains, Fast process, High quality results

Financial Support: FAPERJ, CNPq, CAPES, Instituto Nacional de Neurociência Translacional

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Resumo:22-052

STUDY OF ENVIRONMENTAL ENRICHMENT ON DENDRITIC ARBORIZATION IN HIPPOCAMPAL NEURONS FOLLOWING NEONATAL HYPOXIA-ISCHEMIA IN THE RAT

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Objectives:

Hypoxic–ischemic (HI) injury in term neonates can lead to severe brain damage and it is a frequent cause of neurological handicaps (cerebral palsy, mental retardation, and learning disability) in adulthood. In an effort to find interventions able to
alleviate cognitive deficits provoked by the HI, we have tested the potential neuroprotective effect of environmental enrichment (EE). Previous studies from our group showed the neuroprotective effects of environmental enrichment (EE) in rats submitted to the neonatal hypoxia-ischemia (HI) in behavior tests; however, it didn’t cause any effect on hippocampal atrophy. The aim of this study is to analyze the basal and apical composition of the dendritic tree of neurons in the dorsal CA1 hippocampus of rats submitted to HI and exposed to EE.

Methods and Results:

Male Wistar rats were obtained from the Central Animal House of the Instituto de Ciências Básicas da Saúde (UFRGS) and were randomly assigned to one of four groups: control maintained in standard environmental (CTSE); control exposed to environmental enrichment (CTEE); submitted to hypoxia-ischemia and maintained in standard environmental (HISE); submitted to hypoxia-ischemia and exposed to environmental enrichment (HIEE). For HI procedure, seven-day old rats were anesthetized with halothane, had the right common carotid artery occluded and were then subjected to a hypoxic atmosphere with 8% oxygen, for 90 minutes. A daily enrichment began when rats reached 22 days old and continued during 9 weeks, 6 days per week, 1 hour per day, in groups of 7-10 animals. Two days after the end of EE the animals were transcardially perfused with fixative solutions and their brains were submitted to Golgi staining. The morphological analysis of the dendritic arborization was performed using a camera lucida coupled to an optic microscope that allows simultaneous visualization and design of the sample. For our first analysis, we use only the left hemisphere of the rat (contralateral to arterial occlusion), and for counting of dendritic branches, neurons were chosen randomly from the hippocampal CA1 region, with an average of three neurons per group. The observed variables in the basal and apical dendritic tree were the numbers of primary, secondary and tertiary branches, counted manually after the sample design. For statistical comparisons, we used the two-way analysis of variance (ANOVA) test by Statistica statistical software. There was no effect (P>0.05) referent to the lesion and environment in the left hemisphere. In the apical neurons, the mean of numbers of primary branches (1), secondary branches (10.58), and tertiary branches (51.91) did not differ between groups; as well as in the basal neurons (5.2, 7.2, 23.52, respectively).

Conclusions:

Considering these preliminary results, we conclude that neither hypoxia-ischemia nor environmental enrichment did not change significantly the dendritic arborization of hippocampal neurons in male rats. However, the results are based on a very small n (3), needing to be increased to get more accurate results, and it should also be made by analysis of the two cerebral hemispheres.

Keywords: Hypoxic–ischemic , environmental enrichment , dendritic arborization

Financial Support: CAPES, CNPQ and FAPERGS

Resumo:22-053

GRANULOCYTE-COLONY STIMULATING FACTOR (G-CSF) INCREASES REACTIVITY OF DYSTROPHIC MDX ASTROCYTES IN VITRO

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Objectives:

G-CSF is a glycoprotein normally used for treatment of neutropenia after bone marrow transplants as well as for increasing stem cells harvesting. However, G-CSF has also been unveiled as a neuroprotective molecule that stimulates regeneration at the central nervous system after injury. Such neurotrophic property includes anti-apoptotic and anti-inflammatory activities, stimulation of revascularization and neurogenesis. In this context, G-CSF may be useful stimulate glial and synaptic plasticity in the spinal cord of dystrophic MDX mice. Therefore, the present work aimed to investigate the effects of G-CSF treatment on purified astrocyte
cultures obtained from dystrophic MDX mice, which present a decreased immunoreactivity against glial fibrillary acidic protein (GFAP). We have also evaluated the G-CSF receptor (G-CSFR) expression of such cells and its regulation after G-CSF treatment.

Methods and Results:

Primary cortical astrocyte cultures were isolated from MDX (animal model for studying Duchene’s muscular dystrophy) and C57BL/10 mice. For this purpose, the cerebral cortices of neonatal mice (day 1 or 2 after birth) were extracted and the astrocytes purified by differential centrifugation. Cells were seeded in 24 wells culture plates and maintained at 37°C with 5% of CO2. After 24 hours of culture, G-CSF was added at concentrations of 10 µg/ml, 100 µg/ml, 500 µg/ml and 1000 µg/ml for five days. Non-treated cultures were used as control. All the experiments were performed in triplicate. The cells were then fixed with 4% paraformaldehyde in DMEM and washed in PB buffer without calcium and magnesium followed by incubation, for 2 hours, with primary antibodies against GFAP (1:100, Santa Cruz) and G-CSFR (1:200, Santa Cruz). The secondary antibodies, conjugated with Cy2 and Cy3, were incubated for 45 minutes and the fluorescence was visualized in a Nikon inverted microscope equipped with a high resolution camera. The immunolabeling was measured by calculating the integrated density of pixels of representative pictures obtained from the different experimental groups using the IMAGEJ software (1.33u version, National Institutes of Health, USA). The results demonstrated that G-CSF treatment increased the expression of GFAP and G-CSFR in cultures from both MDX and C57BL/10 mice. Such increase was more pronounced in the highest treatment dose as compared to the untreated cultures (GFAP immunolabeling: MDX untreated - 5361.64 ± 451.09, MDX + 1000 µg/ml - 11296.65 ± 653.77, p

Conclusions:
The present results demonstrate that G-CSF treatment increases GFAP and GCSF-R expression in astrocytes from MDX mice. Such increase in glial reactivity may stimulate synaptic plasticity in vivo, positively contributing to the rewiring of the spinal cord circuits during the course of the disease, in response to the cycles of muscular degeneration/regeneration.

Keywords: G-CSF, ASTROCYTES, MDX

Financial Support: CNPq, CAPES and FAPESP

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Resumo:22-054

ONTOGENY OF MICROGLIA WITHIN THE POSTNATAL SUBVENTRICULAR ZONE

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Objectives:
The determination of the cellular components and their interactions within neurogenic niches is essential to understanding the mechanisms underlying neurogenesis. This study is aimed to characterize the ontogeny of microglia cells - macrophages of the CNS - and their distribution along the subventricular zone (SVZ), an important postnatal neurogenic niche maintained throughout life

Methods and Results:

After anesthesia, the animals, Swiss mice at postnatal ages P3, P7, P15 and P30 were perfused transcardially with saline followed by 4% paraformaldehyde in phosphate buffered saline (PBS 0.1 M, pH 7.4) and brains dissected. Vibratome parasagittal slices (50 µm) were processed for immunohistochemistry. The sections were incubated with the following primary antibodies: anti-CD68 (1:100; AbDSerotec); anti-F4/80 (1:100; AbDSerotec), anti-GFAP (1:400, Dako), anti-BLBP (1:300, Millipore); and the biotinylated isolectin B4 (1:100, Vector Laboratories) at 4°C overnight, and labeling revealed by appropriate secondary
antibodies (AlexaFluor 647, 488, 546; 1:400; Molecular Probes, Invitrogen) or with Cy3 conjugated streptavidin (1:100; Molecular Probes, Invitrogen) for 2 hours at room temperature and nuclear staining was performed with DAPI (Sigma Aldrich). Sections were analyzed on an epifluorescent microscope (Nikon TE200) and a confocal microscope (Leica SP5).

Conclusions:

At P3 and P7 most cells within the cortical SVZ were consistently amoeboid, irrespectively if labeled with CD68, F4/80, or IB4. Interestingly, there was only partial overlap between IB4+ and F4/80+ cells. We found a consistent distribution of microglia cells, associated with blood vessels and GFAP+ radial extensions. Distribution of microglia within the SVZ is non uniform, with a clear accumulation at the interface between the SVZ and adjacent white matter. At early ages, along the length of the rostral SVZ (RMS, rostral migratory stream), we found few microglial cells, which exhibit typical migratory morphology. In the olfactory bulb, CD68+ and F4/80+ were high in the pia mater. In P15 and P30, we observed a dramatic decrease of immunoreactivity for CD68 and F4/80 in the cortical SVZ, in contrast to an intense staining of IB4 revealing complex branched morphology throughout the length of the SVZ/RMS. CD68+ and F4/80+ are predominantly found in the various layers of the olfactory bulb. These results indicate that microglia cells comprise a fairly large, conspicuous and heterogeneous population within the pottnatal SVZ/RMS. These results indicate a possible underestimation of the presence of these cells within the SVZ/RMS and a strong indication that these cells are more diverse than previously believed.

Keywords: microglia, ontogeny, subventricular zone

Financial Support: Faperj

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Resumo:22-055

INTRAVITREAL INFLAMMATION INDUCES PLASTICITY OF INTACT RETINO-COLLICULAR PATHWAY: A POSSIBLE ROLE FOR IL-2 ON MODULATION OF THE RETINAL CHOLINERGIC AND GLUTAMATERGIC SYSTEMS

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Objectives:

Intravitreal inflammation has been used as an induction model of axonal regeneration in post-lesion visual system, but their effects on formation of connection in the intact visual pathway have not been explored. Interleukin-2 (IL-2) is a component of inflammation which participates in regulation of neuritic outgrowth and synaptic plasticity. Recent data showed that in early development of rat retino-collicular pathway, the intravitreal administration of IL-2 induces sprouting of retinal afferents. The formation of retino-collicular topography displays during the first three postnatal weeks and requires a pattern of cholinergic activity among neighbor retinal cells followed by glutamatergic activity. Therefore, the aim of this work was to study the effect of zymosan-induced intravitreal inflammation upon distribution of retino-collicular projection and the influence of IL-2 on the neurotransmission systems required for pathway refinement.

Methods and Results:

Lister Hooded rats were submitted to a single intravitreal injection of IL-2 (1X 156U/ 1,5μL), zymosan (62,5μg/ 3μL) or PBS at PND10 and PND21. For morphological analysis of uncrossed retino-collicular projections, animals received HRP injection in the treated eye. Additionally, retinas were processed by Western blot for PSD95, CHAT and β2 subunit of nicotinic receptor. The cells types in retina were visualized by Nissl staining in order to confirm the inflammation. Our results demonstrated that intravitreous treatment with zymosan induces an inflammatory recruitment in retina in parallel to a robust sprouting of the ipsilateral retinotectal pathway for inappropriate targets in superior colliculi. Matched-control animals exhibited refined retinal
afferents and retinas without inflammation. Moreover, IL-2 treatment downregulated PSD95 and β2 expression, not affecting CHAT content compared to PBS treatment.

Conclusions:

Therefore, these data suggest that zymosan-induced intravitreal inflammation interferes with the ganglion cells development, disrupting their connections refinement in the target in favor to sprouting. IL-2, as an element of the inflammatory response, may be involved in this mechanism, through downregulation of important synaptic proteins from retinal neurotransmitter systems, resulting in the weakening of connections, which leads to the sprouting of retinotectal projections in search of new targets.

Keywords: neuroinflammation, interleukin-2, plasticity, retino-collicular

Financial Support: CNPq, PROPPi-UFF, FAPERJ, CAPES, UFF-PROAP, PRONEX-MCT

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Resumo:22-056

NEUROBLAST MIGRATION TO THE RAT NEOCORTEX FOLLOWING ENDOTHELIN-1-INDUCED FOCAL GRAY MATTER ISCHEMIA

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Objectives:

Neuroblasts migrate from the subventricular zone (SVZ) to the rat striatum following middle cerebral artery occlusion (MCAO). Nevertheless, neuroblast migration to the neocortex has not been consistently reported by all groups. We hypothesized that white matter (WM) matter induced by MCAO may impair neuroblast migration to the neocortex and that a focal cortical gray matter ischemia would facilitate the neuroblast migration to this central nervous system region. We aimed to investigate whether a focal gray matter ischemia induces neuroblasts migration to rat neocortex in the first two weeks posts ischemia.

Methods and Results:

24 male Wistar rats, weighing 250-300 g, received microinjections of the constrictor peptide endothelin-1 (ET-1, 40 pmol/µl, n=15) or sterile saline (n=9) into the motor cortex. Animals were perfused at 3 (n=5), 7 (n=5) and 14 (n=5) days postinjury. Gross histopathology was assessed in 50 µm sections using cresyl violet staining. Migrating neuroblasts were immunolabeled using an anti-doublecortin (DCX) specific antibody. The numbers of DCX+ cells (3 fields/section, 3 sections/animal) were counted in all experimental groups. The microinjections of ET-1 induced focal cortical ischemia restricted to the gray matter. There was conspicuous tissue loss in all investigated survival times. There was an increase in the number of DCX+ cells in the ipsilateral SVZ of ischemic animals, compared to the contralateral side and SVZ of saline-injected animals. Neuroblasts were present in the ischemic neocortex in all investigated survival times, but mainly at 7 days posts ischemia (5.5 ± 0.6, 14.1 ± 2.0, 5.4 ± 0.7, p

Conclusions:

A focal ischemia restricted to the cortical gray matter increases neuroblast numbers in the SVZ and induces neuroblast migration to the rat neocortex. Further studies should investigate whether up regulation of white matter inhibitors like NOGO A impairs neuroblast migration to the neocortex following focal ischemia

Keywords: FOCAL GRAY MATTER ISCHEMIA, NEUROBLAST MIGRATION, NEUROGENESIS

Financial Support: Fundação de Amparo e Desenvolvimento a Pesquisa do Estado do Pará
THE ROLE OF STRESS INDUCIBLE PROTEIN 1 (STI1) IN THE MICROGLIA-GLIOMA INTERACTION

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Objectives:

Microglial activation is also controlled by tumor cells, supporting their progression and infiltration. Our group previously demonstrated that the co-chaperone STI1, a PrPc ligand, is secreted by glial cells and promotes glioblastoma (GBM) proliferation. In the context of microglia-GBM interaction, the present study aimed at investigating the influence of STI1 and PrPc in the tumoral growth and invasion.

Methods and Results:

Highly pure cultures of microglial cells from neonatal mice and tumor cells from GBM95 human cell line were realized. We verified by immunocytochemistry and western blotting analyses that microglial cells express and secrete STI1. In GBM95 cell cultures, the [3H]-thymidine incorporation assays showed that proliferation increased 100% when these cells were cultured with microglial conditioned medium (MCM), compared to control condition (medium without serum). The proliferative effect was reverted when STI1 was depleted from MCM by immunoprecipitation. Moreover, when recombinant STI1 was added to MCM depleted of STI1, the proliferation ratio was restored. As control, no effect was observed when irrelevant IgG was depleted from MCM. Still, in GBM95 cell cultures, we verified that STI1 promoted significant proliferation even if anti-PrPc neutralizing antibody or recombinant STI depleted of the site of binding with PrPc were added. In the migration assays, recombinant STI1 and MCM favored migration of GBM95 cells but failed when STI1 was depleted from MCM. In addition to STI1, we also detected the metalloproteinase MMP-9 in the MG CM. Furthermore, when microglia were treated with anti-STI1 antibodies, MMP-9 was not detected.

Conclusions:

Our results suggest that STI1 is secreted by microglia and favors tumor growth and invasion through the participation of MMP-9 in a PrPc-independent manner. This project was approved by the Ethic Comitee of CCS-UFRJ (Protocol nº DAHEICB015).

Keywords: glioblastoma, microglia, STI-1, migration, proliferation

Financial Support: CNPq, CAPES, FAPERJ, FAPESP

Reactive Zinc in Central Nervous System of Adult Zebrafish

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Objectives:

Reactive or chelatable zinc (Zn) is stained by Neo-Timm method in hippocampal regions of vertebrates. Consistent with a synaptic function, reactive Zn is related to many neuropathies, which zebrafish (*Danio rerio*) has been used as a potential animal model. For this reason, the aim of the present study was to characterize the reactive Zn-sensible Neo-Timm staining in the central nervous system (CNS) of adult zebrafish.

Methods and Results:

Neo-Timm staining revealed yellow-to-brown-to-black granules (0.5 – 4.0 µm), as well as conspicuous brown-to-black neuronal projections in the CNS of zebrafish. Through analysis by optic density, coronal CNS slices obtained regions slightly stained in the telencephalon, whereas the caudal part of the brain showed abundant Neo-Timm positive areas. The rhombencephalic structures predominantly presented high level of staining. Although stained granules were observed surrounding the cell bodies, rhombencephalic structures presented essentially a neuropil staining pattern. Moreover, addition of chelating ligand N,N,N',N'-Tetrakis (2-pyridyldimethyl) ethylenediamine and trichloroacetic acid did modify and decrease the Neo-Timm staining in the zebrafish CNS. Overall, the results indicated that the chelatable Zn is located close to the axon terminals.

Conclusions:

The Neo-Timm staining is the first report of reactive Zn pool in CNS of zebrafish. Its specific and heterogeneous staining revealed more abundant granules in the rhombencephalic structures than in other brain areas. Even though zebrafish has shown an unusual chelatable Zn arrangement, its cellular localization seemed to preserve the presynaptic pool of the other vertebrates.

Keywords: chelatable zinc, glutamatergic synapses, Neo-Timm

Financial Support: CAPES, CNPq/INCTEN and FINEP

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Resumo:22-059

STUDY OF THE NNOS RECEPTOR IN NEURONS OF THE SMALL INTESTINE OF RATS EXPERIMENTAL INTESTINAL MUCOSITIS INDUCED BY 5-FLUOROURACIL (5-FU)

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Objectives:

This study aims to analyze the expression of substance P (SP), neuronal nitric oxide synthase (nNOS) and nitric oxide synthase neuronal receptor in the myenteric plexus during inflammatory phase in experimental intestinal mucositis induced by 5-FU.

Methods and Results:

Intestinal mucositis was induced by intraperitoneal (i.p) administration of 5-FU (450 mg/kg) or saline (control group) in male
rats. During 3 days, diarrhea was evaluated. After 3 days of the treatment, rats were sacrificed and sections of duodenum, jejunum and ileum were removed for evaluation morphometric, assessment of MPO activity, nitrite level, and immunohistochemistry for neuronal nitric oxide synthase (nNOS) and substance P (SP). In order to study tissues of duodenum were prepared by immunohistochemical methods for double staining of receptor with neuronal nitric oxide synthase (nNOS) with pan-neuronal marker anti-HuC/D. The qualitative analysis was obtained from fluorescence microscopy and confocal scanning laser. On the third day of treatment, 5-FU induced severe diarrhea (2 and 3 score) and leucopenia (2698.00±486.32 mm3) in rats, when compared to control group (0 score; 7859.00±985.25 mm3). 5-FU administration significant decreased in villus/crypt ratio in duodenum (1.61±0.07 µm), jejunum (2.08±0.24 µm) and ileum (1.45±0.22 µm), when compared to control group (3.14±0.23; 3.36±0.23; 2.55±0.13 µm, respectively). 5-FU induced MPO activity in duodenum (15.8±0.6 U de MPO/mg), jejunum (13.43±0.6 de MPO/mg) and ileum (12.20±0.8 U de MPO/mg), when compared with control group (2.20±0.3; 1.67±0.23; 2.04±0.76 U de MPO/mg, respectively). There was also increased nitrite/nitrate level in duodenum (160.7±35.9 µM de NOx), when compared to control group (91.4±11.1 µM de NOx). Increased neurotransmitter activity was detected by immunostaining for nNOS and SP on the duodenum of animals submitted to 5-FU-induced intestinal mucositis. The qualitative result showed increase nNOS receptor immunoreactivity in neurons of the myenteric plexus in duodenum when compared to control group.

Conclusions:

These results suggest an important participation of neuronal neurotransmitter, neuronal NO and SP in experimental intestinal mucositis induced by 5-FU, as well as changes in expression of the nNOS receptor in myenteric neurons during inflammatory phase in experimental intestinal mucositis induced by 5-FU, which can cause intestinal motility disorder.

Keywords: Mucositis intestinal, Neuromorphology, myenteric neurons, Inflammation

Financial Support: CNPq

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Resumo:22-060

ENTERIC GLIAL CELLS IN CULTURE

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Objectives:

The enteric nervous system (ENS) contains two ganglioneated plexuses, the submucous and the myenteric plexuses which are mainly composed by neurons and glial cells. Recent studies suggest that enteric glial cells (EGC) play an important role in the maintenance of gastrointestinal functions, but its role in human diseases remains unknown. Further, thyroid hormones (T3 and T4) actively interact with the gastrointestinal tract. It is well known that the gut acts as a reservoir of thyroid hormones, regulating their functional activity. However, the effect of these hormones on enteric glial cells has not been described. Thus, the purpose of this work is firstly to characterize in vivo and in vitro EGC and compare with glial cells from central nervous system (CNS).

Methods and Results:

In vivo and in vitro EGC was obtained using 2-3 months old Swiss male mice. The colon was removed and the myenteric plexus was dissected and submitted to in vivo analyses by cryo freezing samples and in vitro studies by cell cultures in DMEM-F12 at 37 °C and 5% CO2 atmosphere. After two weeks, EGC were passed by trypsinization. To confirm the presence of EGC in the culture, we evaluated the expression of glial fibrillary acidic protein (GFAP) by immunocytochemistry. We observed that most cells in vivo or in vitro express GFAP, showing that these cells have glial origin, as we verified with astrocytes from CNS. Further another cells were stained by antibodies to endothelial or pericyte cells.
Conclusions:

We have well established this EGC characterization which permits now to study the functional properties of these cells in vitro and in vivo. We will then analyze the effects of thyroid hormones T3 and T4 in the modulation, differentiation and proliferation of the enteric glia cell.

Keywords: enteric glial cells, enteric nervous system, thyroid hormone

Financial Support: CAPEs, FAPERJ e CNPq-MCT:INNT

DISTRIBUTION OF NITRIDERGIC NEURONS OF RAT PRIMARY SENSORY CORTEX.

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Objectives:

In isocortex, neurons are organized in horizontal layers and columnar modules. In primary somatosensory cortex (S1) of rats, the barrels of layer IV define the limits cortical functional units, responsible for processing of peripheral sensory information. NADPH-diaphorase histochemistry (NADPHd), reveals the enzyme of synthesis of nitric oxide, producing a neuropil diffuse staining that allows the identification of cortical layers and barrels. It also reveals a subpopulation of strongly labeled neurons (nNADPHd) (Braz.J.Med.Biol.Res. 28:787-790, 1995). In the present work, we characterized the distribution of nNADPHd in the different compartments of area S1 of the rat.

Methods and Results:

Serial 200 μm-thick coronal sections from one of the hemispheres of three adult Wistar rats were processed for NADPHd histochemistry. The sections were reconstructed using a 10x objective lens of a microscope coupled to the NeuroLucida system (MBF Biosciences). The cell body of nNADPHd in S1 and the limits of the different cortical compartments were drawn in these reconstructions. These drawings were visualized on the Neuroexplorer software and individually exported in DXF format for the Canvas X (ACD System) software. Drawings of individual sections were saved in TIFF format, allowing area measurement in ImageVision 6.0 (National Instruments). Area measurements were made in the following laminar compartments of the barrel field in S1: SG - supragranular compartment, corresponding to the area of the supragranular layers; GR - granular compartment, corresponding to cortical layer IV; IG - infragranular compartment, corresponding to the area of infragranular layers V and VI. Additionally, the areas of the barrel and septal columns were defined by a vertical projection from each barrel. We then calculated the mean nNADPHd density in each compartment dividing the mean number of cells obtained in each compartment by the mean value of the area of the compartment in each hemisphere. In case 1, where the barrel fields were fully reconstructed, we found the following mean laminar and columnar densities: SG=21.55 cells/mm2; GR=10.12 cells/mm2; IG=15.96 cells/mm2; barrel=14.94 cells/mm2; septum=18.08 cells/mm2. In case 2 and case 3 selected sections were sampled to calculate the mean density in each compartment. Mean density values in case 2 were: SG=20.38 cells/mm2; GR=12.33 cells/mm2; IG=13.47 cells/mm2; barrel=13.40 cells/mm2; septum=16.87 cells/mm2. In case 3, the following average densities were obtained: SG=20.55 cells/mm2; GR=12.85 cells/mm2; IG=10.36 cells/mm2; barrel=14.43 cells/mm2; septum=10.01 cells/mm2.

Conclusions:

In all cases, supragranular layers (SG) presented the largest nNADPHd density. The granular layer (GR) was the less populated in cases 1 and 2. Comparison between barrel and septal columns revealed a larger density of nNADPHd in the septum. Again, this was observed both in cases 1 and 2, but not in case 3. In conclusion, distribution of nNADPHd is heterogeneous along the
different cortical compartments of the rat barrel field.

Keywords: CORTEX, NITRIDERGIC NEURONS, nNADPHd, RAT

Financial Support: CNPq, CAPEs, FAPERJ and PRONEX

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Resumo:22-062

SKILLED REACHING TRAINING INCREASE MICROTUBULE-ASSOCIATED PROTEIN 2 (MAP-2) IN THE RAT MOTOR CORTEX (M1) AND FACILITATED SENSORIMOTOR RECOVERY AFTER COLLAGENASE-INDUCED INTRACEREBRAL HEMORRHAGE

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Objectives:

Spontaneous intracerebral hemorrhage (ICH) is the most devastating type of stroke and a leading cause of disability and mortality worldwide. Although rehabilitation improves recovery after ICH the cellular mechanisms involved are poorly understood. We decided to examine if skilled (SK) and unskilled (US) training after sham or intracerebral hemorrhage (ICH) surgeries would induce MAP-2 changes in the primary motor cortex (M1) and whether these modifications occurred in association with functional improvement.

Methods and Results:

Initially, forty-eight male Wistar rats (n=48) were habituated in the Staircase test (during three weeks) and all animals received a session for habituation in skilled and unskilled tasks prior to surgery. Before the surgery rats were grouped according to their reaching success (baseline Staircase evaluation) and designed equally to one of six groups: sham no task (S no task, n=08), sham skilled task (S-SK, n=08), sham unskilled task (S-US, n=08), ICH no task (ICH no task, n=12), ICH skilled task (ICH-SK, n=12), and ICH unskilled task (ICH-US, n=12). Then, animals were anesthetized with halothane and positioned in the stereotaxic frame for ICH or sham surgeries. For ICH induction, bacterial collagenase type-IV (0.2 U/1.0ìL) was infused into the striatum over 5 min. Sham animals received the same volume of sterile saline. The injury was induced in the contralateral hemisphere to the preferred paw (determined according to Staircase baseline evaluation). Seven days after Sham/ICH surgery, S-SK and ICH-SK rats received daily skilled reaching session (40 min / 5 days per week); S-US and ICH-US rats receive daily unskilled (treadmill) session (40 min / 5 days per week); S and ICH animals remains in standard cages. After four weeks of training, rats were deeply anesthetized and perfused for morphological analysis. Our results showed that skilled reaching task induced an increased MAP2 immunoreactivity for sham and ICH animals (S-SK and ICH-SK groups). This occurred in both M1 sides (ipsilateral and contralateral to injury). Moreover, only ICH-SK animals showed an improvement in skilled reaching recovery (P

Conclusions:

We concluded that skilled reaching training may induce dendritic plasticity in M1 cortex bilaterally, which occurred in the normal and injured brain. Additionally, ICH-SK group improves forelimb recovery. Thus, skilled reaching may be an interesting strategy of rehabilitation to promote plasticity and forelimb recovery after ICH.

Keywords: intracerebral hemorrhage, Skilled reaching, Striatum, MAP-2, Staircase
Objectives:

Brain edema occurs as the result of several diseases that affect the Central Nervous System (CNS), including hepatic encephalopathy (HE). It manifests as a range of behavioral, neuropsychiatric and neuromuscular symptoms related to the incapacity of the liver disease patients of metabolizing toxic waste. Among these toxins, ammonia has its conversion to urea impaired in cirrhotic liver, and it is detoxified in the brain mainly by astrocytes during the conversion of glutamate to glutamine. Increase of ammonia levels and amount of glutamine in astrocytes cause osmotic imbalance, resulting in cell swelling (cytotoxic edema) and, ultimately, in brain edema. The aim of this work was to investigate by behavioral tests the exploratory activity and degree of neurotoxicity in animals submitted to experimental HE.

Methods and Results:

Adult male Wistar rats were divided into four groups. HE was induced in BDL group (n=12) by bile duct ligation and section surgery (bile duct ligation; BDL). The animals were maintained with standard diet for 21 days. The animals from BDL+diet group (n=14) were submitted to bile duct ligation and section and maintained with standard diet during 14 days. From 15th to 21th day of treatment, the rats were fed with hyperammonemic diet (20% ammonium acetate). In SHAM group (n=12), the rats were just submitted to surgical procedure (without biliar duct connection and section), and were fed with standard diet during the whole treatment. In SHAM+diet group (n=12), the rats underwent the same surgical procedure (but without connection and section of the biliar duct) were submitted to hyperammonemic diet in the last seven days of treatment. The neurotoxicity assessment and the exploratory activity were performed by Functional Observation Battery (FOB) and Open Field tests, respectively. At the end of 21 days, the animals were anesthetized and perfused with the fixative solution of paraformaldehyde 4% in PBS 0.1 M pH 7.4. The degree of hepatic damage was assessed via histopathological analysis, while evidence of encephalopathy induced by hyperammonemia was obtained by determination of plasma levels of ammonia. The histopathological analysis showed livers with severe disorganization of the lobular cytoarchitecture, supporting the diagnosis of biliary cirrhosis in the BDL and BDL+diet groups. Significant Increase in plasma ammonia concentration was observed between SHAM and BDL (p

Conclusions:

Animals submitted to hepatic encephalopathy have demonstrated high index of neurotoxicity, coupled with decreased motivational exploratory behavior, which is independent of locomotor function impairment (reflexes and muscle tonus).

Keywords: Encefalopatia Hepática, Comportamento, Hiperamonemia, Neurotoxicidade

Financial Support: CNPq and FAPESP
MORPHOLOGICAL EVIDENCES OF THE BLOOD-BRAIN BARRIER BREAKDOWN DURING ACUTE PHASE OF PILOCARPINE MODEL OF EPILEPSY.

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2 Dep. de Neurologia e Neurocirurgia – UNIFESP, São Paulo/ SP, UNIFESP

Objectives:

The Pilocarpine Model of Epilepsy (PME) reproduces the main features of human temporal lobe epilepsy in rats. Our quantitative studies have demonstrated that blood-brain barrier (BBB) breakdown to macromolecules occurs around five hours after the status epilepticus (SE) onset in the PME. The aim of this work was to investigate, by morphological analysis of Evans blue (EB) staining, the consequences of the increased BBB permeability in the neural tissue during acute phase of the PME.

Methods and Results:

Wistar male adult rats were injected with a single dose of pilocarpine (320-350 mg/kg i.p.). The BBB integrity was assessed using the EB dye (80 mg/kg) injected intravenously at the beginning of the SE. It binds to serum albumin forming a complex of 68,500 Da, which has been used as a marker of macromolecules extravasation. The animals received an injection of diazepam (7-10 mg/kg i.p.) three hours after the onset of seizures. Rats of the control group received EB injection, but not pilocarpine. The rats were transcardially perfused at 5 and 24 hours after EB injection (n=3 of each group), using a fixative solution. The brains were cut in a cryostat and the EB distribution in the neural tissue was analyzed in a fluorescence microscope. The EB dye extravasation was widely distributed in the brain tissue of pilocarpine-treated rats in a time-dependent manner. Five hours after the SE onset (SE 5h), small group of EB-positive cells was observed in the hippocampus (dentate gyrus), thalamus, somatosensory cortex and amygdala. However, the EB staining was not found in all animals from this group. In the SE24h group, all animals showed dye extravasation into the brain. The EB staining was distributed over several brain regions and a massive number of positive cells were observed. The brain regions preferentially affected were the hippocampus (dentate gyrus and CA3), thalamus (laterodorsal, mediadorsal, ventrolateral, posterolateral, posterior, reuniens and ventromedial nucleus), somatosensory cortex, amygdala and piriform cortex. These areas are involved in defensive, motor, memory and emotions behavior.

Conclusions:

BBB breakdown to macromolecules during acute phase of the pilocarpine model of epilepsy occurs from five hours after SE onset. The cerebral areas preferentially affected are the hippocampus, thalamus, somatosensory cortex, amygdala and piritform cortex.

Keywords: barreira hematoencefálica, epilepsia, pilocarpina

Financial Support: FAPESP (08/06450-0 and 10/05858-5)

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Resumo:22-065

MOTOR NEURON LOSS AND MYELIN IMPAIRMENT IN THE RAT SPINAL CORD AFTER CHRONIC INTOXICATION WITH ETHANOL


Universidade Federal do Pará, UFPA
Objectives:
To investigate the effects of chronic intoxication with ethanol on the gray (GM) and white matter (WM) of rat spinal cord.

Methods and Results:
Female Wistar rats (n=10) were intoxicated (6 g/kg/day), by gavage, with ethanol (n=5) from 35 to 90 postnatal days. Control animals (n=5) received sterile saline only. Animals were perfused with heparinized saline and 4% paraformaldehyde after the last intoxication day. Spinal cords (SC) were removed, post-fixed in 4% paraformaldehyde by 12 hours, cryoprotected, embedded in Tissue Tek and coronally cut at 40 µm thick using a cryostat. Sections from cervical, thoracic and lumbar segments were stained with Cresyl violet for gross histopathological analysis and immunolabeled by an anti myelin basic protein (MBP) antibody. Motor neurons from the ventral horn of all SC segments were counted in 5 sections per animal (3 fields/sections) in both control and ethanol-intoxicated group. Comparisons between groups were performed using the Student’s t test at P<0.05).

Conclusions:
Chronic intoxication with ethanol induces white matter impairment and motor neuron damage in the spinal cord of adult rats.

Keywords: ETHANOL, MOTOR NEURON LOSS, MYELIN IMPAIRMENT

Financial Support: Fundação de Amparo e Desenvolvimento à Pesquisa do Estado do Pará (FAPESPA)
(EtOH gavage + ET-1 stereotaxic injection + minocycline injection). Animals were perfused, brains were post-fixed, cryoprotected, included in Tissue Tek and were sectioned in coronal sections of 50µm and stained with Cresyl violet. For counting, we used light microscope Olympus CX31, with square grid of 0.00625mm2; and 3 fields were counted in four sections of each animals. Data were submitted to statistical analysis with Tukey test with p

Conclusions:

Minocycline treatment do not showed same neuroprotection pattern in ischemic animals, intoxicated with EtOH as in ischemic non-treated animals.

Keywords: EtOH, FOCAL ISCHEMIA, MINOCYCLINE

Financial Support: Fundação de Amparo e Desenvolvimento à Pesquisa do Estado do Pará (FAPESPA)

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Resumo:22-067

D-SERINE REGULATES THE FORMATION OF EXCITATORY SYNAPSES THROUGH NMDA RECEPTOR BINDING IN THE CEREBRAL CORTEX.

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Instituto de Ciências Biomédicas , ICB

Objectives:

Astrocytes are described as the most abundant glial cells in the cerebral cortex and are known as the third element of the synapses. It is known that these cells are capable of secreting various molecules such as ATP, glutamate and D-Serine (D-Ser), known as gliotransmitters that influence synaptic transmission and synaptogenesis. D-Ser is a D-amino acid synthesized by astrocytes that predominantly serves as glutamatergic coagonist of the NMDA receptors. D-Ser is involved in several cellular events such as neuronal migration, long-term potentiation (LTP) formation, synaptic plasticity and memory. We investigated the role of D-serine (D-ser) in the formation of excitatory synapses.

Methods and Results:

Primary cultures of cerebral cortical neurons were treated with D-Ser (0.4 mM) on days 0, 3, 6, 9 and analyzed at 3 and 12 days in vitro. Immunocytochemistry assays for presynaptic and postsynaptic molecules revealed an increase of 122% and 41% in the number of puncta for synaptophysin and PSD-95 proteins, respectively. Furthermore, we found a 219% increase in the number of puncta double labeled for synaptophysin and PSD-95, indicating an increase in structural functional synapses. This increase was corroborated by ultrastructural analysis using transmission electron microscopy (133%). These data were supported by identification of 100% increase in the levels of synaptophysin and PSD-95 in D-Ser-treated neurons by Western blotting assays. Neuritic outgrowth (Cont, 149 ± 10.34, n= 3, D-Ser; 160 ± 13.56, n= 3), and neuronal survival (Cont, 85.23 ± 3.979, n= 3; D-Ser, 96.08 ± 5.389, n= 3) were not affected by D-Ser. D-Ser main action is to potentiate NMDA receptor-mediated transmission by selective stimulation of its glycine site. Inhibition of D-Ser binding by the NMDA antagonist DCK (5,7-dichlorokynurenic acid, 10 micromolar) abolished the synaptic induction previously observed. Further, knocking down of the serine racemase enzyme (responsible for D-Ser production) in neuronal cultures impaired synaptic formation by 32%.

Conclusions:

Our findings describe a new mechanism by which glia cells regulate excitatory synapse formation. Our data support the emerging concept that astrocytes are dynamic partners of the brain signaling through D-Ser and shed light on potential compounds for
Effects of morphine exposure in early life on brain-derived neurotrophic factor (BDNF) levels in the hippocampus.

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2 PPG Medicina: Ciências Médicas, UFRGS
3 Unidade de Experimentação Animal, HCPA

Objectives:

The abuse drugs, such as morphine, decreases neurotrophic factors and negatively impacts the neurogenesis of the hippocampus in adult life. However, effects of this treatment in early life on neurotrophic factors and possible changes in physiological systems are still poorly studied. Knowing that the hippocampus is one of the structures associated with withdrawal symptoms and neurochemical changes after opioid withdrawal, the objective of this study is evaluate the effects of morphine exposure in neonate on BDNF levels in hippocampus at short, medium and long term after the spontaneous withdrawal.

Methods and Results:

were utilized neonate male Wistar rats, which were divided in 2 groups: control (C, n=19) and morphine (M, n=18). The C and M groups received saline or morphine (5 μg s.c., in the mid-scapular area), respectively, at postnatal day 8 (P8), once a day for 7 days. The rats were killed and hippocampus were removed and homogenized at a ratio of 1:10 with a standard solution containing antiproteinases. The homogenates were centrifuged for 30 min (at 14,000 x g) and supernatants were used for assays. The analyses of BDNF level were performed at P16, P30 and P60 by ELISA and were expressed as picogram (pg). Statistical analysis were made by Student’s t test and data were expressed as mean ± standard error of the mean (SEM), considering P<0.05), but at P50 and P60 M group presents an increase in BDNF levels compared to control group (P30: C= 193.83 ± 15.9, M= 252.57 ± 19.54; P60: C= 200.52 ± 13.79, M= 275.09 ± 22.31; Student’s t test, P<0.05).

Conclusions:

This study show that morphine exposure in early life can potentiate the neurogenesis at medium- and long-term, but not in short-term. In contrast, other studies shown that the hippocampus of adult rats presented a decrease of the neurogenesis and BDNF level after chronic morphine exposure. However, we highlight that in neonatal period the synaptic connections are immature and there is an intense neurogenesis in brain structures, particularly in hippocampus. This neonatal treatment was capable to positively modulate neurogenesis until adult life, and this neurochemical change may be related to the phenomenon known as ontogenetic plasticity after drug exposure.

Keywords: MORPHINE, BRAIN-DERIVED NEUROTROPHIC FACTOR, HIPPOCAMPUS, NEONATE

Financial Support: CAPES, GPPG-HCPA, FAPERGS, Propesq-UFRGS
ATORVASTATIN PREVENTS COGNITIVE, EMOTIONAL AND MOTOR DEFICITS INDUCED BY INTRANASAL ADMINISTRATION OF MPTP IN RATS, AN EXPERIMENTAL MODEL OF PARKINSON’S DISEASE.

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2 Bioquímica/Universidade Federal de Santa Catarina, UFSC

Objectives:
Atorvastatin is a [3-hydroxy-3-methylglutaryl-coenzyme A (HMGCoA) reductase] inhibitor with cholesterol-lowering, anti-inflammatory and antioxidant properties. There is increasing evidence that atorvastatin may act as protective agent against different insults of the CNS. Here we evaluated the potential of chronic pretreatment with atorvastatin to prevent cognitive, emotional and motor deficits induced by intranasal infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in rats, an experimental model of Parkinson’s disease developed recently in our laboratory.

Methods and Results:
Male Wistar rats (3 months old) were divided in four groups: control/control, atorvastatin/control, control/MPTP and atorvastatin/MPTP. Animals were pretreated orally with atorvastatin (10 mg/kg/day) or vehicle (NaCl 0.9%) once a day during 7 consecutive days. Four days after initiation of pretreatment, the animals were infused intranasally with a single bilateral dose of MPTP (1 mg/nostril). Animals were evaluated in social recognition, forced swimming and activity chamber tests at 7, 14 and 21 days after intranasal MPTP infusion in order to assess, respectively, cognitive, emotional and motor functions. CEUA-UFSC: PP00551. In the social recognition task, the pretreatment with atorvastatin prevented the MPTP-induced short-term social memory deficits in adult rats, protecting their ability to recognize a juvenile rat after a short period of time (control/control: 0.55 ± 0.05; #control/MPTP: 1.03 ± 0.07; ator/control: 0.60 ± 0.15; *ator/MPTP: 0.53 ± 0.14). Moreover, MPTP infused rats displayed increased immobility time in the forced swimming test suggestive of a depressive-like behavior that was prevented by the pretreatment with atorvastatin (control/control: 9.667 ± 2.00; #control/MPTP: 23.71 ± 2.94; ator/control: 9.57 ± 2.78; *ator/MPTP: 5.12 ± 0.78). Finally, intranasal MPTP infusion promoted a later (21 days after MPTP administration) reduction on locomotor activity of the animals in the activity chamber that was attenuated by the pretreatment with atorvastatin (control/control: 189.80 ± 9.16; #control/MPTP: 146.90 ± 4.40; ator/control: 157.80 ± 9.11; *ator/MPTP: 177.00 ± 9.63). (# p

Conclusions:
Altogether, our results demonstrate that the repeated treatment with atorvastatin prevents cognitive, emotional and motor alterations induced by intranasal MPTP administration in rats. These findings reinforce and extend the neuroprotective effects of atorvastatin observed previously in other models of CNS damage.

Keywords: MPTP, atorvastatin, Parkinson’s Disease, Behaviour, statins

Financial Support: CNPq, CAPES, INCT-EN, FAPESC, UFSC

EFFECTS OF NITRIC OXIDE SYNTHASE INHIBITORS ON DEFICITS IN PREPULSE INHIBITION CAUSED BY NMDA RECEPTOR ANTAGONISTS.
Objectives:

Considering the deficits in sensory-motor filter present in several disorders such as schizophrenia, the importance of interneurons of nitric oxide synthase (NOS) in the regulation of dopaminergic and glutamatergic neurotransmission and on the evidence of the modulatory role of nitric oxide (NO) in behavioral and cognitive activities it is suggested that a nitrergic system hyperactivity may act in parallel to the increase in dopamine neurotransmission and decreased glutamate in schizophrenia. This work investigated the ability of a NOS inhibitor, NG-nitro-L-arginine (L-NOARG) in preventing deficits in prepulse inhibition (PPI) caused by non-competitive antagonists of NMDA receptors, memantine, ketamine and dizocilpine (MK-801)

Methods and Results:

Male Wistar rats (180-300g) received a pretreatment injection i.p. of L-NOARG (40 mg / kg) or saline 1 h before the test and a second treatment of saline or memantine (10 or 17 mg / kg, i.p., 30 min later, N=10-15) in Experiment1, ketamine (6 or 10 mg / kg, s.c., 45 min later, N=12-16) in Experiment2, MK-801 (0.3 mg / kg, i.p., 45 min later, N=7-8) in Experiment3. Each rat was tested with PPI protocol which consisted on five minutes of acclimation (background noise of 65 dB or 57dB), followed by 10 presentations of pulse (white noise of 100 dB or 110 dB, 30s of interval, 40 ms) for habituation and then the PPI test itself consisting on pseudorandom presentations of 64 stimuli: pulse (P), pre-pulse (PP, pure tone, 3 kHz, 69, 73 and 81 dB, 20 ms), PP+P (100 ms between stimuli) and null (no stimuli). The level of PPI in each rat was determined by expressing the PP+P startle response (ASR) as a percentage (%PPI) decrease from pulse-alone ASR. Statistical analysis of %PPI and ASR were performed with repeated measures ANOVAs with stimulus intensity (69, 73, 81 dB) as within factor and treatment as between factor. All procedures were previously approved by the Animal Care and Use Committee of University of São Paulo (229/2005). Analysis of %PPI of experiment1 showed a significant main effects of intensity (F[2.64]=14.548, P

Conclusions:

The non-competitive antagonist of NMDA receptor MK-801 and memantine were able to impair PPI in rats and L-NOARG was able to prevent the memantine effect but not that of MK-801. Results support previous data suggesting that the nitrergic system might modulate glutamatergic mediation of sensory-motor filter.

Keywords: Glutamate, Nitric Oxide, NMDA receptor, Prepulse inhibition, Schizophrenia

Financial Support: Universidade Federal do ABC

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Resumo:23-211

EFFECTS OF NEONATAL HANDLING ON TASTE REACTIVITY IN FEMALE RATS

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Objectives:
Handled animals are known to increased consumption of sweet food when adults, and a different hedonic response has been suggested to explain this phenomenon. Therefore, the purpose of the present study was to determine the effect of neonatal handling on affective facial reactions to sucrose solutions using female rats.

Methods and Results:

Pregnant female Wistar rats were randomly divided into two groups. In the handled group (H), pups were removed from their dams during 10 min/day (incubator at 32°C) from day 0 to post natal day (PND) 10 of life. In the non-handled group (NH), pups were left undisturbed with the dam until weaning (at PND 21). Only females were used in this study. Before puberty, at PND 28, a maximum of two females from each litter were used for taste reactivity test. Rats were placed on top of a mirror for 1 min, in order to habituate the animal. During the following 5 days, a 70 μl volume of water was delivered into the animal’s mouth through a pipette with a plastic tip. On the following day (test), a solution of 0.1M sucrose and 1M sucrose was delivered at different times (30min of interval between tests). Affective facial reactions (tongue protrusions) were recorded on a digital camera for further analysis. Different animals from each litter were used when adults. At PND 60, the stage of the estrous cycle was determined by vaginal smears, and only rats showing regular 4 to 5 days cycle were used. Afterwards, the same protocol described above for testing taste reactivity was performed. Estrous cycle stage was verified soon afterwards. Results reveal that young handled animals (PND 28) showed less positive hedonic reactions (total amount of tongue protrusions) than non-handled ones, when exposed to sucrose 0.1M solution [Student’s t-test, t(23)=2.18, p=0.04, mean +/- S.E.M, NH.= 45.0 +/-4.4 and H = 30.7 +/-4.7], tongue protrusion frequency showed no difference for this concentration [Student’s t-test, t(23)=1.85, p=0.078]. There was no difference for sucrose 1M solution [tongue protrusion frequency Student’s t-test, t(23)= 0.15, P>0.1; total amount of tongue protrusions, t(23)= 0.36, P>0.1]. Adult females were compared according to the stage of the estrous cycle; they showed no difference between groups [two-way ANOVA using estrous cycle and neonatal handling as factors; handling: F(1,21) = 1.80 for protrusion time F(1,21) = 1.35 for protrusion frequency; estrous cycle: F(2,21) = 0.06 for protrusion time and F(2,21) = 0.08 for protrusion frequency; no interactions were observed; P > 0.05 for all cases].

Conclusions:

These results suggest that young rats handled during the neonatal period are less responsive to sucrose in low concentrations. The absence of difference for sucrose 1M solution could be due to a different threshold to sweet taste perception. Adults showed no difference, and further studies are needed.

Keywords: Female rats, Handling, sucrose solution, Taste Reactivity

Financial Support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and FAPERGS

QuebraPagina

Resumo:23-212

MOTOR SIDE EFFECTS INDUCED BY FREE HALOPERIDOL AND HALOPERIDOL NANOENCAPSULATED IN RATS

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2 Faculdade de Ciências Farmacêuticas, UFRGS

Objectives:

The objective of the present study was to evaluate the adverse motor side effects induced by free and nanoencapsulated haloperidol in rats.
Methods and Results:

Drugs: Haloperidol-loaded nanocapsules (H-NC) and blank nanocapsules (B-NC) suspensions were prepared by interfacial deposition of preformed polymer (Int. J. Pharm. 55:1, 1989). A free suspension of haloperidol (0.25 mg/mL) was prepared in water using 5% (w/v) of polysorbate 80. In vivo experiments: motor side effects induced by acute haloperidol administration: 28 adult male Wistar rats were divided in the following groups: C (control), B-NC (blank nanocapsules), FH (free haloperidol) and H-NC (haloperidol-loaded nanocapsules). In the acute study, the rats were treated with their respective formulations (vehicle, free haloperidol, blank nanocapsules and haloperidol nanocapsules – 0.2mg/Kg-IP) and 1h after they were evaluated according with vacuous chewing movements, catalepsy time and locomotor activity (crossing and rearing number). In the subcronic study the rats were treated daily with the formulations during 28 days. Once a week the same behavioral parameters were analyzed.

Results: Duncan’s test performed after one-way ANOVA showed an increase of 95% in the VCM frequency and a decrease in locomotor activity (crossing and rearing) after the acute administration of FH when compared to the C group. Furthermore H-NC treatment decreased the immobility time (31%) in relation to the FH group. Subchronic administration of FH in rats caused motor adverse effects such as VCM, catalepsy and reduction in locomotor activity, while the subchronic treatment with H-NC no developed VCM, and caused lower catalepsy than FH. However, this formulation did not prevent the decrease in locomotor activity.

Conclusions:

Our data demonstrated that the haloperidol nanoencapsulated caused lower motor side effects than that the free drug, demonstrating the advantage of this formulation.

Keywords: haloperidol, motor side effects, nanocapsules

Financial Support: CNPq, CAPES, PRPGP (PROAP-UFSM) and S.S. Guterres - UFRGS

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Resumo:23-213

NICOTINE-INDUCED BEHAVIORAL SENSITIZATION IN ADOLESCENT RATS ENDURES UNTIL ADULTHOOD

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FACULDADE DE CIÊNCIAS FARMACÊUTICAS, UNESP

Objectives:

The aim of this study was investigate whether nicotine-induced behavioral sensitization during adolescence would persist until adulthood.

Methods and Results:

Adolescent rats were injected with saline (1mL/kg s.c.) or nicotine (0.4mL/kg s.c.) once a day for seven days. Three or sixty days after the last nicotine or saline injection, animals were allowed a 20-minute adaptation period to the activity chamber, immediately after this period they were injected with a challenge dose of saline or nicotine (0.4 mg/kg, s.c.) and were returned to the activity chambers where their locomotor activity was recorded during a 20-min testing session. Thus three or sixty days after the last nicotine or saline injection there were three groups (n= 6-8 per group): SAL+SAL; SAL+NIC; NIC+NIC. Data were analyzed by two-away ANOVA followed by Newman-Keuls test. The data are expressed as means ± SEM. Three and sixty days after the last nicotine or saline injections the group NIC+NIC showed a significantly higher locomotor when compared to other groups (Three days: SAL+SAL: 284.5±54; SAL+NIC: 376.6±46; NIC+NIC: 1029.6±145; sixty days: SAL+SAL: 645.2±88; SAL+NIC: 298.4±87; NIC+NIC: 847.8±134; p<0.05).
Conclusions:

Nicotine caused behavioral sensitization that endured until adulthood. Thus nicotine exposure during the adolescence may increase the vulnerability to development of nicotine addiction in adulthood.

Keywords: behavioral sensitization, nicotine, ontogeny

Financial Support: PIBIC/CNPq
Data show that mother deprivation and learned helplessness have opposite effects on DPAG-evoked somatic defensive responses. Therefore, whereas they support the predisposing influence of childhood separation anxiety in panic attacks, they do not support the predisposing effect of reactive depression.

Keywords: Deprivation mother, Learned helplessness, Dorsal periaqueductal gray matter, Depression, Rat

Financial Support: FAPES

Resumo:23-215

CHRONIC FLUOXETINE FAILED TO MODIFY WATER AND FOOD INTAKE, BODY WEIGHT AND DEFENSIVE BEHAVIOURS IN FREE-FEEDING PIGEONS (COLUMBA LIVIA).

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Objectives:

Chronic and acute administrations of Fluoxetine (FLX), a selective 5-HT reuptake inhibitor (SSRI), usually impair the expression of stress-induced behavioral inhibition as well as food intake in non-stressed mammals. In pigeons, under 24-fast-refeeding schedule, acute treatment with zimelidine (also a SSRI) reduced water and food intake. In free-feeding pigeons, acute FLX produce a 24 h-long hypophagic effect. However, the effects of this class of drug on behavioral inhibition were not examined in pigeons. In the present study we investigate the effects of acute or chronic FLX administration on water and food intake as well as the effects of chronic treatment on body weight and behavioral inhibition in free-feeding pigeons.

Methods and Results:

Eighteen pigeons (adults, both sexes, 420-500g bw), individually housed in standard cages (50cmX50cmX50cm) were subjected to 12:12 h light/dark cycle with food and water ad libitum. After a 7-day adaptation period, the animals received daily injection of FLX (s.c., 2.5 or 10mg/kg: n=6/group) or vehicle (0.85 % NaCl, n=6) during the following 15 days. Water and food intake were daily measured, as well as body weight, to calculate the total intake in 24 h per gram of body weight. Independent of the s.c. treatment, all animals lost weight during experimental period. No effects of FLX administration on body weight, water or food intake were observed after acute or chronic treatments. Thirty min after the last injection, pigeons were gently arrested (by the experimenter’s hand for 20 s, maximum of 5 attempts) to induce tonic immobility (TI, minimum of 10 s and maximum of 12 min). Chronic treatment with different doses of FLX did not altered the duration of TI (Kruskal–Wallis p>0,05) or the number of attempts to obtain the immobility (Kruskal–Wallis p>0,05).

Conclusions:

Different to data previously collected in free-feeding pigeons, acute administration of FLX failed to alter food intake. Similar to previous results water intake remained unaffected after acute FLX. Chronic FLX did not change significantly food or water intake. It was not possible to observe alterations in the body weight induced by chronic FLX probably due to the strong effect of s.c. injection on this variable. Furthermore, chronic FLX failed to attenuate the behavioral inhibition (TI) in pigeons. This may suggest that behavioral inhibition in birds may not be sensitive to modifications in the 5-HT reuptake system. Alternatively 5-HT reuptake mechanisms of pigeons may be less vulnerable to fluoxetine.

Keywords: Fluoxetine, Food intake, Defensive behaviours
Objectives:
The elevated T maze (ETM) is an animal model that evaluates two behaviours related to anxiety disorders: acquisition of open arm avoidance, related to generalized anxiety, and escape from the open arm, related to panic disorder. Since cannabinoids may interfere with anxiety-related responses, the aim of this study was to evaluate the participation of cannabinoid CB1 receptors in this model. The synthetic cannabinoid WIN55,212-2 and the CB1 antagonist AM251 were used in this study.

Methods and Results:
Male Wistar (200-300g) were gently handled by the experimenter for 3 min during three consecutive days, on the fourth day, each animal was pre-exposed to one of the open arms of the model for 30 min. Twenty-four hours later, the animals were divided in four experimental groups and received the following treatments (n=7-9/group): vehicle + vehicle (Chremophor, ethanol and saline 1:1:18); vehicle + WIN 55-212 (1.0mg/kg); AM251(1.0mg/kg) + vehicle and AM251 + Win 55-212. Thirty minutes after intraperitoneal injection, each animal was placed at the distal end of the enclosed arm of the ETM facing the intersection of the arms. The time taken by the rat to leave this arm with the four paws was recorded. The same measurement was repeated in two subsequent trials at 30-s intervals. Following avoidance test (30 s), rats were placed at the end of the open arm where they had been previously exposed and the latency to leave this arm with the four paws was recorded for three consecutive times (escapes 1–3) with 30 seconds inter-trials intervals. Two-way ANOVA was used to analyze both avoidance and escape data followed by the Bonferroni post hoc test. Two-way ANOVA revealed a significant trial effect (p

Conclusions:
Our results demonstrate that the anxiolytic-like effects of cannabinoids in the elevated T maze are mediated by CB1 receptors. This result corroborates data obtained in the elevated plus maze.

Keywords: Anxiety, Cannabinoid, Elevated t maze, Panic

Financial Support: Capes; FAPEMIG
Objectives:

Reconsolidation is a dynamic and adaptive process allowing the incorporation of new information into consolidated memories. Typically, this event is triggered by memory reactivation that occurs during its retrieval, rendering the original memory once more susceptible to pharmacological interferences. Based on this premise, that reconsolidation would be impaired by the administration of an amnesic drug after the memory reactivation, the objective of the present study was to determine whether scopolamine, a non-selective muscarinic cholinergic receptor antagonist, would disrupt the reconsolidation of the contextual memory acquired by fear conditioning in rats.

Methods and Results:

The protocol consisted of familiarization (3 min), fear conditioning (0.5 mA, 3 s, inter-shock interval: 30 s), reactivation (3 min) and test (3 min) in the context chamber A. In order to evaluate a possible generalization process, animals were exposed to the context B, a neutral chamber that was also used in the non-reactivated protocol, 24 h after the test in A. Contextual fear memory was inferred from the percentage of freezing behavior. In experiment 1, independent groups (n= 7-9/group) of male Wistar rats, aged three-months, received a systemic administration of vehicle (NaCl 0.9%) or scopolamine hydrobromide (SCO, 0.175-3.0 mg/kg) immediately after reactivation, and were tested in A 24 h later. In experiment 2, other groups (n= 10/group) were submitted to B, received vehicle or SCO (1.5 mg/kg) and tested in A 24 h later. The experimental design aforementioned was approved by the local Ethical Committee in Animal Research (23080.016341/2010-30/CEUA/PRPe/UFSC). One-way repeated-measure analysis of variance followed by Newman Keuls test showed that animals administered with 0.75, 1.5 or 3.0 mg/kg of SCO after the reactivation decreased (p

Conclusions:

The present results suggest that SCO attenuates the expression of freezing, possibly owing to its disruptive effect on the reconsolidation of a contextual fear memory.

Keywords: RECONSOLIDATION, SCOPOLAMINE, FEAR CONDITIONING, MEMORY, FREEZING

Financial Support: CNPq, CAPES, FAPESP and FAPESC

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Resumo: 23-218

GHRELIN PREVENTS COGNITIVE, EMOTIONAL AND NEUROCHEMICAL ALTERATIONS IN A MOUSE MODEL OF ALZHEIMER’S DISEASE

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Objectives:

Increased brain deposition of amyloid β protein (Aβ) and cognitive deficits are classical signs of Alzheimer’s disease (AD) that have been widely associated to oxidative stress and cholinergic neurotransmission alterations. On the other hand, recent evidences indicate that Ghrelin (Ghr), a 28 amino acid peptide hormone produced from the stomach and hypothalamus that promotes positive energy balance, presents neuroprotective and memory-enhancing properties that may be useful in AD.
Methods and Results:

In the current study, we assessed the molecular mechanisms, mainly the modifications in oxidative stress parameters and acetylcholinesterase (AchE) activity, whereby the pretreatment with Ghr prevents the Aβ1–40-induced depressive-like behavior and cognitive impairments in mice. Swiss adult mice (3 months old) received a single intracerebroventricular (i.c.v.) administration of Ghr (3 nmol/μl) or PBS (1 μl) 15 min before the i.c.v. infusion of Aβ1–40 (400 &μmol/mice) or PBS (1 μl) and 10 and 14 days later they were evaluated, respectively, in the tail suspension and step-down inhibitory avoidance tasks. Independent groups of animals, submitted to the same treatments, were sacrificed 24 h after Aβ1–40 infusion and cortical and hippocampal AchE activity and oxidative stress parameters were analyzed. The pretreatment with Ghr prevented the development of depressive-like behavior and spatial learning and memory impairments in Aβ1–40-infused mice. Moreover, Aβ1–40 reduced significantly the cortical activities of the antioxidant enzymes glutathione peroxidase, glutathione reductase and catalase while promoted a marked increase in the cortical AchE activity and hippocampal lipid peroxidation. Of high importance, the pretreatment with Ghr prevented all these neurochemical changes induced by Aβ1–40 administration in mice.

Conclusions:

Altogether, our findings demonstrate that Ghr can ameliorate Aβ-induced cognitive and emotional impairments associated with oxidative stress and cholinergic neurotransmission alterations in mice. Therefore, Ghr may represent a promising therapeutic agent for the treatment of AD.

Keywords: ghrelin, Alzheimer’s disease, cognitive impairments, oxidative stress, acetylcholinesterase

Financial Support: CNPq, CAPES-SPU, FAPESC

QuebraPagina

Resumo:23-219

PROTECTIVE EFFECTS OF ENVIRONMENTAL ENRICHMENT ON STRESS-INDUCED ANXIETY IN RATS CAN BE MEDIATED BY CHANGES ON EXPRESSION OF MINERALOCORTICOID AND GLUCOCORTICOID RECEPTORS IN FRONTAL CORTEX, HIPPOCAMPUS, AND BASOLATERAL AMYGDALA.

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DEPARTAMENTO DE FARMACOLOGIA, ICB / USP

Objectives:

Environmental enrichment (EE) is an experimental paradigm that increases sensory, motor, and cognitive stimulation of animals when compared to those living in standard housing. Reported benefits of EE include improvements in learning and memory, and in motor tasks; and decrements in stress-induced anxiety-like behavior. It is broadly known that different types of stressors cause endocrine responses mediated by the hypothalamic-pituitary-adrenal (HPA) axis, with increased releasing of corticosteroids. However little is known about the molecular mechanisms by which these hormones exert their effects on anxiety-like behavior. Nevertheless, recent studies have shown changes in the expression of both mineralocorticoid (MR) and glucocorticoid (GR) receptors in the limbic system of rats submitted to stress, but it is unclear if the protective effects of EE is due to alterations in the expression of these two receptors. Here, we sought to verify whether the protective effects of EE on stress-induced anxiety could be related to changes in expression of MR and GR on the frontal cortex (FC), hippocampus (HP), and basolateral amygdala (BLA) of male adult rats.

Methods and Results:
To examine the effect of EE on anxiety-like behavior in stressed adult male Wistar rats, animals were housed in enriched cages or in standard cages during 14 days. On fifteenth day, half of the enriched group was submitted to acute immobilization stress (ES) for 1h and then tested in the elevated plus maze paradigm (EPM) for 5 minutes, while the other half was tested without suffering stress (EC). The same was made with animals housing in standard cages (SS and SC). In the EPM, EC and ES animals, as well as SC animals, spent more time in the open arms and had higher percentages of entries into the open arms compared to the SS animals (%. p < 0.05). Moreover, no differences in these parameters were found among SC, EC and ES groups. Additionally, we investigated the possible changes induced by EE on the expression of MR and GR on FC (nuclear extracts), HP (nuclear extracts), and BLA (total fraction) in stressed rats. For this, the same EE paradigm was performed but the animals were subjected to 2 hours acute immobilization stress. By Western blot analysis, we found that ES animals had increased expression of GR and MR compared to SS animals on both FC and HP. However, on the BLA of ES animals, the GR expression was increased while the MR expression was decreased. Statistical analysis: all data were expressed as the mean ± SEM. Experiments were subjected to one-way ANOVA, followed by Student’s t posthoc test for behavioral experiments (N = 6-8 per group), or by Newman-Keuls test for biochemical analysis (N = 4-6 per group). The level of statistical significance was set at P < 0.05.

Conclusions:
Overall, the present data suggest that the protective effect of EE on anxiety-like behavior in rats exposed to acute stress could be due to modulation of corticoid receptors in different areas of the brain, such as FC, HP, and BLA.

Keywords: ENVIRONMENTAL ENRICHMENT, ANXIETY, STRESS, CORTICOSTERONE RECEPTORS, GLUCOCORTICOID

Financial Support: FAPESP and CNPq

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Resumo:23-220

EFFECT OF ACUTE EXPOSURE TO ARGININE ON ECTONUCLEOTIDASES AND ADENOSINE DEAMINASE ACTIVITIES IN ZEBRAFISH BRAIN MEMBRANES (DANIO RERIO).

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Objectives:
The tissue accumulation of arginine is a biomarker for inborn error of metabolism called hyperargininemia caused by deficiency of the enzyme arginase in the liver. The main symptoms of patients affected by this disease are: progressive dementia, mental retardation, hyperactivity, short stature, and epilepsy. Several mechanisms have been proposed to describe the neurotoxicity of hyperargininemia, such as changes in antioxidant enzyme systems and neurotransmission. Zebrafish is a small teleost of the Cyprinidae family that has been widely used for neurotoxicity studies. Considering that zebrafish is an useful model for studying neurological diseases through development and the purinergic system is altered in animal models of inborn errors of metabolism, the aim of this study was to evaluate ectonucleotidases and adenosine deaminase activities in an acute model of hyperargininemia in zebrafish brain membranes.

Methods and Results:
For acute treatment, animals were exposed to different concentrations of arginine (0.1, 1, 1.5 mM) in 3L-tanks for 1h. After the treatment, the animals were crioanesthesized and their brains were dissected. A sample containing five brains was homogenized
in 60 volumes (v/w) of a Tris-citrate buffer (50 mM Tris, 2mM EDTA and 2mM EGTA, pH 7.4) for each membrane preparation. The analysis of NTPDase, ecto-5'-nucleotidase, ADA activities was performed (Life Sci. 5;73(16):2071-82, 2003, J Leukoc Biol. 83(5);1079-87, 2008). There was no significant changes on NTPDase [ATP: F (3.20) = 0.219; ADP: F (3.20) = 1.096, P> 0.05] and ecto-5'-nucleotidase [F (3.19) = 0.118, P> 0.05] activities after acute exposure to arginine when compared to the control group. The results showed that arginine decreased the ecto-ADA [F (3.20) = 6.44, P < 0.05] (n = 6) compared with the control group (11.33 ± 2.2 nmol NH3 min-1 mg protein-1). The analysis of NTPDase, ecto-5'-nucleotidase, ADA activities was performed (Life Sci. 5;73(16):2071-82, 2003, J Leukoc Biol. 83(5);1079-87, 2008). There was no significant changes on NTPDase [ATP: F (3.20) = 0.219; ADP: F (3.20) = 1.096, P> 0.05] and ecto-5'-nucleotidase [F (3.19) = 0.118, P> 0.05] activities after acute exposure to arginine when compared to the control group. The results showed that arginine decreased the ecto-ADA [F (3.20) = 6.44, P < 0.05] (n = 6) compared with the control group (11.33 ± 2.2 nmol NH3 min-1 mg protein-1).

Conclusions:

These results indicate that acute treatment with arginine was unable to significantly alter NTPDase and ecto-5'-nucleotidase. However, the data showed that arginine was able to inhibit adenosine deaminase, suggesting a modulatory role on extracellular adenosine levels during acute treatment with arginine.

Keywords: Adenosine deaminase, Arginine, Ectonucleotidases, Zebrfish

Financial Support: CNPq, FAPERGS.

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Resumo:23-221

PROLONGED EXPOSURE TO CAFFEINATED ALCOHOLIC SOLUTIONS PREVENT THE ALCOHOL DEPRIVATION EFFECT IN RATS

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Objectives:

There is growing evidence showing that the consumption of alcohol mixed with highly caffeinated beverages may increase the risk for heavy drinking and of alcoholism (Hum Psychopharmacol Clin Exp. 24:473, 2009). Several studies have shown that caffeine can increase the behavioral effects of a variety of drugs, including alcohol. The purpose of this study was evaluate the pattern of ethanol consumption/preference and the propensity to show an alcohol deprivation effect in rats exposed to voluntary intake of caffeinated alcoholic solutions.

Methods and Results:

Male Wistar rats (approximately 2 months of age) were individually housed and given unlimited access in a two-bottle choice procedure adapted from Yoneyama and collaborators (Alcohol. 42(3):149, 2008). Rats given free access to two-bottle, one bottle containing tap water and another contain increasing concentration of ethanol solutions (EtOH 0%, 3%, 6%, 10% v/v). They were divided into four groups (7-8/group): EtOH; EtOH + 1% saccharin; EtOH + 1 g/L caffeine and EtOH + 1% saccharin + 1 g/L caffeine. Each EtOH concentration was made available for 4 days to reach the 10% level, reaching a total duration of 54 days. Alcohol deprivation effect, considered a predictive measure of relapse to alcohol (Heyser et al., 1997), was assessed after an abstinence period of 7 days, when the animals were again exposed to their respective alcoholic solutions for 24 hours. It was observed that all animals drank more saccharin solution in free-choice with water, indicating that the presence of caffeine in the solutions did not alter the preference to saccharin. None of the experimental groups preferred to drink ethanol compared to regular tap water. Curiously, it there was no significant difference in alcohol consumption between the groups exposed to sweetened solutions with or without caffeine. Overall, both groups drank significantly more alcohol during whole free-choice procedure (mean of EtOH consumption: 3.84±0.8 and 4.29±1.2 g/kg/day; EtOH + 1% saccharin and EtOH + 1% saccharin + 1 g/L caffeine, respectively) compared to other groups (mean of EtOH consumption: 1.07±0.6 and 0.96±0.4 g/kg/day; EtOH and EtOH + 1 g/L caffeine, respectively). Only the group exposed to EtOH + 1% saccharin solutions showed a slight increase in the ratio of alcohol intake/total fluid intake, when access to alcohol is reinstated. This result suggests that long period of caffeine-exposure possibly prevented the alcohol deprivation effect. Furthermore, rats exposed to caffeinated alcoholic solutions without
saccharin showed a reduction of ethanol intake compared to animals that consume EtOH plus caffeine and saccharin (P≤0.05).

Conclusions:

Our findings indicate that presence of caffeine in alcoholic solutions did not increase EtOH consumption, but prevent the alcohol deprivation effect in rats. Sensory modalities (taste, odor, and chemosensory irritation) play an important role on the acceptance or rejection of oral alcohol consumption in humans and rodents, and they also may have contributed for these results. Certainly further studies are needed to clarify whether the association of energy drinks and alcohol as a risk factor for development of alcoholism.

Keywords: alcohol consumption, caffeine, deprivation, saccharin

Financial Support: CAPES and CNPq

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**Resumo:**

**INVOLVEMENT OF 5-HT1A RECEPTORS IN THE ANTIDEPRESSANT-LIKE EFFECT OF ESCITALOPRAM IN THE FORCED SWIMMING TEST**

Universidade Federal de Santa Catarina, UFSC

Objectives:

Escitalopram is a specific serotonin reuptake inhibitor used in the treatment of depression and anxiety disorders. The present study was aimed at investigating the effect of escitalopram administered by oral (p.o.) route in the forced swimming test (FST) in mice and the involvement of 5-HT1A receptors in the antidepressant-like effect.

Methods and Results:

Female Swiss mice (30-40 g, n = 6-8/group) weighing 30–40g maintained at 21-23°C with free access to water and food, under a 12:12 h light:dark cycle (lights on at 7:00h) were used. In the FST, animals were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm), containing 19 cm of water at 25°C and the immobility time was registered during 6 min. In order to rule out non-specific influence of locomotor activity in the antidepressant-like effect of escitalopram alone or in combination with the pharmacological antagonists, mice were submitted to the open-field test. Comparisons between treatment groups and control were performed by ANOVA followed by Tukey’s HSD test when appropriate. A value of P

Conclusions:

Escitalopram produced an antidepressant-like effect after p.o. administration in the FST in mice, at doses that did not affect locomotor activity. Moreover, the results indicate that the antidepressant-like effect of escitalopram seems to be direct or indirectly mediated by an interaction with 5-HT1A receptors.

Keywords: depressão, escitalopram, receptor 5-HT1A , teste do nado forçado

Financial Support: CNPq, CAPES, UFSC, FINEP-IBN-Net

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**QuebraPagina**
Objectives:

We examined if a single dose of cocaine (10 mg/kg; i.p.) would alter the availability of excitatory amino acids analyzing the mechanisms of uptake of [3H]-aspartate.

Methods and Results:

Swiss mice of 10 days post natal received a single dose of cocaine 10 minutes before sacrifice. Cerebral cortex was dissected and samples were incubated for 1 h in 1 ml of Minimum Essential Medium (MEM) containing 1µCi [3H]-D-Aspartate (1.2 x 10^-6M) buffered to pH 7.4 with 20mM HEPES at 37°C or 4°C (to block aspartate active transport). In other experiments H-89 (1 &muM) or SQ22536 (1&mu M) were added during the incubation. To measure de AMPc levels in saline and cocaine groups, samples were dissected and incubated with 500µ M phosphodiesterase inhibitor(IBMX 500µ M; 30min). The reaction was stopped with trichloroacetic acid (final concentration 10%) and cAMP accumulation was assayed. We show that a single intraperitoneal administration of cocaine produces significant decreases in [3H]-aspartate uptake in the mouse frontal cortex (4,11 ±0,2114 pmol/mg ptn, n=6 control versus 2,496±0,2779 pmol/mg ptn, n=8, cocaine) . The decrease in [3H]-aspartate uptake is associated with elevated dopamine levels and requires dopamine D1-receptor signaling, since the blockage with its antagonist, SCH 23390, prevented de effects of cocaine (3,253±0,4362 pmol/mg ptn; n=4 e 2,496±0,2779 pmol/mg ptn, n=8) and adenylyl cyclase activation. Inhibition of PKA inhibits cocaine’s effects on the [3H]-aspartate uptake (4,518±0,4767 /pmol/mg ptn n=5 and 2,496±0,2779 pmol/mg ptn, n=3).

Conclusions:

Cocaine’s effects are mediated via cAMP-induced PKA-driven phosphorylation of aspartate/glutamate transporter. The rapid and short-lived decreases in [3H]-aspartate uptake may facilitate drug-environment pairing as a result of rapid elevation of excitatory amino acid content in the frontal cortex.

Keywords: cocaine, cortex, D1, glutamate, transporters

Financial Support: FAPERJ, CNPq, Pronex, INCT

QuebraPagina

Resumo:23-224

STRIATAL 6-OHDA-INDUCED LESION PROMOTES EMOTIONAL AND COGNITIVE CHANGES IN RATS

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Objectives:

The evaluation of animal models of Parkinson's Disease (PD) during pre-motor stage is important to examine the progression of the disease and it can lead to the improvement of existing therapies. The aim of this study was to assess the effect of low dose of 6-OHDA-induced lesion on behavioral tests assessing cognition, emotional reactivity and motor function, one to three weeks after surgery in rats.

Methods and Results:

Male Wistar rats were submitted to stereotaxic surgery to intrastriatal administration of 6-OHDA as described previously in our group (Neurosci 156:830, 2008). Initially, oral sucrose consumption was tested as a measure of anhedonic-like behavior two weeks after the surgery. The animals were kept into single home cages with free access to food. After 24-h training phase (with two water bottles), one bottle was switched to contain 0.8% sucrose solution, and 24 h later, the bottles were reversed, totaling 48-h testing period. The fluid consumption was measured by weighing the bottles. Independent rat groups were subjected to object location memory task in order to assess spatial memory and locomotor activity one week after the surgery. This test consisted of a period of habituation (without objects) 24 h before trials, a trial T1 (presentation of two identical objects placed in two adjacent corners for 2 min) and a discrimination trial T2 (one of the two similar objects was moved to a different location) with intertrial of 20 min. During habituation, the animals were free to explore the apparatus, which consisted of an open field arena for 10 min. Their locomotor activity and anxiety-like behavior (distance travelled, average speed and time in central zone), during the initial 5 min, was analyzed with the ANY-Maze program, and repeated two weeks later. The time spent by rats exploring each object during T1 and T2 were recorded. The discrimination between familiar location (FL) and new location (NL) during T2 was measured and a discrimination index (D) was calculated (D=NL−FL/NL + FL). Rats injected with 6-OHDA drank significantly less sucrose than sham group (178.1 ± 14.35 vs. 131.5 ± 8.53 ml/kg; sham vs. 6-OHDA, respectively; P<0.02 ± 0.16 D; sham vs. 6-OHDA, respectively; P

Conclusions:

Together, these data indicate that striatal 6-OHDA-induced lesion promotes emotional alterations and cognitive impairments without affecting locomotor activity in rats. Our results confirm and extend the previous data that partial degeneration of dopaminergic neurons of substantia nigra may resemble premotor PD model and can be useful to evaluate possible pharmacological manipulations and to study the neurobiological mechanisms underlying the initial symptoms of PD.

Keywords: 6-OHDA, Anhedonia, Memory, Parkinson disease, Striatum

Financial Support: CAPES and CNPq.

QuebraPagina

Resumo:23-225

THE MONOTERPENIC KETONE PULEGONE POSSESS PSYCHOSTIMULANT BUT NOT REWARD PROPERTIES

INSTITUTO DE CIÊNCIAS BIOMÉDICAS, UFU

Objectives:

Pulegone, a monocyclic monoterpenic ketone present in the peppermint oil, increases mice locomotor activity. This psychostimulant effect is sensitive to dopamine receptor antagonists, suggesting the involvement of this neurotransmitter in the
ambulation promoted by pulegone. Thus, considering the important role of dopamine in the regulation of both movement and reward, we assessed the hypothesis that pulegone has psychostimulant, as well as reward properties.

Methods and Results:

The experiments were conducted using male Swiss mice (35-45 g; n=6-8) treated with pulegone (100 – 800 mg/Kg, i.p.) or vehicle (olive oil). The locomotor activity (number of floor units entered by the animal with all four legs in 5 min) was determined in the open-field. The motivational properties of the drug were evaluated by pairing pulegone effects with the mice less preferred compartment (previously determined) of a conditioned place preference (CPP) apparatus. An increase in the time (s) spent by animals on the non-preferred compartment after drug treatment defines a CPP. Pulegone increased the locomotor activity only at the dose of 200 and 400 mg/Kg (146±12 vs. 210±19 and 244±21, vehicle, 200 and 400 mg/Kg of pulegone, respectively; P<0.05). The dose of 400 mg/Kg of pulegone was not tested in the CPP paradigm since it was extremely toxic (mortality within 24 h: 60%).

Conclusions:

Our results suggest that pulegone has psychostimulant but not rewarding properties.

Keywords: conditioned place preference, Pulegone, Reward

Financial Support: CNPq

QuebraPagina

Resumo:23-226

GABA SYSTEM CHANGES IN METHYLPHENIDATE SENSITIZED FEMALE RATS

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Objectives:

Methylphenidate (MPD) is a psychostimulant that is prescribed to treat attention-deficit/hyperactivity disorder and has been used as a recreational drug. In animal models, repetitive exposure to MPD can induce a behavioral sensitization that is similar to cocaine and methamphetamine. Stimulants are able to change neuronal circuits in the mesolimbic pathway, and the GABA system is one of the most involved neurotransmitter systems in this process. Women represent a risk group for psychostimulant abuse because they respond more strongly to the drug, which is probably due to the influence of sex hormones. The objective of the present study was to investigate the influence of sex hormones on behavioral sensitization and changes to glutamic acid decarboxylase (GAD65 and GAD67) isoenzymes and α2 GABAA receptor subunit mRNA expression in the prefrontal cortex and the striatum of rats, as induced by MPD administration.

Methods and Results:

Female adult Wistar rats were randomly assigned to the ovariectomized (OVX) or intact ovary (INT) animal group. Rats were subdivided in controls (CTR), acute (ACT) or repeated (RPT) methylphenidate (MDP) treatment, with 8 rats for group. For 5 consecutive days, rats of the RPT group were daily administered with 2.5mg/kg of MDP solution and ACT and the CTR groups received 1mg/kg of saline solution, via intraperitoneal. After a 7-day washout period (day 12° of the experiment), rats from the ACT and RPT group received the challenge dose of MPD, and the CTR group received saline and the horizontal activity was scored for 60 min and behavior were recorded. The photocell cage was coupled with a digital counter that counted the consecutive interruption of adjacent beams. At the end of the behavior sensitization test, rats were euthanized by decapitation and the dorsolateral striatum (dSTR) and the prefrontal cortex (PFC) were dissected and stored at -80ºC for further mRNA analysis. Relative RT-PCR was performed to measure gene expression of GAD65 and GAD67 mRNA and the presence of the α2 GABAA
subunit. The statistical analysis was performed using a two-way ANOVA followed by the Tukay test. A p value of less than 0.05 was considered significant. Our results showed repeated MPD treatment led to behavioral sensitization, which was stronger in females with circulating hormones (INT group), with a significant (F(2.66) = 10.287; P = 0.002) interaction between treatment MPD and hormonal condition. The analysis of mRNA levels in the dSTR, in both groups, showed a decline in GAD65, (F(2.25) = 7.056; P = 0.006) but not GAD67, transcription after repeated MPD treatment compared to the CTR group. In the PFC, both treatment group, ACT and RPT (F(2.11) = 6.131; P = 0.005), showed an increase in GAD65 mRNA expression compared to CTR and GAD67 mRNA levels were increased in the RPT group (F(2.34) = 5.632; P = 0.008). There was also no difference between O VX and INT in neurochemical analysis and no change in the transcription level of α2 GABA A receptor subunits.

Conclusions:

Here we showed that sex hormones were able to modify behavioral sensitization to MPD, and MPD affected the GABA system in brain areas known to be involved in the development of drug dependence. In closing, needs more investigation with respect to mechanisms of action of MDP in both males and females, and a simple comparison with other psychostimulants will not suffice.

Keywords: GABA system, gonadal steroid hormones, prefrontal cortex, sensitization, striatum

Financial Support: CAPES, CNPq, UFCSPA

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Resumo:23-227

EFFECT OF PINEALECTOMY IN MPTP AND 6-OHDA-LESIONED RATS

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Objectives:

To determine the effects of pinealectomy (Px) in MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and 6-hydroxydopamine (6-OHDA)-lesioned rats we used the open field test and forced swimming.

Methods and Results:

Rats were randomly divided in six groups (n=8/group): Sham+Sham, Sham+MPTP, Sham+6-OHDA, Px-Sham, Px-MPTP and Px-6-OHDA. All animals were submitted two surgical procedures with 10 days of interval. First, the pinealectomized rats were anesthetized and then the pineal gland was removed using the standard procedure. Rats in the Sham-operated groups underwent similar surgical procedures without the removal of the pineal gland. After 10 days, these animals received bilaterally 1μl of MPTP, 6-OHDA or saline within the substantia nigra pars compacta. One, seven and fourteen days after surgery the rats were evaluated in the open-field test. Fifteen days after the surgery the rats were evaluated in the forced swimming test. One day after the surgery, 6-OHDA lesioned rats (both groups Sham and Px, respectively) exhibited increased immobility time (72.7 ±12.54; 72±16.27) and low locomotion frequency (23.37±6.40; 34.60±7.68) in comparison to Px-Sham group (immobility time: 14±3.83; locomotion frequency: 84±8.33). Besides, Px-6-OHDA animals showed increased latency to start the movement time (16.5±2.46) when compared with Sham-Sham group (4.87±0.74) and Px-Sham (4.16±0.70). Sham-MPTP group exhibited low rearing frequency (3.33±0.72) and increased latency to start the movement time (16.1±3.25) compared to Sham-Sham (1±0.51; latency 35.5±8.24). Fifteen days after surgery the rats were evaluated in the forced swimming test. Seven days after the stereotaxic surgery Px-6-OHDA group exhibited increased latency to start the movement time (9.83±2.02) in comparison to Sham-Sham (4.05±0.74) and Px-Sham (4.05±0.74). Fourteen days after surgery 6-OHDA-lesioned rats (Sham and Px, respectively) showed increased latency to start the movement time (6.14±0.91 and 5.33±1.02) and immobility time (93.33±20.53) compared to Sham-Sham group (latency 1±0.51; immobility 35.5±8.24). Fifteen days after
surgery in the forced swimming test Px-6-OHDA animals showed low swimming time (82.74±5.03) in comparison to Sham-Sham (171.22±9.96), Sham-MPTP (138.12±8.65), Px-Sham (176.80±6.73) and Px-MPTP (136.48±13.14) groups. Besides Px-6-OHDA group exhibited increased immobility time (124.66±12.11) when compared both Sham groups (Px-Sham 49.20±13.19; Sham-Sham 54.57±10.57). Sham-6-OHDA group showed similar behavior with low swimming time (107.5±9.15) in comparison to Sham-Sham and Px-Sham.

Conclusions:

The MPTP and 6-OHDA-lesioned rats exhibited reduced motor function 24h after the surgery in comparison to both Sham groups in the open field test. Seven and 14 days after surgery Px-6-OHDA animals maintained behavioral alterations. Besides 6-OHDA-lesioned rats, but no MPTP-lesioned rats showed behavioral alterations at forced swimming test suggesting that 6-OHDA neurotoxin change the neurotransmission involved in depression signal in PD.

Keywords: 6-hidroxidopamine, MPTP, Neurotoxicity, Parkinson's disease, Pinealectomy

Financial Support: CAPES and CNPq

QuebraPagina

Resumo:23-228

FACILITATION OF NITRERGIC NEUROTRANSMISSION WITHIN THE MEDIAL PREFRONTAL CORTEX PRODUCES ANXIOGENIC-LIKE EFFECTS IN MICE EXPOSED TO THE ELEVATED PLUS-MAZE.

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Objectives:
The medial prefrontal cortex (mPFC) is a limbic structure that has been markedly related to the neurobiology of anxiety states. Among the neurotransmitters involved in the mediation of emotional responses in the mPFC, the atypical neurotransmitter nitric oxide (NO) has been recently investigated due to its proaversive actions in different brain structures of rodents. Considering that the mPFC contains nitrergic neurons, it becomes relevant to investigate whether NO exerts anxiogenic-like actions in this structure. The present study investigated the effect of intra-mPFC microinjection of a NO donor, NOC-9 (6-(2-hydroxy-1-methyl-2-nitrosohydrasino)-N-methyl-1-hexanamine), on the anxiety of mice exposed to the elevated plus maze (EPM).

Methods and Results:

Swiss male mice received surgical implantation of stainless steel guide cannulae intra-mPFC. Five days after surgery, they received intra-mPFC injection of vehicle (Veh, 0.2 µl, n= 6) or NOC-9 (N9, 75 nmol/0.2 µl , n= 5) and five minutes later each mouse was placed at center of the EPM to record anxiety indices [percentage of open arm entries (%OE) and percentage of open arm time (%OT)] and locomotion (frequency of closed arm entries). Results showed that intra-mPFC NOC-9 produced a borderline effect on %OT (p= 0.07; veh: 16.6±4.9, N9: 5.5±1.2) without changing locomotor activity (veh: 9.0±1.7, N9: 10±2.4, p= 0.78). Intra-mPFC NOC-9 did not significantly alter %OE (veh: 34.0±4.9, N9: 24.6±4.0, p = 0.17)

Conclusions:
The present results suggest that the facilitation of nitrergic transmission within the mPFC through local infusion of NO donor NOC-9 leads to an increase in the anxiety-like behavior in mice exposed to the EPM. However, it is necessary to increase the sample size of both experimental groups to confirm this assumption.

Keywords: anxiogenic-like effects, defense reactions, elevated plus-maze, medial prefrontal cortex, nitrergic neurotransmission
ANALYSIS OF TWO PROTEINS RELATED TO PLASTICITY (EGR-1, SYNAPSIN) IN THE STRIATUM AFTER AN EMOTIONAL MEMORY TASK.

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Objectives:

Previous studies from our laboratory showed that dorsal striatum (DS) is related with tone fear conditioning (TFC). However, we could not completely rule out the involvement of the hippocampus as the contextual features of the training can still influence the contextual learning process. In order to exclude a possible interference of context on the proteins expression in the striatum, the dorsal hippocampus was inactivated during training with AP-5 (antagonist NMDAr). Thus, the purpose of the present study was to evaluate the effect of TFC training on the expression of Egr-1 and Synapsin in the striatum. Egr-1 is immediate-early gene used as marker for neuronal activity and synapsin is a protein involved in neurotransmitter release.

Methods and Results:

Male Wistar rats (3 months old) underwent surgery to receive a guide cannula into the hippocampus. All behavioral tasks were conducted after a recovery period of 10 days. Five minutes before the training, the animals were injected with AP-5 (2.5 μg/μl) or saline and then subdivided into 3 groups, accordingly to training type: injection control; non-paired (context and sound without foot-shock) and paired (5 tone foot-shock pairings; 0.8mA; ITI 30s). Thirty minutes (experiment 1) or 90 minutes (experiment 2) after training, the striatum was dissected for WB analysis. The striatum was homogenized using a lysis buffer and protein inhibitors. The samples were separated in a 10% acrylamide gel and transferred to a nitrocellulose membrane. Egr-1 and synapsin were detected using specific antibodies and Odyssey Infrared imaging system. The expression level of each protein was normalized by β-actin. The results were analyzed using two-way ANOVA. Two-way ANOVA test showed that the relative expression of Egr-1 were not altered between animals that received saline or AP-5 (drug factor) (F(1,30)=0.66212, p=0.42), by the training type (F(2,30)=0.36582, p=0.70) nor interaction drug X training type (F(2,30)=0.62997, p=0.54) after 30 minutes. Similar results were observed after 90 minutes (drug (F(1,30)=0.00452, p=0.95); training type (F(2,30)=0.48583, p=0.62); interaction drug X training type (F(2,30)=1.0155, p=0.37)). Synapsin relative expression by two-way ANOVA did not report a significance between drugs (F(1,24)=0.01987, p=0.89), training type (F(2,24)=1.3463, p=0.28) and interaction drugs X training type (F(2,24)=0.64068, p=0.54) for 30 minutes and did not report a significance between groups (F(1,24)=0.57894, p=0.45), type of training (F(2,24)=1.1637, p=0.33) and interaction group X type of training (F(2,24)=1.0588, p=0.36) for 90 minutes.

Conclusions:

These preliminary results suggest that these protein expressions in the striatum are not related with TFC after 30 or 90 minutes after training.

Keywords: striatum, tone fear conditioning, egr-1, synapsin

Financial Support: FAPESP, AFIP, CAPES
DIFFERENTIAL EFFECTS OF ATORVASTATIN TREATMENT AND WITHDRAWAL ON PENTYLENETETRAZOL-INDUCED SEIZURES

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Objectives:
Statins are selective inhibitors of 3-hydroxyl-3-methyl-glutaryl coenzyme A reductase, the rate limiting enzyme of the mevalonate pathway for cholesterol biosynthesis. Increasing evidence indicates that statins, particularly atorvastatin, are neuroprotective in several conditions, including stroke, cerebral ischemia and traumatic brain injury. However, only a few studies have investigated whether statins modulate seizure activity. In the current study we investigated whether atorvastatin and simvastatin chronic treatment and withdraw alters seizures induced by pentylenetetrazol (PTZ), a classical convulsant agent widely used in discovery of novel anticonvulsant drugs.

Methods and Results:
Adult male Wistar rats (250-300 g, n=10-12 per group) were treated with atorvastatin (10 mg/kg/day, p.o.), simvastatin (10 mg/kg/day, p.o.) or vehicle (0.9 % NaCl, p.o.) for seven days. On the seventh or eighth day after starting the treatment the animals were injected with PTZ (60 mg/kg, i.p.) and observed for 15 min for the appearing of clonic and generalized seizures. Seizure activity and its modulation by statin treatment was confirmed electrographically in a subset of animals (n=5 per group), which were anesthetized with equithesin and had two electrodes for electroencephalographic recordings positioned over the cerebral cortex. Cerebral cortex and plasma cholesterol levels were determined by a standard spectrophotometric method. We found that oral atorvastatin treatment increased the latency to PTZ-induced generalized-seizures [U=20.50; P

Conclusions:
We conclude that atorvastatin, but not simvastatin, treatment and withdrawal have differential effects on PTZ-induced seizures, which are not related to changes in plasma or cerebral cortex cholesterol levels. Additional studies are necessary to evaluate the molecular mechanisms underlying our findings as well as its clinical implications.

Keywords: STATIN, CHOLESTEROL, EEG, EPILEPSY, WITHDRAWAL

Financial Support: CNPq, CAPES and FAPERGS.
Objectives:

Recent evidences suggests that inflammation may play an important role in the pathophysiology of depression. A promising development in this regard is the emergence of inflammation as a common mechanism of disease, indicating that inflammation may also be involved in neuropsychiatric diseases, as well as depression. This study aimed to investigate whether anti-inflammatory drugs have antidepressant activity.

Methods and Results:

Male Wistar rats from the Federal University of the Parana were used. The protocols were approved by the Ethics Committee in Animal Experimentation (CEEA) of UFPR (number 0479). The rats were treated with acute administration of anti-inflammatory: nimesulide, piroxicam, and celecoxib. The open field test was used to evaluate motor behavior, and for the evaluation of depression the forced swimming test was performed. The animals were randomly divided into 5 groups, saline, nimesulide, piroxicam, celecoxib and imipramine. The evaluation of the locomotor activity used the test of open field. The animals were gently placed in the right corner of the open field and were allowed to freely explore the area for 5 min, and then the following aspects were determined: locomotion speed and distance traveled. This procedure was performed 1 hour after the drug administration. The forced swimming test consisted in putting the animal in a container with water (30 cm and 20 °C) for 15 minutes and, after 24 hours, the animal was re-exposed to the test for 5 minutes, when the swimming time, escalation and the animal immobility were evaluated. This proceeding was carried 1 hour after the drug administration. The results were expressed by mean ± S.E.M, using ANOVA followed by Tukey test, p < 0.05. In the open field the groups did not demonstrate significant differences in all the evaluated parameters in comparison to saline group (p

Conclusions:

In accordance with the obtained results celecoxib and piroxicam animals presented exhibited increased in the time of swimming and reduction the time of immobility. In conclusion, these anti-inflammatory have demonstrated an antidepressant activity after acute administration.

Keywords: Inflamation, Depression, Celecoxib, Nimesulide, Piroxicam

Financial Support: CNPq, REUNI

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Resumo:23-232

EFFECTS OF PIRACETAM AND ENDOCANNABINIODS IN MEMORY PROCESSING

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Objectives:

Aim: Memory is a mnemonic system which is responsible to evoke information that was acquire through experiments. The processing starts with acquisition, the period when the person is in contact with experience stimulus. Then there are the consolidation which involve molecular events to occur retention. Lots of substances can affect this system, as piracetam, a nootropic drug related with neuroprotective effects and cognitive stimulation, and anandamide, an endogenous endocannabinoid that has receptors on cognitive area. So, this study tried to analyze the influence of this substances on memory acquisition and cosolidation.

Methods and Results:
Methods: Memory acquisition and consolidation were evaluated at the behavioral test Elevated Plus Maze (EPM) and the parameter used to determine if the animals learnt or not was the latency of transference (LT) from an open arm to one of the closed arms, in seconds. In a first moment, the animals were exposed to one train session and the time they spent to arrive in the closed arm was recorded (LT1). 24 hours after, they were positioned on EPM for a second time and they had their times registered (LT2). Generally, memory process induces a decrease of LT2 related to LT1. LT2 was used as mnemonic measure because it was not observed statistically significant differences in LT1 among the groups. Male Swiss mice were distributed in groups of eight and four experimental groups were employed for each test (saline+saline, saline+anandamide, piracetam+saline, piracetam+anandamide) and every one of the drugs were injected intraperitoneally (saline 10 ml/kg, piracetam 300 mg/kg and anandamide 1.0 mg/kg). In the first assay to evaluate acquisition, animals received the first drug injection 30 minutes and the second one 15 minutes before the training session. For consolidation tasks, the animals received the first injection 15 minutes and the other 30 minutes after train. Results were expressed as mean±S.E.M of the time (seconds) of Transfer Latency and analyzed by two-way ANOVA followed by Bonferroni post-hoc test (p

Conclusions:

Conclusion: Finally, we can conclude that anandamide in acute administration only interfere on memory consolidation provoking prejudice. Also we conclude that the piracetam has an important effect against the damage caused by anandamide, so it is also necessary understood what molecular mechanisms are responsible for this.

Keywords: acquisition, consolidation, endocannabinoids, piracetam

Financial Support: Universidade Federal de Uberlândia

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Resumo:23-233

LONG-TERM FISH OIL ADMINISTRATION PREVENTS ISCHEMIA-INDUCED AMNESIA IN RATS: INFLUENCE OF THE TREATMENT SCHEDULE

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Objectives:

Cognitive impairment and hippocampal neurodegeneration are the major outcomes of transient, global cerebral ischemia (TGCI) as it is the case of reversible cardiac arrest. Fish oil (FO) constitutes a rich dietary source of omega-3 polyunsaturated fatty acids, mainly docosahexaenoic acid (DHA). We aimed to investigate whether long-term treatment with commercial, high concentration, DHA-containing FO could be effective in alleviating both the cognitive and neurodegenerative deficits caused by TGCI in rats, and whether the administration schedule could influence the efficacy of FO.

Methods and Results:

Naive rats were trained for 10 days in an aversive eight-arm radial maze task and then subjected to TGCI for 15 min (4-VO model) 3 days later (day 13). Retention of the previously acquired cognition (i.e., memory) was assessed weekly on days 27, 34, 41, 48 and 55, and measured by three behavioral parameters: (i) latency to find the goal box, (ii) number of reference memory errors, and (iii) number of working memory errors. The extent of pyramidal cell death in the hippocampus was examined at the end of the behavioral analysis. Fish oil (300 mg/kg DHA, p.o.) administration occurred once daily according to different schedules (S). S1: 4 days pre-ischemia + 37 days post-ischemia, S2: 4 days pre-ischemia + 7 days post-ischemia, and S3: 21 days post-ischemia starting at the 15th day post-ischemia. TGCI markedly disrupted memory performance measured by all three parameters ($P < 0.0001$ vs. sham). This amnesic effect of ischemia persisted until the end of the behavioral analysis. Treatment with FO according the schedules S1 and S2 progressively reversed the TGCI-induced retention deficit until rats achieved control levels ($P < 0.001-0.01$ vs. vehicle). FO was no longer efficacious, however, when administered according the schedule 3. This
protective effect of FO on learning/memory function was clearly observed after both daily and cumulative data analysis. Such memory improvements remained statistically significant even after cessation of FO treatment, indicating a sustained effect of FO (P < 0.001 vs. vehicle). The treatment with FO failed, however, to prevent ischemia-induced hippocampal damage in areas CA1, CA2, or CA4, whatever the treatment schedule used.

Conclusions:

The present findings suggest that long-term FO treatment is able to prevent the cognitive sequelae caused by TGCI, an effect that occurred only when FO administration covered the acute period of ischemia (S1 and S2). Moreover, the anti-amnesic effect of FO seems to be independent of neurohistological protection, at least in respect to the CA1 pyramidal cells of the hippocampus.

Keywords: Fish oil, Global cerebral ischemia, Neurodegeneration

Financial Support: Fundação Araucária, CNPq and Universidade Estadual de Maringá (UEM).

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**EFFECT OF EBSELEN AND CAFFEIC ACID OF OXIDATIVE STRESS IN NEURO-2A CELLS "IN VITRO"**

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Objectives:

Aging is defined as a progressive and multifactorial process of the diminution of cell function and physiology and cognitive damage. One important factor present in these alterations is the oxidative stress. This is characterized by an accumulation of reactive species leading to cell damage and being important to aging development and in the pathogenesis of neurodegenerative diseases. Oxidative stress occurs due to an imbalance of antioxidant and pro-oxidant levels in cells. Thus, it’s relevant to study the antioxidant and neuroprotective effect of ebselen and caffeic acid, important antioxidants found in nature. *In vitro* techniques have been widely used to understand molecular mechanisms involved in aging process caused by oxidative stress. The aim of this study was to verify the in vitro antioxidant effect of ebselen and caffeic acid in neural cells (Neuro-2A) in oxidative stress conditions.

Methods and Results:

Neuro-2A cells were incubated with hydrogen peroxide (to mimic oxidative stress conditions) and different concentrations of ebselen (0.5; 5; 10 e 20 µM) or caffeic acid (25, 50, 100, 200 e 500µM) for 20 minutes on 37°C. After incubation, chemiluminescence assay, neutral red and cellular dye reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT) were performed to verify the antioxidant capacity, cell viability and reduction power of the described substances, respectively. Our results show that ebselen presented an antioxidant capacity in the 5µM concentration and the caffeic acid had an antioxidant power in all concentrations. Cell viability wasn’t amended when using both substances. Ebselen didn’t show a reduction power, but caffeic acid showed a significant decrease (p

Conclusions:

Antioxidant substances may have a pro-oxidant effect and may alter the antioxidant/pro-oxidant balance in cells. This alteration may generate an augmentation of oxidative stress in the nervous system level, worsening the pathologies related to aging process. Thus, we recommend more attention when using these antioxidants, because they may become pro-oxidants, losing their neuroprotective effect.
Objectives:
The CB1 receptors modulate synaptic plasticity in the hippocampus, as well as consolidation and retrieval of memory. This study investigate the roles of the metabotropic CB1 receptors infusing the agonist CP55,940 into the dorsal hippocampus of rats.

Methods and Results:
Rats were cannulated bilaterally in the dorsal hippocampus and trained in the context fear conditioning task. Animals received vehicle or CP 55,940 pre and post training. The test was performed 24 hours after training. Immediately after training, the animals were bilaterally infused into the dorsal hippocampus with CP55,940 (0,01μg/μl, 0,1μg/μl, 1μg/μl, 5μg/μl e 10μg/μl). We found a significant difference between groups (one-way ANOVA, F(4.46)=4.021, P=0.007, N=10,10,10,12 and 9, respectively). The post hoc Tukey test showed significant difference only in the concentration of 5μg/μl compared with the control group (P=0.006), i.e., that was an amnestic effect on memory consolidation. Posttraining infusion of CP55, 940 (5μg/μl, or CP55,940 5μg/μl + 0.2 mM of CB1 antagonist AM251) caused a significant difference between groups (one-way ANOVA, F(2,18)=4.421, P=0.027, N = 7, 6 and 8, respectively). The post hoc Tukey test showed significant difference for CP55,940 on the 5μg/μl concentration (P=0.022), i.e., a subeffective concentration of AM251 reverted the amnestic effect of CP55,940. Infusion before of the test, with CP55,940 (0.1 g / ul, and 1μg/μl 5μg/μl): there was a significant difference between groups (one-way ANOVA, F(3,26)=7.838, P=0.001, N=7, 7, 8 and 8, respectively) and the post hoc Tukey test showed significant difference for each concentration of CP55,940 compared with the control group: CP0,1μg/μl (P=0,002), CP1μg/μl (P=0,015) and CP5μg/μl (P=0,001), were amnestic upon memory retrieval. Pretest infusion of CP55,940 5μg/μl, or CP55,940 5μg/μl concomitant with a higher concentration (1,0μM) of the CB1 antagonist AM251 reverted the effect of CP: there was a significant difference between groups (one-way ANOVA, F(2,18) = 4.504 P=0.026, N=7,8 and 6, respectively) and the post hoc Tukey test showed significant difference of CP55,940 5μg/μl compared to the control group (P=0.031), while the group CP 5μg/μl + AM251 1.0 mM had only a tendency to differ from control (P=0.076). The electrophysiological procedure was performed by long-term potentiation(LTP) protocol with theta-like frequency stimulation. Hippocampal slices were perfused with artificial cerebrospinal fluid or CP55,940. The CP55,940 at concentrations equivalent to 5μg/μl intra-hippocampal infusions of 10 mM was prepared in 20 ml of buffer solution and used to perfuse slices during recording. After LTP induction, we can see a reduction in the magnitude of the potentiation (P=0.031, t test).

Conclusions:
The intrahippocampal administration of CP 55,940 impaired both the consolidation and the retrieval of memory in concentrations that were similar to those capable of LTP induction inhibition. The quite selective CB1 receptor agonist CP 55,940 exhibited a very particular profile in terms of behavioral effects, differing from what we and other groups have observed, e.g., for anandamide or Win55 before. This variability of effects may be attributed to the different selectivities, afinities and even target.

Keywords: AGING, CAFFEIC ACID, EBSELEN, NEUROPROTECTIVE, OXIDATIVE STRESS

Financial Support: FAPEMIG, UFMG, CAPES, CNPq

Resumo:23

MODULATION OF THE ENDOCANNABINOID SYSTEM IN DIFFERENT STAGES OF PROCESSING OF AVERSIVE MEMORIES IN RATS: INTERACTIONS AND SYNAPTIC PLASTICITY HIPPOCAMPAL STUDIED WITH THE AGONIST CP55,940

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aimed by each drug.

Keywords: Endocannabinoid System, Memory, Long-Term Potentiation

Financial Support: CAPES, CNPq, FINEP, FAPERGS, IFS

EXCESSIVE GABAERGIC STIMULATION IS ASSOCIATED WITH NEURONAL DEATH AND INCREASED NICOTINIC RECEPTOR FUNCTION

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Objectives:

The nicotinic neuronal receptors (nAChR) are ion channels involved in the modulation of the synaptic release of gamma-aminobutyric acid (GABA), glutamate and other neurotransmitters in the brain. Recent studies have shown that in several neurological diseases including Alzheimer's, schizophrenia and epilepsy, the number and function of hippocampal alpha7 nAChRs are altered. We have previously shown that GABAergic drugs promote the increase of alpha7 nAChRs currents in rat primary hippocampal cultures (J. Pharmacol. Exp. Therap. 319: 376, 2006). Vigabatrin (VGB), an antiepileptic drug that inhibits the catabolism of GABA, is toxic to retinal neurons and we recently found that it also potentiates nAChR currents. Thus, we have investigated the effects on cell viability of VGB and other GABAmimetics and the possible relationship with nAChR signaling.

Methods and Results:

Primary hippocampal cultures from E19 Wistar rats were maintained for 14 days and then treated for 7 days with 1, 10 and 100 microM VGB, 100 microM muscimol, a GABA-A selective agonist, and 100 microM GABA. Pictures were taken using an Olympus IX-71 inverted microscope before the treatment and in the first, second and last day of treatment. All animal procedures were approved by the institutional ethics committee. The cell numbers are expressed as mean percentages relative to control (vehicle) of two different experiments each run in duplicate in independent cultures. The imaged area contained a minimum of 80 neurons. All GABAergic treatments caused a time-related reduction in the number of cells, relative to control. The results were analyzed by the ANOVA statistical test. 1 and 10 microM VGB treatments had milder effects with reductions of 12% and 16%, respectively, after 7 days of treatment. 100 microM VGB, muscimol and GABA treatments showed more pronounced effects with reductions of 18%, 16% and 15%, respectively, already on the first day of treatment and 40%, 39% and 26% after 7 days. Treatment with 1 nM methyllycaconitine, a selective alpha7 nAChR antagonist, was markedly neuroprotective.

Conclusions:

These results show that excessive GABAergic stimulation is neurotoxic, possibly through the activation of GABA-A receptors, and alpha7 nAChR activation contributes to neuronal cell death. As we have previously shown, GABAergic treatment leads to an increase in nAChR currents, so it is reasonable to hypothesize that the toxic effect of GABAergic drugs is associated with the increase in nAChR function.

Keywords: GABA, vigabatrin, nicotinic receptor, hippocampus, neurotoxicity

Financial Support: CNPq, CAPES and FAPERJ
EVALUATION OF POSSIBLE NEUROPROTECTIVE EFFECT OF ADENOSINE RECEPTOR ANTAGONIST
AFTER ACUTE ETHANOL ADMINISTRATION

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Objectives:

Ethanol affects several neurotransmitters in CNS and causes oxidative stress (OS) in brain. Aminophylline is an adenosine receptor antagonist, which has been described as having neuroprotective effects (Yang et al., Chin Med Sci J, 22:62, 2007). This work investigated the association of acute ethanol and aminophylline administration on OS tests, and was approved by the Ethical Committee of the University of Ceara with the protocol number 084763361.

Methods and Results:

Male Swiss mice received ethanol via oral(E: 2 g/kg) or aminophylline intraperitoneally(A5: 5 mg/kg; A10: 10 mg/kg) alone or in association (EA5; EA10); then OS (TBARS, catalase, nitrite/nitrate) tests were performed. Ethanol increased TBARS [in hippocampus (HC)] and catalase activity [in prefrontal cortex (PFC), HC and striatum (ST)], but did not alter nitrite/nitrate concentration [PFC: F(5,50) = 5,168; p=0,0008]; [HC: F(5,55) = 3,920; p=0,0045]; [ST: F(5,54) = 19,14; p

Conclusions:

These data support the involvement of adenosinergic system on ethanol effects and a possible protective effect of aminophylline, an adenosine receptor antagonist, against ethanol-induced oxidative stress.

Keywords: adenosine, aminophylline, ethanol, oxidative stress

Financial Support: CNPq; FUNCAP

LACK OF BEHAVIORAL EFFECTS OF NEUROPEPTIDE S IN HALOPERIDOL-INDUCED PARKINSONISM IN MICE

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Objectives:

Neuropeptide S (NPS) is a peptide discovered in 2004, and it is the endogenous ligand of a G-protein coupled receptor named
NPSR. NPS induces arousal and prolonged wakefulness as well as anxiolytic-like behavior (Neuron, 43:487, 2004). Additionally, the finding that NPS administered centrally induces hyperlocomotor activity has been repeatedly demonstrated in both mice and rats, suggesting that the hyperlocomotor action of NPS is a common and robust phenomenon. The mechanisms supporting this effect are not clearly understood, especially in concern of the relationship between NPS and dopaminergic neurotransmission. Considering the limitations of the available pharmacological treatment of Parkinson’s disease, the aim of this study is to investigate the effects of NPS in the haloperidol-induced parkinsonism in mice.

Methods and Results:

Female Swiss mice (25–30 g) were housed in groups of fifteen per cage (33x40x17 cm) with food and water ad libitum. Animals were maintained under constant temperature (23±2°C), and under a 12-h light/dark cycle (lights on at 06:00 h). To assess akinesia in mice injected with haloperidol (1 mg/kg, ip), time spent immobile (i.e. cataleptic) in a bar elevated 4 cm from the floor was recorded. Haloperidol increased the time which mice remained immobile in the bar test, and the administration of L-DOPA and benserazide (100 mg/kg, po, and 25 mg/kg, ip, respectively; 30 min after haloperidol administration) attenuated haloperidol-induced akinesia [this effect was particularly evident at 60 (Halo=90±15 s, n=12; Halo+L-DOPA=62±10 s, n=11) and 90 min after L-DOPA administration (Halo=98±14 s, n=12; Halo+L-DOPA=56±7 s*, n=11, *p

Conclusions:

These findings suggest that NPS, at the dose tested, did not improve motor performance of mice treated with haloperidol. Further studies aiming to test higher doses of NPS, under the same experimental conditions, are in progress.

Keywords: Dopamine, Haloperidol, Mouse, Neuropeptide S, Parkinson’s disease

Financial Support: CAPES, CNPq

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**Resumo:**

**BRAIN PRECONDITIONING IS DUE TO AN ADENOSINE A1 RECEPTOR BOLSTERING FUNCTION IN GABAERGIC NEURONS**

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**Objectives:**

Adenosine is a neuromodulator mainly acting through inhibitory A1 receptors (A1R). A1R constitute a hurdle for the spreading of brain damage, and bolstering A1R is envisaged as a promising prophylactic strategy to prevent brain disorders. Preconditioning can be viewed as one such prophylactic neuroprotective strategy whereby a sub-maximal noxious stimulus can limit subsequent neurodegeneration triggered by a more intense noxious stimulus through a process related to A1R. However, it is unclear if other mild noxious apart from ischemia can trigger brain preconditioning and which might be the underlying mechanisms.

**Methods and Results:**

Male adult Wistar rats (2 months old) subject to a 2 hours period of restraint stress displayed 24 hours later a 22% enhanced expression (gauged by qPCR) and a 21-28% enhanced density of A1R in their hippocampus and cerebral cortex (evaluated by 3H-DPCPX binding and Western blot analysis, 95% significant differences, Student’s t test). When rats were subject either to kainate (10 mg/kg, ip)-induced convulsions or focal ischemia upon distal occlusion of the middle cerebral artery, the rats subject
to restraint stress 24 hours earlier displayed an extent of kainate-induced hippocampal lesion (FluoroJade-labelling 24 hours later) 64-73% lower (in CA1 and CA3 regions, n=4) than injured rats that were not previously subjected to restraint stress (control rats) and an extent of ischemia-induced cortical damage (2,3,5-triphenyltetrazolium chloride, TTC, labelling 24 hours later) similar to rats not subject to ischemia (n=8-12). Treatment with the A1R antagonist DPCPX (1 mg/kg, ip) abrogated this ability of restraint stress to reduce subsequent neuronal damage by kainate (n=4) or by ischemia (n=8-16) (95% significant differences with Duncan test after two ways ANOVA). Albeit A1R are mainly synaptically-located, restraint stress enhanced the density of extra-synaptic rather than synaptic A1R; accordingly, restraint stress enhanced the activation of pathways know to be involved in cardiac preconditioning (e.g. PKCε, PI3K and Erk) in total rather than synaptosomal extracts, a feature abrogated by DPCPX (n=4-7; 95% significant differences with Duncan test after two ways ANOVA), and the A1R-mediated modulation of glutamatergic transmission was unaffected by restraint stress (n=4-5). Instead, restraint stress enhanced the coupling between oxidative and phosphorylation activities in isolated cortical mitochondria with the consequent decreased production of free radicals (n=4). Finally, isotopomeric 13C-NMR revealed that restraint stress selectively affected neuronal GABAergic (but not glial) metabolism (n=4).

Conclusions:

This shows that activation of extra-synaptic neuronal A1R plays a key role in stress-induced preconditioning against subsequent convulsions-induced and ischemia-induced cortical damage.

Keywords: adenosine, metabolism, mitochondria, preconditioning, stress

Financial Support: FCT, CAPES-FCT

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**EFFECTS OF AMINOPHYLLINE ON AMINO ACIDS CENTRAL LEVELS IN ANIMALS ETHANOL TREATED**

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**Objectives:**

The aim of this study was assessing relationship between aminophylline, a nonselective antagonist of adenosine receptors with excitatory effect on CNS, and ethanol on amino acids levels in three different brain areas (Prefrontal cortex – PF; Hippocampus – HC; and Striatum – CE), to check possible reversal effect of this drug.

**Methods and Results:**

Methods: It was used male Swiss mice, 2-4 months old, 25-30 g. They were divided into six groups of six animals. They were treated with saline (Control – C; Ethanol – E6) or aminophylline in different doses (A5 – 5 mg/kg or A10 – 10 mg/kg, i.p.) in single dose for seven consecutive days and thirty minutes later the animals received water (C, A5 and A10) or ethanol 6g/kg (E6, A5E6 and A10E6, p.o.). On the seventh day, animals were sacrificed and dissected 60 minutes after last administration, separating the areas (PF, HC and CE). Levels of different amino acids (aspartate – ASP, glutamate – GLU, glycine – GLI, taurine – TAU, and GABA) were determined by HPLC. We used ANOVA and Dunnett as post test to compare E6, A5 and A10 to C and ANOVA and Bonferroni as post test to compare the associated groups and their respective controls (A5E6, A5 and E6, and A10E6, A10 and E6). This work was approved by the Ethical Commite Of Estadual University of Ceara, with protocol number 08476336-1. Results were expressed as M/mg of tissue. Results: It was found that ethanol did not alter ASP and GLU levels on three areas when compared to respective C (ASP - C: 1.600±132.9 (PF); C: 3.052±191.8 (HC); C: 1.615±334.8 (CE)), except for ASP on PF (ASP – E6: 5.551±1.014(PF)). When
Drugs were associated, aminophylline at different doses did not alter ethanol effect on these two amino acids levels. Regarding inhibitory amino acids, ethanol increased ones levels in all areas (TAU - C: 2.113±216.3; E6: 4.769±435.7 (PF); C: 1.784±216.1; E6: 2.715±71.1 (HC); C: 2.252±375.9; E6: 4.274±533.6 (CE); GABA – C: 1.193±84.1; E6: 12.673±1.641 (PF); C: 3.494±115.9; E6: 10.276±1.491 (HC); C: 1.526±216.6; E6: 4.403±354.8 (CE)), except to GLI on CE (GLI - C: 1.243±93.7; E6: 7.878±340.2 (PF); C: 2.207±321.8; E6: 3.565±196.3 (HC); C: 1.903±205.8 (CE)). Aminophylline in different doses reversed ethanol effects when associated in both PC and HC, approaching of respective C levels.

Conclusions:

It was noticed that aminophylline interferes on ethanol action path on inhibitory levels amino acids, such glycine, taurine and GABA, but this interference was not observed on excitatory ones (aspartate and glutamate).

Keywords: Amino acids levels, Aminophylline, CNS, Ethanol

Financial Support: CNPq, CAPES and FUNCAP

THE DORSOLATERAL PERIAQUEDUCTAL GRAY MATTER AND THE DORSAL PREMAMILLARY NUCLEUS INTERPLAY TO SUPPORT OLFATORY FEAR CONDITIONING

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Objective:

Memory for odors is often associated with highly emotional experiences. In rodents, olfaction is the most important sensory system and olfactory cues can represent a reasonable choice to explore questions about brain mechanisms of emotional processing. Behavioral studies have already employed the olfactory stimuli as conditioning stimulus (CS) in a fear conditioning paradigm and have found similarities with the cat odor model, a more realistic model to study natural fear responses (Neurosci Biobehav Rev. 32:1228, 2008). Preliminary data from our laboratory have shown that olfactory fear conditioning (OFC) is an useful paradigm able to detect the aversive nature induced by a chemical stimulation of the dorsolateral periaqueductal gray matter (dPAG) or the dorsal premamillary nucleus (PMd) functioning as a unconditioned stimulus (US). The present study was outlined to verify possible relationship between the dPAG and PMd stimulation functioning as a US in an OFC acquisition paradigm.

Methods and Results:

Two sets of experiments were performed in Wistar male rats (350g) with cannulas implanted into the PMd and the dPAG. Experimental procedure consisted of 2 phases: acquisition and expression of OFC. The acquisition phase consisted of 2 sessions: familiarization (5 min) and conditioning (10 min, 24 h after), where amyl acetate odor (AMYL) and the dPAG or the PMd stimulation were associated. The expression phase consisted of 2 consecutive sessions (10 min each): familiarization and test, performed in an odor box comprising an open and an enclosed communicating compartment. During the expression phase the defensive behavior was represented by: % time approaching the AMYL source (%AT) and % time hiding in the enclosed compartment (%HT). To test the interplay between the dPAG and PMd in the OFC acquisition, in experiment 1, rats pre-treated with beta-adrenoceptor antagonist (atenolol; ATE 40 nmol) or PBS into the PMd were microinjected within the dPAG with the n-methyl-d-aspartate receptor agonist (NMDA 100 pmol), after 5 min. Regardless the behavioral measure, no statistical differences were detected among the groups’ performance during the familiarization session. ANOVA followed by Newman-Keuls test revealed that ATE pre-treatment into the PMd significantly (p
Conclusions:

The results showed that PMd beta-adrenergic blockade severely impaired OFC promoted by chemical dlPAG stimulation. In the same vein, NMDA receptor antagonist AP5 infused into the dPAG blocked the OFC acquisition promoted by ISO microinjection into the PMd. Therefore, this study has demonstrated the important integration between dlPAG and PMd, suggesting that the feedback pathway between these brain structures is critical to support olfactory fear conditioning.

Keywords: Defensive behavior, dorsolateral periaqueductal gray matter, dorsal premammillary nucleus, Fear, olfactory fear conditioning

Financial Support: CNPQ, PROEX/CAPES, FAPESP.

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Resumo:23-242

ASSESSMENT OF THE EFFECT PANICOLYTIC AND ANXIOLYTIC TO AQUEOUS EXTRACT OF GUARANA IN THE T-MAZE.

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Objectives:

Paullinia cupana (H.B.K. var. sorbilis (Mart.) Ducke), belongs to the Sapindaceae family, popularly known as guaraná, is mainly grown in the central Amazon basin, in Brazil (1). Guarana has been popularly used as a stimulant of the central nervous system in cases of intellectual and physical stress (2). Hydroalcoholic extract of guaraná produced similar effect to antidepressant compounds in rats in forced swimming test (3). The aim of this study was to evaluate the panicolytic and anxiolytic effect of the aqueous extract of guaraná (PEA) in rats subjected to the elevated T maze (ETM). The ETM is a model of anxiety which demonstrates two defensive responses in the same rat, namely inhibitory avoidance and one-way escape, which have been related to generalized anxiety disorder and panic disorder, respectively (4). The selective serotonin (5-HT) reuptake inhibitor (SSRI), paroxetine, was used as a positive control. Locomotion was assessed in a circular arena following each drug treatment, as a control for nonspecific motor effects.

Methods and Results:

Male Wistar rats (n=6), were separated into three groups. Each group was treated with oral injections for 21 days with PEA (8mg/kg), paroxetine (3mg/kg) or saline (NaCl 0.9%). After 30 min of the last administration, the animals were exposed to the ETM where inhibitory avoidance (baseline, avoidance 2 and 3) and escape from the open arm (escape 1, 2 and 3) latencies were recorded. Repeated-measures analysis of variance (RM-ANOVA) or one-way was used to analyze data. When appropriate, the post hoc Duncan’s multiple comparison test was used. Differences between groups were considered significant if \( p \leq 0.05 \).

Conclusions:

This study showed that chronic treatment with PEA produced a panicolytic and anxiolytic effect in ETM, suggesting that the aqueous fraction of guaraná could be an important alternative therapy for psychiatric diseases.

Keywords: Guarand, panicolytic, anxiolytic, T maze
INFLUENCE OF FEMALE SEXUAL HORMONES IN THE BEHAVIORAL SENSITIZATION TO COCAINE

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Objectives:

Chronic intermittent administration of cocaine produces behavioral sensitization that is relevant to addictive behaviors. The hormonal differences between males and females may influence cocaine use and its effects, with females experiencing with more intense behavioral effects than males, including higher behavioral sensitization. Estrogen has been associated with increased response to psychostimulants among females, whereas progesterone is associated with a lower potential effect. The aim of this study was verify the influence of female sexual hormones in the behavioral sensitization to cocaine.

Methods and Results:

120 female Wistar rats (~300 g) were used, 96 of these were submitted to bilateral ovariectomy. Sham surgery was realized in 24 rats (SHAM). The rats submitted to ovariectomy were randomly assigned to progesterone 0.5mg/kg (PRO), estrogen 0.05mg/kg (EST), estrogen+progesterone (PRO+EST) or ovariectomized (OVX) groups. These rats also were assigned to control (CTR), acute (ACT) or repeated (RPT) cocaine treatment groups. Sensitization protocol started ten days after ovariectomy/sham surgery, when CTR and ACT animals received saline 1 ml/kg i.p. and RPT rats received 15 mg/kg cocaine hydrochloride i.p. for eight consecutive days. After ten days of pause on administrations, ACT and RPT animals received the same dose of cocaine, while CTR received saline. The hormone treatment started 24h before the first drug administration, according to the hormonal group. In the challenge day, locomotor activity of rats was monitored for 35 minutes and the truncal blood was collected immediately after the finish of the locomotion test to realization of hormonal dosage with Elisa test. The results show that repeated treatment with cocaine in female rats of different hormonal conditions induces augment in their locomotor activity when compared to CTR and ACT drug treatments (CTR = 123.02 ± 36.22; ACT = 184.51 ± 35.61; RPT = 755.22 ± 32.72; F(2,74)=105.98; p

Conclusions:

Repeated treatment with cocaine resulted in behavioral sensitization of female rats in different hormonal conditions. Also, the concomitant presence of estrogen and progesterone on SHAM and PRO+EST hormonal groups seems to improve the behavioral sensitization of repeatedly treated cocaine rats.

Keywords: cocaine, sensitization, female, sexual hormones, behavioral

Financial Support: CAPES; CNPq; UFCSPA
Objectives:

Parkinson's disease is a neurodegenerative disease, whose symptoms include bradykinesia, tremor at rest and postural instability associated with depression. The prevalence of depression in these patients is between 40% to 70%, with an incidence of 1.86% per year and a cumulative risk of 8.5% over life. The current study investigated and compared depressive symptoms among the animal models of Parkinson’s disease induced by 6OHDA in different time.

Methods and Results:

Male Wistar rats from the Federal University of the Parana were used. The protocols were approved by the Ethics Committee in Animal Experimentation (CEEA) of UFPR (number 0470). The rats were infused bilaterally in the substantia nigra with 6-OHDA. The open field test was used to evaluate motor behavior, and for the evaluation of depression the forced swimming test and the sucrose preference consumption test was performed. The animals were randomly divided into 2 groups, SHAM (n=8-9/group) and 6-OHDA (n=8-9/group). The evaluation of the locomotor activity used the test of open field. The animals were gently placed in the right corner of the open field and were allowed to freely explore the area for 5 min, and then the following aspects were determined: locomotion speed and distance traveled. This procedure was performed 1, 7, 14 and 21 days after the stereotaxic surgery. The forced swimming test consisted in putting the animal in a container with water (30 cm and 20 ºC) for 15 minutes and, after 24 hours, the animal was re-exposed to the test for 5 minutes, when the swimming time, escalation and the animal immobility were evaluated. This proceeding was carried out 7, 14 and 21 after the stereotaxic surgery, although those groups were submitted to the sucrose preference consumption test on days 7, 14 and 21 after neurotoxin exposure. The results were expressed by mean ± S.E.M, using ANOVA followed by Tukey test, p < 0.05. In the open field the groups 6-OHDA presented a decrease in locomotion and rearing frequencies 1 day after surgery in comparison to control and SHAM groups (p

Conclusions:

In accordance with the obtained results 6OHDA animals presented hypolocomotion 24 h after the surgery. Moreover, 6OHDA caused depressive signs in rats evaluated in the forced swimming test 7, 14 and 21 days after the surgery. In conclusion, the animals infused with 6OHDA present depression since the 7 days after surgery.

Keywords: Parkinson's Disease, Depression, 6OHDA

Financial Support: REUNI, CNPq
this study was to investigate the effects of dorsal intra-hippocampal microinjections of Chlorpheniramine (CPA), a histaminergic H1 antagonist, on anxiety-related behaviors in male mice, using elevated plus-maze (EPM) test of anxiety.

Methods and Results:

71 male Swiss Albino mice of body weight 25-35g were pre-treated with saline (SAL) i.p. or L-Histidine (LH – 500mg/Kg) i.p. After two hours they were treated with dorsal intra-hippocampal microinjections of SAL or CPA (0,006nmol; 0,052nmol; 0,06nmol/0,1µl). Five minutes after intra-hippocampal microinjections the animal were exposed to EPM. The animals were randomly assigned to 8 groups based on drug treatment: SAL/SAL (n=9), SAL/CPA1 (n=9), SAL/CPA2 (n=10), SAL/CPA3 (n=8), LH/SAL (n=10), LH/CPA1 (n=8), LH/CPA2 (n=8) and LH/CPA3 (n=9). All three doses of intra-hippocampal microinjections of CPA did not change the open arm activity (%OAT) - SAL/CPA1 (%OAT) (46,13±4,45); SAL/CPA2 (%OAT) (47,59±4,89); SAL/CPA3 (%OAT) (44,30±6,65) - when compared to control group SAL/SAL (%OAT) (35,84±2,77) and did not change the locomotor activity (enclosed arm entries - EAE) - SAL/CPA1 (EAE) (8,56±1,06); SAL/CPA2 (EAE) (9,70±1,10); SAL/CPA3 (EAE) (9,38±1,25) - when compared to control group SAL/SAL (EAE) (10,56±1,11). Intrapertitoneal injections of LH increased open arm time (%OAT) – LH/SAL (%OAT) (59,79±4,71) – when compared to control group SAL/SAL (%OAT) (35,84±2,77), but not locomotor activity LH/SAL (EAE) (9,20±1,78), SAL/SAL (EAE) (10,56±1,11), thus showing an anxiolytic response. When animals were treated with LH and CPA3, the anxiolytic-like effect of LH was reversed: LH/CPA3 (%OAT) (32,25±4,81).

Conclusions:

Dorsal intra-hippocampal microinjection of Chlorpheniramine (0,006nmol; 0,052nmol; 0,06nmol/0,1µl) has no effect on anxiety-related behaviors in male mice. Intrapertitoneal injection of L-Histidine (500mg/Kg) has an anxiolytic-like effect in male mice exposed to elevated plus-maze, that is reversed by the higher dose of Chlorpheniramine (0,06nmol/0,1µl).

Keywords: CHLORPHENIRAMINE, L-HISTIDINE, ANXIOLITIC, Mice, elevated plus-maze

Financial Support: CAPES/PROEX, CNPq

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Resumo:23-246

ARIPIPRAZOLE, A PARTIAL DOPAMINE AGONIST, INHIBITS ACUTE EFFECTS OF ALCOHOL IN ANIMAL MODELS

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Objectives:

Aripiprazole is an atypical antipsychotic that acts as a partial agonist at the dopamine D2 receptors. This antipsychotic has a low capacity to induce extra-pyramidal side-effects. In addition, aripiprazole had anxiolytic effects in animal models of anxiety. Furthermore, this drug was able to inhibit the hyperactivity induced by cocaine in mice. Considering that alcohol induces several behavioural changes, including motor hyperactivity, the aim of this study is to test the hypothesis that aripiprazole would prevent some effects of this drug in mice.

Methods and Results:

In the first experiment, male Swiss mice (n=8/group) received intraperitoneal (i.p.) injections of vehicle or ethanol (1,5; 2,5; 3,5 mg/kg) in a volume of 10 mL/kg, immediately before exposure to a circular arena (40 cm in diameter with a 50 cm high Plexiglas wall). The distance moved was automatically analyzed with the help of the AnyMaze software. The data were analyzed by ANOVA followed by the Newman-Keuls test. The values (mean/SEM) for the total distance moved during 20 minutes were as
follows: Vehicle, 21.3±2.5 cm; ethanol 1.5 mg/Kg, 38.1±6.3 cm; ethanol 2.5 mg/Kg, 80.2±44.6 cm; ethanol 3.5 mg/kg, 8.7±2.5 cm. The psychostimulant dose was 2.5 mg/kg. The second experiment was designed to analyze the effects of aripiprazole on the hyper-locomotion induced by this dose of alcohol. This antipsychotic (0.1; 1; 10 mg/Kg; n=8/group) was administered via i.p. 30 min before alcohol. The values for the total distance moved during 10 minutes were: Vehicle + Vehicle, 9.0±1.8 cm; Vehicle + Ethanol 76.4±11.5 cm; Ethanol + Aripiprazole 0.1 mg/Kg, 18.3±7.8 cm; Ethanol + Aripiprazole 1 mg/Kg, 15.0±5.0 cm; Ethanol + Aripiprazole 10 mg/Kg, 17.0±7.5 cm. All the doses were able to completely prevent ethanol effect.

Conclusions:
Aripiprazole was effective in preventing the effects of alcohol on locomotion. Further experiments are in course to test whether this antipsychotic may also interfere with other effects induced by acute or chronic alcohol treatment.

Keywords: ARIPIPRAZOLE, ALCOHOL, ANTIPSYCHOTIC

Financial Support: Capes

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Resumo: 23-247

EFFECT OF MATERNAL SEPARATION ON THE EXPRESSION OF AMPA GLUTAMATE RECEPTORS IN THE HIPPOCAMPAL FORMATION OF RATS.

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Objectives:
Early life stress seems to put an individual at a greater risk for many mental disorders such as depression and posttraumatic stress. Glutamatergic neurotransmission has been shown to be involved in these disorders through different receptors in several brain areas. The aims of this study were to investigate the changes induced by maternal separation (MS) on the expression of AMPA receptors, GluR1 and GluR2, in the hippocampal formation of rats.

Methods and Results:
Male Wistar rats have been used. Pups (n=10) underwent a daily-3h separation from their mothers from PND1-21 and the controls (n=11) were left undisturbed. On PND21 the rats were housed (4/cage) for 5 weeks when were deeply anaesthetized, perfused with paraformaldehyde 4% and their brains removed. The expression of GluR1 and GluR2 subunits was evaluated by immunohistochemistry (n=6/group). The immunopositive cells (IC) were quantified in the hippocampal formation (hippocampus, amygdala and entorhinal cortex) in 3 sections/rat, bilaterally, by 3 examiners independently. The average was compared by Student t-test and the level of significance was set at p

Conclusions:
Considering the high calcium permeability of the GluR1 subunit, the decreased expression of this subunit in all areas of the hippocampal formation induced by MS suggest a reduction on the neuronal excitability of the glutamatergic loop involved on the brain stimulation triggered by entorhinal cortex in response to stress. The increased expression of the AMPA subunit impermeable to calcium, GluR2, in the hilus of dentate gyrus supports this conclusion as the dentate gyrus is the first area stimulated by the perforant path from entorhinal cortex in response to stress.
THE INVOLVEMENT OF Na+,K+-ATPASE ACTIVITY AND FREE RADICAL GENERATION IN THE SUSCEPTIBILITY TO PENTYLENETETRAZOL-INDUCED SEIZURES AFTER EXPERIMENTAL TRAUMATIC BRAIN INJURY

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Objectives:

Traumatic brain injury (TBI) is a devastating occurrence that commonly causes disability and strongly affects the quality of life of patients. The sequelae of TBI, such as posttraumatic epilepsy, represents a societal problem. Nevertheless, clinical trials aiming the prevention of epilepsy following TBI have failed. In this sense, there is great need for the identification of biomarkers that provide quantitative measures of the process of post-traumatic epileptogenesis and predict which TBI patients are likely to develop epilepsy. In this context, oxidative stress, an imbalance between oxidants and antioxidants, contributes to the pathogenesis of TBI as well as the inhibition of some selected targets such as Na+,K+-ATPase. Accordingly, we decided to investigate the involvement of free radicals and Na+,K+-ATPase activity in the process of post-traumatic epileptogenesis after FPI model.

Methods and Results:

Were used adult male Wistar rats (270–300 g) maintained under controlled light and environment (12:12 h light/dark cycle, 24 ± 1 °C, 55% relative humidity), with standard laboratory chow and water ad libitum. The animals were subjected to Fluid percussion injury (FPI) or FPI surgery (sham) as described previously by D’Anbrosio (2004). After 4 weeks of FPI procedure, all animals were deeply anesthetized and two screw electrodes were placed bilaterally over the parietal cortex, along with a ground lead positioned over the nasal sinus, 5 days after surgery procedure, animals were subjected to a subeffective dose of PTZ (30 mg/kg; i.p.) and observed for the appearance of generalized tonic-clonic convulsive episodes for 20 min and EEG recordings were visually analyzed for seizure activity. After the EEG and behavioral evaluation, the animals were killed by decapitation and cortical tissues surrounding the injured core were removed to perform biochemical analysis for protein carbonyl content, TBARS and Na+,K+-ATPase activity. Statistical analysis revealed that 5 weeks after FPI, the subeffective dose of PTZ decreased latency to the first clonic seizures [U=8.0; p

Conclusions:

FPI episode in rat parietal cortex decreases Na+,K+-ATPase activity with a concomitant increase in the levels of oxidative stress markers (protein carbonylation and TBARS), 5 weeks after the injury. In addition, the early posttraumatic seizures caused by injection of subeffective dose of PTZ suggest that failure of some selected targets, such as Na+,K+-ATPase elicted by ROS generation, may increase cellular excitability and facilitate the appearance and/or propagation of convulsions after TBI.

Keywords: Traumatic brain injury, seizures, Na+,K+-ATPase, oxidative damage,

Financial Support: CAPES, CNPq, FAPERGS
OBJECTIVES:
In mice, when dopamine (DA) D2 neurotransmission is blocked, REM sleep is practically abolished for 4 hours following injection. Here we set out to test the hypothesis that post-training REM sleep suppression by DA D2 antagonist haloperidol (HALO) impairs memory consolidation. Also we checked if hyperdopaminergic transgenic mice would present a basal impairment of memory consolidation, that could be reverted by Halo in different protocols and dosis, and if halo in high dose could block REM sleep in a group of WT mice.

METHODS AND RESULTS:
C57BL-6 mice, including Heterozygous DAT-KO mice (DAT-Hz; expressing higher basal DA levels) were used evaluate memory consolidation in the Novel Object Test (NOPT). Basically, all groups received i.p injection immediately (a priori injection: PI) before, and immediately after (immediate injection: II) the first exposure to new objects, or 6 hours later (late injection, LI) considering the first exposure to novel objects, with re-exposure 24 h later at 10:00 -12:00 a.m; On the second day, ratios of familiar/unfamiliar object exploration time were obtained. All comparisons were performed with student’s T test (corresponding halo x vehicle, n= 7-8 per group),and groups were studied as follows. II groups: Wild-Type VH (2.50 xx SD 0.55) and WT Halo 0.3mg/kg (1.51 SD 0.55), p = 0.001 ; DAT-Hz VH (1.07 SD 0.27) and DAT-Hz Halo 0.3mg/kg (0.96SD0.33); non-significant p= 0.5. LI groups: WT Halo 0.3mg /Kg( 1.10SD0.57) and WT VH (2.70SD0.25Y), p = 0.6. PI groups: DATHz VH (1.38 SD 0.49 ) and DAT Hz Halo 0.05 mg/kg (2.25SD 0.59); p = 0.01. Also , comparing II Hz VH and Hz Halo with II WT VH mice (in ANOVA test followed by Tukey) we observed a deficit in learning in both Hz groups (p=0.05). Comparing PI Hz Halo in a low dose (0.03 mg/kg) with II Hz Halo or VH, we observed an increase in memory consolidation that was not observed with hyperdopaminergic basal D2 activity (Hz VH)(p=0.05), and also was maintained with low D2 activity in a high dosis of Halo (Hz Halo 0.3 mg/kg)(p=0.05). Another two groups of VH were submitted to multielectrode implant in hippocampus and bidimensional 12h state-map evaluation of sleep-wake cycle in which we confirmed Halo action in blocking REM sleep in the first four hours after injection (eletrophysiology groups).

CONCLUSIONS:
We observed that supress of D2 receptor activity caused by Halo in high dosis can block REM sleep in the first four hours, and also memory consolidation in wild type mice. Heterozygous mice with basal hyperdopaminergia or with block of D2 activity did not disclose memory consolidation in NOPT, but with mild dopaminergic activity at D2 receptor (low dose of Halo) they presented memory consolidation similarly to WT levels. Our data supports the hypothesis that the anti-mnemonic role of the D2 receptor antagonist HALO is mediated by REM sleep suppression, and that both lack or excess of basal DA activity in D2 receptors may impair memory consolidation.

KEYWORDS: mice, dopamine transporter, haloperidol, REM, memory consolidation

FINANCIAL SUPPORT: CAPES, CNPQ, AASDAP
LONG-TERM PROLINE EXPOSURE INDUCES BEHAVIORAL CHANGES IN ZEBRAFISH.

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Objectives:

Zebrafish have emerged as a promising experimental model for neurobehavioral studies. Hyperprolinemia is an inherited disorder of proline metabolism and patients affected by this disease can present neurological manifestations, including seizures and cognitive dysfunctions. Moreover, it has been reported an association between adulthood schizoaffective disorders and moderate hyperprolinemia. However, the mechanisms related to these behavioral phenotypes remain still unclear. In the present study, we investigated the effect of proline treatments on behavioral parameters in zebrafish, such as locomotor activity, anxiety, and social interaction.

Methods and Results:

For the behavioral studies, the adult zebrafish (Danio reio) were exposed at two proline concentrations (1.5 and 3.0 mM) during 1 hour or 7 days (short- or long-term treatments, respectively). Short-term proline exposure did not promote significant changes on behavioral parameters examined. However, after long-term exposure, proline at 1.5 mM induces significant changes on parameters of zebrafish swimming activity. As indicated by the number of line crossings in the apparatus, the locomotor activity increased (47%; P

Conclusions:

These findings demonstrate that proline is able to induce changes in the behavior performance of zebrafish, which reinforce the use of the zebrafish as a complementary vertebrate model for studying behavioral phenotypes associated with inborn errors of amino acid metabolism.

Keywords: Zebrafish, Hyperprolinemia, Locomotor activity, Anxiety, Social interaction

Financial Support: CNPq; FAPERGS.

EFFECTS OF NICOTINE PROGRAMMING DURING LACTATION ON ANXIETY, NOVELTY-SEEKING AND LEARNING/MEMORY BEHAVIORAL TRAITS OF WISTAR RATS

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DEPARTAMENTO DE CIÊNCIAS FISIOLÓGICAS - IBRAG DA UERJ, UERJ

Objectives:
Maternal nicotine exposure during lactation in rats programmes for hyperleptinemia and hypothyroidism in the adult offspring. Changes in the levels of these hormones are known to affect behavior. Hence, here we investigated the short- and long-term behavioral effects of neonatal nicotine exposure in Wistar rats.

Methods and Results:

Lactating rats implanted with minipumps either containing nicotine (NIC=6mg/Kg/dia) or a solution with equivalent concentration of sodium bitartrate (C) during the first 14 postnatal (PN) days. Sixty two NIC and 48 C rats were tested according to the following protocol: 1) Either at PN30 or PN75, anxiety levels were assessed in elevated plus-maze (EPM). Entries into each area of the maze (open and closed arms and centre square) were recorded over a period of 10 min; 2) One hour, animals were tested in the hole board arena (HB) in order to assess novelty-seeking behavior. Animals were allowed 10 min to explore. The number of explored holes was noted; 3) Either at PN31 or PN76, learning/memory was assessed in the radial arm water maze (RAWM). Each animal was tested 4 times a day for 4 consecutive days. They were allowed 2 min to find the hidden escape platform. The latency to find the platform was noted. The percentage of time spent in the open arms of the EPM by PN30 NIC animals (6.4±1.1 %) was significantly (ANOVA: P<0.1). No differences (ANOVA: P>0.1) were observed between groups regarding the number of closed arm entries at both ages. As for the HB test, no differences were observed between groups at both ages (ANOVA: P>0.1). In the RAWM, PN75 NIC animals (274±24 s) displayed a significantly (ANOVA: P<0.1).

Conclusions:

Neonatal nicotine programming results in a short term effect on anxiety-like behavior, observed by a high anxiety levels during adolescence. No difference was observed regarding novelty-seeking behavior. However, neonatal nicotine programming causes a long-term late emerging effect on cognitive-behavioral, observed by a better performance in learning/memory task at adulthood.

Keywords: NICOTINE PROGRAMMING, LACTATION, ANXIETY, NOVELTY-SEEKING, LEARNING/MEMORY

Financial Support: FAPERJ, CNPq, CAPES, SR2-UERJ

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Resumo:23-252

BEHAVIORAL EFFECTS OF VARENICLINE TREATMENT IN MICE EXPOSED TO NICOTINE DURING ADOLESCENCE.

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Objectives:

Nicotine is the main bioactive substance present in tobacco smoke that is responsible for addiction. Epidemiological studies have been showing that adolescents are more likely to use nicotine and that this exposure causes a greater risk of long-term tobacco addiction. Varenicline is FDA-approved for smoking cessation that is recommended for all smokers, including those with psychiatric or addictive comorbidity. Less is known, however, concerning the benefit-risk profile of this medication in adolescents. The aim of our study was to evaluate behavioral changes in mice resulting from a period of exposure to nicotine during adolescence followed by nicotine withdrawal associated with varenicline treatment.

Methods and Results:

From postnatal day (PN) 30 to 45, 140 Swiss mice (both sexes) either had a 50μg/ml nicotine-free base (Sigma, St. Louis, MO) in 2% saccharin solution (NIC) or a 2% saccharin solution (VEH) to drink. Three procedures were adopted for the withdrawal period (PN45 to PN55): 1) Varenicline (Sigma, 0.1 mg/kg/day - VAR), 2) Exposure to the nicotine solution used between PN30
Exposure to the saccharin solution (VEH). Accordingly, male and female mice from each litter were distributed into five treatment groups: VEHVEH; VEHVAR; NICVEH; NICNIC; NICVAR. Groups received treatment (varenicline+DMSO or DMSO only) by gavage. On PN55, four hours after the last gavage, animals were submitted to the elevated plus maze (EPM) test. Animals were allowed 5 min to explore the EPM. The % of time spent in open arm (Time OA) and % of open arm entries (Entries OA) were used as anxiety measures; total number of arm entries (Entries CA) was used as a locomotor activity measure. Two hours after the EPM, the animals were tested in the Hole Board arena (HB) in order to assess novelty-seeking behavior. The floor of the HB has 16 uniformly spaced holes that the animal can explore. Animals were allowed 5 min (divided into four consecutive 1 min 15 s intervals) to explore. The number of head-dips was used as measure of exploring activity. No differences were observed between groups regarding the analysis of behavior in the EPM. As for the HB, a significant (ANOVA: P=0.034) interval×exposure×treatment interaction was observed regarding the number of nose pokes. The main finding was that while VEHVEH group increased the number of holes explored throughout the intervals, the NICVEH group presented an initial increase that was rapidly followed by an stabilization, and the treatment of NIC animals with Varenicline (NICVAR group) reverted this pattern in a way that made the exploration profile of this group similar to that of the VEHVEH group. For example, significant differences between groups were observed for the fourth interval (ANOVA: P=0.004): the number of nose pokes of the VEHVEH (14.2±1.4) and NICVAR (14.1±1.3) groups were higher (FPLSD: P

Conclusions:

Exposure to nicotine followed by treatment with Varenicline did not affect anixiety-like behavior. On the other hand, novelty-seeking behavior was restored to normal in animals exposed to nicotine that were subsequently treated with Varenicline.

Keywords: NICOTINE, VARENICLINE, ANXIETY, NOVELTY-SEEKING BEHAVIOR, MICE

Financial Support: FAPERJ, CNPq, CAPES and SR2-UERJ.
The present results provide evidences that the antagonist of CB1 and TRPV1 receptors induces changes in depressive-like behavior in mice, which is supported by the fact that endocannabinoids and endovanilloids participates in lipopolysaccharide-induced behavioral changes.

Keywords: sepsis, sickness, cannabinoid, lipopolyssacaride

Financial Support: Fapemig, CNPq, CAPES, FINEP

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**EFFECTS OF INTRAHIPPOCAMPAL ADMINISTRATION OF OUABAIN IN THE WNT SIGNALING PATHWAY IN RATS.**

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**Objectives:**
It has been shown that ouabain (OUA) can activate the Na, K-ATPase complex and mediate intracellular signaling in the central nervous system. The present work wants to verify if ouabain (OUA) is able to modulate the Wnt signaling pathway in rat hippocampus and monitor the modulation profile of the NFκB signaling pathway by OUA.

**Methods and Results:**
Methods: Adult Male Wistar rats (300-350g) were positioned in a stereotaxic instrument and guide cannulae were lowered to the dorsal hippocampus, bilaterally. One week after the surgical procedure, each animal received bilateral infusions of 2μl of OUA (10 nM) or 2μl of vehicle (0.9% saline) in different sides of hippocampus over 2 minutes. One group was sacrificed after 1 hour of the end of the infusion and another group, after 2 hours of infusion. For the biochemical tests were performed Western Blot for proteins as β-CATENIN, GSK-3β, pGSK-3β, IκB, pIκB, iNOS and p65. PCR assays were performed for the principal genes modulated by the NFκB signaling pathway as IκB, iNos, Bdnf, Tnf and for genes that are involved in programmed cell death as Bax/Bcl2. Groups of 8 animals. Results: By Western blot, it was found that the OUA 10 nM was not able to modulate the Wnt pathway in 1 and 2 hours. However, after 1 hour of OUA infusion, activation of NFκB signaling pathway was observed by an increase in the nuclear translocation of p65 subunit, increased phosphorylation of IκB and decrease of total IκB amount. By PCR, we observed an increase in gene transcription of iNos, Tnf, Bdnf and Bcl2. There was no change in the level of IκB in 1 hour and neither in levels of gene transcription of Bax.

Conclusions:
Ouabain at a dose of 10 nM was not able to modulate Wnt signaling at 1 and 2 hours of treatment. However, the same dose of the OUA was able to activate the signaling pathway NFκB, to activate the production of neurotrophins like BDNF and of the anti-apoptotic factor BCL2, suggesting that the OUA could have a neuroprotective role in the central nervous system.

Keywords: ouabain, Wnt, NFkB

Financial Support: CAPES, FAPESP and CNPq
CAFFEINE MODULATES EXCITATORY AMINO ACID TRANSPORT IN THE DEVELOPING RAT RETINA

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Objectives:
The retina is an integral part of the Central Nervous System (SNC), and expresses many neurotransmitter systems (such as glutamate) involved in several events during development, such as differentiation, neuroprotection and cell death. Adenosine is a purine able to modulate glutamatergic synapses in the SNC. In this context, investigation of substances that modulate excitatory synapses during retinal development is likely to reveal important mechanisms regulating neurochemical and morphological tissue differentiation. This work investigated whether acute exposure to caffeine alters extracellular levels of excitatory amino acids (EAA) in the developing rat retina.

Methods and Results:
METHODS:
[3H]D-Aspartate uptake. Rat retinas were incubated for 1h in 1mL of MEM containing 1Ci [3H]D-Aspartate. Caffeine at different concentrations (100, 200, 500µM), ZM241385 (10nM), Forskolin (10µM), SQ22536 (1µM) and CGS21680 (10µM) were added during incubation. After several freeze-thaw cycles, cellular radioactivity was assayed using a scintillation counter and protein content assayed by the Lowry method. Cyclic AMP assay. Rat retinas were incubated for 1h in 1mL of MEM containing 0.5mM Rolipram. The reaction was stopped by adding 10% TCA. The cAMP was purified and assayed by methods previously described (Gilman, 1970), and the results normalized for protein content. RESULTS: Characterization of EAA transport: (A) Temperature (T4ºC) and sodium (Na+)-dependent (CTRL=7.14±0.78,n=9; T4ºC=1.53±0.28,n=4; Na+=0.825±0.45, n=4), but independent of chloride ions (6.72±0.097,n=4). (B) Temporal profile of [3H]D-Aspartate uptake in the rat retina at different ages postnatal (P) (P3=10.4±0.73,n=4; P7=7.69±0.97,n=4; P14=3.84±0.62,n=4). Caffeine modulates EAA transport: 200µM caffeine (CAF) is already maximally blocking EAA uptake (CTRL=7.72±0.49,n=6; CAF=3.89±0.61,n=7). (B) The maximal inhibitory effect induced by caffeine (200µM) was observed at 60 minutes. Caffeine modulates EAA uptake during the development: (A) Exposure to caffeine 200µM at P3 and P7 (undifferentiated retinas) decreases aspartate uptake (P3=6.26±0.33,n=4; P7=4.56±0.35,n=4), but not at P14 (differentiated retina) (P14=3.19±0.29,n=4). (B) Inhibitory effect in aspartate uptake induced by caffeine is reversed following its removal (8.92±0.82,n=4). Preincubation with the A2A receptor antagonist, ZM241385, reduces [3H]D-Aspartate uptake (2.6±0.08,n=4). Caffeine effects on EAA uptake is reversed by application of adenyl cyclase activator (forskolin 10µM) (8.23±0.25, n=4), and mimicked by the adenyl cyclase blocker SQ22536 (10µM) (2.9±0.49, n=4). Study of the functionality of A2 receptors by Cyclic AMP assay, activating these receptors with specific agonist A2 (CGS21680, 100nM) at ages P7 and P14 (Basal P7=1.05±0.85,n=4; CGS P7=5.49±0.19,n=4; Basal P14=13.7±3.77,n=4; CGS P14=18.7±3.82,n=4).

Conclusions:
Our results suggest that caffeine blocks EAA uptake via A2 receptors and cAMP. Adenosine modulation of extracellular glutamate levels in the developing retina could ultimately influence cell differentiation and synaptogenesis.

Keywords: ADENOSINE, CAFFEINE, EAAT, GLUTAMATE, RETINA

Financial Support: CNPq, FAPERJ, PROPPi – UFF, PRONEX, UNIC

EFFECT OF CASSIA OCCIDENTALIS LINN (LEGUMINOSAE) AGAINST 3-NITROPROPIONIC ACID INDUCED
BEHAVIORAL ALTERATIONS IN RATS
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Objectives:
Huntington’s disease (HD) is a progressive neurodegenerative disorder characterized by motor impairment, cognitive decline and psychiatric symptoms. 3-nitropropionic acid (3-NP) is a mitochondrial toxin that irreversibly inhibits the activity of the mitochondrial metabolic enzyme succinate dehydrogenase (SDH). Lesions produced by systemic administration of 3-NP is striatal specific, develop spontaneously and the changes are similar to those described in the striatum in HD (J. Neurosci. 13; 4181, 1993 and J. Neurochem. 95; 1521, 2005). Cassia occidentalis (CO) popularly called fedegoso in folk medicine it has been used because of its properties antibacterial, anti-fungal, analgesic, anti-inflammatory and antioxidant. The present study has been designed to explore the possible role of dry extract of stems and leaves of CO on behavioral parameters induced by intraperitoneal (i.p.) administration of 3-NP.

Methods and Results:
Males rats Wistar (250-300g) were used and divided into four groups experimental (n=6/group). Group 1 served as the control group and received pretreatment with a vehicle (NaCl 0.9% i.p.); Groups 2, 3 and 4 received 3-NP (30 mg/kg, i.p.) for 5 days. In addition, after the vehicle or 3-NP treatment for 5 days, the group 1 and 2 received water (10 mL/kg, p.o.) route and group 3 and 4 received CO in daily doses of 400 and 800 mg/kg (p.o.) for 14 days. After the final treatment, the rats were tested for behavioral parameters on day 14. Behavioral assessments were performed using the models of open field, rotarod and elevated plus maze. Data are expressed as mean±s.e.m. Differences between groups were analyzed by ANOVA and Tukey’s test (p

Conclusions:
Treatment of rats with Cassia occidentalis attenuate behavioral changes in 3-NP induced neurotoxicity. The clinical relevance of these findings in HD remains unclear and warrants further studies.

Keywords: Behavioral parameters, Cassia occidentalis, Huntington’s disease, 3-nitropropionic acid
Caffeine is a mild stimulant of the central nervous system and improves memory, particularly at low doses. Caffeine can improve cognitive function through antagonism of adenosine receptors, which induces the release of acetylcholine, or even the induction of synaptic changes, due to prolonged use (Nature 31-39; 361, 1993; Ann. Rev. Neurosci. 149; 21, 1998). Recently, caffeine administration has also been associated with changes in the rate of neurogenesis in adult rodents hippocampi (Neurophamacol. 1-7; 30, 2009), what could affect memory and learning (Science 325, 210-213, 2009). In this study, we investigated the effects of treatment with low and high doses of caffeine on two episodic-like memory tasks, which have been shown to be hippocampal-dependent (Behav. Brain Res. 1-8; 300, 2009; Hippocampus 955-964; 18, 2008).

Methods and Results:

Eighteen three-month-old male Wistar rats (200 - 350g) were treated i.p. with saline, 15 or 30 mg/kg caffeine once a day for 45 days, and episodic-like memory behavioral tasks were started on the 29th and 41th days of treatment, respectively. The first one recollects what-when and what-where aspects of memory. The rats were exposed to four copies of an object on the first trial and then to a second sample of four copies of a new object on the second trial (after 1 h interval). The objects were placed on different positions on two trials. In the test, after 24h, the animals were exposed to two objects from each sample trial, located on the same position as before, except for one copy of the first sample (displaced old object). The second task consists of three 5-min-trials with 3-min-intervals between them in which the animal is exposed to the same object located in different positions in each trial. In the test trial, after 30 min, two copies of the object are presented (one of them in the same position as the first sample and the other in the same position as the third sample trial. Test trial is performed upon two conditions: high and low interference, with the two copies of the object distant 42 or 84 cm apart, respectively. Our results showed that in the first task rats treated with saline failed to present increased exploration of old objects (mean ± SE: 0.43 ± 0.11) compared to recent ones (0.57 ± 0.11), while rats treated with caffeine (15 mg/kg) explored more the old (0.77 ± 0.09) compared to recent objects (0.24 ± 0.09, paired samples t-test, p = 0.033). However there were no differences between exploration rate of displaced and non-displaced objects, in any of the groups. In the second task, under high interference condition, there was no difference in the exploration time of the first object compared to the third object in both control (2.72 ± 1.79; 4.72 ± 3.76) and caffeine 15 mg/kg (4.23 ± 1.99; 2.63 ± 1.04) groups. However, the caffeine 30 mg/kg group explored more the first (11.08 ± 3.79) object than the third one (4.68 ± 1.95; ANOVA and Bonferroni post-hoc, p = 0.050)

Conclusions:
Our results suggest that chronic caffeine administration improves temporal aspects of episodic-like memory in object recognition tasks. It should be noted that caffeine, even at high doses could improve memory on conditions of greater difficulty.

Keywords: caffeine, memory, rats

Financial Support: CAPES, CNPq and FAPERN.
expression of AMPA glutamate receptors induced by intrahippocampal NMDA injection.

Methods and Results:

Male Wistar rats (250g) were divided in two main groups, control (0.6 µL saline) and NMDA (7.2µg/0.6µL) bilaterally injected in hippocampus. The NMDA group was further divided in 3 subgroups: 24 hours, 2 weeks and 4 weeks (time after injury when animals were tested and sacrificed). Spatial memory was evaluated on Morris Water Maze and the expression of GluR1 and GluR2 subunits analyzed by immunohistochemistry. Groups compared by oneway anova and Newman Kewls (significant when p≤0.05) Spatial memory test showed that groups 2 or 4 weeks post-injury had higher latencies (110%) to find the platform compared to control and this increase persisted during the four days of training (p< 0.001]. However, in CA3 an increase was observed 2 weeks (22%) and 4 weeks (21%) after injury while no difference was found after 24hours (p< 0.001).

Conclusions:

The results suggest that glutamate is involved on the mechanisms of injury triggered by intrahippocampal NMDA injection in different ways considering: i) GluR1 or GluR2 subunit, ii) specific area of the hippocampus and iii) time after injury. Specific changes on GluR1 and/or GluR2 expression by NMDA may contribute to either neurodegeneration by excitotoxicity due to a higher Ca2+ inflow or neuroregeneration by the mechanisms of plasticity.

Keywords: NMDA, hippocampus, neurodegeneration, AMPA

Financial Support: Capes/PROEX, Fapesp

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Resumo:23-259

WHY DOES MEMORY BECOME LABILE AND RECONSOLIDATE AFTER RETRIEVAL?

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Objectives:

An established memory reactivation might undergo a labile state, requiring a reconsolidation process in order to persist. The result of this plastic state is that memory can be changed after the destabilization induced by reactivation. Although much has been learned regarding the molecular and cellular mechanisms of reconsolidation process in different species and experimental paradigms, little is known about its biological function. Here, we provide a wider comprehension on the possible functional roles of memory reconsolidation. Specifically, it was investigated if memory updating, precision maintenance overtime and strength are mediated by reconsolidation mechanisms.

Methods and Results:

Rats were fear conditioned (context A, day 1), exposed (or not) to a reactivation session (context AB, day 3) and tested in the context B (day 5). Animals that were not exposed to the reactivation session or treated with nimodipine i.p. (LVGCC blocker, acting on impairing memory destabilization, but not retrieval) expressed significant less freezing than reactivated rats (one way ANOVA followed by Tukey post hoc test (p0.05; n=7-8 per group). Finally, in order to check if reactivation can strength memory, rats were fear conditioned (day 1), reactivated or not (day 14) and tested for 12 min to induce extinction (day 28), always in the same context. Repeated measure ANOVA revealed that the non-reactivated group and reactivated+nimodipine group have extinguished memory (p

Conclusions:
These results show that memory reactivation play an important role in updating and keeping memory precision (qualitative contend) and memory strength (quantitative contend) over time. Furthermore, we provide evidences that these adaptive features are mediated by memory reconsolidation. Thus, we have shown that memory reconsolidation has a crucial biological function in order to keep memory contend or change it, depending on the situation. That is, if the original information is the same, then memory is strengthened, but if it changes, memory reconsolidation update this information in order to guide accurately the behavior response.

Keywords: memory, reconsolidation, updating

Financial Support: CAPES, CNPQ, UFRGS
INCREASED GABAERGIC NEUROTRANSMISSION IN AMYGDALA INTERFERE WITH AVERSIVE MEMORY IN FEMALE RATS

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Objectives:
GABA neurotransmission has been implicated in several aspects of mood disorders and memory and learning. The amygdala is an essential structure for emotional memory modulation and it has been one of the major focuses for research on GABAergic participation on memory. However, most of the animal studies on this issue are held with male subjects, but there is evidence that female mice have an increased GABAergic transmission when in proestrus or estrus (j.psyneuen. 34:84,2009). In the present study, we investigate the consequences of an increased GABAergic transmission in the amygdala on aversive memory retrieval and extinction in female rats in proestrus and estrus.

Methods and Results:
Three-month old female Wistar rats about 230g were implanted with bilateral cannula aimed at the basolateral amygdala (BLA). Estrous cycle phase was determined by vaginal cytology for two weeks before behavioral tests and experiments were scheduled so that females were in proestrus or estrus during test. We used the discriminative avoidance task in the elevated plus-maze to access memory retrieval and extinction. During training the animals learned to avoid one of the closed arms, in which they receive an aversive stimulus, consisting of a bright light and a loud noise. Fifteen minutes before the test session (held 24h later) animals received bilateral intra-amygdala 0.2 μL injections of saline (n=8) or GABAR agonist muscimol (0.05 mg/ml, n=7). As expected animals showed acquisition of the task, indicated by the lower relative time in aversive arm during the final minutes compared to the initial minutes of training (saline 1st-3rd min: 7.82±1.60; 8th-10th min: 1.61±0.73 – muscimol 1st-3rd min: 10.22±3.08; 8th-10th min: 0.63±0.61). Saline group, both in proestrus and estrus, retrieved but did not present memory extinction, as indicated by similar low relative time in aversive arm during all test (Proestrus 1st-10th min: 12.66±3.04; n=4 – Estrus 1st-10th min: 8.08±5.54; n=4). In contrast, both proestrus and estrus muscimol groups showed high aversive arm exploration during all test (Proestrus 1st-10th min: 31.71±2.49; n=3 – Estrus 1st-10th min: 42.23±0.58; n=4), indicating impaired evocation. Innate fear was not altered in any group, as showed by similar relative time in open arms during the initial minutes of the test (Saline proestrus 1st-3rd min: 24.44±6.75; n=4 – Saline estrus 1st-3rd min: 19.38±11.10; n=4 – Muscimol proestrus 1st-3rd min: 18.22±12.90; n=3 – Muscimol estrus 1st-3rd min: 30.55±9.78; n=4), suggesting that the effects of muscimol were not due to an overall fear decrease, but by a specific interference in the learned fear memory.

Conclusions:
Increased GABAergic transmission was able to disturb aversive memory retrieval. This indicates that in females the amygdala is able to influence memory retrieval in this task and that a normal GABAergic transmission is important to properly retrieve memories. More studies will be necessary to confirm those results and clarify the role of female gonadal hormones in the relationship of amygdala GABAergic system and emotional memory.

Keywords: amygdala, estrous cycle, females, GABAergic, memory

Financial Support: CNPq, CAPES, PROPESQ-UFRN
GLUTAMATERGIC MECHANISM IS INVOLVED ON THE SUPRESSION OF LPS-INDUCED FEVER BY PARACETAMOL IN RATS.

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Objectives:

Glutamatergic neurotransmission in several brain areas is involved on the febrile response. Specifically in hypothalamus the concentration of glutamate and the core temperatures were simultaneously increased following systemic administration of LPS in rabbits (Eur. J. Pharmacol., 2008, 593:105). The aim of this study was to investigate the involvement of glutamate AMPA receptors on the brain mechanisms activated by pretreatment with the antipyretic paracetamol in rats after i.p. injection of LPS.

Methods and Results:

Methods: Male Wistar rats (200g; n=3-6) received vehicle (ethanol 10% in saline plus 20µl tween 80) or paracetamol (200mg kg-1) i.p. 30 min before (0.5 ml) of LPS (50µg kg-1) or saline (control group). The rectal temperature (oC) was measured every 30 minutes for up 3h by telethermometry. The fever induced by LPS (3 h: 1.4 ± 0.06 oC) was reduced by treatment with paracetamol (3 h: 0.3 ± 0.08 oC). Saline injection did not alter the basal temperature of the animals (3 h: 0.1 ± 0.08 oC). After 3 hours the animals were deeply anaesthetized (50ml/kg of urethane 25%), perfused with paraformaldehyde 4% and their brains removed. 40-µm sections were used for immunohistochemistry. The immunopositive cells (IC) were counted by 2-3 examiners independently, bilaterally, in 3 sections/rat, in lateral hypothalamus (LH), anteroventral thalamic nucleus (AV) and ependymal and subependymal layer/olfactory ventricle (E/OV). Data were compared by one-way ANOVA followed by Duncan test (p<0.05). A reduction of 29% was induced by LPS only in GluR1 expression in E/OV, but it was not significant when compared to saline (p>0.05).

Conclusions:

Glutamatergic mechanism in LH through GluR1 AMPA receptors is involved on the antipyretic effect of the paracetamol during LPS induced fever. However, this mechanism in AV or E/OV is not involved on the paracetamol antipyretic effect.

Keywords: Fever, LPS, AMPA Receptors, Paracetamol, Immunohistochemistry

Financial Support: FAPESP and FPA.
Objectives:

Tryptophan is an essential amino acid precursor of serotonin (5-HT). The 5-HT may promote changes in the growth of tissues that influence the body structure of animals. Researchers have observed that malnutrition can also cause changes in mice’s body growth. This study evaluated the effects of the administration of L-Tryptophan 15mg on body weight and growth of the neonatal rat tail or not undergone to protein restriction during the lactation period (1th to 21st postnatal days).

Methods and Results:

Newborn male Wistar rats were divided according to treatment group, distilled water (DW) n = 20, tryptophan 15mg (TRYP = 15mg/kg/day sc) n = 14, distilled water malnutrition (DW MN) n = 17, tryptophan 15 mg malnutrition (TRYP MN = 15mg/kg/day sc) n = 17, during breastfeeding, where the mothers of the malnourished group were undergone to protein restriction (8% protein) during this same period. In the groups there were evaluated the parameters body weight (BW) and tail length (TL). Data were analyzed using ANOVA - Two Way Repeated Measures followed by Holm-Sidak’s test. There were used a Starret Caliper (0.01 cm of accuracy) for the body measurements and semi-analytical digital scale Mars, AS 2000C model, capacity of 2000g (0.01 g of accuracy). The group TRYP, compared with the DW MN group, showed a reduction of the TL, F (3.1406) = 36.270, p

Conclusions:

This study demonstrates that the treatment, during the period of 21 days postnatal, with tryptophan in the group undergone to protein restriction reduced BW and TL when compared to nourished. When compared with the malnourished control group it was increased the BW and TL. In the TRYP group increased BW and TL decreased compared with the control group malnourished. More studies are needed to elucidate the present findings.

Keywords: GROWTH OF SOMATIC, MALNUTRITION, NEONATAL RATS, TRYPTOPHAN, WISTAR

Financial Support: CAPES, FAPESB, CNPQ, PIBIC, UFBA

QuebraPagina

Resumo:23-264

EFFECT OF BONE MARROW MONONUCLEAR CELLS IN MPTP-LESIONED RATS

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Objectives:

To determine the effects of mononuclear cells transplantation in MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)- lesioned animals 3 and 7 days after neurotoxin infusion using the open field test and forced swimming test in the eighth day.

Methods and Results:

In this study, rats were randomly divided in four groups: Sham+Saline (n=10), Sham+Mononuclear cells (n=10), MPTP+saline (n=10), and MPTP+Mononuclear cells (n=10). The MPTP and Sham groups were anesthetized with sodium thiopental (40 mg/kg), then arranged in stereotaxic equipment and were infused bilaterally with 1 µl of MPTP or saline within the substantia nigra pars compacta. After surgery, the animals were left in a temperature-controlled chamber until they had recovered from
anesthesia. Twenty four hours after surgery the rats were evaluated in the open-field test and then saline or bone marrow mononuclear cells isolates from animals by ficoll method’s were labeled with CM-Dil cell tracker and then were injected intravenously in the concentration of 5 x 106 cell/ml. In the third and seventh days after surgery the rats were evaluated in the open field test and in the eighth day forced swimming test. Data from the open field test twenty four hours after surgery were analyzed by unpaired t test. Data from the open field test third and seventh days after surgery and forced swimming test were analyzed by ANOVA followed by Tukey-Kramer test. The values were expressed as mean ± S.E.M. Twenty four hours after the surgery, MPTP lesioned rats exhibited reduced locomotion (29.73±6.75), low rearing frequency (4.63±1.04) and more latency to start the movement (17.70±4.21) and immobility time (85.84±18.47) in comparison to Sham animals (locomotion 67.57±7.36; rearing 10.94±2.25; latency to start the movement 7.41±0.81; immobility time 16.68±5.38). Three days after cells transplant and seven days after surgery MPTP-lesioned rats treated with mononuclear cells didn’t differ of the other groups at the open-field test. However, seven days after surgery MPTP+mononuclear cells group exhibited reduced swimming time (138.12±12.58) and more immobility time (92.54±16.17) in comparison to MPTP-saline group (swimming time 221.92±6.11; immobility time 30.44±6.71).

Conclusions:
The MPTP-lesioned rats exhibited reduced motor function twenty four hour after the surgery in comparison to Sham animals when evaluated in the open field test. Three and seven days after surgery MPTP-treated animals didn’t showed behavioral alterations observed twenty four hours after surgery. These results weren’t modified by bone marrow mononuclear cells transplant at open field test and forced swimming test. Thus, our data demonstrated that bone marrow mononuclear cells intravenous transplant twenty four hours after MPTP infusion wasn’t able to prevent the behavioral alterations induced by MPTP intranigral infusion and suggest a depression of catecholamine production letting the animals transplanted more lethargic.

Keywords: Forced swimming test, Mononuclear cells, MPTP, Open field test, Parkinsonism

Financial Support: CAPES and CNPq

QuebraPagina

Resumo:23-265

POSSIBLE DORSAL HIPPOCAMPUS NMDA RECEPTORS/ NITRIC OXIDE PATHWAY MEDIATING AUTONOMICS RESPONSES IN RATS SUBMETED TO RESTRAINT STRESS

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Objectives:
The dorsal hippocampus (DH) is a limbic structure involved with modulation of cardiovascular responses. Injection of L-glutamate (L-glu) in DH evokes tachycardia and hypertension in unanesthetized rats. NMDA receptors are presents in the DH and nitric oxide synthase (NOS) has been associated with NMDA responses. Acute restraint is an unavoidable stress situation that evokes autonomics changes, characterized by elevated mean arterial pressure (MAP), intense heart rate (HR) increases and decrease in the tail temperature.

Methods and Results:
Male Wistar rats (250-270g) were used. Guide cannula was implanted bilaterally in the DH for drug injection and a polyethylene catheter was implanted in the femoral artery for MAP and HR recording using a computerized acquisition system. Tail temperature was measured at the initial portion of the rat tail, using a thermal camera (Multi-Purpose Thermal Imager IRI 4010). The animals were submitted to restraint, which was initiated by introducing animals into a small plastic cylindrical restraining tube (diameter =6.5cm and length =15cm) and lasted for 60 minutes. All drugs were administrate 10 minutes before the restraint stress and were dissolved in saline (vehicle).The restraint stress increase MAP (F35, 468 =5.095, P<0.001).
Conclusions:

The present results indicate that glutamatergic neurotransmission present in DH modulate autonomic responses during restraint stress. They also indicate that a DH NMDA-nitric oxide pathway mediates those responses.

Keywords: Cardiovascular, Defense Reactions, nNOS

Financial Support: CNPq, FAPESP and FAEPa

QuebraPagina

Resumo:23-266

POTENTIAL PROTECTIVE ROLE OF CB2 RECEPTORS IN DIFFERENT ANIMAL MODELS OF ALCOHOLISM

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Objectives:

Despite the well established role of endocannabinoid system in drug addiction, notably via CB1 receptor signaling, the role of CB2 receptors in this condition remains to be clarified. Previous studies described an important individual variability regarding the locomotor sensitization induced by ethanol (EtOH_Sens x EtOH_Nsens). Likewise, our group found similar variability concerning the rewarding effects of ethanol in the conditioned-place preference: EtOH_Cpp (preferent - positive reinforcement memory), EtOH_Cpa (aversive - aversive memory) and EtOH_Neither (indifferent – ethanol leads neither rewarding nor aversive memory). Here we analyzed the expression of CB2 receptors in these two animal models.

Methods and Results:

Adult male Swiss mice were used in both paradigms. For locomotor sensitization, they were daily injected with ethanol (2 g/Kg, i.p. 15 % v/v), for 21 consecutive days. Control mice were similarly injected with saline. After 4 days of withdrawal, all mice were challenge with ethanol (1.4 g/kg, i.p.). The locomotor activities were measured during 15 min in an active cage, before ethanol treatment (baseline), after the first and last ethanol injections, and after ethanol challenge. According to the locomotor activity after ethanol challenge, the animals were allotted to one of two groups: EtOH_Sens and EtOH_Nsens. The animals were perfused 24 hours after challenge with ethanol. Immunohistochemistry analysis showed that EtOH_Nsens group had increases in CB2 immunoreactivity in the hippocampus CA1 [F(2,15)=5.61, p=0.015], CA2 [F(2,15)=7.96, p=0.004], CA3 [F(2,15)=3.78, p=0.046] and dentate gyrus [F(2,15)=3.88, p=0.043]; in the infra limbic [F(2,15)=9.39, p=0.002] and pre limbic cortex [F(2,15)=4.12, p=0.037]; in the nucleus accumbens core [F(2,15)=5.6, p=0.015] and shell [F(2,15)=5.04, p=0.021], when compared with the other groups. For place preference paradigm, mice were injected with saline (at morning) and ethanol 2g/kg; i.p. (at afternoon) for 5 consecutive days. Control group received saline at morning and afternoon. The score of preference was obtained by measuring the time spent in compartment paired with the drug before and after the pairing. According to the score of preference, animals were classified as EtOH_Cpp, EtOH_Cpa, EtOH_Ind. The CPA group had increases in CB2 immunoreactivity in the infra limbic [F(3,20)=4.9, p=0.01] and pre limbic cortex [F(3,20)=3.75, p=0.02] and in the nucleus accumbens core [F(3,24)=4.0, p=0.019] when compared to the other groups.

Conclusions:

CB2 receptor could play an important role in the development of locomotor sensitization, as well as, in the aversive hedonic state.
induced by ethanol.

Keywords: alcoholism, CB2 receptors, drug abuse, endocannabinoid

Financial Support: CNPq and CAPES

QuebraPagina

Resumo:23-267

THE ROLE OF STRESS IN MEMORY PERSISTENCE

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Objectives:
In this study we investigated the interactions between the stress by immobilization and its effects on the persistence of memory and also a possible effect mediated by β-adrenergic modulation of stress on memory persistence.

Methods and Results:
Male Wistar rats (n = 140) were obtained from breeding colony of Universidade Federal do Rio Grande. The animals were maintained in groups of five in each cage, with a 12-h light/dark cycle, at a temperature of 22°C, with food and water ad libitum. The stress through immobilization procedures were carried out with a plastic tube with an anterior orifice for respiration, sealed with a plaster tape on the outside of the tube. The animals were maintained on movement restriction for 1 or 3 hours. In a second series of experiments, the animals were injected with β-adrenergic blocker propranolol (25mg/Kg, IP), while the control group received an IP injection of saline. For the reversion experiment, a stress 1h group received IP injection of saline or propranolol prior to stress session. All the treatments were performed 12h after the training session in Inhibitory Avoidance task (IA). After a week of acclimatization, the animals were submitted to IA. On the training section, each animal was placed over the platform and, immediately after stepping down with its four paws on the steel floor, the animal received three 0.7mA, foot-shock. In the test session no foot-shock was given and the step-down latency was cut off at 180s. Memory retention was tested 2 or 7 days post training, different groups of animals were used to test 2 or 7 days to avoid extinction of memory. The animals were also submitted to an elevated plus-maze (EPM), 2 or 7 days after the treatments, to determine if the treatments affected mobility, locomotion or caused pro- or anti- conflict behaviors. When the stress was induced 12h after de training session during 1h, the persistence of memory tested 7 days after the training was improved, (p = .0076, n = 10-12, Mann Whitney test), but not 2 days after the acquisition in IA. No differences were found on animals exposed to stress for 3h on 2 or 7 days IA test (p = .4532, n = 10, Mann Whitney test). The propranolol treated group presented a normal memory formation on a 2 days test (p = .8421, n = 10, Mann Whitney test). When tested 7 days after the IA training session an impaired memory retention was observed (p = .0030, n = 10, Mann Whitney test). Propranolol was infused immediately before the 1h immobilization stress in an attempt to revert the previously observed facilitation of memory retention at 7 day test. The propranolol/stress group showed a significant difference from stress 1h group (p = .0250, n = 10, Mann Whitney test), and no difference was observed from control group, while the saline/stress group demonstrated a memory facilitation (p = .4698, n = 7-10, Mann Whitney test). Moreover propranol/stress group showed no difference from the control group (p = .4698, n = 7-10, Mann Whitney test). There were no alterations in the percentage of time spent on open arms or total of entries among groups (p = .0206 on One-way analysis of variance, with no post test significance).

Conclusions:
These findings suggest that moderate stress facilitates the persistence of memory and β-adrenergic receptors are involved in this enhancement. Moreover, it is suggested that β-adrenergic receptors themselves participate in the process of memory persistence.
Objectives:

Rats reared in isolation from weaning have been used as an experimental model of affective disorders like schizophrenia. Several evidence have shown that this isolation induces behavioral changes like hyperactivity in a novel environment. However, in a previous study we did not found this alteration when the animals were tested for five minutes in the arena. In this study we evaluate the pattern of locomotor and exploratory activity during twenty minutes in the arena showed by rats submitted to social isolation for 10 weeks from weaning.

Methods and Results:

Two groups of Wistar rats (n=12 each) were used. In both groups the pups remained with their mothers (6 pups per mother) until weaning (21 days - 40g) when they were allocated randomly to one of two conditions: 1) grouped, housed 3 per cage and handled 3 times a week; 2) isolated, housed individually and handled once a week for cleaning purpose. After 10 weeks all animals were tested in a circular open arena. The animals were put into the middle of the arena and the behavioral responses scored every minute for 20 minutes: Number of crossings (horizontal exploration), number of rearings (vertical exploration) and time spent at the center or at the periphery of the arena. Groups were compared by ANOVA and Student t-tests and the level of significance was set at p

Conclusions:

These results suggest that Wistar rats reared in isolation from weaning should be exposed to a novel environment for longer time to present hyperactivity. This behavior has been shown to occur for others rat strain in five minutes of exposition. Wistar rats may present less susceptibility to develop this behavioral response after isolation. Rats reared in isolation from weaning show a pattern of exploration with a clear preference for the periphery of the arena, which probably reflect the emotional aspects of schizophrenia.

Keywords: Social Isolation, Schizophrenia, Chronic Stress, Locomotory Behavior, Exploratory Behavior

Financial Support: CAPES, FAPESP and FPA.

RAPID ESTROGEN TREATMENT ACTIVATES MAP KINASE AND AKT IN NEURONAL PRIMARY CORTICAL
CULTURES AND THE MAINTENANCE OF THIS ACTIVATION IS DEPENDENT OF GLIAL CELLS.

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Objectives:

Estrogen (E2), the female sex hormone, plays an important role in homeostasis, protection, and plasticity in the central nervous system (CNS). E2 can act through classical, nuclear-initiated and/or non-classical, membrane-initiated mechanisms. These classical nuclear actions are well known and are mediated by the activation of intracellular receptors (ER), which act as transcription factors. The membrane-initiated, rapid E2 actions are believed to be mediated by the G-protein coupled membrane receptor newly discovered, GPER-1. In breast cancer cell lines, GPER-1 is thought to act through the activation of protein kinases, such as MAP kinase (mitogen activated protein, ERK) and Akt kinase, but little is known about GPER actions in the CNS. In this study we sought to analyze the rapid E2-effects on the activation of MAPK and AKT in primary neuron-enriched and mixed (neuronal and glial cells) cortical cells.

Methods and Results:

Primary cortical cultures were obtained from newborn rats (P1-P4) as described previously (Ahlemeyer, J Neurosci Methods, 149:110, 2005). Mixed cultures were maintained in DMEM (High Glucose media) supplemented with 10% Fetal Bovine Serum, 10% Horse serum and 0.1% penicillin/streptomycin. Enriched neuronal cultures were maintained in DMEM supplemented with 2% B27 and 0.1% penicillin/streptomycin. On the 5th day after culturing, the media was replaced by phenol-free media and on the 8th day after culturing, cells were treated either with vehicle (ethanol) or E2 (10uM and 10nM) for 5, 10, 15 or 30 minutes. Cell viability was measured with the CellTiter-Blue® Cell Viability Assay (Promega). The content of phosho-ERK and phosho-Akt, an index of kinase activation, were addressed by Western blot. Our results showed that E2 rapid-treatment did not modulate cell viability neither in mixed nor in neuron-enriched cultures in any concentration analyzed (n = 4 independent experiments; ANOVA one-way post hoc Newman-Keuls test, p>0.05). Western blot results indicated that in mixed cultures, E2 (10uM or 10nM) activated MAPK (measured as phosho-ERK/total ERK ratio) after 15 minutes and this effect persisted until 30 minutes (n = 4 independent experiments; ANOVA one-way post hoc Newman-Keuls test, p

Conclusions:

Our results showed that rapid E2 treatment activated MAPK and AKT in cortical primary cells and the persistence of this activation was mediated by glial-cell.

Keywords: estrogen, GPER, glial cells, neuron

Financial Support: FAPESP, CNPq and CAPES

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Resumo:23-270

MODULATORY EFFECTS OF CAFFEINE ON GABAERGIC SYSTEM IN EMBRYONIC AVIAN RETINA

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Objectives:

We are investigating the modulatory effects of caffeine, non-selective adenosine receptors antagonist (A1R and A2R), on NMDA receptors regulating GABA release in embryonic chick retina.

Methods and Results:

Retinas from 13-day-old chick embryo (E13) were dissected in CMF (calcium magnesium free solution) and incubated with 3H-GABA or 3H-D-Aspartate in 1 mL of Hanks solution with 4mM of glucose (Hanks 4) or in a modified Hanks 4 solution (without magnesium ions) during 1 hour in 37°C. After this period, the medium was washed out with cold Hanks 4 to 3H-GABA or 3H-D-Aspartate uptake assay or to proceed 3H-GABA release assay. The results were analyzed by GraphPad software with t test or ANOVA for 3 or more experimental groups followed by Bonferroni test. The values were expressed as Mean±SEM and the significance levels were considered as p-value

Conclusions:

The presented data suggest that caffeine potentiates D-Aspartate induced GABA release in a magnesium free medium by activation of NMDA receptor. This effect is mimicked by the activator of the AC but is not mediated by A2a receptor.

Keywords: caffeine, GABA release, NMDA receptor, retina

Financial Support: CNPq, Capes, Proppi, INNT, Pronex, Faperj.

Malnutrition and Maternal Lactation in Treatment with L-Tryptophan (15 mg):
Implications in the Maturation of Reflections in Wistar Rat Neonates.

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Objectives:

Aim: The nutritional deficiency alters morphogenetic events occurring during the critical period of development of the nervous system, with consequences in the maturation of physiological patterns of the system. The aim of this study was to evaluate the application of isolated tryptophan and how it influences in the maturation of the reflexes in rats malnourished during lactation.

Methods and Results:

Materials and Methods: Groups of newborn male Wistar rats were divided into four groups (n = 11) according to the administration of distilled water or saline (SAL) or L-tryptophan 15 mg/kg/day (TRIP) of 7 to 21 days postnatal (PN) and maternal diet during lactation (standard 23% protein: NUT or hypoproteic with 8% protein: DES) and called: DW NUT, DW DES, NUT TRIP, DES TRIP. The groups were observed until Day 21 PN as: Palmar-Pressure (PP), Recovery of the decubitus (RD), Negative-Geotaxis (NG), Free-fall righting (FR), Vibrissa-placing (VP), Cliff aversion (CA) and Reaction to Scare (RS). Used the statistic test Sigma stat version 3.5. Results: The DW group when DES was to compare DW NUT, appearance acceleration in the onset of RD, p < 0.001. There was no difference between groups towards VP, FR and the reflexes FR, RD e PP were not assessed. Compared to DW DES: Palmar-Pressure (PP) 7 (4-10) lying down Recovery (RD) 2 (1-5), Vibrissa-Placing (VP) 12 (10-13); Cliff Aversion (CA) 12 (8-14), negative geotaxis (NG) 16 (14-17); startle response (SR) 12 (11-13), Cliff Aversion (CA) 15 (8-16) group NUT TRIP 15 presented in anticipation disappearance of PP 4 (4-6) (p = 0.003). There was no difference between groups for the reflex VP, CA, RS, FR. While the reflexes NG and RD were not evaluated. The group
presented 15 NUT TRIP PP 15 4 (4-6), RD 2 (1-4), VP 13 (10-15), CA 9.5 (6-15) NG 15 (12-16) RS 11.5 (10-12), FR 12 (8-13) and compared to DES TRIP 15, presented early in the onset of RS and FR, p

Conclusions:

Conclusion: Alone or in combination, malnutrition and implementation trip. seem to anticipate the late RD and NG. Tryptophan malnutrition was associated with delayed onset of RS. Malnutrition associated or not to show trip delay in the maturation of some reflexes.

Keywords: L-Triptophan, Malnutrition, Maturation of reflections

Financial Support: PIBIC, FAPESP, CAPES, UFBA

QuebraPagina

Resumo:23-272

LEARNING AND MEMORY: POSSIBLES ALTERATIONS IN COGNITION BY USE MONOTHERAPY OR COMBINATION WITH OF TWO ANTIDEPRESSANTS IN RATS

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Objectives:
The present study aimed to investigate the possibles alterations on memory by chronic use monotherapy or combinations of two antidepressants.

Methods and Results:
In the protocol, male rats (250g -315g, g=7-10/group) chronic treatment for 14 days three groups were treated with different antidepressants 10mg/Kg, i.p in different ways: monotherapy (2 groups – venlafaxine e paroxetine); combination therapy [1 group – venlafaxina (5mg/Kg)+ paroxetine (5mg/Kg)] and one group with saline 24 h after the last dose the rats were trained on the 8-arm radial maze up to on accuracy level in choice 80%. After training of learning memory rats were tested. Statistical analysis was performed using ANOVA with Student Newman Keuls as post hoc test and differences were considered when p< 0.005. In training of learning rats the group treated with venlafaxine (monotherapy) and venlafaxine + paroxetine (combination drugs) presented significantly increased number trials when compared to control group. (Training of learning: control – 5,1 ± 0,4547; Paroxetine – 4,143 ± 0,83; Venlafaxine – 11 ± 1,983; Venlafaxine + Paroxetine – 10,88 ± 1,875. In test of memory the treatment chronic of groups did not present significantly changes in the number of errors. Memory test: Short-term memory: control – 3,1 ± 0,564; Venlafaxine – 2,167 ± 0,6; Venlafaxine + Paroxetine – 2,857 ± 0,5084; Long-term memory: control – 3,1 ± 0,45; Paroxetine – 4,4 ± 0,48; Venlafaxine – 4,5 ± 0,68; 2,7 ± 0,33.

Conclusions:
These findings suggest that both the treatment - monotherapy and combination drugs antidepressant – doesn’t adversely affects working memory performance.

Keywords: antidepressants, cognition, learning, memory, radial maze

Financial Support: CAPES
BDNF LEVELS IN WOMEN WITH POSTPARTUM AFFECTIVE DISORDER AND SUICIDALITY BDNF, POSTPARTUM MOOD AND SUICIDALITY

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2 Departamento de Ciências Fisiológicas, UFCSPA  
3 Departamento de Bioquímica, UFRS

Objectives:
Investigate serum brain-derived neurotrophic factor (BDNF) levels in postpartum affective disorder (PPAD) women who were at risk for postpartum suicide.

Methods and Results:
Methods A cross-sectional study was carried out with women between 45 to 90 days after delivery. PPAD (depression, manic and mixed episode) and suicide risk were assessed using the Mini International Neuropsychiatric Interview (MINI). BDNF was assessed using a commercial ELISA kit. Linear regression and Poisson regression were used for multivariate analyses. Results A hundred ninety women participated in the study, 15.3% had PPADs, 7.4% showed PPAD with suicide risk. BDNF levels were lower in subjects with three or more Stressful Life Events (p=0.01). The serum BDNF levels of women with PPADs presenting suicide risk were significantly lower than those of women without suicide risk (1.50 ± 1.38 ng/ml and 2.33 ± 1.28 ng/ml, p=0.02). In the adjusted Poisson regression PPAD and suicidality showed a risk of 10.73(CI 2.09 to 55.03) for lower serum BDNF levels.

Conclusions:
Clinicians should enquire postpartum women about their history of stressful life events, PPAD, and suicidality. This study shows the potential role of BDNF in the neurobiology of association of PPAD and suicidality. This may contribute in psychiatryclinical decisions.

Keywords: BDNF, POST-PARTUM, SUICIDALITY

Financial Support: CNPq, CAPES, PRONEX- FAPERGS, and FINEP IBN-Net andINCT-EN
Objectives:

The aim of this study was evaluate the effects of L-Tryptophan 15mg (TRYP) administration on the immune response of young rats undergone to the protein restriction during lactation, by counting the total and the differential of leukocytes.

Methods and Results:

Male Wistar’s rats were divided into two groups (n = 11 each) according to the Distilled Water’s Treatments (DW) and Tryptophan 15mg (TRYP = 15mg/kg/day sc) whose mothers were submitted to the protein restriction (8% protein) during the lactation period (1st to 21th postnatal days). After 50 days of life these animals were undergone to blood collection to perform the count. For analysis of WBC was extracted from 0.5 ml blood and deposited in 5 ml tube previously incremented of one drop (20µl) of anticoagulant (EDTA - ethylene diamine tetra-acetic to 3%-acid) and performed total and differential count of leukocytes (neutrophils, monocytes, lymphocytes). It was used Turk’s solution, optical microscope with a 40x magnifying glass and a Neubauer chamber, kit Quick Panoptic LB - Laborclin Ltd., optical microscope with 100X objective and immersion manual cell counter DigiTimes’s branded with 8 buttons for each type of cell. The total and differential count of leukocytes was not significantly different between the groups.

Conclusions:

Preliminary results suggest that administration of tryptophan, in the period of lactation, does not alter the immune response in young animals undergone to protein restriction. More studies are needed to elucidate the presents such findings.

Keywords: Immune Response , Leukocytes, Protein restriction , Tryptophan, Wistar’s rats

Financial Support: CAPES, FAPESB, CNPQ, PIBIC, UFBA

QuebraPagina

Resumo:23-275

MALNUTRITION AND MATERNAL TREATMENT WITH L-TRYPTOPHAN (10 MG): EFFECTS ON MATURATION OF REFLEXES IN RATS WISTAR NEONATES.

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Objectives:

The serotonin (5-HT), synthesized by the essential amino acid tryptophan, can influence the maturation of reflexes. The aim of this study was to evaluate effects of neonatal treatment with 10 mg TRIP on the maturation of reflexes in the offspring of rats undernourished during lactation.

Methods and Results:

Methods: Groups of rats newborns were divided into four groups (n = 10) according to the administration sc of distilled water (AD) or L-tryptophan 10 mg / kg / day (TRIP) of 7 to 21 days post natal (PN) and maternal diet during lactation (standard 23% protein; NUT) or (hipoproetic with 8% protein; DES) and called: NUT AD, AD DES, NUT TRIP, TRIP DES. The groups were observed until Day 21 PN regarding: handgrip (PP); recovery of recumbency (DR), placing the vibrissae (CV), aversion to the cliff (AP) negative geotaxis (NG) response to shock (RS ) and acceleration (AC). We used table ramp with an inclination of 45 °, 30 cm ruler attached to the polyester pillow, container, metal stick and stopwatch to assist in the observations. Data were
analyzed using Mann-Whitney Rank Sum Test and the number of days of life, are expressed as median (maximum and minimum). Results: Group 4 TRIP NUT PP (3-6) RD 5.5 (2-7), CV 9 (6-12) PA 7 (5-10) GN 12.5 (8-16); RS 12 (11-13) AC 13 (10-15) as compared to group AD des PP 7 (7-12) DR 5.5 (9.3), (CV) 13.5 (8-17) AP 12.5 (11-17) GN 14.5 (13-17) RS 13.5 (12-17), BC 14.5 (13-17), presented early in the disappearance of PP, appearance of HP, AP, NG and RS (p

Conclusions:
As the tryptophan the precursor of 5-HT, there could have been increased serotonin synthesis, incurring changes in the reflex maturation. Literature data are scarce, and studies are needed to further elucidate the present findings.

Keywords: L-TRYPTOPHAN , MALNUTRITION, NEONATES. , REFLEXES IN RATS, MATURATION

Financial Support: CAPES, FAPESB, PIBIC, UFBA

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Resumo:23-276

MATERNAL BEHAVIOR AND OPIOID GENE EXPRESSION OF OPIOID RECEPTORS ON PERIAQUEDUTAL GRAY OF FEMALE RATS TREATED CHRONICALLY WITH MORPHINE.

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Objectives:
This study evaluated the effects of chronic morphine treatment on maternal behavior and gene expression of female lactating rats.

Methods and Results:
60 female rats with 90 days of life and 220 g of body weight were used, according Experimental Animal Use Comittee certificate(nº 112). These animals were divided into two similar groups with 6 animals. The animals mated with experienced male rats and the presence of spermatozoa in vaginal smear is indicative of pregnancy. From 17 to 21 days of gestation the animals received one injection of morphine (3.5 mg/kg). The delivery was considered day 0 of lactation. On the 5th day, groups were formed according previous treatment and challenge dose and thirty minutes after injection (morphine at 3.0 mg/kg) the animals were evaluated for parameters of maternal behavior as: retrieving each pup (sec), grouping (sec), crouching (sec) and full maternal behavior. The nest building quality and indirect pups parameters were also analyzed. After behavioral analysis the rats were decapitated, the periaqueductal gray placed on ice and treated for RNA total extraction for further gene expression investigation (Oprm1, Oprd1 and Oprk1 from mu, kappa e delta opioid receptors respectively). The results of opioid gene expression showed that the morphine treatment increase the Oprk1 expression (p< 0.001 compared to saline group). The results showed that the morphine chronic treatment was able to inhibit the maternal behavior (full maternal behavior in morphine group p< 0.001 when compared to saline group), as well as pups care (retrieve of first and second pups, p

Conclusions:
The morphine treatment was able to block the maternal behavior in lactating female rats, that were previously treated or not with this opioid agonist.

Keywords: morphine, periaqueductal grey, female rat, opioidergic system, molecular biology
ROLE OF β2-ADRENOCEPTOR IN SKELETAL MUSCLE REGENERATION

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Objectives:
The aim of this study was to investigate the role of β2-adrenoceptor in skeletal muscle regeneration.

Methods and Results:
Male wild-type FVB mice and β2-adrenoceptor knockout mice (2 months-old, both) were evaluated at 1, 3 and 10 days after cryolesion of tibialis anterior muscle. The cryolesion procedure consisted of one freeze-thaw cycle of the muscle in situ. Animals were sacrificed and the left and right tibialis anterior muscles were removed and weighed. Each muscle was immediately frozen in melting isopentane and stored in liquid nitrogen. Frozen muscles were cut in 10 µm cross-sections on a cryostat. Unfixed histological sections were stained with a solution of aqueous toluidine blue and borax to reveal the general morphology. For mechanical measurements mice were anaesthetized with tribromoethanol (20 mg/100 g BW, i.p.). The sciatic nerve was then exposed and an electrode was connected on it. At 10 days post-cryolesion, twitches were generated continuously for 2 min, and tetanic contractions were subsequently induced (at 200 Hz) for 2 s in each tibialis anterior muscle analyzed. The body weight did not change in FVB and β2 groups. The muscle weight at 1 day post-cryolesion had an increase in both FVB and β2 mice compared with their respective controls [43.62% and 35.48% respectively, P < 0.05]. On post-cryolesion day 3 and day 10 the muscle weight in FVB group decreased compared with that from post-cryolesion day 1 [29.16% and 50% respectively, P < 0.05]. On post-cryolesion day 10 the muscle weight in FVB group decreased compared with that from post-cryolesion day 3 [29.80%, P < 0.05]. Histological cross-sections of cryolesioned muscles analyzed at 10 days post-injury from β2 mice showed deficit in regenerative process with apparent decreased diameters of myofibers when compared to their controls and FVB group. Control muscles from FVB mice did not present difference in maximum tetanic force, however muscles at 10 days post-cryolesion showed a reduction of maximum tetanic force (5.79%, P < 0.05). The control muscles and cryolesioned muscles analyzed at 10 days post-injury from β2 group showed a decrease of maximum tetanic force (36.82% and 31.19%, respectively, P < 0.05).

Conclusions:
Our results suggest that the β2-adrenergic receptor may be important to the process of skeletal muscle regeneration.

Keywords: adrenoceptor knockout, muscle function, skeletal muscle regeneration

Objectives:
Investigate the effects of aerobic training of high intensity in the expression of genes involved in lipid metabolism.

Methods and Results:
Male adult Wistar rats (200 g) were housed in collective cages under controlled lights (05:00-19:00 h) and room temperature (23 ± 2°C) conditions, with food and water provided ad libitum. The animals were familiarized to exercise on a motor-driven treadmill for 4 days. Each daily session consisted of running for 5 min at a constant speed of 18 m.min⁻¹ and grade of 5%. Then, the rats were randomized to either the control (n=10) or training (n=10) group. Progressive tests of running speed (PVmax) were performed at the beginning, during and at the end of the training period in order to determine the maximal velocity, to adjust the training parameters and to determine the effect of training at maximal velocity, respectively. On five consecutive days, during ten weeks, the rats performed 45-60 min of exercise at 75% of maximal velocity. Study was approved by the “Ethics Committee in Animal Experimentation of the Federal University of Minas Gerais” (protocol number 29/2009) and was conducted in accordance with the regulations described in the Committee’s Guiding Principles Manual. Muscular peroxisome proliferator-activated receptor α (PPAR α), carnitine palmitoyltransferase 1 (CPT 1), sterol regulatory element-binding proteins (SREBP-1c) and tribbles 3 (TRB3) mRNA expression were determined by qRT-PCR. Data are means ± SEM and statistical significance was tested using unpaired Student’s t-test. Significance levels were set at P < 0.05. The training protocol improved the performance of animals in the last PVmax (26 min until to fatigue, control vs 45 min until to fatigue, trained group; p=0.00), without changes in body weight (414± g control vs 407± g trained). It was observed a 126% increase in CPT1 and 100% SREBP-1C mRNA expression in the trained group. In relation to PPAR α gene expression, no difference was observed between the groups, and a 57% reduction was found in the expression of TRB 3 in the trained group. Intramyocellular lipid content did not differ between the groups (7.03 ± 0.64 mg/dL/100 g, control vs 6.21 ± 0.54 mg/dL/100g, training group).

Conclusions:
The results of our study show that high intensity training protocol evokes changes in expression of genes involved in lipids metabolism. The adaptative response to exercise protocol seems to involve an increased turnover of lipids substrates, since the expression of oxidative and lipogenic genes increased without changes in the intramyocellular lipid content. Furthermore, our results have shown that the target gene expression was not synergistic with that of the transcription factor.

Keywords: Metabolic adaptations, lipids metabolism, aerobic training

Financial Support: CNPq, CAPES and FAPEMIG
Objectives:

The aim of this study was to evaluate the effect of neonatal malnutrition and the effect of insulin on myotubes mechanical profiles in culture.

Methods and Results:

We used 12 male Wistar mice that were divided into 2 groups, 6 animals in each group, according to their mother’s diet used during the lactation period. One group, Nourished (N), was fed with a regular diet during this period with casein 17%. At the same time, another group, Undernourished (U), received a low protein diet, casein 8% based. After this period, all animals were given a standard laboratory diet until the day of the experiment. The animal weight was measured every 5 days during lactation and once a week until they completed 60 days of life. Between 60 and 70 days, primary muscular cells were collected from the soleus muscle. These cells were grown in triplicate (25,000 cells/cm²) in complete growth environment (Dulbecco’s Modified Eagle Medium - DMEM - 10% Fetal Bovine Serum and 10% horse serum) and every 2 days, the medium was replaced by a differentiation environment (DMEM with 5% horse serum) with and without insulin (10μU/mL), in order to stimulate cell fusion to form myotubes. By the tenth day of culture, the myotubes formed were analyzed for their contraction potential on 30 seconds films, performed three times in all culture cells that were grown. The mechanical parameters analyzed, using the images, were frequency of contractions per minute, amplitude (mM) and duration of contraction (s). The nonparametric Mann-Whitney test ($\alpha = 95\%$) was used to compare the results of mechanical parameters analyzed between groups N and U. For frequency, the results showed no difference between N and U. However, in the presence of insulin, myotubes from both groups had a higher frequency of contraction in relation to non-stimulated myotubes (about 12 contractions / min). Regarding amplitude of contraction in the presence of insulin, there was no difference between groups N and U, 7μm and 16 μm, respectively, which was not observed between groups in the absence of insulin. Concerning the duration of contraction, it was lower in U group stimulated with insulin around 0.4 s, whereas in absence of insulin there was no difference.

Conclusions:

Results showed that undernutrition during neonatal period probably does not interfere in frequency and amplitude of contraction of primaries muscle cells. However, the same diet was able to reduce duration of the myotubes’ contraction time, in culture. Regarding presence of insulin in the cultures, it is important to improve the frequency and the amplitude of contraction of myotubes.

Keywords: INSULIN STIMULATION, MYOTUBES, MALNUTRITION

Financial Support: Capes-COFECUB

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Resumo:24-112

EFFECTS OF A LOW PROTEIN DIET DURING PERINATAL PERIOD ON OXIDATIVE STRESS IN THE DORSAL AND VENTRAL SURFACE OF THE MEDULLA OF RATS.

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Objectives:

It is well described that animals submitted to protein undernutrition during pregnancy (in utero) and lactation period (perinatal
period) develop hypertension in adult life. One suggested underlying mechanism is related to earlier oxidative stress in the ventral and dorsal medulla. Thus, herein we evaluated the level of oxidative stress and the catalase activity in those areas of the medulla.

Methods and Results:

Male wistar rats received during perinatal period either a normoproteic (18 % casein; NP group, n=4) or a hypoproteic (7 % casein; HP group, n=4) diet. After weaning, both NP and HP groups received a control diet with normal amount of protein. At 90th day of life, animals were sacrificed by decapitation and the medulla were collected and stored at - 80°C. Protein concentration (by Bradford; Anal Biochem. 72:248-254, 1976), level of oxidative stress (by Malondialdehyde-MDA, Draper & Hadley in Methods Enzymol 186:421-31, 1990) and activity of a cellular antioxidant enzyme (catalase activity; Aebi et al. in Methods Enzymol 105:121, 1984) were determined in the samples of the dorsal and ventral surface of the medulla from NP and HP groups. At 90th day of life, NP and HP animals presented similar values for body weight (295 ±27 vs. 301±3.9, NP vs. HP, P=0.853). In addition, we observed that the level of oxidative stress in the dorsal surface of the medulla of the HP rats was similar to those observed in NP rats (62.9±28.9 vs. 43.2±32.9 umol/mg of protein, P=0.668). Likewise, catalase activity did not change between groups (65.7±14.6 vs. 62.6±16.7 nmol/min/mg of protein, P=0.899). In the ventral surface of the medulla, no difference was observed between NP and HP groups in oxidative stress production (22.7±6.7 vs. 21.1±1.2 umol/mg of protein, P=0.789) and in catalase activity (75.0±18.6 vs. 39.5±11.5 nmol/min/mg of protein, P= 0.147).

Conclusions:

Our data showed that the formation of reactive oxygen species was not altered in dorsal and ventral surface of the medulla of 90-days-old animals submmitted ealier to low-protein diet during perinatal period.

Keywords: hypertension, low protein diet, medulla, oxidative stress

Financial Support: FACEPE (APQ-1365-2.07/10)

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**DEVELOPMENT OF TYPE I - OXIDATIVE MUSCLE FIBER SPECIFIC VECTOR CONTAING A GREEN FLOURESCENT PROTEIN MARKER**

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Objectives:

Skeletal muscle has been appointed as a promising site of energy expenditure on treatments against obesity. Type I fibers – slow and oxidative – preferentially express enzymes which act in lipid oxidation, using fatty acids as an energetic substrate. We believe that promoting hypertrophy on this type of fiber will increase basal energy demand and, consequently, lead adipose tissue to atrophy. In order to prove our point, a specific expression vector of a target protein is necessary. In this study, we aimed to build a muscle-specific vector regulated by the slow human Troponin gene I controlling expression of bi-cistronic mRNA codifying a target protein and Green Fluorescent Protein (GFP) as a marker.

Methods and Results:

Genomic human DNA was extracted from lineage U937 cells (mutants to p53) with TriReagent following standard procedures. Primers were designed to amplify the slow human Troponin gene I promoter region specific to type I fibers (TNIS) approximately 1 Kb upstream from TSS (5’-ATGCCAGATCTTTAGGCAGCGAGAAATGTCGTTG-3’ 3’–ATGCCGGATCGCGGAGGCTATGAAAATCTGTTG -5’ ) with the addition of linkers BGL-II e Asis-I. They were tested at
temperatures of 54ºC, 56ºC, 58ºC, 60ºC and 62ºC for their annealing. 58ºC was the temperature chosen to amplify the fragment into a new PCR. The reaction was submitted to electrophoresis and fragment recovered from the agarose gel with PureLink Quick Gel Extraction Kit. The recovered fragment that corresponds to the TNIS was cloned in a pGEM-t vector system and expressed in competent bacteria by heat shock, selected by ampicillin. The plasmids were recovered with Pure Link Quick Miniprep Kit. The cloning was confirmed by digestion of plasmids containing Sal-I. Two clones were obtained, amplified and recovered with Pure Link Quick Midiprep Kit. The promoter TNIS, after double digestion with Bgl-II and Asis-I, was subcloned into the pCINEO- IRES- EGFP, which was previously obtained by combining final expression vector pCINEO, the gene codifying the Green Fluorescent Protein (GFP) and the internal ribosome entry site (IRES). The plasmids were digested with Bgl-II and Asis-I for cloning confirmation. We obtained 100% efficiency in the subcloning process a positive clone was confirmed by sequencing in ABI 3730 DNA Analyser using BigDye Terminator v3.1 Cycle Sequencing Kit. The final construct contains the TNIS promoter in pCINEO IRES EGFP, a tool of specific expression in type I mammal muscular fibers containing molecular marker GFP.

Conclusions:

A suitable type I muscle specific vector was obtained using standard molecular protocols.

Keywords: Molecular Biology, Tissue Specific, Skeletal muscle, Expression Vector, Obesity

Financial Support: FAPESP 08/10700-1 e UFABC/PIC

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Resumo:24-114

DIETARY SUPPLEMENTATION WITH FISH OIL AND VITAMIN E REDUCES PULMONARY INFLAMMATION AND RE-ESTABLISHES TRACHEAL SMOOTH MUSCLE FORCE IN ASTHMATIC RATS.


Depo. de Fisiologia/Universidade Federal do Paraná, UFPR

Objectives:

The aim of this study was to investigate the effects of dietary supplementation with fish oil (FO) associated to vitamin E on the tracheal smooth muscle (TSM) force production and in the asthma pulmonary inflammation.

Methods and Results:

Adult male Wistar rats were separated into groups: control, supplemented, asthmatic, asthmatic supplemented with FO (1 g/kg body weight, AS), asthmatic supplemented with FO (1 g/kg b.w.) and vitamin E (100 mg/kg b.w., ASE). Dietary supplementation was given for 21 consecutive days. On the 5th and 12th day of supplementation the animals were immunized by receiving ovalbumin (1mg/mL, sc). All groups were nebulized with ovalbumin in the 19th, 20th and 21st day of supplementation. Lethal doses of urethane were administered one day after the last nebulization. Eight tracheal cartilage rings were dissected and kept in Normal Ringer (NR). Bronchoalveolar lavage fluid (BALF) was obtained with 50 mL of PBS and both the total and differential cell types were quantified. The force of the TSM from a single ring was measured by using a transducer. The TSM was stretched to a 20% of the original length and the maximum force of was obtained by adding acetylcholine into the Ringer solution. After that, the preparation was transferred to a NR containing isoproterenol (10 ng/mL) to obtain the maximum relaxation. The total amount of cells present in the BALF of asthmatic and in the control group was 1.98 x 106 ± 0.47 and 1.12 x 106 ± 0.39 (p

Conclusions:

FO supplementation associated with vitamin E reversed both pulmonary asthma inflammatory effects and the TSM force
production in asthmatic animals.

Keywords: asthma, fish oil, force production, tracheal smooth muscle, vitamin E

Financial Support: UFPR/TN

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Resumo

THE APLICATION OF LOW-LEVEL LASER THERAPY PROVIDES DECREASE OF BIOCHEMICAL PARAMETERS AND INCREASE OF ADIPOSE TISSUE IN RATS WISTAR.

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1 PROGRAMA DE PÓS-GRADUAÇÃO EM BIOTECNOLOGIA - UFSCAR, PPGBIOTEC - UFSCAR
2 DEFMH - LABORATÓRIO DE NUTRIÇÃO E METABOLISMO - UFSCAR, DEFM- LANUM - UFSCAR
3 PROGRAMA DE PÓS GRADUAÇÃO EM CIÊNCIAS FISIOLÓGICAS - UFSCAR, PPGCF - UFSCAR
4 INSTITUTO DE FÍSICA DE SÃO CARLOS - USP, IFSC - USP

Objectives:

To investigate the effects of low-level laser therapy (LLLT), 830 nm, on biochemical parameters and relative weight of several tissues sedentary rats.

Methods and Results:

Were analyzed sixteen males Wistar rats with 12 weeks, weight 295.22±0.95g. The animals had free access (ad libitum) to tap water and food (PRIMOR™, Brazil). They were housed in individual cages in a constant temperature of 23 ± 20C, 12 hour cycle lilight/dark. The study was approved by the Ethics Committee for Animal Experimentation at the UFSCar. The rats were randomized into two groups with eight animals each: sedentary (S) and sedentary irradiated with LLLT (SL). Low-energy Ga–Al–As laser (Thera laser, DMC, Equipment of São Carlos, SP, Brazil), 830 nm, continuous wave, 0.6 mm beam diameter, 60 W/cm², 100 J/cm², with an irradiation time of 47 s was used. The point of applied was the gastrocnemius muscle, during eight weeks/five days by week. The biochemical parameters analyzed was glucose, cholesterol, HDL-cholesterol and triglycerides. While the tissues analyzed was heart, liver, gastrocnemius muscle, soleus muscle, brown adipose tissue and three white adipose tissues: epididymal, retroperitoneal and visceral. The respective means and standard deviations of biochemical parameters: glucose (S)71.62±1.84mg/dl - (SL)70.06±2.17mg/dl; cholesterol (S)201.46±20.95mg/dl - (SL)136.81±44.37mg/dl*; HDL-cholesterol (S)56.11±8.74mg/dl (SL)52.90±7.93mg/dl; triglycerides (S)113.37±26.33 mg/dl - (SL)89.59±7.58 mg/dl**. The respective means and standard deviations of relative weight of tissues: heart (S)0.28±0.10g - (SL)0.35±0.03g; liver (S)0.46±0.10g - (SL)0.49±0.03g;soleus muscle (S)0.04±0.01g - (SL)0.04±0.01g; brown adipose tissue (S)0.07±0.02 - (SL)0.06±0.01g; epididymal (S)0.73±0.18g - (SL)1.03±0.29g; retroperitoneal (S)0.74±0.17g - (SL)1.37±0.26g**; visceral (S)0.65±0.19g - (SL)0.81g±0.11g; Was significant difference *P<0.05.

Conclusions:

The values found show that some biochemical parameters like cholesterol and triglycerides decrease and the values like white tissue retroperitoneal increase, indicating that somehow there was increase of fat´s deposition. This mechanism has not yet been elucidated but with more analysis will be possible to know the actual connection systemic, knowing the metabolic pathway that provides this fact.
EFFECTS OF HIGH-FAT-DIET ON BODY AND ADIPOSE TISSUE WEIGHT IN WISTAR RATS TREATED WITH DEHYDROEPIANDROSTERONE.

Depto. Fisiologia - ICBS, UFRGS

Objectives:

The objective of the present study was to examine the effects of dehydroepiandrosterone (DHEA) treatment on body and adipose tissue weight in high-fat-diet-fed healthy Wistar rats.

Methods and Results:

Adult male (n=24) and female (n=23) Wistar rats weighing 220–300g at the start of experiment, were randomly assigned into standard diet (Nuvilab CR-1, Nuvital) or high-fat diet (70% fat, Pragsoluções). Both groups received 10mg/kg DHEA (dehydroepiandrosterone; Calbiochem) or vehicle (vegetal oil) once a week, subcutaneously, for 4 weeks. Males and females were divided into four experimental groups: standard diet + vehicle (SDV); standard diet + DHEA (SDD); high-fat diet + vehicle (HFDV); high-fat diet + DHEA (HFDD). In females, the vaginal smears were taken daily and only those rats showing regular estrous cycles were used. Animals were housed in plastic cages (maximum 4 per box) and maintained under controlled temperature of 21°C, 12 h light/dark cycle. At the end of the experiment, the measures of body weight (g) were performed. The animals were killed by decapitation and the measures of epididymal and retroperitoneal adipose tissue weight (g) were performed. Data were analyzed using one-way ANOVA test followed by Newman-Keuls Multiple Comparison Test, p

Conclusions:

There was no increase in body weight in rats fed the high-fat diet. However, rats fed a high-fat diet had higher adipose tissues weight despite not differ in body weight. DHEA had no effect on body and adipose tissues weight. Other measurements will be performed to clarify the metabolic mechanisms involved in this data.

Keywords: adipose tissue, dehydroepiandrosterone, high-fat diet

Resumo:24-116

HIGH FAT DIETS INDUCE ATHEROSCLEROSIS MIRRORED BY PLASMA LIPIDS AND TRANS FATTY ACIDS ADVERSELY LEAD TO ADVANCED LESIONS CHARACTERIZED BY FIBROUS PLAQUE IN LDLR-KO MICE

Machado, R. M. 1; Nakandakare, E. R. 1; Quintão, E. C. D. R. 1; Cazita, P. M. 1; Afonso, M. S. 1; Bombo, R.
Objectives:
We evaluated the effect of high fat diets (40% of energy as fat), enriched with trans (TRANS), polyunsaturated (PUFA) or saturated (SAFA) fatty acids, on the development of atherosclerosis in the aortic root of LDLr-KO mice (C56/BL-6).

Methods and Results:
We evaluated the effect of high fat diets (40% of energy as fat), enriched with trans (TRANS), polyunsaturated (PUFA) or saturated (SAFA) fatty acids, on the development of atherosclerosis in the aortic root of LDLr-KO mice (C56/BL-6). Weaned male mice after 16 weeks of high fat diets (40% of energy as fat), enriched with trans (TRANS), polyunsaturated (PUFA) or saturated (SAFA) fatty acids on the development of atherosclerosis in the aortic root. Plasma and aortic root total cholesterol (TC; n=13-15 on plasma; n=8-13 on aortic root) and triglycerides (TG; n=13-15 on plasma; n=8-13 on aortic root) concentrations, lesion areas (oil red-O; n=5), ABCA1 content and macrophage infiltration (immunohistochemistry; n=7-9), collagen content (Picrosirius red; n=4) and colocalization of ABCA1 and macrophage (confocal microscopy) were measured. Plasma TC (TRANS:27.17±11.01;PUFA:4.92±2.10;SAFA:10.39±3.29) and TG (TRANS:4.17±1.40;PUFA:1.11±0.57;SAFA:1.81±0.55) concentrations were higher in TRANS compared to SAFA and PUFA (mean±SD;PA>PUFA and related to plasma cholesterol and triglyceride concentrations mirroring the contents of TC, CE and FC in the aortic root.

Conclusions:
In LDLr-KO mice atherosclerotic lesion areas were elicited by dietary fats in the order TRANS>SAFA>PUFA and related to plasma cholesterol and triglyceride concentrations mirroring the contents of TC, CE and FC in the aortic root.

Keywords: atherosclerosis, cholesterol, dietary fatty acids, lipids, trans fatty acids

Financial Support: State of São Paulo Research Foundation (FAPESP #06/55516-8)
Preliminary data were analyzed on 41 adult voluntary women, selected from a waiting list for bariatric surgery performed in the service of Obesity Surgery of Hospital das Clínicas, Faculdade de Medicina de Ribeirão Preto - SP (HC/FMRP/USP). The survey was conducted in two phases: before surgery and four months after it: Dutch Eating Behaviour Questionnaire (DEBQ), Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI) were applied and Figure Rating Scale (FRS) was applied after the surgery. Anthropometric evaluation was performed according to the parameters of the World Health Organization (1995) to obtain the Body Mass Index (BMI). It was used a descriptive statistics (percentages and mean ± Standard Deviation) and t test for independent samples (p

Conclusions:

Despite the decrease in BMI, the subjects were dissatisfied with their body shape and would like to weigh less. There was a reduction in levels of anxiety and depression after surgery. In relation to feeding behavior, a lack of knowledge about nutritional habits was suggested. However, there was an improvement in the control of food related to the attractive aroma and flavor from food, as well as the emotional state and social situations after the surgery.

Keywords: EATING BEHAVIOR, EMOTIONAL BEHAVIOR , BODY IMAGE

Financial Support: Coordenação de Aperfeiçoamento do Ensino Superior - CAPES

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Resumo:24-119

EPINEPHRINE IN VITRO REDUCES THE PROTELYTIC EFFECT OF FASTING ON SKELETAL MUSCLE FROM EPINEPHRINE-DEPLETED RATS.

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2 Bioquímica/Faculdade de Medicina de Ribeirão Preto-USP, Bioquímica/FMRP-USP

Objectives:

In a previous study, we demonstrated that depletion of plasma epinephrine by adrenomedullation (ADMX) in rat muscles amplifies the catabolic effects induced by fasting on the activity of proteolytic systems (lysosomal and Ub-proteasome) and the mRNA expression of atrophy-related genes (atrogin-1 and MuRF1) and autophagic genes (LC3 and GABARAP). Thus, the goal of the present work was to test whether epinephrine in vitro could revert such deleterious effects on skeletal muscle.

Methods and Results:

Materials and methods: Four groups of animals were used: (1) fed control; (2) fasted control; (3) fed adrenomedullated and (4) fasted adrenomedullated. The removal of adrenal medulla was performed 10 days before the experiments. The group of fasted rats was left without food for 2 days. cAMP content muscle was determined by radioimmunoassay. Extensor digitorum longus (EDL) muscles of rats (~85g) were isolated and incubated in the presence of epinephrine (10^-5 M) for 2 h and the overall proteolysis and the mRNA and protein expression of atrogin-1, MuRF1, LC3 and GABARAP were measured. All animal protocols were approved by Animal Care and Use Committee (087/2010). Results: cAMP levels decreased (30%) in EDL from fasted ADMX rats (518.2 ± 37.6 vs. 739.2 ± 73.1 fmol/mg muscle in fasted rats). The rate of protein breakdown (nmol Tyr.mg^-1.2h^-1) in EDL muscle was increased by fasting (0.287 ± 0.018 vs. 0.236 ± 0.014 in fed rats), an effect that was 50% increased by ADMX. The addition of 10^-5M epinephrine to the incubation medium reduced the overall proteolysis in muscles from fed (0.209 ± 0.014 vs. 0.236 ± 0.014) and fasted ADMX rats (0.346 ± 0.016 vs. 0.404 ± 0.013). Although the ADMX promoted an additional increased in the mRNA expression of all genes evaluated in muscles from fasted rats, the in vitro addition of this hormone was unable to reverse such effect. The protein expression of atrogin-1, MuRF1, LC3 and GABARAP was increased (~3-fold) by fasting, but no further increase was observed in muscles from fasted ADMX rats.
Conclusions:

The data support the hypothesis that epinephrine, probably via cAMP signaling, exerts a direct anti-catabolic effect on muscle protein metabolism, which prevents excessive activation of proteolysis during fasting.

Keywords: EPINEPHRINE, SKELETAL MUSCLE, PROTEOLITIC SYSTEMS, FASTING, ATROGENES

Financial Support: FAPESP (Proc. 10/12206-4 and 2010/11015-0)

QuebraPagina

Resumo:24-120

GROWTH INDICATORS OF YOUNG RATS SUBMITTED TO PALATABLE/HYPERLIPIDIC DIET IN PERINATAL LIFE AND/OR AFTER WEANING

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Objectives:

To evaluate weight gain and growth indicators evolution in rats aged 30 or 60 days old, that were submitted to a palatable/hyperlipidic diet during perinatal life and/or post-weaning.

Methods and Results:

During gestation and lactation dams (Wistar Rats) were fed with a palatable/hyperlipidic diet (4.2 Kcal/ g; distribution of nutrients in relation to the total energy = 49% carbohydrate, 20% protein, 32% lipids), or normocaloric diet based on AIN-93G recommendations (3.6 kcal/ g - 63% carbohydrate, 18% protein and 19% lipids). Pups from these dams formed two groups: Palatable/hyperlipidic (H) and control (C), respectively. After weaning, a half of the offspring in each group remained with the same diet received by the dams during pregnancy, while the rest changed the diet, forming four groups: hyperlipidic-hyperlipidic (HH); control-control (CC); hyperlipidic-control (HC) and control-hyperlipidic (CH), each group with seven male rats. Analyses of growth indicators included body weight, longitudinal axis of the body, body mass index (BMI) and Lee Index, measures on the ages of 30 and 60 post-natal days. In all the cases, diet and water were offered ad libitum. For comparison between samples, was used ANOVA two way RM, considering as statistical significance p

Conclusions:

The palatable/hyperlipidic diet post weaning induced to an increased body weight at the age of 60 days, without in growth indicators. Therefore, we suggest that both perinatal diet and post-weaning palatable/hyperlipidic diet can alter body weight in young rats with the evolution of age. However, a more detailed evaluation on growth indicators is needed to determine if physical changes could happen and its correlation with body protein, total fat and water.

Keywords: GROWTH INDICATORS, HYPERLIPIDIC DIET, PALATABLE DIET, PERINATAL

Financial Support: Support: CAPES and LNE laboratory Department

QuebraPagina
Objective:
To assess the somatic growth and ontogeny reflex of pups whose mothers were subjected to a 40% food restriction during pregnancy.

Methods and Results:
Wista rats pregnant for 100 days divided into 2 groups of 4-6 animals, as the diets offered; Control (GC): 17% casein diet; Restricted (GR) casein-based diet with 17% offering 40% of the amount of food consumption in relation to the control group. Were performed: body weight (BW), naso-anal length (CNA), lateral-lateral axis of skull (ELLC) Antero-posterior axis of skull (EAPC) and tail length (CC). For development analysis evaluated the consequences of recovery in the supine position (RD), placing the vibrissae (CV), cliff aversion (AP), negative geotaxis (GN), response to shock (RS) and righting to free fall (QL). For statistical analysis we used the teste t de Student ou Mann-Whitney depending on the normality of the data with = 5% critical level. Data were expressed as mean ± SD or median (25th percentile and 75th). The project was approved by members of the Ethics Committee of the Centre for Biological Sciences, Federal University of Pernambuco in protocol for use of experimental animals No 23076.004773/2008-42. In relation to PW, GR showed greater weight than the GC from day 15 (32.37 ± 5.68, 35.8 ± 3.52). The GR had ANC greater than the GC in the 3rd (62.27 ± 1.75, 58.69 ± 2.7), 6 (72.91 ± 1.92, 69.45 ± 3.38), and from 15 ° day (102.73 ± 4.12, 105.66 ± 4.19). The CC was higher in RG from the 1st day as compared to GC (20.34 ± 1.13, 17.42 ± 0.77). The EAPC was higher in RG than in CG from Day 1 (15.59 ± 0.65, 14.86 ± 0.65, 14.05 ± 1.22). The ELLC was higher in RG than in CG at 1 (10.56 ± 0.32, 10.08 ± 0.34) and 3 days (12.13 ± 0.4, 11.33 ± 0.54). GR presented the progress of reflexes RD [4(4,0-6,0); 3 (2,0-3,0)], AP [5(3,2-6,7); 2 (2,0-3,5)], GN [13(13-14); 11,5 (10,0-13,0)], RS [13(13-0-14,0); 12 (11,0-12,0)]; QL [16(13,7-16,0); 14 (13,0-15,0)] on the GC.

Conclusions:
The results that the restriction imposed during pregnancy followed by lactation balanced diet, promoted persistent weight gain in pups until weaning and higher anthropometric parameters. There was no impairment in growth or in the longitudinal axis skull and there was progress of reflexes.

Keywords: FOOD RESTRICTION, ONTOGENY REFLEX, PREGNANCY, SOMATIC GROWTH

Financial Support: FACEPE
Objectives:

Early exposure to different Interventions, as nutritional variation and exposure to stress, are associated with persistent alterations in metabolism and behavior, and can be considered a trigger of psychiatric disorders in adulthood. We aimed at determining the interaction between early stress and chronic nutritional deficiency of n-3 polyunsaturated fatty acids (PUFAs) in adult behavioral and metabolic outcomes.

Methods and Results:

Ten litters were randomized into non-handled (NH, n=5 litters) and separated (S, n=5 litters) groups (incubator at 34°C, 3 hour/day from 1st to 10th postnatal days - PND). Weaning was on 21 PND. At 35 PND, forty three male Wistar rats were randomized into adequate or deficient diet in n-3 PUFAs groups. Animals’ body weight and food consumption were measured weekly. After 15 weeks of diet exposure, 1% sucrose preference test and open field test were performed. Then, animals were sacrificed, abdominal fat was weighed, plasma and hepatic triglycerides (TG) were measured. Besides, plasma insulin was measured using a commercial ELISA kit (Millipore). In heart tissue, parameters related to oxidative stress were analyzed. Statistical tests used were two-way or repeated ANOVA. Significance levels were set at p

Conclusions:

Taken together, these data suggest that neonatal environment can alter rat metabolism and behavior, and alter the response to a diet deficient in n-3 PUFAs. This model can also be a useful tool for studying the interaction between the early environment and the life-course nutrition on different outcomes.

Keywords: Wistar rat, n-3 polyunsaturated fatty acid depletion, BDNF, hippocampus, oxidative stress

Financial Support: CNPq, CAPES, FIPE/HCPA

RISK FACTORS FOR OBESITY IN A RURAL POPULATION

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4 Hospital de Clínicas de Porto Alegre, HCPA

Objectives:

The objective was to determine risk factors for obesity in a rural population in South of Brazil. 6,506 subjects predominantly composed of Europeans descending ainly Germans and Italians, participated on the study.
Methods and Results:

All participants signed the informed consent. It was used demographic questionnaire, Beck Inventory to assess depressive symptoms and Munich Chronotype Questionnaire to access chronotype (MSF-sc). One-way ANOVA and Chi-square test were used. Linear regression was carried out in the course of assessing the effects of confounding variables and of reporting an association between BMI and the other variables. 67% were female, aging 42.01 ± 0.18, with BMI = 25.49 ± 0.06. When sample was divided by BMI 32% of the sample was overweight and 17% were obese. One-way ANOVA showed that obese group were older (46.94 ± 12.27), presented less years of education (6.03 ± 2.79) and were formed predominately by women then normal BMI (38.05 ± 15.82; 7.29 ± 3.33 respectively; P < 0.05). When overweight and obese were grouped and compared with normal BMI by Chi-square (P < 0.05), it was observed that be woman (64%), be ex-smoker (85%) and presence of disease (52%) are associated with overweight and obesity. Linear regression showed that gender, age, years of formal education, presence of disease, smoke, exposure to agrotoxics and use of medicines (P < 0.05) are the main factors associated with overweight and obesity in this population.

Conclusions:

These results are important since this is a very specific population, with habits linked to rural life and ethnicity and must be in target for public health and prevention.

Keywords: Obesity, BMI, Rural population, Risk factors

Financial Support: PROPESQ-UFRGS, FIPE-HCPA, PROBRAL-CAPES, PNPD-CAPES

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Resumo: 24-124

ALTERATIONS OF ANTIOXIDANT BIOMARKERS AND TYPE I COLLAGEN DEPOSITION IN THE PAROTID GLAND OF STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Objectives:

The aim of this study was to evaluate the effect of acarbose treatment on antioxidant parameters and deposition of type I collagen in the parotid glands of diabetic rats.

Methods and Results:

Diabetes mellitus was induced by intravenous injection of streptozotocin in 40 male Wistar rats weighing approximately 160–210 g, and rats were divided into four groups (n = 10 in each group): non-diabetic (NDM), diabetic (DM), diabetic treated with 25mg/kg acarbose (DMA) and non-diabetic treated with acarbose (NDMA). Changes in enzymatic antioxidant systems, such as the activity of SOD and GPx enzymes, were evaluated, and the specific staining pattern of the type I collagen fibres was investigated in the rat parotid glands. A one-way analysis of variance (ANOVA) was used to compare the values amongst groups, and a p value of < 0.05 was considered significant. The DM group (n=10) presented high levels of SOD and GPx enzymes, which were reduced by acarbose treatment by 15% and 8%, respectively (p<0.01).

Conclusions:
These results suggest that the diabetic state influences the type I collagen concentration in the parotid glands of rats. In addition, acarbose treatment was helpful in preventing the deposition of such fibres, as well the increase in oxidative stress induced by hyperglycemia.

Keywords: DIABETES MELLITUS, PAROTID GLAND, OXIDATIVE STRESS, ACARBOSE, TYPE I COLLAGEN

Financial Support: FAPEMIG, CAPES, CNPQ

### EFFECTS OF CAFETERIA DIET DURING PREGNANCY AND LACTATION ON THE GLYCEMIC CURVE IN RATS OFFSPRING


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Objectives:

This study analysed the effects of cafeteria diet during pregnancy and lactation on the glycemic curve and body weight gain in newborn rats.

Methods and Results:

Female Wistar rats were fed with cafeteria diet (20% fat) or control diet (4% fat) during pregnancy and lactation. The male offspring were divided in two groups: control group (CG, n= 8) rats fed with control diet and test group (TG, n= 8) of rats fed with cafeteria diet. During the lactation (21 days) the body weight gain were measured on alternate days. Blood glucose were analysed (22 days old) after 4 hours of fasting in 0, 30, 60 and 120 minutes. In the end of lactation were calculated average body weight. The difference between groups was analysed using Student’s t test and differences were considered statistically significant at p< 0,05. During the lactation the body weight increased in test group (p=0,018) (TG = 30,24 ± 1,36 > CG = 27,80 ± 2,18) while not observed differ on the concentrations of glucose in fasting (p= 0,546) between groups, so as 30’ (p= 0,267); 60’ (p= 0,425) e 120’ (p= 0,212).

Conclusions:

The feeding of cafeteria diet during pregnancy and lactation increased body weight gain when compared with the animals of control group. Blood glucose levels did not differ between groups. The intake of cafeteria diet during perinatal period didn’t influence on the glycemic curve. Other studies are necessary to clarify this findings.

Keywords: CAFETERIA DIET, GLYCEMIC CURVE, LACTATION, PREGNANCY, RATS

Financial Support: CNPq, CAPES, FAPESB, UFBA.
EVALUATION OF THE EFFECTS OF INFliximab (ANTI TNF-α) ON SOME PLASMATIC AND HEPATIC METABOLIC PARAMETERS AFFECTED BY WALKER-256 TUMOR

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Objectives:
Cachexia occurs in many chronic diseases and it is present in approximately 80% of patients with cancer being the main cause of mortality among these individuals. Several compounds, including cytokines, have been implicated as mediators of cachexia. TNF-α seems to be the major cytokine mediator of the cachectic process, and it is involved in various metabolic disorders. The objective of this study was to investigate the effect of infliximab (anti TNF-α) on some plasmatic and hepatic metabolic parameters that are affected by Walker-256 tumor.

Methods and Results:
Wistar male rats were used, either healthy or Walker-256 tumor-bearing not treated (WK) or treated with infliximab (WK+infliximab). The animals were submitted to 24 hours of food deprivation. For tumor implantation 8x10^7 Walker-256 cells were inoculated subcutaneously in the right rear flank of the animals. In the healthy group, PBS was inoculated at the same place. WK+infliximab animals were submitted to daily intraperitoneal injection of infliximab (0.5 mg/kg) twice a day during 12 days, starting on tumor cells inoculation day. The healthy and WK animals were injected with saline. On the 12th day after treatment with infliximab or saline, plasmatic metabolic parameters (glucose, lactate, triglycerides and urea) and hepatic parameters (glycolysis and gluconeogenesis) were assessed. The hepatic gluconeogenesis from L-alanine (2.5 mM), and glycolysis from exogenous glucose (20 mM) were assessed in in situ liver perfusion. The normal distribution (Shapiro-Wilk and Lilliefors) and homogeneity of variances (Levene’s test and Brown Forsythe) were analyzed by t-Student test. Analyses were performed using the program STATISTICA 5.1 and GraphPad Prism 4.0. Data were expressed as mean ± standard deviation and significance level of 5% (p

Conclusions:
The results suggest that treatment with infliximab was not effective in mitigating the metabolic changes produced by the Walker-256 tumor. The absence of effects on the parameters examined, after treatment with infliximab, suggests that TNF-α is not the unique mediator of these metabolic changes.

Keywords: cancer, glycolysis, gluconeogenesis, metabolic disorders, Walker-256 tumor

Financial Support: Fundação Araucária

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Resumo:24-127

HIGH-FAT DIET INCREASES OXIDATIVE DAMAGE AND DELAYS CUTANEOUS WOUND HEALING

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Objectives:
Obesity is associated with significant changes in skin and is characterized by increased serum concentrations of proteins combined with metabolic alterations such as insulin resistance, hypertension and lipotoxicity (Skin Res. Technol. 8: 22, 2002). Our aim was to investigate the effects of metabolic disorders such as obesity, insulin resistance and hypertension induced by high-fat diet on cutaneous wound healing.

Methods and Results:

Male C57BL/6 were fed with standard (SC group) (6% lipids, 16% protein, 78% carbohydrates) or high-fat chow (HFC group) (42% lipids, 14% protein, 44% carbohydrates) for 30 weeks. On day 0, all animals received subcutaneous implants of polyurethane sponges and an excisional wound was made (1 cm²). Fourteen days later glucose tolerance and insulin resistance were evaluated by oral glucose tolerance test (OGTT) and intraperitoneal insulin tolerance test (IPITT), the mice were euthanized and blood and implants were collected. The lesions were removed and divided into two fragments. One fragment was formalin-fixed and paraffin-embedded, and the other was frozen to perform hydroxyproline assay. Sections were stained with hematoxylin–eosin and with Sirius red. Sections were immunolabeled with anti-NOS 2 for quantification of NOS 2-positive cells. Nitrite assay and myeloperoxidase (MPO) activity were determined in implanted polyurethane sponges. Lipid peroxidation (TBARS) and carbonyl were evaluated to obtain an index of oxidative damage. Alpha-SM actin (α-SMA) and transforming growth factor-β (TGF-β) expression were detected through immunoblot. From the 8th week the HFC group had significantly higher (P<0.001) were observed in higher in HFC group (P<0.001) and MPO activity (>60%) were also higher in HFC group (P<0.001).

Conclusions:

These findings support the hypothesis that high fat diet besides inducing metabolic disorders also induces oxidative damage and both lead to delayed cutaneous wound.

Keywords: metabolic alterations, Obesity, insulin resistance, wound healing, oxidative damage

Financial Support: FAPERJ

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Resumo:24-128

L-ARGININE REDUCES SERUM INFLAMMATORY MARKER EXPRESSION AND IMPROVES RENAL DYSFUNCTION IN INTRAUTERINE UNDERNOURISHED RATS

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Objectives:

Maternal undernutrition can induce a range of fetal adaptations, which can lead to permanent alterations in adulthood. Interleukin (IL)-18 play an integral role in tubular injury and the development of renal dysfunction during a variety of inflammatory processes. In this work, we have investigated the impact of intrauterine undernutrition on the inflammatory markers, and the correlation of these markers with the renal dysfunction; the effect of L-arginine administration on those parameters also was investigated.

Methods and Results:
All procedures used in this study were approved and performed in accordance with guidelines of the Ethics Committee of Biomedical Institute, Federal University of São Paulo and were conducted following the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No.85-23, revised 1996). Female pregnant Wistar rats were fed either normal or 50% of the normal intake diets, during the whole gestational period. After birth, each litter was left with the mother for 28 days, and then, some of the offspring received a 2% L-arginine solution in drinking water: nourished group + L-arginine and intrauterine undernourished group + L-arginine. Proteinuria and glomerular filtration rate (GFR) were determined. IL-18 and cytokine induced neutrophil chemoattractant-2 (CINC-2) were evaluated by Bioplex, in serum. Statistical evaluation of the data was carried out using the t-test or the One Way Analysis of Variance (ANOVA) followed by Tukey’s post-test. A value lower than 0.05 was considered to be significant. In intrauterine undernourished group, the proteinuria (63%), IL-18 (121%) and CINC-2 (61%) were increased, when compared to nourished group, and the L-arginine treatment abolished these effects; the GFR was decreased in intrauterine undernourished group (43%), and the L-arginine treatment prevented this reduction.

Conclusions:

In intrauterine undernourished group, the inflammatory markers, such as IL-18 and CINC-2, are present in an early stage after birth, probably contributing for the development of renal injury in this model. The lower expression of these markers seems to be directly related to improvement of renal function. Our results suggest that these inflammatory markers can be attenuated by L-arginine.

Keywords: Programação fetal, inflamação, Oxido Nitrico, desnutrição

Financial Support: FAPESP, CNPq, and INCT Complex Fluids

Objectives:

Several studies conducted over the past years in vivo and in vitro have provided considerable evidence that the vertebrate kidney possess sufficient gluconeogenic enzyme and glucose-6-phosphatase activities to enable them to release glucose into the circulation as a result of gluconeogenesis. Stanniocalcin 1 (STC1) is a glycoprotein of 50kDa present in high levels in kidney and described as an endocrine-paracrine-autocrine factor related to fuel production in mammalian kidney and liver. Since STC1 is an important hormone which regulates ATP production and that gluconeogenesis is an important source of glucose into the kidney, the aim of this work was investigate the role of STC1 on the gluconeogenesis pathway in the rat kidney.

Methods and Results:

Male Wistar rats, with weight between 250 and 300 grams, were housed in plastic cages, and received water and pelleted food ad libitum. They were maintained under standard laboratory conditions (controlled temperature of 21°C, 12h light/dark cycle). All animal procedures used in this study were in accordance with the Principles of Laboratory Animal Care (COBEA Brazilian College of Animal Experimentation), and the experimental protocol was approved by the UFRGS Animal Care Committee. The
rats were killed by decapitation and the kidneys were excised, weighed and placed in Petri dishes with cold Krebs-Ringer Bicarbonate buffer (RB), pH 7.4. The kidney was sliced free-hand with a slicer apparatus, and the renal cortex (CX) and medulla (MD) were separated. Three slices of about 1000 micrometres of thickness were obtained from each kidney. These tissues were then placed in separate tubes containing RB plus 0.01ng/ml (group T1) or 0.1ng/ml (group T2) of human STC1, incubated in a metabolic bath during 1h at 37°C and assayed for gluconeogenesis, phosphoenolpyruvate carboxykinase (PEPCK) activity and mRNA expression (Pck1 gene) by RT-PCR. Gluconeogenesis using 14C-glutamine as glucose precursor was significantly reduced (P

Conclusions:

STC1 is able to regulate the gluconeogenesis pathway in the MD but not in the CX. The reduced activity in the MD was paralleled by an increase of PEPCK mRNA expression. These results demonstrate for the first time that STC1 has a functional role on the gluconeogenesis pathway.

Keywords: gluconeogenesis, stanniocalcin, kidney

Financial Support: INCT-EN, CAPES-GRICES, CNPq

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Resumo:24-130

NUTRITIONAL RECOVERY WITH OKARA DIET PRODUCED GLUCOSE INTOLERANCE DESPITE NORMAL INSULIN SENSITIVITY

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Objectives:

Aim: Okara is the residue obtained from soybean after extracting soymilk, that possess high nutritional value and contains beneficial health components. Large quantities of okara produced are discarded due the difficult conservation, and its disposal has become a significant environmental problem. Because early malnutrition is associated to diabetes in adulthood and okara may have beneficial effects on its prevention and treatment, this study evaluated glucose homeostasis in adult rats subjected to protein restriction during the intrauterine and lactation stages and recovering on an okara diet.

Methods and Results:

Methods and Results: Four groups of the Wistar rats were used: CC, offspring born to and suckled by mothers fed a control diet and fed the same diet after weaning; CO, offspring born to and suckled by mothers fed a control diet and fed an okara diet with 17% protein after weaning; LC, offspring of mothers fed a low protein diet, but fed a control diet after weaning; LO, offspring of mothers fed a low protein diet, but fed an okara diet with 17% protein after weaning. Intraperitoneal glucose tolerance test (GTT) and insulin tolerance test (ITT) were performed after 12h fast. Glucose tolerance was calculated by estimating total areas under the glucose curve during GTT (f́G) and insulin sensitivity was estimated by the determination of rate constant for the serum glucose disappearance (Kitt) during ITT. Liver glycogen concentrations were determinate in fed and fast states. Basal serum glucose levels (mmol/L) and EGG (mmol.L/120min) were higher in LO (5,2±0,6, n= 4 and 941±21, n=4, respectively) and CO (5,9±0,2, n=4 and 953±22, n=4, respectively) groups compared to LC (3,9±1,4, n=6 and 668±185, n=6, respectively) and CC (3,9±1,7, n=4 and 494±262, n=4, respectively) groups. In the fed state, liver glycogen concentrations (mg/100mg) were lower in LO and CO (3,2±1,2, n=5 and 2,7±1,0, n=6, respectively) rats in relation to LC and CC (6,1±2,6, n=6 and 6,1±2,5, n=6, respectively) rats. Basal serum insulin concentrations, Kitt and liver glycogen concentration in fast state were not significantly different among groups. The results were expressed as mean ± standard deviation, two-way and one-way analyses were used to compared data, p<0.05 indicated statistical significance.
Conclusions:

Conclusion: Thus, nutritional recovery with okara diet produced glucose intolerance possibly due to increased breakdown liver glycogen, despite to normal insulin sensitivity.

Keywords: glucose metabolism, malnutrition, nutritional recovery, okara, rat

Financial Support: CAPES, CNPq, FAPEMAT and UFMT

MORPHOMETRIC ADJUSTMENTS IN THE ADIPOSE TISSUE IN RESPONSE TO FOOD RESTRICTION AND REFEEDING IN RATS WITH DIET OBESITY-INDUCED

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Objectives:

Evaluate possible changes in the area of adipocytes from the white adipose tissues of rats with obesity-induced diet undergoing two types of food restriction.

Methods and Results:

Males Wistar rats (90 days old) from the breeding colony of Federal University of São Carlos (UFSCar), SP, Brazil, were used with an initial body mass of approximately 250±12.73g. The animals were kept in individual cages at a constant temperature of 23±2°C and had a cycle of 12 h light/12 h dark, with light from 08:00 to 20:00 hours. The animals were assigned in five groups: control (C), severe caloric restriction (S), severe caloric restriction plus refeeding ad libitum (S-r), moderate caloric restriction (M), moderate caloric restriction plus refeeding ad libitum (M-r). The severe and moderate caloric restriction groups were restricted to 50% and 25% respectively of food consumption with chow diet. The calculation was based on data of the amount consumed by control group (C) the previous day. Except for group C, all the other groups were fed with a high-fat diet ad libitum to develop exogenous obesity and dyslipidemia. (3 wk) before caloric restriction. All animals were killed by decapitation and the white adipose tissues retroperitoneal (RET), visceral epididymal (EPI), and visceral omental (VIS) were immediately removed and weighed. A fragment (100 mg) of RET, EPI and VIS was fixed in 0.2 M collidine buffer, pH 7.4, that contained 2% of osmium tetroxide at 37°C. After 24 hours, they were washed with warmed saline as described by Hirsch & Gallian (1968). Histologic sections of the adipose tissue were photographed with a CCD-Iris camera (Sony Corp., Tokyo, Japan) interfaced with an Olympus BX60 optic microscope (Olympus Corp., New York, NY, USA) and a computer. The adipocyte area (approximately 60 cells from each animal - n=5/group) was measured using image analysis software (Image Pro Plus 3.0, Media Cybernetics, Silver Spring, MD, USA) and was expressed as μm². The moderate and severe caloric restriction promotes decreased in adipocyte area in all adipose tissues analyzed in relation to control group (p≤0,001). Besides, the adipocyte area in all tissues was less in the groups submitted the severe caloric restriction than groups submitted to moderate caloric restriction (p≤0,001). In the other hand, the refeeding increased the adipocyte area in all adipose tissue in both groups in relation to restricted groups (p≤0,001). Nevertheless EPI adipocyte area before refeeding was bigger than control groups (p≤0,001).

Conclusions:
The caloric restriction can be an important strategy for control of obesity, but without food education, the rebot effect happens increasing the chances of developing obesity and associated diseases.

Keywords: adipocyte, caloric restriction, Obesity

Financial Support: FAPESP, CNPq

MATERNAL HIGH FAT DIET IN RATS ALTERS BREAST MILK COMPOSITION AND THYROID FUNCTION OF THE OFFSPRING AT WEANING

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Objectives:

Environmental, hormonal and nutritional changes in critical periods of life (gestation and lactation) may alter the physiology of several tissues in a short and long-term manner. This phenomenon is named programming. Maternal nutritional status is determinant for future development of the offspring. Both, undernutrition and overnutrition in early life may program for obesity in adult rats. Here, we evaluated the effect of maternal high fat diet on breast milk composition and its impact upon leptinaemia and thyroid function of the offspring at weaning, an important period for programming.

Methods and Results:

Wistar female rats were fed with normal (9% fat; C group) or high fat diet (29% fat; HF group) for 8 weeks before matting, and during pregnancy and lactation. Body composition was evaluated before matting and at weaning by using DEXA. Breast milk samples were collected in the middle (day 11) and at the end of lactation (day 21), when dams and pups were killed. Leptinaemia, TSH, T4 and T3 serum concentration of the pups were determined by radioimmunoassay. HF group presented higher body fat content after 8 weeks of treatment (+27%, p

Conclusions:

As expected, HF diet increased maternal body fat and this additional energy seems to be transferred to the offspring during gestation and/or lactation, since at weaning dams showed normal fat and pups displayed obesity. The higher content of fat and protein in the breast milk seems to induce early overnutrition in the offspring. Because leptin stimulates the hypothalamus-pituitary-thyroid axis, we expected to find alterations in the thyroid function of the pups. The higher T4 levels may be a consequence of a direct leptin effect upon thyroid, since the gland presents functional leptin receptors. It is possible that thyroid function be programmed for other alterations in adult life, as we have shown in other experimental models of programming.

Keywords: OBESITY, PROGRAMMING, LEPTIN, THYROID

Financial Support: CAPES, CNPQ, FAPERJ
EFFECTS OF TOPICAL APPLICATION OF OLIVE OIL ON CUTANEOUS WOUND HEALING

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Objectives:

Olive oil consists of monounsaturated fatty acids that are involved in cutaneous wound healing. The aim of this study was to investigate the effects of topical application of olive oil during different stages of cutaneous wound healing.

Methods and Results:

We used 12 healthy male rats, about 3 months old, weighing 220-350g, divided into two groups: mineral oil (OM) (n = 6) and olive oil (OO) (n = 6). On day 0 an excisional lesion was performed (1cm²) and following surgery, the wound was immediately treated with topical application of olive oil (200μl) or the same volume of mineral oil for 6 days, the wound was covered with bandages. Dressings were changed alternately and the oils were reapplied at each change. From the seventh day the lesions were kept open. The contraction and re-epithelized wound area was determined by measurements on the day of injury (d0), 2(d2), 5(d5), 7(d7) and 14(d14) days after wounding. Fourteen days later the rats were euthanized, the lesions with adjacent normal skin were removed and divided into two fragments. One fragment was formalin-fixed and paraffin-embedded, and the other was frozen to perform hydroxyproline assay. Sections were stained with hematoxylin–eosin and with Sirius red. In order to evaluate the blood vessels and volume density of myofibroblasts immunolabeled against α-SMA was performed. Sections were immunolabeled with anti-NOS 2 for quantification of NOS 2-positive cells. Transforming growth factor-β (TGF-β) expression was detected through immunoblot. Seven days after wounding was observed a greater wound contraction in OO group (P

Conclusions:

Therefore, the use of olive oil topic accelerates cutaneous wound healing in rats.

Keywords: olive oil, wound healing, topical

Financial Support: Faperj

LACTACIONAL STRESS INCREASES ANXIETY AND PALATABLE DIET INTAKE IN RATS

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Objectives:

Maternal separation is a potent stress that can alter the level of palatable food intake in adult rats by increasing the hormones like corticosterone and by changing mechanisms responsible for regulating appetite and anxiety. (Neuropharmacology 42; 421-427; 2002, Curr Opin Pharmacol. 9; 787-793; 2009, Behav Brain Res. 156; 297-310, 2005). In this study, we investigate in rats, the effects of lactational stress on food intake of palatable diet and anxiety.

Methods and Results:

The lactational stress was performed by the model of maternal separation. This was from the 1st to the 14th day of lactation in two cycles of luminosity: between 18:00 and 00:00 hr (light cycle) and from 6:00 to 12:00 hr (dark cycle). Wistar rats were divided into eight groups: Light control males (LCM, n=10); Light control females (LCF, n=10); Dark control males (DCM, n=10); Dark control females (DCF, n=10); Light maternal separation males (LMSM, n=10); Light maternal separation females (LMSF, n=10); Dark maternal separation males (DMSM, n=10); Dark maternal separation females (DMSF, n=10). At age 180 days, the groups were tested in the elevated plus maze. Subjects were placed in the center of a cross-shaped platform located 50cm above the floor, containing two enclosed arms by side walls and two open. We analyzed the time (min) and number of entries in each arm of the maze. The intake of palatable diet (g) was conducted for one hour after food deprivation for eight hours. Groups DMSM (118,1± 18,4) and LMSM (27,4±11,8) remained less time in the open arms of the maze, opposite results were observed in LMSF (115,4± 23,6) compared with LCF (101,7±16,08). Intake of standard diet at 120 days in response to deprivation in groups LMSM (8,26± 0,2), LCF (6,79 ± 0,45), DCM (8,0 ± 0,39), DCF (6,48±0,56). Contrary results were observed for the intake of palatable diet, which was higher (p< 0,05) for males LMSM (16,32±1,97), DMSM(15,77± 0,57), and females LMSF(11,58± 1,74), DMSF(10,18 ± 0,68) compared to controls LCM(11,30±1,25), DCM(11,97± 0,99), LCF(8,54± 0,71), DCF(7,71 ± 0,66). Data are expressed as mean ± standard deviation (±SD). One way analysis of variance (ANOVA) was performed in all experiments.

Conclusions:

Animals separated from their mothers during the period of development were more anxious in adulthood, increasing responsiveness to palatable diet independent of the cycle of luminosity and gender.

Keywords: Ansiedade, Ciclo de luminosidade, Dieta palatável, Estresse, Separação materna

Financial Support: FACEPE

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Resumo: 24-135

MATERNAL HIGH FAT DIET ALTERS OFFSPRING'S EXPRESSION OF SRBI IN THE LIVER AND IN THE PLACENTA

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Objectives:

Scavenger Receptor Class B Type I (SRBI) is an HDL receptor protein expressed in various organs such as liver, intestine, gonads and adipose tissue. Because of its ability to mediate the selective cholesterol uptake through the reverse cholesterol transport, this receptor has an anti-atherogenic activity (Science, 271:518, 1996). The current study investigates whether maternal high fat diet intake alters the SRBI protein expression in placenta and liver in the offspring rats at different ages of development.

Methods and Results:

The study was approved by institutional Ethical Committee. Virgin female Wistar rats at 21 days were divided into two groups: 1) Control (CTL, n=6), fed with rodent standard diet and 2) experimental (DHL, n=6), which received high fat diet. The females were maintained in isolated cages and, after 8 weeks of feeding, were mated. Placenta tissues were collected from pregnant rats on 17th day of gestation and liver samples were obtained from male offspring at ages of 17th prenatal day (E17), 12 days (P12d), 8 (P8s) and 16 weeks (P16s) postnatal. Liver and placenta protein expression of SRBI were analyzed and measured by immunoblotting and immunohistochemistry. Data obtained over time were analyzed using appropriate ANOVA and Student t test. Post hoc comparisons between selected means were made by Student-Newman-Keuls test considering P

Conclusions:

It may conclude that expression of SRBI in the liver of offspring decreases with the age of the animal, so older animals may have the reverse cholesterol transport decreased, raising the chances of increasing cholesterol levels in peripheral tissues. Furthermore, we may suppose that pups from mothers that received high-fat diet has a tendency to decrease the expression of this protein, since in the fetal phase all animals received a greater amount of cholesterol carried by the placenta that showed increased expression of SRBI. These results indicate that a maternal high-fat diet induce changes in the lipid metabolism of offspring by altering the function of lipid binding proteins as SRBI.

Keywords: Scavenger Receptor Class B Type I, High fat diet, Liver, Placenta, Fetal programming

Financial Support: FAPESP and CNPq

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Resumo:24-136

SERUM PROFILE OF RATS FED HIGH-FRUCTOSE DIET IS IMPROVED BY THE ACTION OF AVE 0991, NON-PEPTIDE AGONIST OF THE MAS

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Objectives:

It is well known that the administration of a high-fructose diet contributes to the development of insulin resistance. It has been demonstrated that angiotensin-(1-7), other renin-angiotensin system peptide, acting through the G protein-coupled receptor encoded by the Mas protooncogene, increases glucose tolerance and insulin sensitivity. However, the role of AVE 0991in glycemic and lipidic profile is not established. Thus, the effects of AVE on weight-related and serum biochemical parameters were evaluated in Sprague-Dawley male rats.

Methods and Results:

The animals were randomly distributed into: control, fed a commercial diet with (CA) or without (C) 1 mg / kg AVE, and treated with 10% fructose in drinking water with (FA) or without (F) 1 mg / kg AVE. The oral glucose tolerance test (OGTT) was
performed in fasted animals. Serum concentration of total cholesterol, triacylglycerol, free fatty acids, and insulin were evaluated. For the data analysis used variance two-way test (Two-way ANOVA) followed by Bonferroni comparison test. The level of significance was p

Conclusions:

The data suggest that AVE intake increase insulin sensitivity and improves glycemic control and serum lipid profile of rats fed a fructose-rich diet.

Keywords: ave 0991, fructose, glicemic profile, lipidic profile, MAS receptor

Financial Support: CNPq, CAPES, FAPEMIG

PROTEIN RESTRICTION DURING PREGNANCY INCREASED THE CASPASE 3 PROTEIN CONCENTRATION BUT DID NOT MODIFY THE ISLET SIZE

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Objectives:

Pregnancy and protein restriction are commonly associated with opposite alterations in the structure and function of pancreatic islets. Expansion of islet mass occurs during pregnancy to accommodate for insulin resistance. In malnutrition reduction beta-cell mass appears to result from changing the balance between proliferation and apoptosis. We investigated the effect of protein restriction during pregnancy on pancreatic islet size and pro-apoptotic genes and protein expressions.

Methods and Results:

Methods: Adult control non-pregnant (CN) and control pregnant (CP) rats were fed a normal-protein diet (17%), whereas low-protein non-pregnant (LPN) and low-protein pregnant (LPP) rats were fed a low-protein diet (6%) for 20 d. Islets were isolated by collagenase digestion. Pro-apoptotic genes were assessed by real time PCR and protein content was detected by immunoblotting. The pancreas sections were counterstained with hematoxylin-eosin for morphometric analysis. The results were expressed as mean ± standard deviation with the number of rats or determinations indicated in parentheses. Two-way ANOVA (nutritional status and physiological status) were processed. When necessary, analyses were complemented by the LSD test. The level of significance was set at P < 0.05. Results: Pregnancy increased the Bad mRNA expression in LPP and CP groups in relation to LPN and CN groups (0.04±0.01; 0.04±0.009; 0.02±0.007; 0.02±0.01, n=4, respectively). However, Bad protein concentration was similar in all groups. In contrast, Caspase 3 mRNA levels did not differ among groups, but protein contents (arbitrary unit) were higher in islets from LPP than in islets from LPN and CP rats (29452±6238; 19704±5657; 18636±3644, n=4, respectively) and similar to islets from CN group (23896±3849, n=4). Caspase 8 mRNA and protein levels did not differ among groups. The frequency distribution of lowest, intermediate and highest islets size class did not differ among groups.

Conclusions:

Expansion of islet area was preserved in rats protein restricted during pregnancy, despite increased Caspase 3 protein expression.
EFFECTS OF CAMELLIA SINENSIS TEAS IN STRENGTH AND BONE MINERAL DENSITY OF YOUNG RATS.

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Objectives:

Objectives: determine the effect of Camellia sinensis teas in bone mineral density (BMD) and bone strength of young male rats.

Methods and Results:

Methods and results: 30 rats Wistar males (40 days old) had been randomly divided in 3 groups: C - control; CV – treatment with green tea infusion (125mg/100g body weight) by gavage once a day for 8 weeks; CB - treatment with white tea infusion (125mg/100g body weight) by gavage once a day for 8 weeks. All the animals had free access to deionized water and standard diet. The rats were housed in temperature controlled room (21±2°C) with a 12 hours light/dark cycle. After this period the animals were anesthetized with xylazine (10 mg/Kg BW) and ketamina (100 mg/Kg BW), and killed by abdominal aorta puncture. The right femurs were removed cleaned of adherent muscles and others tissues, frozen in saline solution at –20 °C until analysis. The cleaned bones were scanned using dual energy X Ray absorptiometry (DXA) to determine bone mineral density (BMD). Radiographs were obtained and scanned in a DIGORA digital imaging system (Soredex, Tuusula, Finland) coupled to a computer. Standardized femoral epiphyseal and diaphyseal areas were determined in the bone image on a computer monitor to obtain the bone density. Data presented as mean ± standard deviation were evaluated for normality (ANOVA) and compared using the Tukey test, using the program Graph-Pad Prism. It was established as 5% significance level. The study protocol and all procedures involving animals were in compliance with the São Paulo State University/Araçatuba School of Dentistry Animal Care and Use Committee rules and regulations contained in their Ethical Principles of Animal Experimentation (protocol number:3286-2009). DXA measures showed no differences in BMD between the groups; however analyses for density radiographic images had shown positive effect of C. sinensis teas in the BMD in areas with predominantly trabecular bone. Femoral head BMD increase 9.27% (CV) and 8.74% (CB) compared to the control group. Significant increase (8.45% in CV group and 6.55% in CB group) was observed in trochanter. No significanct difference was observed in the strength values of maximum force in flexion or compression, however rigidity was observed in the femurs of rats treated with teas during mechanical assay of compression.

Conclusions:

Conclusion: we conclude that treatments with green tea or white tea improve bone strength, however do not improve the fracture resistance in young rats. Additional analysis will be needed to understand the action of the teas in the growth phase.

Keywords: bone mineral density, camellia sinensis, rats

Financial Support: fundunesp
MATERNAL HIGH FAT FEEDING DURING LACTATION IS CRUCIAL TO THE PROMOTION OF AN OBESE PHENOTYPE AND LEADS TO ALTERATIONS IN SKELETAL MUSCLE MORPHOLOGY IN WEANING RATS.


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Objectives:

Previous reports have found that a maternal high fat diet during pregnancy and lactation causes body lipid accumulation and impairment in the skeletal muscle development of offspring. Despite this available information, it is still unclear if maternal gestational or post-gestational high-fat feeding alone could lead to these disturbances. Therefore, this study aimed to evaluate the effects of a maternal high-fat diet during gestation, lactation or both in nutritional status markers and on skeletal muscle morphology of offspring at weaning.

Methods and Results:

All the proceedings were approved by the ethics committee on animal experimentation of the Faculty of Pharmaceutical Sciences, University of Sao Paulo, according to the guidelines of the Brazilian College on Animal Experimentation (protocol number: 214). Ten days prior to conception, 39 female wistar rats were randomly assigned to either a control [CON (n=19)] or a high-fat [HF (n=20)] diet. After birth, six pups per litter were placed with the mothers who, in turn, were distributed into four groups according to gestational and post-pregnancy diets: gestational and post-pregnancy control diet [CON/CON (n = 8)]; gestational control and high-fat post-pregnancy diet [CON/HF (n = 9)]; gestational and post-pregnancy high-fat diet [HF/HF (n = 8)]; gestational high-fat diet and post-pregnancy control diet [HF/CON (n = 7)]. At the end of lactation, pups were euthanized and samples were collected. The body composition of the animals was determined by chemical analysis of the carcass. Serum glucose, cholesterol, triacylglycerols and leptin concentrations were measured through using commercial kits. Muscle morphology was evaluated by histology. Two-way ANOVA was performed to determine any significant effects and interactions of the gestational diet and postnatal diet. Firstly, we observed influence of maternal post-gestational high-fat diet in promoting an obese phenotype, characterized by body fat accumulation (P = 0.001) and high serum leptin (P = 0.041), glucose (P = 0.05) and lipids (P < 0.001) levels. We also detected alterations on skeletal muscle morphology that included reductions in the relative masses of soleus (P = 0.027) and gastrocnemius (P = 0.039) muscles and decreased number of muscle fibers per field (P = 0.001), resulting in a lower density of myocytes. The reduced myofiber density indicates possible increase in the interstitial space, accumulation of intramuscular fat and/or monocytes infiltration.

Conclusions:

We could demonstrate that postnatal high-fat maternal diet, rather than high-fat gestational diet, was crucial to the development of an obese phenotype and to alterations in skeletal muscle morphology in weaning rats. These results highlight the importance of maternal diet during lactation on morphology and on metabolic and physiologic adaptations of lactating rats.

Keywords: lactation, gestation, high-fat, offspring

Objectives:

OBJECTIVE: To evaluate the effects of hypocaloric diet in perinatal life with or without water activity on early somatic growth and locomotor activity of young rats.

Methods and Results:

METHODS: Wistar rats (n = 17) (246.99 ± 23.20 g) received water and food ad libitum during the perinatal period. The dietary manipulation was started from the third week of gestation until the end of lactation, resulting in two groups: Normocaloric Group (NG) (3.6 kcal/g) and Hypocaloric Group (HG) (2.3 kcal/g). From 8th to the 52th day of life, offspring of each group (NG and HG) carried out or not water activity (swimming), forming the groups: Inactive Normocaloric (IN) and Active Normocaloric (AN), and Inactive Hypocaloric (IH) and Active Hypocaloric (HA). In total, four groups were formed, composed of 18 rats each. The swimming protocol consisted of daily sessions of free movement in water. The animals were adapted to the aquatic environment, remaining in water for 2, 5 and 10 min, on 8th, 9th and 10th days, respectively. From the 13th to the 17th day, they performed movements of free swimming by 15, 20, 25 and 30 min per day, consecutively. They continued the free swimming of the 20th until the 52nd day of life by 30 min per day, 5 days per week. Animals in inactive groups remained under the same conditions of active ones, with water added to the vats in insufficient quantity to make physical activity. Body weight was measured on days 21, 30 and 60 to calculate the percentage of weight gain (%WG). The somatic growth was evaluated at 30 and 60 days through the longitudinal axis of the body (LA) and length of tail (LT). The locomotor development was evaluated by test of the open field from the following physical magnitudes: distance (m), average speed (m/s), average power (mW), immobility time (s) and number of charts in 8th, 14th, 17th, 21th, 30th and 60th days of life. The project was approved by the Ethics Committee (No 23076.006025/2009-85). For statistical purposes, we used two-way RM ANOVA followed by Holm-Sidak test.

Conclusions:

CONCLUSION: We can infer that the hypocaloric diet used in the perinatal period showed to be deleterious to the growth of the offspring, but did not affect locomotor activity. The undertaking of aquatic activity initiated at the time of nutritional aggression proved to be an environmental factor to be explored. It is suggested that even hypocaloric diet with balanced nutrients in relation to energy, when used in the perinatal period, does not maintain appropriate growth of the offspring, and that physical activity acts favorably on early somatic growth.

Keywords: hypocaloric diet, locomotor activity, perinatal, somatic growth, water activity

Financial Support: CAPES, CNPq
Objectives:

Three metabolic pathways are known to contribute to generation of glycerol-3-phosphate (GP3) in white adipose tissue (WAT): glycolytic via, glyceroneogenetic via and the direct phosphorylation of glycerol by the enzyme glycerol kinase (GyK). Although this last process was thought being inexistent in WAT, recent data from our research group have shown a modulation on the activity of this enzyme by the sympathetic nervous system (SNS). We had previously observed that sympathetic denervation of WAT changes GyK activity in fasted, diabetic and cafeteria fed rats. Thus, we investigated if the in vivo, infusion of noradrenaline could also modulate GyK activity in these situations.

Methods and Results:

To verify if noradrenaline was able to change GyK activity in WAT, we used male Wistar rats (220-230g) submitted to different experimental situations: normally fed, fasted, diabetic and cafeteria fed. Before GyK activity measurement, animals were surgically implanted with osmotic minipumps (Alzet 1007D) to noradrenaline infusion (40ug . h^{-1}) at a rate of 0,5uL . h^{-1} for three days. To each experimental situation the control group was formed by animals implanted with osmotic minipumps infusing saline. After this period the animals were killed, blood collected to determine catecholamines plasma levels and epididymal WAT was surgically removed to posterior analysis. The measurement of GyK activity (nmol GP3 . mg of protein^{-1} . h^{-1}) was performed by a glycerol-C^{14} based assay, according to Newsholme et al. (1967), followed by a liquid chromatography to separate the formed GP3 from remaining glycerol, as described by Kawashita et al. (2002). Statistical analyses were performed by Student’s T-test (p

Conclusions:

These results show that GyK activity in WAT may be regulated by insulin levels, but also suggest that further noradrenaline administration induce an increase on GyK activity regardless of insulin or catecholamine levels.

Keywords: Glycerol Kinase, noradrenaline, Adipose Tissue

Financial Support: CAPES; CNPq; FAPESP

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Resumo:24-142

EARLY WEANING ATTENUATES ACUTE PANCREATITIS IN ADULT RATS


FISCLINEX, UERJ

Objectives:

Overweight has been associated with several morbidities. In the other hand, survival to chronic or acute emergencies sometimes is higher in the overweighed. The present study aimed to evaluate acute pancreatitis in adult rats programmed to overweight by early weaning (EW) treated with a diet with a higher proportion of sugar

Methods and Results:

The use of the animals according to our experimental design was approved by the Animal Care and Use Committee of the Biology Institute of the State University of Rio de Janeiro (CEA/017/2009). Ten lactating Wistar rats were separated into two groups: EW – dams were wrapped with a bandage to interrupt suckling in the last 3 days of lactation; C – control – dams whose
pups had free access to milk during all lactation (21 d). The number of pups in each litter was reduced for six male rats, one rat of each litter was randomly assigned into four groups (n=10/group), which were all submitted to acute pancreatitis (AP) when they were 180 days-old by an injection of 5% taurocholate sodium (0.2 ml) into the common bile duct and were killed at 181 days after 20 hours of AP induction: (C) control group, (EW) early weaned, (CS) control group + higher sugar diet (HSD – 72% sugar) between 60 and 180 days and (EWS) – EW + HSD between 60 and 180 days. Data were analysed by ANOVA and differences of the means were significant at P < 0.05. CS, EW and EWS groups had higher body weight (489.2 ± 9.2; 521.9 ± 14.9; 507.1 ± 18.7) than C (457.3 ± 7.6) at 181 days. EW and EWS groups had higher mesenteric (8.87 ± 0.91; 9.83 ± 1.95), retroperitoneal (12.93 ± 1.57; 17.89 ± 3.31) and epididymal fats (10.2 ± 0.90; 14.47 ± 2.09) than C e CS groups (5.96 ± 0.49; 7.80 ± 1.32), (11.12 ± 1.62; 14.32 ± 1.85) (8.50 ± 0.89; 13.93 ± 1.63) respectively. Regarding spontaneous deaths, only 1 occurred at EW and EWS groups each, compared to 4 deaths in the C group. No deaths were observed in CS group. Higher serum cholesterol and Low Density Lipoprotein (LDL), while lower High Density Lipoprotein (HDL) and triglycerides was found in all groups after AP, compared with these values before AP induction. However, the increase in LDL in EW and EWS groups was much smaller, 70% and 72%, respectively, when compared to C (146%) and CS (170%) groups. All groups showed a rise in serum amylase after AP. However, in C (364%) and CS (514%) groups the increase was higher when compared to EW (216%) and EWS (320%) groups.

Conclusions:
Our results suggest that early weaned animals possible had a better evolution of AP, as well as the ingestion of higher sugar diet in control animals. These findings suggest that elevated body weight and fat compartments can be protective against some immediate consequences of acute pancreatitis, as well as a higher sugar diet

Keywords: Early weaning, sugar diet, pancreatitis

Financial Support: FAPERJ

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Resumo:24-143

EFFECT OF MODERATE PHYSICAL TRAINING AND HYPERLIPIDIC DIET ON THE RATE OF PHAGOCYTOSIS OF ALVEOLAR MACROPHAGES IN TRAINED ADULT RATS.

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Objectives:
To evaluate the effect of physical training of moderate intensity and hyperlipidic diet on the rate of phagocytosis of alveolar macrophages in adult rats.

Methods and Results:
Male albino Wistar rats (n = 40), up to 125 days were used. After weaning, the animals were divided into two groups according to the dietary regimen. Each group comprised 20 animals. The control group was fed with a standard diet from the vivarium (Labina Purina-Brazil S/A) and the hyperlipidic group was fed with a palatable diet consisting of a hypercaloric mixture (normal protein and hyperlipidic) of commercial feed LABIN ®, roasted peanuts, milk chocolate and biscuit in the proportion of 3:2:2:1. The caloric density of the hyperlipidic palatable diet was 4.8 kcal/100g (24.5% of lipid) whereas the standard diet was 2.7 kcal/100g (4% of lipid). Therefore two experimental groups were obtained, the standard diet group (SD) and the hyperlipidic diet group (HD). The administration of the diets lasted 18 weeks, period in all animals were weighed on alternate days. At 90 days of life, the animals of both groups were divided and subjected to swimming training (5 times per week for 45 min / day for 8 weeks), forming the following groups: Standard Diet Control (SDC), Standard Diet Training (SDT), Hyperlipidic Diet Control (HDC), Hyperlipidic Diet and Training (HDT). After 24h of the last training, a bronchoalveolar lavage was collected to calculate
the rate of phagocytosis. The analysis of variance (ANOVA) was used to compare the results from the different groups. The results were expressed as mean ± standard error. After 18 weeks of the administration of diets, mean body weight in both groups were considered similar, SD equal to 273.6 ± 8.9 g and HD equal to 268.7 ± 5.5 g. The dietary consumption of the SD group (15 ± 1.4 g) was 32% higher than the HD group (22 ± 3.2 g), p = 0.001. However, the daily caloric consumption of the HD group was 11 calories higher than the SD. The mean rate of phagocytosis from alveolar macrophages was 15,500 in SDC group and 13,500 in SDT group (p = 0.792) with no statistically significant difference. There was not either statistically significant difference (p = 0.324) in the rate of phagocytosis between HDC group, which was 10,000 and HDT group, which was 12,000. In the comparison between control groups, there were statistically significant differences. The SDC group rate of phagocytosis was 15,500 and in HDC group was 10,000 (p = 0.032). However, similar results were not observed in trained groups. The rate of phagocytosis in SDT group was 13,500 and in HDT was 12,000 (p = 0.356).

Conclusions:

The results obtained regarding the rate of phagocytosis from alveolar macrophages in response to physical training and exclusive consumption of a hyperlipidic diet showed that exercise did not change the phagocytic activity of macrophages in trained adult rats.

Keywords: nutrition, physical training, hyperlipidic diet, rate of phagocytosis, obesity

Financial Support: FACEPE, CNPq, PROPESQ.
hemolytic assay, a 2% mouse erythrocyte suspension was used. After incubation for 1h with compounds (0.78-250 μg/mL), the supernatant containing hemoglobin was measured at 540nm. To further understand the mechanism underlying the cytotoxicity of compounds, differential morphology analysis with acidine orange/ethidium bromide staining were performed in ACP-02 cell line. Sequencing of TP53 revealed that ACP02 have several mutations on exon 8 (L289F and H296C) but not in exon 9. Unfortunately, sequencing of exons 5 to 7 was not conclusive. Most of the TP53 mutations are located between exons 4 and 9, which contain the DNA binding site of the p53 protein, suggesting that this lineage has a mutated TP53 status, whereas NIH-3T3 has a wild type TP53 status. Cytotoxic studies showed that compound 1 presented IC50 of 0.8 μg/mL (0.64 to 1.0) whereas DOX showed IC50 of 1.083 μg/mL (1.139 to 1.451) in ACP02 cell line, after 72h of treatment. As for NIH3T3, compound 1 showed IC50 of 1.519 (1.339 to 1.723) whereas for DOX showed IC50 of 0.23 μg/mL (0.19 to 0.28). Differential morphology staining indicated that compound 1 and DOX induce apoptosis in ACP02 cells.

Conclusions:
These findings points out to the potential of compound 1 as lead molecules to produce new compounds with the same efficacy but less side effects to normal cell when compared to doxorubicin, and with anticancer properties to cells with mt-p53.

Keywords: GASTRIC ADENOCARCINOMA, ANTICANCER, BENZOTHIAZOLES, MUTATION, TP53

Financial Support: CNPq, CAPES, FINEP, FAPESPA, FAPEAM

QuebraPagina

Resumo:25-091

CORRELATION BETWEEN CD44 AND ABCB1 MEMBRANE EXPRESSION IN LEUKEMIC CELLS.

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Objectives:
The superexpression of transmembrane transporter ABCB1 is one of main causes of multidrug resistance (MDR), and its function is dependent on ATP hydrolysis. This transporter is present in a number of different tumors, being capable of extruding a wide variety of structurally unrelated drugs and, as a result, reducing the drug intracellular level to a non-toxic concentration. Other proteins are involved in the MDR phenotype, and a correlation between the expression of ABCB1 and CD44 has been described in some tumors, including leukemia, but this conection is not clear. CD44 protein regulates growth, survival, differentiation and migration and are involved in tumor progression and metastasis. The aim of this work was to verify the membrane expression of CD44 comparing to ABCB1 expression in the cell lines K562 (human erythroleukemia), and the K562-derived cell lines: Lucena and FEPS (these cells were selected with vincristine and daunorubicine, respectively).

Methods and Results:
Using flow cytometry, the cell lines were stained with anti-ABCB1 and anti-CD44 antibodies to analyze the expression of both proteins. ABCB1 expression was shown to be null in K562, as described previously, while Lucena and FEPS cell lines expressed high levels of this protein (the later showing a two-fold increase over the cell line Lucena). Only K562 cell line had membrane expression of CD44 when compared with Lucena and FEPS, and this expression appeared in, approximately, 50% of cells. In order to analyze if the exposure to drugs made a negative selection of CD44 in cell lines Lucena and FEPS, the cell line K562 was cultured with low concentrations of vincristine or daunorubicine and the result showed that after 72h the expression of CD44 decreased.

Conclusions:
These results suggest a negative correlation between membrane expression of ABCB1 and CD44 proteins in leukemic cells.
Furthermore, the selection of resistant cell lines from K562 seem to select CD44 negative cells.

Keywords: ABCB1, CD44, leukemia

Financial Support: CNPq and INCT

QuebraPagina

Resumo:25-092

MIRNAS: GENERAL VIEW IN MURINE MELANOMA MODEL

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Objectives:

Aim. Melanoma is a complex disease rising from the malignant transformation of melanocytes. Despite being rare, it is responsible for the great number of deaths promoted by all skin cancers. One reason for this scenario is that metastatic melanoma is usually incurable. Five-year survival rate for advanced melanoma is estimated less than 10%. miRNAs are non-coding RNAs that bind to mRNA targets and disturb their stability and/or translation, thus acting in the post-transcriptional regulation. It is predicted that over 30% of mRNAs are regulated by miRNAs. Therefore these molecules are considered essential in the processing of many biological responses, such as cell proliferation, apoptosis and stress responsiveness. The loss or gain of function of miRNAs may contribute to tumor progression. Little is known about the regulation of miRNAs and understanding the events that lead to changes in their expression may provide new perspectives for the treatment of cancer. Evidences have shown that epigenetic events may be involved in miRNA expression. Moreover, epigenetic changes observed in malignant transformation of tumor cells may be a consequence of the deregulation of miRNA molecules that bind at components of epigenetic machinery, like DNA methyltransferases. In this regard, the aim of the present study is identify miRNAs differentially expressed during the malignant transformation of murine melanocytes, determine which miRNAs have their expression regulated by epigenetic mechanisms and related them, by informatic analyses, with mRNA targets.

Methods and Results:

Methods and Results. It was used a murine model of melanoma genesis in which several cell lineages (i.e., 4C, 4C11-, and 4C11+) were obtained after submitting melan-a melanocytes to sequential cycles of anchorage blockade. The expression profile of miRNAs in melan-a melanocytes, non-tumorigenic 4C cells (melan-a cells submitted to 4 deadhesion cycles) and tumorigenic 4C11- (non-metastatic melanoma) and 4C11+ cells (metastatic melanoma) as well as the miRNAs which expression is regulated by epigenetic mechanisms in these same lineages were analyzed by TaqMan® MicroRNA (miRNA) assay (Applied Biosystems). Among miRNAs analyzed, 25 miRNAs are progressively expressed along melanoma progression model. It was also noted that miRNAs are targets of epigenetic regulation. Several oncogenes and tumor suppressor genes were identified as targets of deregulated miRNAs.

Conclusions:

Conclusion. miRNAs found in this study represent strong candidates in the contribution of melanoma genesis because they present oncogenes and tumor suppressor genes as targets. Besides, they may be useful as therapeutic targets on interventions against cancer. Supported by FAPESP.

Keywords: melanoma, epigenetics, miRNA

Financial Support: FAPESP
TISSUE DISTRIBUTION AND PRE-CLINICAL PHARMACOKINETIC EVALUATION OF A MONASTROL ANALOGUE LASOM 65 AFTER DOSING TO RATS

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Objectives:
The compound ethyl 6-methyl-4-(3-nitrophenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-carboxylate (LaSOM 65) revealed more than 70% antitumoral activity in murine Sarcoma 180 model after 90 mg/kg i.p./day for 7 days. In this context, the aims of this study were: to investigate the pre-clinical pharmacokinetics (PK) of LaSOM 65 after intravenous (i.v.), intraperitoneal (i.p.) and oral (p.o.) administration to rats and to determine its plasma protein binding and tissue penetration after i.v. dosing.

Methods and Results:
The study protocol was approved by UFRGS Ethics Commission on Animal Use (2008196). LaSOM 65 was administered to Wistar rats (300–350 g) as single 1 mg/kg i.v. bolus (n = 7), 10 mg/kg (n = 8) and 30 mg/kg (n = 6) p.o., and 30 mg/kg (n = 7) and 90 mg/kg (n = 6) i.p. doses. After dosing, blood samples were collected from lateral tail vein at scheduled times up to 12 h. Plasma drug concentration was quantified by previously validated HPLC/UV method. LaSOM 65 tissue distribution (liver, heart, lung, brain, kidney, and fat) was evaluated after 1 mg/kg i.v. bolus (n = 3/time point) up to 4.5 h. After harvesting, tissues were frozen at -80 °C until assayed. Protein binding was determined by ultrafiltration (drug range 0.4–8 μg/mL) using Centrifree® devices (Millipore®). Individual plasma and tissue concentration-time profiles were evaluated by non-compartmental approach to determine PK parameters. Parameters from different doses and routes of administration were compared by ANOVA (α= 0.05). After i.v. dosing the compound showed a short half-life (t1/2) (1.70 ± 0.39 h), high clearance (CL) (0.82 ± 0.12 L/h/kg) and high volume of distribution (Vd) (1.76 ± 0.33 L/kg). The PK parameters were dose and route independent except for the 90 mg/kg i.p. that showed higher Vd and t1/2. The bioavailability was 58.6% and 49.2% for the 10 mg/kg and 30 mg/kg after p.o. dosing, and 70% and 60.8% for the 30 mg/kg and 90 mg/kg after i.p. dosing, respectively, indicating that the absorption process may be dose-dependent. LaSOM 65 protein binding was 84.7 ± 1.6%, concentration independent. The compound presented similar penetrations ratio (ASCtissue/ASCplasma) in the majority of tissues investigated (liver 0.4, kidney 0.6, heart 0.4 and brain 0.4), with a tissue tmax of 45 min. Lung concentrations were very high, with a penetration ratio of 2.7, followed by fat, with a penetration ratio of 1.4. This disposition is expected for high lipophilic drugs such as LaSOM 65.

Conclusions:
The investigation LaSOM 65 PK in rodents revealed that the compound distributes greatly into tissues with a high affinity for lung and fat, probably due to its high lipophilicity. The compound presents a high bioavailability after oral and i.p. dosing, with a tendency to dose-dependent absorption. The drug, which is rapidly eliminated from the body, showed linear pharmacokinetics in the dose range investigated. Further investigation is needed to determine the route of elimination and the formation of metabolites.

Keywords: LaSOM 65, pharmacokinetics, pre-clinical, protein binding , tissue penetration

Financial Support: INCT-if, CNPq/Brazil and CAPES/Brazil
TRANSCRIPTION PROFILE DIFFERENCES FROM FIBROBLASTS OBTAINED FROM THE PRIMARY TUMOR, LYMPH NODES AND BONE MARROW FROM BREAST CANCER PATIENTS

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Objectives:

In breast cancer, there is increasing evidence that stromal cells, including fibroblasts, may influence tumor development in the primary site, local and distant metastasis. Our goal was to compare fibroblasts from the primary tumor, lymph nodes and bone marrow of breast cancer patients, by means of their expression profile. Our second aim was to identify differential transcripts between fibroblasts obtained from involved (macro or micrometastasis) or uninvolved sites (lymph node or bone marrow) as compared with fibroblasts from the primary tumor, which might influence the metastatic development.

Methods and Results:

Fibroblasts primary culture was established from 14 patients with invasive breast carcinoma: 4 were obtained from the primary tumor, 6 from the axillary lymph nodes (three from positive and three from negative nodes) and 4 from the bone marrow (two with and two without micrometastasis, identified by RT-PCR for cytokeratin 19). Expression analysis was performed in a cDNA microarray platform containing 4,800 ORESTES, assembled at Ludwig Institute for Cancer Research, São Paulo, and analyzed using SAM multiclass test (TMEV software), FDR 0% and 5000 permutations. At first, we compared the expression profile of fibroblasts obtained from the three sites (breast tumor, lymph node and bone marrow) and identified 527 genes differentially expressed. These transcripts appropriately clustered fibroblasts from the primary tumor, lymph node or bone marrow in three different branches. Using LIMMA test (p = 0.001), 273 genes were differentially expressed between fibroblasts from the primary tumor and lymph node; 384 genes between fibroblasts from the primary tumor and bone marrow, and 418 genes between fibroblasts from lymph nodes and bone marrow. Genes sets (GSEA analysis) enriched in fibroblast from lymph nodes included genes up-regulated by interferon beta; mitochondrial genes; and in fibroblasts originated from bone marrow, genes up regulated by butyrate. To identify potential differences between fibroblasts obtained from sites with or without previous contact with macro or micrometastasis (nodes or bone marrow) and fibroblasts from the primary site we have performed a SAM multiclass analysis (FDR 0% /5000 permutations). This analysis resulted in 87 differentially expressed genes, which are mainly involved in transcription regulator activity and protein transmembrane transporter activity.

Conclusions:

In breast cancer patients, our results indicate that fibroblasts obtained from different sites present a differential gene expression profile, which may influence tumor behavior. Despite the origin of the fibroblasts, small differences may be due to a previous contact with malignant cells on the metastatic sites.

Keywords: breast cancer, fibroblast, gene expression, metastasis

Financial Support: FAPESP (process number 2009/10088-7) and CAPES
Objective:
The epidermal growth factor receptor (EGFR) is a transmembrane tyrosine kinase receptor, which plays an essential role in organ development and growth by regulating the differentiation of cells and tissues. EGFR is frequently overexpressed, and elicits unrestricted proliferation, in many tumors, including breast cancer. The polymorphism R497K (rs11543848), located in the exon 13 of the EGFR gene, has attenuated functions in ligand binding, tyrosine kinase activation and growth stimulation. The R497K polymorphism is associated with favorable prognosis in colorectal carcinoma and with reduced metastasis in non–small cell lung cancer. Other EGFR polymorphism is a (CA)n dinucleotide repeat sequence in intron 1 (rs72554021), ranging from 14 to 25 repeats. Shorter sequences of this (CA)n dinucleotide repeat sequence are associated with increased gene expression of EGFR in breast tumors. The aim of the present study was to investigate the association between EGFR polymorphisms and histopathological variables with prognostic value in breast cancer.

Methods and Results:
The study protocol was approved by the Ethics Committee of the Brazilian National Cancer Institute (#129/08) and consisted of a prospective cohort of Brazilian women (≥18 years old) with a confirmed diagnosis of unilateral non-metastatic breast cancer. Genomic DNA was extracted from blood samples and the polymorphism R497K was identified by PCR-RFLP (N = 427), whereas the fragment length of the intron 1 was determined by capillary electrophoresis (N = 63). The histopathological profile was determined after tumor resection based on the following parameters: histological type (invasive or in situ); tumor grade (G1 - well differentiated, G2 - moderately differentiated, G3 - poorly differentiated); tumor size (based on the largest tumor dimension reported by the pathologist after surgical excision); lymph node status (N0 – no metastasis; N1, N2 and N3 – number of affected nodes were, respectively: 1 to 3, 4 to 9 or > 9); hormone receptors (estrogen and progesterone) and Her2 status. The association between EGFR genotypes and histopathological features was evaluated by the Chi-square method. Eighteen different (CA)n alleles were found, with frequencies ranging from 0.01 to 0.19. The major allele was (CA)18 (0.436, 95%CI = 0.357–0.523). The genotypic distribution of the polymorphism R497K was in Hardy-Weinberg equilibrium, with 62% of the patients being homozygous for the Arg allele, 35% heterozygous (Arg/Lys), and 4% homozygous for the Lys allele. The frequency of the Lys allele was 0.258 (95%CI = 0.222–0.295). Patients with Arg/Arg genotype showed a higher chance (OR =1.957 95%CI = 1.089–3.517, p=0.023) of presenting tumors classified as high-risk (N1 and estrogen and/or progesteron receptor negative, or N ≥ 2). The association between (CA)n polymorphism and histopathological features could not be evaluated due to the small number of patients genotyped until now.

Conclusions:
Our data suggest that the R497K polymorphism may be associated with better prognosis in breast cancer.

Keywords: breast cancer, dinucleotide repeat , EGFR, polymorphism, R497K

Financial Support: MS/FAF, CNPQ, FAPERJ, CAPES
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Objectives:
Thymidylate synthase (TS) is a key enzyme in the nucleotide biosynthesis, essential in proliferating cells and an important target for several drugs such as methotrexate, 5-fluorouracil and oral prodrugs of 5-fluorouracil. TS catalyzes the methylation of deoxyuridine-5'-monophosphate (dUMP) to deoxythymidine-5' monophosphate (dTMP). TS provide the only source for de novo thymidylate production in the cell being critical for DNA replication and repair. TS overexpression has been reported to correlate with the responses and toxicity to TS-directed chemotherapeutics (TSCT). A polymorphic tandem repeat in the 5'UTR of the TYMS enhancer region (TSER) acts as an enhancer to the TS promoter and consists of 28 bp repeat units and suggests an increase TYMS RNA and protein expression, being associated with a worse response to TSCT, however more studies have to be done.

Methods and Results:
High-quality blood samples for genetic studies were obtained from a series of 225 healthy brazilian subjects from Belém/PA and TS1494del6 polymorphism was analyzed by PCR followed by sequencing in ABI 3130 using GeneMapper ID v.3.2. The Ethics Committee of UFPA approved this study and the written consent form. In this study, 16.8% of individuals have a homozygosis deletion and 37.33% presented a homoyzgosis insertion genotype. The observed mutated allele frequency is 60% in this population. This may be due to high frequency of heterozygosis genotype (45%).

Conclusions:
Since insertion is associate to drug toxicity, resistance and increase risk of death to patients in TSCT, pharmacogenetic analysis in the clinic, prior to the administration of TS-targeted chemotherapy, would help to avoid unnecessary toxicity and expense, especially in patients with homozygous insertion. More studies have to be performed to elucidate this issue.

Keywords: Thymidylate synthase, Polymorphic, Mutated Allele, Toxicity Chemotherapeutics, Overexpression

Financial Support: CAPES, CNPq, FINEP, FAPESPA

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Resumo:25-097

RECOMBINANT HUMAN NAT2*7 (GLY286GLU) AND ITS ASSOCIATION WITH CANCER RISK AND DRUG TOXICITY/RESPONSE IN HEALTHY AND CANCER SUBJECTS FROM THE NORTH OF BRAZIL

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Objectives:

N-acetyltransferase is an enzyme that catalyses the transfer of acetyl groups from acetyl-CoA to arylamines. It isoform, NAT2 (N-acetyltransferase 2), functions to both activate and deactivate arylamine and hydrazine drugs and carcinogens. NAT2 polymorphisms modifies individual cancer risk and drug response, or susceptibility to adverse drug reactions. Recombinant human NAT2*7 (Gly286Glu) leads to a variable reductions in catalytic activity associated with slow acetylator phenotype. Pharmacokinetic studies have shown that G857A (G268E) reduces Km for some N-acetylation substrates (such as sulfamethazine and dapsone) but not for others (such as 2-aminofluorene and isoniazid), suggesting this phenotype may be related to substrate. Residue G286 is located on the C-terminal tail, directly adjacent to the active site of the NAT2 enzyme. Replacing glycine with a much larger glutamate residue at codon 286 could significantly alter the conformation of the C-terminal tail adjacent to the active site. Since the C-terminal tail residues play a significant role in defining the size and shape of the active site opening, the G286E variant protein is likely to have an altered active site and substrate selectivity. Thus, the addition of a bulky glutamate side-chain at residue 286 in the NAT2 (G286E variant) might be expected to alter substrate selectivity and/or catalytic activity of target drugs currently used in clinics. Individuals who are slow acetylators are at higher risk of drug side effects, such as isoniazid-induced peripheral neuropathy, drug-induced lupus erythematosus, sulphonamides-induced hypersensitivity among HIV patients and also susceptibility to cancer development. In this way, the aim of the study was to evaluate the NAT2 G286E polymorphism in health and cancer subjects in Belém/Pará, Brazil.

Methods and Results:

High-quality blood samples were obtained from a series of 372 subjects (313 health, 12 gastric cancer (GC) and 33 breast cancer (BC) and NAT2 G286E polymorphism were analyzed by PCR followed by sequencing in ABI 3130 using GeneMapper ID v.3.2. The Ethics Committee in UFPA approved this study. In this study, no correlation was observed for NAT2 G286E polymorphism and cancer (p>0.05). The mutated allele frequency was 9.5%, 10% and 12% for health, GC and BC subject, respectively, suggesting that in this sized and studied population, the mutated allele is not a risk for cancer development. Although the mutated allele is present in only 9.5 to 12% of the subjects, 20% of them are heterozygous for NAT2 G286E polymorphism, what may contribute to mild to slow acetylator phenotypes and thus a predisposition drug toxicity in patients treated with different drugs.

Conclusions:

The application of NAT2 genotyping may be important in optimising the treatment of the several illness in particular those with homozygous mutate allele variant, diminishing drug toxicity and improving response.

Keywords: NAT2, Pharmacokinetic, Polymorphisms, Cancer, Modify

Financial Support: CAPES, CNPq, FINEP, FAPESP

QuebraPagina

Resumo:25-098

FREQUENCY OF DRUG TOXICITY-RELATED POLYMORPHISM ON TP53 GENE (ARG72PRO) IN HEALTHY SUBJECTS FROM THE NORTH OF BRAZIL.


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Objectives:
TP53 gene, located on chromosome 17p13, plays a central role in maintaining genomic integrity and preventing cells from oncogenic transformation. However, over 50% of cancer development is associated with mutation of the TP53 gene. Among known TP53 polymorphisms, Arg72Pro (PEX4), resulting from single nucleotide changed (G/C), is the most studied, and thus is associated with carcinogenesis. Recently, few studies have demonstrated that this polymorphism has a role in p53 on drug toxicity. Platinum drugs are among the most active and widely used agents in the treatment of different cancers. However, the great individual variability in both outcome and toxicity of platinum chemotherapy requires the identification of genetic markers that can be used to screen patients before treatment. In this way, screening for germ line p53 Arg72Pro is needed, especially in a very admixture population found in the North of Brazil. The aim of this study is to screen healthy and cancer subjects for PEX4 polymorphism.

Methods and Results:

High-quality blood samples for genetic studies were obtained from a series of 200 subjects (100 healthy and 100 gastric cancer) and Arg72Pro polymorphism in exon 4 was analyzed by RFLP with BstU1 restriction enzyme, confirmed by direct sequencing in ABI 3130. The Ethics Committee in UFPA approved this study. Pro allele frequency is 0.52 and 0.43 to health and cancer subjects, respectively. Pro allele is related to cell cycle arrest, DNA repair and enhances cell-death potential in cancer cells exposed to hypoxia. Arg allele is correlates with apoptosis induction. Several studies correlate Pro polymorphism with severe neutropenia and TP53 72 Arg/Pro with cancer susceptibility.

Conclusions:

These results show that TP53 72 Pro allele was found in a important frequency in our population. Pro genotypes may have a modifier effect on germline TP53 gene and may provide a useful genetic marker in predicating high-risk individuals for the development of toxicity to platinum drugs like cisplatin. In the future, we should consider the research of this polymorphism as an indicator of therapeutic conduct.

Keywords: Gastric Cancer, TP53 Gene, Polymorphism, Drug Toxicity, Apoptosis

Financial Support: CAPES, CNPq, FINEP, FAPESPA
viability was performed by the method of Trypan Blue. Cell counting was done in the hemocytometer. Cell Cycle Analysis Cell cycle analysis was performed by flow cytometry using propidium iodide. Growth factors MM cultured cells were submitted to the process of RNA extraction according to the procedures of Illustra RNAspin Mini Isolation Kit from GE Healthcare. The extracted RNA was then converted to DNA according to the protocol of the First-Strand cDNA Synthesis Kit, from Amersham Biosciences. The resulting DNA was subjected to RT-PCR technique to verify the expression of growth factors TGF, HMG, IL6, TGFâ1 and VEGF. Treatment with pravastatin 0.6 mM and 0.9 mM showed a significant reduction in cell population when compared with the control after 72h. There was a greater cell cycle arrest in G0/G1 stages in the groups treated with 0.6 µM and 0.9 µM of pravastatin after 72h. The RT-PCR showed a reduction of growth factors in cells treated with pravastatin at a concentration of 0.9 µM.

Conclusions:

The results of this study showed activity of pravastatin on cancer cells of multiple myeloma in vitro, reducing the number of viable cells and therefore is potentially useful as an antiproliferative drug.

Keywords: CULTIVO CELULAR, FATORES DE CRESCIMENTO, MIELOMA MÚLTIPLO, PRAVASTATINA, RT PCR

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**QuebraPagina**

Resumo:25-100

**EFFECTS OF FLAVONOIDs ON TUMOR PROGRESSION**


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Objectives:

Glioblastoma is a malignant brain tumor characterized by rapid cell growth, invasion and production of angiogenic factors. Glioblastoma is considered the most aggressive brain tumor and therapy options are still ineffective. Alternative drugs to combat tumors were recently discovered. In this context, the flavonoids emerged as potential therapeutic agents, acting on the tumor cytotoxicity. Flavonoids are polyphenolic compounds extracted from flowers and leaves. Flavonoids have antioxidant, antimutagenic and antiproliferative effects. Some flavonoids were shown to have antitumor effects and they could induce apoptosis by interacting with proteins that control the cell cycle, change the tumor gene expression and act in metastatic tumor development. This study evaluated the role of the flavonoids apigenin-7-O-â-d-apiofuranosyl-â-d-apiofuranoside, hydroxyluteolin-6-7-O-â-D-apiofuranoside, 5,6,5,3 tetrahydroxy-4 methoxyflavone, Apiina, apigenin-7-O-glucoside on healthy astrocytes and on Glioblastoma cells GBM95 and U87. The cells were treated with 5µM, 10 µM, 50 µM, 75 µM e 100 µM for 24 hours and 48 hours in search of new drugs for therapeutic treatments.

Methods and Results:

Primary cultures of cortical astrocytes obtained from cerebral cortex from P0 mice were cultured in DMEM-F12 supplemented with 10% fetal bovine serum until reaching confluence. Human glioblastoma cells GBM95 and U87 were obtained from patients of the Hospital Clementino Fraga Filho and commercial line (ATCC), respectively. The treatment with the flavonoids 5,6,5,3 tetrahydroxy-4 methoxyflavone and apigenin-7-O-glucoside reduced the viability of GBM95 and U87 tumor cells without modifying the viability of healthy astrocytes. The flavonoids apigenin-7-O-â-d-apiofuranosyl-â-d-apiofuranoside and hydroxyluteolin-6-7-O-â-D-apiofuranoside increased the viability of healthy astrocytes suggesting a neuroprotective effect of these drugs.

Conclusions:
In summary, the effect of different natural products has been evaluated on the viability and progression of glioblastoma cells. Flavonoids 5,6,5,3 tetrahydroxy -4 methoxyflavone and apigenin-7-O-glucoside decreased the viability of Glioblastomas GBM95 and U87, suggesting that these drugs may act to inhibit tumor proliferation. These results are promissory and should be considered to development of treatment protocols. Despite these advances, more studies are necessary to design treatment protocols. Natural products and chemical modification of antitumor substances are important strategies in the search for new anticancer drugs.

Keywords: glioblastoma, flavonoid, tumor

Financial Support: CNPq, FAPERJ, CAPES

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Objectives:

The Curine is an alkaloid Bisbenzilisoquinoline isolated from Chondrodendron platyphyllum. Among the biological activities of this class of molecules are: antimicrobial, analgesic, antitumoral, among others. Recent studies demonstrated that curine and others alkaloids Bisbenzilisoquinoline induce cytotoxicity in tumor cell lines (Chem. Pharm. Bull. Tokyo 58, 986, 2010). In this study, we evaluate the cytotoxic potential and the type of cell death induced by Curine in HL-60 tumor cell line.

Methods and Results:

HL-60 cells (5x10^4 cells/well) were treated with Curine (3.75-100 μM) for 24h. The cell viability was analyzed by the exclusion of trypan blue, MTT, Neutral red uptake, Nucleic Acid Content analysis, and flow cytometry. To assess the type of cell death caused in HL-60, cells were incubated with the Curine (3.75 - 20 μM) for 24 hours. To analyze the cell cycle distribution and mitochondrial depolarization was used flow cytometry using propidium iodide and tetramethylrodamine, respectively. Morphological alterations were determined by acridine orange/ethidium bromide staining using fluorescence microscopy. The IC50 values obtained using the HL-60 were 9.1μM to the exclusion of trypan blue, 7.8 μM to reduce MTT, 7.3 μM to NRU and 10.7 μM for NAC. Analysis by flow cytometry showed a significant reduction in cell viability at concentrations of 15 μM (83 ± 5.9 %; p < 0.05 (15 μM). A small mitochondrial depolarization occurred of 0.7 ± 0.2 (control) to 4 ± 0.6 %, p

Conclusions:

It was concluded that Curine was cytotoxic for HL-60 cells. The molecular mechanism of this cytotoxicity in leukemia cells involves induction of apoptosis and G1 phase cell-cycle arrest. This alkaloid can be considered as a class of molecules with cytotoxic potential.

Keywords: Apoptosis, Cytotoxicity, Curina, Leukemia cells

Financial Support: CAPES/CNPq

QuebraPagina

Resumo:25-101

INDUCTION OF APOPTOSIS OF CURINE IN HL-60 CELL LINE

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Objectives:

The Curine is an alkaloid Bisbenzilisoquinoline isolated from Chondrodendron platyphyllum. Among the biological activities of this class of molecules are: antimicrobial, analgesic, antitumoral, among others. Recent studies demonstrated that curine and others alkaloids Bisbenzilisoquinoline induce cytotoxicity in tumor cell lines (Chem. Pharm. Bull. Tokyo 58, 986, 2010). In this study, we evaluated the cytotoxic potential and the type of cell death induced by Curine in HL-60 tumor cell line.

Methods and Results:

HL-60 cells (5x10^4 cells/well) were treated with Curine (3.75-100 μM) for 24h. The cell viability was analyzed by the exclusion of trypan blue, MTT, Neutral red uptake, Nucleic Acid Content analysis, and flow cytometry. To assess the type of cell death caused in HL-60, cells were incubated with the Curine (3.75 - 20 μM) for 24 hours. To analyze the cell cycle distribution and mitochondrial depolarization was used flow cytometry using propidium iodide and tetramethylrodamine, respectively. Morphological alterations were determined by acridine orange/ethidium bromide staining using fluorescence microscopy. The IC50 values obtained using the HL-60 were 9.1μM to the exclusion of trypan blue, 7.8 μM to reduce MTT, 7.3 μM to NRU and 10.7 μM for NAC. Analysis by flow cytometry showed a significant reduction in cell viability at concentrations of 15 μM (83 ± 5.9 %; p < 0.05 (15 μM). A small mitochondrial depolarization occurred of 0.7 ± 0.2 (control) to 4 ± 0.6 %, p

Conclusions:

It was concluded that Curine was cytotoxic for HL-60 cells. The molecular mechanism of this cytotoxicity in leukemia cells involves induction of apoptosis and G1 phase cell-cycle arrest. This alkaloid can be considered as a class of molecules with cytotoxic potential.

Keywords: Apoptosis, Cytotoxicity, Curina, Leukemia cells

Financial Support: CAPES/CNPq

QuebraPagina
BRADIKYNIN AFFECTS THE INVASIVENESS OF MURINE MELANOMA AND MAMMARY ADENOCARCINOMA CELLS

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Objectives:
Aim: Generation and cleavage of factors by proteolytic enzymes, that may modulate tumor development, is observed at all stages of tumor progression. The nonapeptide Bradykinin (BK) is modulated by proteases present in the tumor microenvironment and may be involved in tumor progression. BK participates as a primary mediator of tumor angiogenesis, and the involvement of this molecule in the induction of innate and adaptive immune responses is now being unveiled. The presence of kinin receptors (B1R and B2R) has been demonstrated in several tumor cell lineages, and interaction with their ligands induces cell signaling cascades, that may interfere with the cell growth in vitro or induce the production of factors involved in the metastatic potential of these cells, as metalloproteases. Our goal was to verify the presence of B1R or B2R receptors in murine melanoma B16F10 and breast adenocarcinoma 4T1 cells, and determine the functionality of these receptors in these cells by verifying primarily the growth and metastatic potential in vitro after incubation with the peptide.

Methods and Results:
Methods and Results: A real-time PCR with murine specific primers showed the expression of B1R and B2R in B16F10-Nex2 and 4T1 murine cell lines. To determine the effect of BK on tumor cell proliferation, 5x10^3 B16F10-Nex2 and 4T1 cells were seeded in 96-well plates and incubated with several concentrations of BK for 24, 48 and 72h. The Cell Proliferation Kit I [Roche, based on MTT (3-(4,5-Dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide) method] and counting viable cells in the presence of Trypan blue in a Neubauer chamber were used to evaluate B16F10-Nex2 and 4T1 cell viability, respectively. The results showed that BK has no effect on the proliferation of both tested cell lines. To verify the effect of BK on the metastatic potential of these cells, in vitro migration and invasion assays were performed. Cells (2x10^5 ) were seeded in transwell chambers precoated with 50 μg of extracellular matrix (Matrigel, BD Biosciences), 10μM or 100μM of BK was added to the bottom well, and incubated for 24h. The number of invading cells was evaluated using the Leica Qwin software. BK inhibited B16F10-Nex2 invasion and had a contrary effect on 4T1 cells, increasing the number of invading cells. The wound healing assay was performed to test the effect of BK on cell migration. B16F10-Nex2 (2x10^5) cells were seeded on 12-well plates, when 90% confluence was reached a wound was inflicted and cells were incubated in the presence of 10μM or 100μM of BK for 24h. Images obtained from 0 to 24h showed that BK reduced significantly the migration of these cells compared with untreated control.

Conclusions:
Conclusion: B16f10-Nex2 cells showed the expression of B1R and B2R, and suggested that the interaction of one or both receptors with BK reduced the invasive potential, but had no effect on in vitro proliferation, of this tumor cell. 4T1 cells also showed the expression of both receptors, and interaction with BK increased the invasiveness, but had no effect on proliferation, of this murine cell line.

Keywords: ADENOCARCINOMA, BRADIKYNIN, CELLS, INVASIVENESS, MELANOMA

Financial Support: FAPESP, CNPq
Objectives:

Identify and quantify lycopene (LYC) present in red guava, even as evaluate the influence of this LYC on human breast cancer cell line (MCF-7) proliferation.

Methods and Results:

LYC was extracted from fresh pulp of guava (“Pedro Sato”), acquired from Tinguá producer of Rio de Janeiro city. Identification of LYC was conducted in detector Shimadzu Class-VP Chromatography, with C30 Waters Symmetry column (Massachusetts, USA) (250 x 4.6mm, 5µm) (Journal of Chromatography. v.1012, p.103-109, 2003) and using all-trans LYC standard from Sigma. Quantification of this carotenoid was performed in spectrophotometric method using an extraction solution of hexane, acetone, ethanol and BHT (Postharvest Biology Technology. v.41, p.151-155, 2006). For cellular viability (CV), cell line (MCF-7) from human breast cancer was routinely grown in medium containing 10% FBS, 1% penicillin 100 UI/ml and streptomycin 100µg/mL and 5µg/mL insulin and stored at 37 °C in a 5% CO2 humidified atmosphere. Later of 70-80% confluence, the cells was treated with 5, 10, 15, 20, 25 µM of LYC extracted from guava and 10 µM of LYC from Sigma dissolved in acetone. After treatment, cells were incubated for 3 h in 0.5 ml of MTT solution (0.5 mg/ml of PBS) at 37 °C in 5% CO2 in an incubator. The medium was removed and 0.5 ml of 0.04 mol/l HCl in absolute isopropanol was added to attached cells. Absorbance of converted dye in living cells was measured at a wavelength of 570 and 650 nm. CV of breast cancer cells cultured in the presence of studied compounds was calculated as a per cent of control cells (Journal of Immunological Methods. v.65, p.55–63, 1983). The experiments were performed in triplicates. The IC50 values were calculated from dose-response curves as the concentration of drugs that reduce the number of viable cells to 50% of control using the GraphPad software 5.0. According to chromatographic data, lycopene found in red guava are mostly present in trans-lycopene, with content of 4.75 + 0.02 mg lycopene/100g fresh weight of pulp. All concentrations used were able to reduce CV by up to 60% of breast cancer cells. There was no significant difference between the rates. Lycopene extracted from guava at a concentration of 10 µM was as efficient in reducing cell viability as lycopene standard in the same concentration, indicating that lycopene extracted from the fruit also has a great potentiality.

Conclusions:

None study had demonstrated the effect of lycopene extracted directly from guava fruit yet. So this search confirm that lycopene by guava have an antitumor effect like the sintetic that seen in others works. Although the physiological relevance is unknown, doses from 5 µM of lycopene, as used in this study, can be achieved through supplementation.

Keywords: Guava Fruit, Lycopene, MCF-7

Financial Support: FAPERJ and CNPQ
Objectives:

**Aim:** The medulloblastomas (MB) are the embryonic neuroepithelial tumors that affect mainly children with 9 years old. These tumors affect the cerebellum and the median survival of patients is 5 years. ATP is proposed as extracellular signaling molecule and shows many functions in the central nervous system (CNS). E-NTPDases and ecto-5’NT/CD73 are enzymes that catalyze the extracellular nucleotide metabolism and present different expression in brain tumors. Some evidences suggest that these enzymes are involved in the malignance process of these tumors. Considering these data, the objective of the present study was to investigate the involvement of the E-NTPDases in MB progression.

Methods and Results:

**Methods and results:** Cell culture - The MB cell lines Daoy, ONS76 and D283 were maintained in standard conditions of temperature with temperature of 37°C, minimum relative humidity of 95% and an atmosphere of 5% CO2 in air. HPLC assay – The cells were incubated with incubation medium plus AMP as substrate. The supernatant was removed in crescent times assayed, centrifuged and applied to a reversed-phase HPLC system. To analyze the nucleotide amount secreted by MB cell lines, cells were incubated with incubation medium in absence of AMP substrate. PCR – Total RNA from MB cell lines cultures was isolated with Trizol LS reagent, the cDNA species were synthesized and the PCR reaction was performed to evaluated the ectonucleotidases and ecto-5’NT/CD73 expression. Flow Cytometry analysis – The cells are cultivated, tripinized and incubated for 1h with Purified Mouse Anti-Human CD73 antibody (1:10) for 1h at 4°C, in the sequence the cells were incubated for 1h with Alexa fluor 555 Rabbit anti-mouse IgG (1:100). With the labeled cells was performed the flow cytometry where 5x104 events in the cell gate were collected.

**Results:** We observe that ATP was the main nucleotide secreted by all MB cell lines (Daoy - 100.04 µM ± 14.99; ONS76 55.61 µM ± 0.145; D283 - 26.48 µM ± 10.54). The analysis of AMP hydrolysis by HPLC showed that Daoy hydrolyzed AMP totally after 30 min of incubation with parallel adenosine accumulation; ONS76 hydrolyzed completely AMP within 60 min of incubation and with inosine accumulation. On the other hand, D283 cell line did not hydrolyze AMP in this incubation condition. The PCR analysis showed that MB cell lines express the E-NTPDase5, that is a proto-oncogene but only Daoy and ONS76 express ecto-5’NT/CD73. In flow cytometry assays we observed that just Daoy and ONS76 showed the ecto-5’NT/CD73 expression in their cell membranes.

**Conclusions:** The results presented here showed that all medulloblastoma cell lines studied secrete mainly ATP to extracellular medium and Daoy and ONS76 express active ecto-5’NT/CD73 that is able to hydrolyze AMP to adenosine. It is possible that, the production of inosine in ONS76 cell line was due to the presence of the adenosine deaminase enzyme. Finally we suggest that ATP accumulation and the adenosine and inosine production could favour the medulloblastoma progression.

Keywords: ecto-5’-nucleotidase, medulloblastoma, ectonucleotidases
Objectives:

Cancer development has been associated with alterations in polyamine biosynthesis and metabolism, which induce cell proliferation, angiogenesis, expression of genes related to tumor invasion and metastasis; whereas inhibit apoptosis. Based on the strong rational to develop novel polyamine depleting molecules, and adding the strategy to have substances that can control cancer through different cellular pathways aiming to bypass the acquisition of drug resistant phenotype by cancer cells; this work aimed to screen, in an ovarian cancer (OVCA) line, 40 novel rationally developed potential anti-cancer compounds, following rapid, high efficient, and low cost synthetic methodologies, then confirmed by spectroscopic techniques. OVCA is the most lethal gynecological malignancy, with high rates of chemoresistance and disease relapse; therefore, supporting the urge to generate novel anti-OVCA agents.

Methods and Results:

Naphthoquinones were rationally designed and synthesized in the Laboratório de Pesquisa em Química Orgânica at UFES. A serous ovarian adenocarcinoma cell line with intrinsic cisplatin resistance (OVCAR-3) was used to evaluate the in vitro citotoxicity of drugs. Cells were cultured in RPMI medium supplemented with 10% FBS, antibiotics and antifungics, at 37°C in 5% CO2. The in vitro cell toxicity of the drugs was determined by IC50 calculation after cellular viability, assessed by MTT assay (Absorbance at 630 nm). Briefly, cells were plated at 1.5x10^5 cell/well, and allowed to recover for 72h; then, treated for 24 hours in a dose-dependent manner with each drug, or cisplatin, the gold anti-OVCA drug, at concentrations of 10^-4, 10^-5, 10^-6, 10^-7, and 10^-8 M. Experiments were performed in quadruplicate. The mean and standard-deviation of the absorbances were calculated using Microsoft Excel. IC50 was calculated using GraphPadPrisma version 5, and compared to cisplatin IC50. We have performed a pilot screening assay to evaluate 40 novel naphtoquinone-derived drugs by means of each molecule proliferation inhibitory effect and IC50 value. When compared to cisplatin, 3 drugs, namely M8, PIC10 and PIC20, have described promising anti-proliferative action. The calculated IC50 (95% confidence interval) were: 3.87x10^-5M (2.13x10^-5 to 7.03x10^-5) to cisplatin; 1.64x10^-5M (8.31x10^-6 to 3.24x10^-5) to M8; 9.13x10^-6M (4.04x10^-6 to 2.06x10^-5) to PIC10; and 5.31x10^-5M (1.77x10^-5 to 1.59x10^-4) to PIC20. Whereas M8 e PIC20 have IC50 values in the same range as for cisplatin, the IC50 value calculated for PIC10 is at one order of magnitude lower than the others; therefore, pointing PIC10 as a more potent drug than M8, PIC20 or cisplatin. Regarding the drugs’ efficacy, we have observed that whereas cisplatin inhibit OVCAR-3 proliferation by 6.8% at 10^-5M, M8, PIC10 and PIC20 inhibit it by 45.8%, 53.2% and 16.2%, respectively, at the same concentration. These drugs will be further evaluated regarding their in vivo toxicity and median lethal dose in mice.

Conclusions:

We strongly believe that the present pre-clinical research project is innovative, as it introduces novel anti-OVCA drugs, economically viable, because we have developed an efficient and low-cost synthetic strategy to obtain the compounds, and socially important, as it might bring hope to put OVCA treatment in a perspective in which the disease control is a real possibility.

Keywords: antiproliferative, naphthoquinones, ovarian cancer

Financial Support: FAPES, PETROBRÁS

Resumo:25-106

EVALUATION OF THE GROWTH OF RECOMBINANT E. COLI TO IN PRESENCE OF DIFFERENT CONCENTRATIONS OF MGSO4 OR MNSO4

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Objectives:

Check the growth of recombinant *Escherichia coli* in Luria-Bertani medium (LB) with the presence of Magnesium Sulfate (MgSO4) or Manganese Sulfate (MnSO4).

Methods and Results:

There were made pre-cultures of recombinant *E. coli* carrying genes of H-Ras G12G or G12V in culture LB medium in the presence of antibiotics. After eight hours of incubation aliquots were transferred to new culture LB medium plus MgSO4 or MnSO4, both at concentrations of 0.03 M, 0.06 M, 0.23 M or 0.5 M. At the end of more eight hours of incubation it was found the growth rate of cultures in a spectrophotometer at a wavelength of 600 nm. The results were statistically analyzed in GraphPad Prism using t test. **Results:** It was observed that *E. coli* recombinant carrier of the gene G12G had a higher growth rate at a concentration of 0.03 M MgSO4, compared to the negative control in the absence of Mg+2, showed a statistically significance with P < 0.05. It was also found that the presence of 0.06 M MgSO4 had no influence on the growth of strains carrying the gene G12G. However at concentrations of 0.23 M, 0.5 M MgSO4, and also at all concentrations of MnSO4 tested, we observed the inhibition of the growth of strains carrying the gene G12G, with P < 0.05. For the G12V strain only the concentration of 0.03 M MgSO4 showed stimulation in growth rate with P < 0.05. Already at concentrations of 0.06 M, 0.23 M, 0.5 M MgSO4 and all concentrations of MnSO4, there was inhibition of growth rate of strains carrying the gene G12V, with P < 0.05.

Conclusions:

At concentration of 0.03 M MgSO4 was observed an upregulation in the rate of growth of *E. coli* carrying the genes G12G or G12V, a statistically significance compared to the negative control.

Keywords: Escherichia coli, H-Ras, Recombinant protein, Salt

Financial Support: CNPq/PIBIC and UFPB

**QuebraPagina**

**Resumo:**

**EXPRESSION OF THE CLOTTING INITIATOR PROTEIN, TISSUE FACTOR, IN CANCER STEM CELLS DERIVED FROM THE HUMAN BREAST CANCER CELL LINE T47D**

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Objectives:

Despite early detection of breast cancer, some patients relapse within five years. Identification of potential biomarkers of poor prognosis may guide the therapeutic approach and improve patient’s survival. Tissue factor (TF), a protein that initiates blood clotting upon binding to coagulation factor VIIa (FVIIa), has been recognized as a marker of tumor progression and metastasis. TF/FVIIa complex also enables the activation of protease activated receptor-2 (PAR-2) in tumor cells. In addition to PAR-1 isoform, also known as the thrombin receptor, PAR-2 have been correlated with several pro-tumoral responses. In breast cancer, cells with the phenotype CD44+CD24low have been described as cancer stem cells (CSC) that originate the more differentiated luminal CD24+ cells. However, recent data suggest that epithelial-mesenchymal transition generates cells with CSC characteristics. Based on CD24 and CD44 expression, our group has recently isolated two subpopulations of the T47D human breast cancer cell line that show distinct properties in vivo and in vitro. Tumorigenicity and invasiveness were restricted to the cells with the CD44+CD24low phenotype. The aim of this study is to investigate the expression of TF and its receptors, PAR-1 and PAR-2, in CD44+CD24low and CD24+CD44+ cells derived from T47D cell line.
Methods and Results:

The procoagulant potential of human breast cancer cell lines was evaluated by plasma coagulation assay. Surface and intracellular expression of TF and its receptors were investigated by FACS and confocal microscopy. Preliminary results showed that, as the invasive cell lines MDA-231 and MDA468, the subpopulation CD44+CD24-low of the cell line T47D has procoagulant activity, while the T47D CD24+CD44+ subpopulation was similar to the non invasive cell line MCF-7. Accordingly, TF was highly expressed by the invasive cell lines. Although PAR-2 was observed in the cytoplasm of all cell lines examined, surface expression was detected only in T47D CD24+CD44+ and MDA468 cells. Expression of PAR-1 was observed just in the cytoplasm, but T47D CD24+CD44+ cells were negative.

Conclusions:

The procoagulant activity and TF expression directly correlated with the invasive behavior of the cell lines. Additional experiments are needed to understand the role of PAR isoforms in this model.

Keywords: blood clotting, breast cancer, cancer stem cells, protease-activated receptors, tissue factor

Financial Support: CAPES, CNPq e FAPERJ.

QuebraPagina

Resumo:25-108

ANTITUMOR PROPERTIES OF A SULFATED POLYSACCHARIDE ISOLATED FROM THE RED SEAWEED GRACILARIA CAUDATA

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2 Departamento de Fisiologia e Farmacologia, UFC

Objectives:

Polysaccharides isolated from seaweeds have been immunomodulatory and anticancer properties. The aim of this study was to investigate the in vitro and in vivo antitumor properties of an sulfated polysaccharide isolated from the red alga Gracilaria caudata J. Agardh.

Methods and Results:

The dried seaweed(5g) was dissolved in distilled water(335 ml) and kept under stirring for 2 hours at 100°C. The extract was filtered in fine cloth and the polysaccharide was precipitated with ethanol (1:4 v/v), filtered, and the pellet washed with ethanol/acetone and dried at 40°C. The material was then resuspended in distilled water (1.5% w/v), reprecipitated with ethanol (1:4 v/v) and dried with ethanol/acetone at 40°C. The in vitro cytotoxicity of the polysaccharide (PGC) was evaluated against HL-60, MDA-MB-435, SF-295 and HCT-8 cancer cell lines by MTT assay. For the in vivo test female Swiss mice(n=8 per group) were used (25-30g). One day after inoculation of Sarcoma 180 tumor(2 x 106 cells), PGC was dissolved in 0.9% NaCl and administered intraperitoneally(25 and 50 mg.Kg-1.day-1) and orally (50 and 100 mg.Kg-1.day-1) for 7 days. 5-fluorouracil(5-FU) was used as a positive control(25 mg.Kg-1.day-1) and the negative control was administered with 0.9% NaCl. Then, PGC(25 mg.Kg-1.day-1) was tested associated with 5-FU(10 mg.Kg-1.day-1). On day 8, blood samples from control and treated mice were collected for hematological and biochemical analyses before sacrifice. Tumor, liver, spleen and kidney were excised, weighed and fixed in 10% formaldehyde. Inhibition ratio(%) was calculated by the following formula: inhibition ratio(%)=(A-B)/A x100, where A is the tumor weight average of the negative control, and B is that of the treated group. The in vitro test demonstrated that PGC had IC50 values greater than 25 ìg.mL-1 for all cells tested, being considered non-toxic. The inhibition
rate (IR) of the in vivo tumor growth by the intraperitoneal route was 47.59 and 53.53% at the doses of 25 and 50 mg Kg⁻¹, respectively. The oral administration of 50 and 100 mg Kg⁻¹ day⁻¹ of PGC caused IR of 37.27 and 35.98%, respectively. 5-FU reduced tumor weight by 74.14%. When PGC was tested combined with 5-FU the inhibition ratio improved from 37.22% for 5-FU (10 mg Kg⁻¹ day⁻¹) to 70.74%, suggesting a synergic effect. There was significant difference (p

Conclusions:

PGC did not show in vitro cytotoxicity, but exhibited in vivo antitumor activity without an expressive toxicity. This polysaccharide enhanced the efficacy of 5-FU, while preventing immunodeficiency induced by 5-FU, suggesting an immunomodulatory property.

Keywords: ANTITUMOR, POLYSACCHARIDE, GRACILARIA CAUDATA, RED SEAWEED, IMMUNUNOMODULATORY

Financial Support: CNPq, CAPES, FUNCAP, FINEP

QuebraPagina

Resumo:25-109

CORRELATION BETWEEN XIAP, PAKT, AND ATP7A EXPRESSION AND CISPLATIN RESISTANCE IN NON-SMALL CELL LUNG CANCER.

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Objectives:

Despite significant advances in non-small cell lung cancer (NSCLC) therapy during the past decade, platinum compounds remain the cornerstone for both early and advanced stages NSCLC management. However, the mechanisms underlying platinum resistance and sensitivity are poorly understood, and no major impact in patient selection or treatment modulation has been achieved to date. Apoptosis inhibition and platinum-efflux are evolving mechanisms, and therefore we evaluated the role of XIAP, pAkt, and ATP7A in this setting.

Methods and Results:

We have analyzed three human tumor cell lines, ACC-LC94, ACC-LC319, and A549, all comprising adenocarcinoma histology and harboring KRAS mutations. The cisplatin IC50 was assessed using MTT assay, after 48 hours incubation. mRNA expression was evaluated by Real-time PCR, while Western blotting was performed to analyse protein expression. Also, two distinct cohorts of NSCLC patients were evaluated. The first was comprised of 39 patients diagnosed as early-stage NSCLC (IA-IIIB), whereas the second included patients with metastatic disease (IV). In the latter, patients were clinically classified as platinum-sensitive (N=4) and platinum-non-sensitive group (N=47, less than 6 months interval to progression). In these two cohorts, we have either analyzed mRNA expression or protein expression by immunohistochemistry. The studied NSCLC cell lines exhibited distinct profiles regarding cisplatin sensitivity. LC94 was especially sensitive to cisplatin (IC50 11.2 mM), in contrast to LC319 (IC50 47.4 mM) and A549 (IC50 63.7 mM). Notably, resistant cell lines (LC319 and A549) showed a positive correlation between high XIAP and ATP7A mRNA expression when compared to LC94. These data was corroborated by XIAP and ATP7A mRNA expression, which also showed a significant positive correlation (R²=0.88) in early-diagnosed patients (IA-IIIB). However, we were not able to correlate these data to response to cisplatin, since only few patients of this cohort received adjuvant therapy. Subsequently, the XIAP protein expression was evaluated in cell lines and in the second clinical cohort. In fact, XIAP showed a
Considerably higher expression in the resistant cell lines (LC 319 and A549) than in the sensitive (LC 94). Among patients with metastatic NSCLC, XIAP was expressed in 45% (21/47) in the platinum-non-sensitive group, while no expression was observed in the sensitive (0/4). Knowing that XIAP is regulated by PI3K/Akt pathway through phosphorylation at the serine 87 residue, which increases XIAP stability and accumulation, we further analyzed the Akt phosphorylation. In the resistant cell lines, with higher XIAP protein expression, we observed superior Akt phosphorylation, in comparison to the lower level in LC 94. We have also analyzed Akt activation after cisplatin exposure. In fact, cisplatin induced Akt phosphorylation in A549, but not in LC 94, confirming the role for Akt phosphorylation in resistance mechanisms of lung tumor cell lines.

Conclusions:

XIAP, pAkt, and ATP7A mRNA expression were positively correlated to cisplatin resistance/sensitivity in NSCLC. This concept was identified in vitro using established human cell lines and in vivo, in two cohorts of patients. Further analyses are warranted to validate these findings and explore their potential as biomarkers in this setting.

Keywords: XIAP, cisplatin, pAKT, ATP7A, lung cancer

Financial Support: Fundação Ari Frauzino - FAF, FAPERJ, INCT-Câncer/CNPq

QuebraPagina

Resumo:

CHARACTERIZATION OF CELLS FROM PRIMARY CULTURE OF A CASE OF DIFFUSE ASTROCYTOMA FIBRILLAR

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2 Universidade Federal da Bahia - UFBA, LabImuno
3 Departamento de Neurocirurgia, HSR
4 Serviço de Anatomopatologia, HSR

Objectives:

Among the central nervous system tumors, gliomas have a fatal prognosis. Gliomas are tumors originating from glial cells and astrocytomas are composed of cells that resemble astrocytes. When produce many fibrils are called glial fibrillary astrocytomas. Molecular alterations frequently occur in tumors and are associated with aggressiveness and disease progression. A molecular subtyping, suggested by some authors, could be used to predict response to treatment. Based on the expression of a set of markers determinants of gliomas, these tumors can be subclassified into three groups: proneural, proliferative and mesenchymal, the latter two with a worse prognosis. OBJECTIVE: to characterize primary cells obtained from a diffuse fibrillary astrocytoma as the expression of glial fibrillary acidic protein (GFAP), oligodendrocyte transcription factor (OLIG-2), proliferating cell nuclear antigen (PCNA) and glycoprotein-39 (GP-39).

Methods and Results:

METHODS: cells were obtained from biopsy of a female patient, 44 years, underwent surgical resection of the tumor by the Department of Neurosurgery, Hospital São Rafael (HSR). The term of informed consent, in accordance with Resolution 196/96 of the National Health Council, was first obtained. The project was approved by the Ethics Committee of HSR and registered under number 15/09. Performed magnetic resonance image of the head showed an expansive lesion in the left temporal lobe. The pathology report was conclusive for diffuse fibrillary astrocytoma grade II (WHO), with foci of anaplasia suggestive of malignant transformation to grade III (WHO). The cells were maintained in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, penicillin (100 IU / ml) and streptomycin (100 μg / ml). The qualitative and quantitative characterization of the population of glial cells was performed by immunocytochemistry and flow cytometry, respectively. Cells with characteristics...
proneurais were identified by antibody against OLIG-2. ANCP antibody was used to mark proliferating cells and subclassified as antibody against GP-39 cartilage was used as a marker of mesenchymal cells. **RESULTS**: the cells showed positivity for GFAP, OLIG-2 and GP-39 by immunocytochemistry technique. According to flow cytometry, the cells showed 33%, 45%, 51% and 88% positivity for GFAP, OLIG-2, ANCP and GP-39, respectively.

Conclusions:  

**CONCLUSION**: the primary cells studied in a diffuse fibrillary astrocytoma cases showed positive staining for GFAP, OLIG-2, ANCP and GP-39. The biggest positive for GP-39 is indicative of a worse prognosis in some studies. Characterization of cells is important for prognosis and contribute to the understanding of disease mechanisms and resistance to chemotherapeutic agents.

Keywords: characterization, gliomas, molecular subtyping, prognosis, therapeutic agents

Financial Support: CNPq

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EFFECTS OF ZEBULARINE IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA CELLS.

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Objectives:

Acute lymphoblastic leukemia (ALL) is the most common hematologic malignancy in childhood and represents a heterogeneous disease regarding its biology and prognosis. Despite the advances in treatment, about 20% of patients relapse and/or die, indicating the need of different therapies for this group. Recently, epigenetic drugs as inhibitors of DNA methyltransferases (iDNMTs) have shown anti-neoplastic effects in different tumors. Zebularine (ZB) is a potent inhibitor of DNA methylation and has been associated with induction of apoptosis and enhancing tumor chemo- and radiosensitivity. However, its effects on childhood ALL cell lines have not been previously reported. Herein, this study aimed to evaluate the effects of ZB on cell proliferation and apoptosis of childhood ALL cells.

Methods and Results:

Functional studies of cell proliferation and apoptosis were performed in triplicate with Jurkat and ReH cell lines. Statistical analysis was made by one or two-way ANOVA and Bonferroni post-hoc. To calculate the doses with 50% inhibition of proliferation (IC50 values) data were analyzed by the median-effect method (Calcusyn software; Biosoft, Ferguson, MO). Both cell cultures were sensitive towards ZB treatment (50, 100, 200, 300µM), showing a dose- and time-effect difference of proliferation (P< 0.05).

Conclusions:

These results indicate that ZB may be a promising drug for the adjuvant treatment of ALL, since it is reported as a less toxic drug among the others iDNMTs. Further studies should be conducted to confirm its potential.

Keywords: acute lymphoblastic leukemia, childhood neoplasia, epigenetics, iDNMT, zebularine

Financial Support: FAPESP (2010/14378-7) and FAEPA.
EFFECTS OF LOW-INTENSITY DIRECT ELECTRIC CURRENT ON VIABILITY AND MORPHOLOGY OF HUMAN PROSTATE CARCINOMA CELL LINE DU-145

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Objectives:
Tumoral Electrotherapy (ETT) consists on the use of low-intensity direct electric current (DC) for the treatment of solid tumors, through insertion of electrodes on tumoral lesions, promoting electrolytic processes. However, the action mechanisms of ETT are not yet clear. It is known that some chemical products generated by electrolysis have important cytotoxic effects. In the present work, we evaluated the alterations produced by DC on viability and morphology of human prostate carcinoma cell line DU-145.

Methods and Results:
The experimental system used in our study allows for the assessment of the separate effects of anodic (AF) and cathodic flows (CF), as well as cell which undergo only the electroionic flow (EIF). DU-145 cells were seeded in 24-well culture dishes and treated with 2 mA of DC for 4, 8, 12 and 16 minutes and compared with untreated cells. For each experimental situation, cell viability was assessed immediately after treatments by two methods: Trypan Blue dye exclusion and MTT. Results show that viability drops proportionally to treatment times with AF and CF, reaching 100% of killing after 16 minutes. MTT method evidenced a 50% viability drop in after 8 minutes of EIF treatment, following with stabilization of the values in further times, while Trypan Blue analysis showed this phenomenon only at 16 minutes. Assessment of morphological alterations induced by DC in this cell line is being carried out by the May-Grumwald-Giemsa staining method for observation by light microscopy.

Conclusions:
Obtained results shows that DU-145 cells are sensible to DC treatment in a dose-dependent manner, and this killing profile is dependent to the proximity with the electrodes. Previous studies indicate that ETT is an effective treatment for malignant tumors and also provides advantages such as safety, low cost and minimal invasiveness and adverse effects.

Keywords: Direct Electric Current, Cancer Treatment, Prostate Cancer, Electrotherapy

Financial Support: CNPQ, FUJB, FAPERJ
Objectives:

Lung cancer is the leading cause of cancer deaths in the world. In lung cancer, K-Ras oncogenic mutations are widespread, and compounds used to target the biological activity of the Ras proteins failed in clinical trials. Therefore, it is imperative to identify novel therapeutic targets that reduce K-Ras-induced lung tumorigenesis. We have previously shown that K-Ras-induced lung tumorigenesis is potentiated by the subunit p65 of the transcription factor NF-κB; and that K-Ras-induced NF-κB activation in the lung requires IKKβ. Therefore, we hypothesized the following: (1) the IKKβ kinase promotes K-Ras-induced oncogenesis; and (2) pharmacological inhibition of IKKβ activity will be beneficial therapeutically.

Methods and Results:

We used genetic and/or pharmacological approaches to inactivate IKKβ in primary lung epithelial cells transformed by K-Ras, as well as K-Ras-positive lung cancer cell lines, and determined that IKKβ inhibition reduces certain oncogenic properties in vitro, such as growth and invasion. IKKβ inhibition also reduces expression of the Ras-induced chemokine IL-8, previously shown to be involved in angiogenesis. In addition we used a highly specific IKKβ inhibitor (CmpA, Bayer) to treat a K-Ras-induced lung cancer mouse model (LSL-K-RasG12D) combined with loss of the tumor suppressor p53. We chose this model for the following reasons: (1) K-Ras activation coupled to p53 loss leads to aggressive lung adenocarcinomas in mice that better resemble human lung tumors; (2) lung cancer patients harboring K-Ras oncogenic mutations frequently display p53 inactivation; and (3) it has been recently demonstrated that p53 loss can enhance NF-κB activation by K-Ras in lung tumors. In our initial round of treatment, we administered the inhibitor daily for 2 weeks immediately following the oncogenic insult. Surprisingly, we found that tumor number is not affected by treatment with CmpdA, which suggests that contrary to p65, IKKβ does not seem to control lung cancer cell survival. Nonetheless, we found that mice treated with CmpdA presented smaller and lower grade tumors than mice treated with placebo.

Conclusions:

These results show that IKKβ promotes lung cancer growth and progression both in vitro and in vivo. In addition, even though tumor regression is unlikely following IKKβ inhibitor therapy, we have shown that pharmacological administration of an IKKβ inhibitor slows lung tumor growth and progression in vivo, supporting our hypothesis that IKKβ inhibition therapy will have clinical benefits in lung cancer. Studies aimed at evaluating how later administration of the IKKβ inhibitor affects lung tumor kinetics and survival are underway and will serve to further validate the use of IKKβ inhibition in lung cancer therapy.

Keywords: K-Ras, Lung cancer, IKKbeta

Financial Support: Flight Attendant Medical Research Institute (FAMRI) and NIH

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Resumo:25-114

ANTITUMOR EFFECT OF JACARANONE DERIVED FROM PENTACALIA DESIDERABILIS


Microbiologia, Imunologia e Parasitologia / UNIFESP, EPM-UNIFESP

Objectives:

Jacaranone is a plant derived phytoquinoid which has already been shown to exert antitumor activity against carcinoma cell line COR-L23. In the present work, jacaranone obtained from Pentacalia desiderabilis was investigated for in vitro and in vivo antitumor activity using the murine melanoma model (B16F10-Nex2 cells). We also studied the in vitro jacaranone induced mechanism of action and cell death in tumor cells.
Methods and Results:

The in vitro cytotoxicity assay was performed using MTT and different jacaranone doses. We observed that jacaranone inhibited growth of B16F10-Nex2 cells with EC50 4.12µg/ml. Tumor cells treated with jacaranone at 4µg/ml induced formation of anion superoxide stained with dihydroethidium (DHE). The cytotoxicity of jacaranone in tumor cells was reduced after previous treatment with an intracellular antioxidant, N-acetylcysteine. To investigate the apoptotic response related to oxidative stress on tumor cells we tested the cytotoxicity of jacaranone at two temperatures, 4°C and 37°C. We observed that the phytoquinoid was not cytotoxic at 4°C, and proposed that its activity was energy dependent. Further we showed that melanoma cells treated with 10µg/ml of jacaranone, stained with Hoechst 33342, indicating condensation of chromatin. DNA degradation in cells treated with 10µg/ml of jacaranone was also shown using the TUNEL assay. Finally, we investigated the in vivo jacaranone antitumor effect using the subcutaneously grafted melanoma model in C57Bl/6 mice. Jacaranone was administered every other day, on the first day after the challenge with 5 x 104 melanoma cells. An increased survival of mice treated with jacaranone at 4mg/kg was obtained.

Conclusions:

Our results demonstrate that jacaranone isolated from Pentacalia desiderabilis presents a cytotoxic activity against murine melanoma B16F10-Nex2 cells in vitro and in vivo. The in vitro cytotoxic effect involves an oxidative stress characterized by abundant anion superoxide production. An apoptotic cell death of tumor cells is consistent with DNA condensation and fragmentation, cell shrinking, rounding up and detaching from the substrate. Additional apoptotic phenotypes are being presently studied.

Keywords: jacaranone, melanoma, apoptosis, antitumor, phytotherapy

Financial Support: FAPESP, CNPq

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Resumo:25-115

EVALUATION OF ECTONUCLEOTIDASES ACTIVITY IN HUMAN MEDULLOBLASTOMA CELL LINES

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Objectives:

Aim: Medulloblastoma are neuroepitelial tumors that occur preferentially in children with a mean age of 9 years old. They are classified as grade IV tumors, which is the highest degree of malignancy. They affect specifically the cerebellum, and the median survival of patients is approximately 5 years. Ectonucleotidases were related to malignant tumors, because they play important roles in the control the amount of extracellular nucleotides that may be involved in tumor progression. These enzymes were responsible for nucleotides extracellular catabolism, especially ATP, which have been demonstrated favoring the tumor progression of glioma and others systemic tumors. The ecto-5'-NT/CD73 is an important enzyme of ATP metabolism, degrading AMP to adenosine in the extracellular medium. Taken these facts, the aim of this work was to characterize the ectonucleotidases in different human medulloblastoma cell lines.

Methods and Results:

Methods and results: Cell culture - The medulloblastoma cell lines Daoy, ONS76 and D283 were maintained in DMEM medium supplemented with fetal calf serum 10% and standard conditions of temperature with temperature of 37 °C, a minimum relative
humidity of 95% and an atmosphere of 5% CO2 in air. Enzymatic assay – The cells were incubated with different substrates, which were specific for each enzyme assayed and the amount of Pi released was determined by staining method of Malaquite Green. To analyze the phosphatases activity, the enzymatic assay was performed in the presence or absence of levamisole, which is considered a phosphatase inhibitor.

Conclusions:

Results: The cell lines evaluated did not show significant values concerning the hydrolysis of ATP and ADP nucleotides. Daoy cell line showed the highest AMPase activity (74.11 ± 8.33 nmol Pi/min/mg protein), followed by ONS76 (9.206 ± 0.730 nmol Pi/min/mg protein) and finally, with less activity, D283 cell line (3.654 ± 0.157 nmol Pi/min/mg protein). The three medulloblastoma cell lines maintained the profile of AMP hydrolysis when in presence of others monophosphate nucleotides. By analyzing the phosphodiesterases activity, it was observed that this enzyme did not show contribute to nucleotide hydrolysis. Unspecific phosphatase showed low enzymatic activity. The AMP hydrolysis was unchanged in the presence of levamisole.

Conclusion: Results showed that medulloblastoma cell lines poorly hydrolyze ATP and ADP. Due to the low activity of unspecific phosphatases and phosphodiesterases, the high hydrolysis of others monophosphate nucleotides in addition to AMP and the incubation enzymatic conditions, we suggest that these cell lines, especially Daoy, present the ecto-5’NT/CD73 active in its cell membrane.

Keywords: cell lines, ectonucleotidases activity, human medulloblastoma

Financial Support: CNPQ

PURIFICATION OF RECOMBINANT PROTEIN BY ION EXCHANGE CHROMATOGRAPHY

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Objectives:

Perform the extraction and purification of recombinant H-Ras wild.

Methods and Results:

Initially it was made cultures of recombinant Escherichia coli in culture medium Luria-Bertani in the presence of antibiotics. After incubation for eight hours was added Isopropyl-β-D-thiogalactopyranoside (IPTG) to induce protein expression. All proteins together with H-Ras were extracted by lysis chemistry, mechanics and centrifugation. The supernatant of bacterial lysate was passed into an ion exchange column using different programs for the flow and volume fractions. In the first chromatographic program used, the H-Ras was eluted in fractions of 0.8 mL for 40 minutes. It was observed that there was a slight of contaminating proteins in relation to the protein under study. In the second program used, the elution of the recombinant protein was performed in fractions of 0.8 mL for 60 minutes. It was observed that the pattern of elution of the proteins remained the same as observed on the first try. In the third program, the protein was eluted in fractions of 0.8 mL for 120 minutes. Through this chromatographic program it was observed that the contaminating proteins were shifted to the left and recombinant H-Ras was shifted to the right, it is possible to elute practically pure protein. All results were verified on SDS-PAGE 12.5% using as a control the molecular weight and Western blot tested with monoclonal antibody specific for a protein sequence.

Conclusions:

We conclude that the level of purity of the recombinant protein H-Ras eluted through ion exchange chromatography is dependent
time on the elution process.

Keywords: Ion Exchange Chromatography, Recombiant Protein, Wild H-Ras

Financial Support: CNPq/PIBIC and UFPB.

RESVERATROL POTENTIATES THE CYTOTOXIC EFFECT OF MELPHALAN IN MCF7 CELLS BY INHIBITION OF CDK7 ACTIVITY

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Objectives:

Melphalan (MEL) is a chemotherapeutic agent used in the breast cancer therapy. However, MEL produces side effects that limit its clinical applications. The use of resveratrol (RSV) in combination with MEL to improve the efficacy of this chemotherapeutic agent was analyzed in this study.

Methods and Results:

RSV and MEL exhibited cytotoxic effects on MCF-7 cells in a dose and time-dependent manner. The IC50 values of treatments with RSV and MEL for 24h were 120 micromolar and 110 micromolar, respectively. The treatment with association of RSV with MEL enhanced the cytotoxic effects of MEL on MCF-7 cells and this increase was dependent on treatment sequence. The sequential treatment with RSV followed by MEL yielded more cytotoxic effects than treatment with MEL followed by RSV. After observing the effects of RSV and MEL on the cell viability, the effect of these drugs on cell cycle distribution was analyzed by flow cytometry. Treatment for 24 hours with 50 micromolar and 200 micromolar of RSV induced significant accumulation of cells in S phase. When the cells were treated with combinations of RSV and MEL, an increase of cells in S phase was also observed. To directly test the role of cell cycle progression in increasing MEL’s cytotoxicity, cells were arrested in G1, S or G2/M phase using the specific cell cycle inhibitors mimosine, thymidine or nocodazole, and treated with MEL. These combinations of drugs also potentiated the effect of MEL in decreasing the viability of MCF-7 cells, as well as combination of RSV with MEL. These findings indicate that RSV-induced cell cycle arrest in S phase can be one of the mechanisms of this molecule in sensitizing MCF-7 cells for treatment with MEL. On the basis of the results of cell cycle distribution, it was investigated whether the cell cycle arrest at S phase by RSV and MEL was related to the expression of cell cycle regulatory proteins which are essential for cell cycle progression at S phase. It was observed that the treatment with RSV or MEL increased the levels of p-Chk2 expression. No alteration was observed in cdc25A phosphatase levels. The levels of cyclin E expression were not significantly changed by treatments. The cyclin A expression was decreased by treatment with RSV and its association with MEL. Moreover, the p21 protein levels, CDK2 inhibitor protein, were significantly increased by treatment with RES or MEL. While the total protein levels of CDK2 remained largely unchanged by treatments, its active form (Thr160-phosphorylated CDK2) was decreased by treatment with RSV and its association with MEL. No effect on the Thr160-phosphorylated CDK2 expression was observed by treatment with MEL alone. Therefore, the CDK7 activity, kinase that phosphorylates CDK2 at Thr160, was analyzed by in vitro kinase assay. RSV inhibited the CDK7 kinase activity with a reduction in phosphorylation of CDK2 substrate at Thr160, supporting the above suggestion. The same effect was found in the cells treated with association of RSV and MEL, but not in the treatment with MEL alone.

Conclusions:

Taken together, our results indicate that RSV may be an adjuvant agent during breast cancer therapy with MEL.
ABNORMAL INFLAMMATORY RESPONSE INDUCED BY OBESITY ENHANCES GROWTH OF PROSTATE CANCER XENOGRAFTS

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Objectives:
Obesity is associated with increased predisposition to some cancers, aggressiveness of others, insulin resistance/hyperinsulinemia as well as a state of abnormal inflammatory response. Recent study focusing on prostate cancer has shown that obesity is an important adverse prognostic factor. However, the molecular mechanisms involved in the increased aggressiveness of prostate cancer in obese individuals are still unknown.

Methods and Results:
In order to investigate the effects of inflammation and hyperinsulinemia induced by high-fat diet (HFD) on prostate cancer growth, SCID mice fed a control or HFD for eight weeks were injected subcutaneously with PTEN positive (DU145) and PTEN negative (PC-3) prostate cancer cell lines. Here, we show that obese mice experienced a higher tumor growth of both DU145 and PC-3 xenografts compared to the control group. Xenografts of mice fed a HFD show an increase in IκB kinase complex and c-Jun NH2 terminal kinase activity, which is prevented by blocking TNF-alpha. Interestingly, pharmacological blockade of TNF-alpha in HFD mice was effective to reduce tumor growth induced by HFD to control levels of both DU145 and PC-3 xenografts. In addition, we show that DU145, when grown as tumor xenografts in mice, are sensitive to the reduction of hyperinsulinemia of obesity induced by octreotide treatment, whereas PC-3 cells, that presents a constitutive activation of PI3K, are resistant.

Conclusions:
Thus, the present study documents that low grade inflammatory response observed in obesity, in an insulin sensitivity independent manner, drives the growth of prostate cancer xenografts.

Keywords: Cancer, Inflammation, Obesity

Financial Support: FAPESP and CNPq

CARVACROL ACTIVITY AGAINST SALMONELLA AND ACTION ON NEUTROPHIL CHEMOTAXIS

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Objectives:

We evaluated the carvacrol (CVL) effect on in vitro cell viability, against *Salmonella* strains from outbreaks and on leukocytes recruitment.

Methods and Results:

CVL was isolated from *Thymus vulgaris* essential oil and identified by CG-MS and RMN. To evaluate the antimicrobial effect of CVL, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) in the micro-dilution antibacterial assay were determined. The *Salmonella* spp were cultured in Tryptic Soy Broth at 35°C during 24 hours. CVL (2500 µg/mL) were diluted twofold along each well and incubated with *Salmonella* spp during 24 hours. The MIC was determined as the lowest concentration that CVL inhibited the microbial growth. To MBC determination, an aliquot without visible growth was spread in Hektoen Enteric Agar incubated at 35 °C during 24 hours and the MBC was the lowest concentration of CVL able to inhibit bacterial growth. In the *in vitro* MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide] assay, the neutrophils (1x10⁶ cells/mL) were treated with CVL (20 µg/mL) and then the cell viability was measured by spectrometry and expressed in terms of relative absorbance of CVL-treated cells in comparison with no treated cells.

To investigate the inhibitory effect of CVL on leukocytes recruitment, neutrophils (1x10⁶ cells/mL) isolated from the peritoneal cavity of mice, 4 hours after injection with zymosan (1mg/cavity, i.p) were treated with CVL (5, 10 or 20 µg/mL) for 30 min. Then, the cells were placed in the chemotaxis Boyden chamber (48 wells; Neuro Probe, MD–USA) and fMLP (10⁻⁶M) was used as chemotactic stimuli and medium RPMI as control of migration. The cells were incubated for 1 h at 37 °C, 5% CO₂ and the membrane was washed and stained using the Instant Prov (Newprove). Each well-associated membrane area was scored using optical microscopy (1000X) to count the intact cells present in 5 random fields. The results were expressed as the number of neutrophils per field. Data were presented as mean ± SEM of 3 separate experiments. The means from different treatments were compared by ANOVA with Tukey’s correction. Statistical significance was set at P ≤ 0.05. The protocol (number 066/2010) regarding this study was approved by the ethical commission in animal research (CEAE/State University of Maringá). The CVL revealed a remarkable antimicrobial effect against all *Salmonella* strains tested with MIC and MBC of 156 µg/mL and 312 µg/mL, respectively. In the MTT assay, it was found 92% of viable cells in the presence of CVL. The chemotaxis induced by fMLP (27.5 ± 6.49 cells/field) was inhibited by CVL₁₀µg/ml: 3.5 ± 0.85 cells/field* and by CVL₂₀µg/ml: 1.2 ± 0.6 cells/field*, but not by CVL₅µg/ml: 19.7 ± 1.5 cells/field. As negative control medium RPMI was used (1.5 ± 0.26 cells/field).

Conclusions:

Our data suggest that although carvacrol in tested concentrations showed inhibitory effect on neutrophil chemotaxis, carvacrol has prophylactic potential to be used in the control of *Salmonella* spp and further studies are need to investigate if the phagocytosis could be also inhibited by carvacrol.

Keywords: ANTIBACTERIAL, CHEMOTAXIS, CYTOTOXICITY, CARVACROL

Financial Support: Capes
Objectives:

Aim: Mansoa standleyi (Bignoniácea) is known in Brazil as “Cipó d’alho” in reference to the strong smell of garlic when crushed leaves. This herb has several uses in traditional medicine and among the most cited are the treatment of flu, fever, pain, inflammation, arthritis and rheumatism. The aim of this study is validate the anti-inflammatory and analgesic activity of this plant.

Methods and Results:

Methods and Results: The anti-inflammatory activity was analyzed in the air pouch model. The rats were randomly located in three groups: Control, Essential Oil of Mansoa standleyi (OEMS) 0, 1% and 0, 01% and the samples were collected and the numbers of cells were quantified. The results showed a decrease in cell migration of macrophages and neutrophils (Lymphocytes: 33.38±9.38; 15±2.02; 27.74±10.18; Macrophage: 16.63±3.47; 11±1.6%; 11±3.3% respectively to Control, 0.1 and 0.01% of OEMS). The macrophage cultures were also used to evaluate the effects of OEMS on peritoneal macrophages. Therefore the level of nitrite (µM, mean ± SD) was measured by Griess reaction and obtained the following result: 16.73 ± 1.25; 3.98 ± 3.6; 5.26 ± 3.38; respectively to Control, 0.1% and 0.01% of OEMS follow the cell viability was analyzed but the oil did not interfere in any concentration. Due to the results, we investigate the action of OEMS in pain models. Writhing test, male mice, 6 to 8 weeks, were divided into five groups (n = 10): control (saline or morphine-10 mg / kg), 0.1% or 0.001%. All mice received the chemistry algic agent, 1% acetic acid. However the results show that treatment with OEMS was able to reduce the writhing as the result exposed ( percentage of inhibition vs control 37%, 34%, 14% respectively to 0,1%, 0,01% and 0,001% of OEMS). All procedures involving animal care and experimentation were performed in accordance with the guidelines of the Ethical Committee for Research with Experimental Animals of the Universidade Federal do Pára (BIO001-09).

Conclusions:

Conclusion: Our results demonstrared the anti-inflammatory activity of OEMS decreasing defferents events of inflammation such as cellular migration of macrophages and production of high levels of nitrite and OEMS also shows analgesic activity confirmed by the writhing.

Keywords: analgesic , anti-inflammatory , Essential oil of Mansoa standleyi

Financial Support: CNPq,FAPESPA,UFPA

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Resumo:26-237

ANTINEOPLASIC ACTIVITY OF SHORT CATIONIC AMPHIPHILIC PEPTIDES FROM CAESALPINIA FERREA.

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Objectives:
Short cationic amphiphilic peptides (SAP), known as members of antimicrobial peptides family are found in all forms of life, like plant seeds, insects and usually they have presented immunomodulatory or antimicrobial activities. A lot of seeds are used in folk medicine in Amazon, Caesalpinia ferrea is used as analgesic and antiinflammatory. In this context, we studied the SAP composition of C. ferrea seeds and tested their antineoplastic effects.

Methods and Results:

The proteins were extracted from seeds flour in phosphate buffer (pH 5.4) and submitted to a dialysis process. The fraction, which is full of SAP, was recovered by lyophilization and then eluted on ion exchange chromatography where another two fractions were collected (D1 – basic peptides; D2 – acid one’s). They were visualized in Tris-Tricine/SDS gel electrophoresis. Later this D1 fraction was submitted to a C8C18 HPLC reverse phase column to assure its purity. Seven peaks of peptides were obtained (P1, P2, P3, P3.1, P4, P5, and P6) and pharmacologically analysed in vitro using C6 glial cell line. Cells were cultured in DMEM and seeded in 96 wells plates. After 24 h of incubation at 37°C, the peptides were added at final concentrations of 2.5 or 5.0 ul/well. The cell viability was evaluated by MTT assay (n=4) in 24, 36 and 48 hours and the nitrite released (n=4) was quantified. The result shows that P1 at 2.5ul concentration is decreasing cell viability in time course (23.92±3.74; 14.38±0.61; 17.97±1.38) and at 5.0ul (18.33±2.68; 10.63±0.7; 10.4±0.38) respectively to 24, 36, 48h compared to control [99.61±0.31(24h); 98.09±1.91(36h); 99.53±0.47(48h)]. To nitrergic pathway P1 also promotes significant release of nitrite at 24 hours (2.5ul/well – 2.05±0.84; 5.0ul/well – 1.88±0.48) compared to control (1.3±0.29). At 5.0ul concentration, P3 activated [2.04±0.46(24h)] this cell type via nitrergic pathway compared to control [1.3±0.29(24h)]. It is also decreasing cell viability [92.03±2.61(24h); 88.91±2.03(36h); 85.79±2(48h)], vs control [99.61±0.31(24h); 98.09±1.91(36h); 99.53±0.47(48h)].

Conclusions:

These short cationic amphiphilic peptides demonstrated an interesting activity by inhibit C6 cell proliferation time course and in a dose-dependent manner. Based on these results we can infer that cellular viability decrease is due to the activation of nitrergic pathway.

Keywords: short peptides, glioblastoma, antineoplastic

Financial Support: CNPq, CAPES, UFPA.

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Resumo:26-238

COMPARATIVE STUDY OF THE MODULATORY EFFECTS OF (–)-CUBEBIN ON THE MUTAGENICITY/RECOMBINOGENICITY INDUCED BY DIFFERENT CHEMICAL AGENTS.

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Objectives:

Piper cubeba L. (Piperaceae) extracts have a broad range of biological activities (anti-inflammatory, anti-type IV allergic, antileishmanial, genotoxic, antineoplastic and molluscicidal). The beneficial effects of these preparations are related to secondary plant metabolites such as lignans, which are produced by the oxidative dimerization of two phenylpropanoid units. (–)-Cubebin (CB), a dibenzylbutyrolactolic lignan, was isolated from dry seeds of P. cubeba. This lignan is known to possess anti-inflammatory, analgesic and antimicrobial properties. Previous studies suggest that CB could inhibit mitochondrial complex I and also deactivate cytochrome P450 (CYP 450) and the NADH oxidase activity of mitochondrial complex I. Several studies have
demonstrated the probability of pharmacokinetic interactions of natural compounds and herbal products with conventional drugs when administered simultaneously. Here, we examine the mutagenicity and recombinogenicity of CB when administered alone or simultaneously with the anthracycline antibiotic Doxorubicin (DXR) and Ethyl-carbamate, also known as Urethane (URE). DXR, a drug that targets topoisomerase II, is one of the most effective anticancer drugs used clinically. URE, a promutagen having a clearly mutagenic potential in *Drosophila melanogaster*, is strongly dependent on metabolic activation by CYP 450.

Methods and Results:

The Somatic Mutation and Recombination Test (SMART) in wing somatic cells of *D. melanogaster*, was performed employing two genetic markers located on the left arm of chromosome 3: multiple wing hairs (*mwh*, 3–0.3) – a homozygous-viable recessive mutation that produces multiple trichomes per cell instead of one trichome; and flare3 (*flr3*, 3–38.8) – a recessive mutation that produces flare-shaped wing hairs. Three *D. melanogaster* strains were used: 1) multiple wing hairs: *y; mwh*; 2) flare-3: *flr3/In(3LR)TM3, ri ppsep l(3)89Aa bx34ee BdS*; and 3) ORR; flare-3: *ORR; flr3/In(3LR)TM3, ri ppsep l(3)89Aa bx34ee BdS*.

Two different crosses were carried out: Standard (ST) cross and High Bioactivation (HB) cross. For the ST cross, virgin flare-3 females were mated with mwh males. For the HB cross, which is characterized by an augmented level of CYP 450, virgin ORR, flare-3 females were mated with mwh males. Third instar larvae obtained from both crosses were fed chronically (48 h) with CB alone (0.25, 0.5, 1.0, 2.0 or 4.0 mM) or in combination with DXR (0.2 mM) or URE (10 mM). CB alone did not induce mutation or recombination. When associated with DXR, at lower concentrations, CB statistically reduced the frequencies of DXR-induced mutant spots. However, at higher concentrations, CB potentiates DXR effects, leading to increased frequency of mutant spots (4.0 mM) due to its toxicity. The results obtained when associated with URE were fairly similar.

Conclusions:

These results suggest that, depending on the concentration, CB may interact with the enzymatic system that catalyzes the metabolic detoxification of DXR, inhibiting the activity of mitochondrial complex I, thereby scavenging free radicals, and that CB may also modulate the metabolic activation of URE, inhibiting the formation of DNA-binding metabolites.

Keywords: PIPER CUBEBA, SECONDARY METABOLITIES, SMART

Financial Support: CNPq (Process 304376-2010-8); CAPES; UFU; UNIFRAN; FAPESP.

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Resumo:26-239

ANTI-INFLAMMATORY AND ANALGESIC PROPERTIES OF THE ETHANOLIC EXTRACT FROM CASEARIA LASIOPHYLLA EICHLER.

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Objectives:

Plants from the genus *Casearia* are popularly used for snake bites, and it is a general belief that this may be related to properties of the extract of this plant to inhibit phospholipase A2. An anti-inflammatory and analgesic activity has been shown for *C. sylvestris* (Esteves et al., *J. Ethnopharmacol.*, 101:191, 2005). *Casearia lasiophylla* is found in the State of Paraná and is popularly known as Guarantú. This study evaluated the analgesic and anti-inflammatory properties of the ethanolic extract from *C. lasiophylla* (EECL).

Methods and Results:
Male swiss mice (20-25g) were used and all experiments were approved by the Institution’s Ethics Committee for Animal Use under protocol # 461. Mice were orally treated with EECL (30–300 mg/kg), dexamethasone (DEX, 1 mg/kg) or vehicle (Tween 80 0.1%) 1 h before the injection of 20μL Cg (300g/paw, s.c.) in the right hind paw. Paw thickness was measured by a digital micrometer 1 h before any treatment and at different time points (0.5, 1, 2 and 4h) after the injection of Cg. EECL reduced dose-dependently the paw oedema induced by Cg (0, 25 and 44 % for 30, 100 and 300mg/kg, respectively, 2h after Cg injection) while DEX reduced 45% at the same time point. Another group of animals were treated with EECL (10–100mg/kg), indomethacin (5mg/Kg) or vehicle (Twee 80 0.1%) by oral route 1h before injection of the 20μl of 2.5% formalin in the right hind paw and the nociceptive behavior (licking and flinching) was observed for 40 min. EECL reduced 36% of the phase I (0-5min) at the higher dose and 64 and 69% of the phase II (15-40min) at 30 and 100mg/kg, respectively. Indomethacin reduced only the phase II (59%). However, at the same doses EECL was unable to modify the latency time in the hot-plate test (54±1°C) while the positive control fentanyl (100μg/kg, s.c.) increased the latency to 98.6% of the maximal possible effect (30s on the plate). EECL(100 mg/Kg) also did not modify the locomotor performance of the animals when submitted to the rotarod task while positive control diazepam (5 mg/kg, s.c.) significantly reduced 98% the time that the animal spent in the rotarod.

Conclusions:

These results suggest that the EECL posses anti-inflammatory and analgesic activities which may be related to its popular use. The analgesic activity may be due to a central effect, but further studies are necessary to clarify this point.

Keywords: oedema, pain, medicinal plant

Financial Support: CNPq and Fundação Araucária

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Resumo:26-240

CYTOTOXICITY OF THE METANOL EXTRACT OF BIXA ORELLANA L. SEEDS IN NORMAL AND NEOPLASTIC GLIAL CELLS

Freitas, V. S. 1; Oliveira, D. M. D. 1; Lima, R. M. F. 1; Sampaio, G. P. 1; Costa, S. L. 1; Costa, M. D. F. D. 1; Velozo, E. D. S. 2; El-bachá, R. D. S. 1

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2 School of Pharmacy, UFBA

Objectives:

Aim: The general objectives of this study were to test the hypothesis that the methanol extract of Bixa orellana L. decreases the viability of human glioblastoma GL-15 cells and murine glioma C6 cells, without being toxic to normal astrocytes in vitro, and to investigate its cytotoxic mechanisms.

Methods and Results:

Methods and Results: After 48 hours of confluence, the cells were treated for the same period with concentrations of the methanol extract of Bixa orellana L. seeds between 60 ands 240 μg/mL. The cytotoxicity was measured by the MTT test. The extract killed cells in a dose-dependent manner. Medians for the minimum cytotoxic concentration (mcc) after 48 hours in GL-15 and C6 cells were 180 μg/mL and 80 μg/mL, respectively. After 48 hours, the median mcc in astrocytes was 240 μg/mL. The morphological analysis showed empty spaces between the neoplastic cells, which increased with concentration. In astrocytes, we observed elongation of the cell body and some cells became round after treatment with 240 μg/mL for 48 hours. Monochlorobimane (MCB) was used to evaluate reduced glutathione (GSH) depletion by fluorescence microscopy and it did not deplete GSH in all cell types after 24 hours of treatment. The type of cell death was investigated by flow cytometry using Anexin V/Propidium iodide (PI). The ability to interfere in cell cycle was evaluated using PI. There were no significant changes in cell

Financial Support: CNPq and Fundação Araucária
cycle relative to control cells, indicating that the reduction of viability is due to a cytotoxic effect rather than a decrease in proliferation. Flow cytometry confirmed the cell death by necrosis in GL-15 cells (57.45%) and late apoptosis in C6 cells (77.10%). Astrocytes showed small values for late apoptosis (29.96%) and necrosis (1.36%) compared to untreated control cells.

Conclusions:

Conclusion: In conclusion, these data suggest the potential cytotoxic effect of the methanol extract of *Bixa orellana* L. seeds and its ability to decrease cell viability in neoplastic cells.

Keywords: Glioblastoma, Cytotoxicity, *Bixa orellana* L., Annato

Financial Support: Supported by CNPq and FAPESB.

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Resumo:26-241

HYPOGLYCEMIC ACTIVITY OF METHANOLIC EXTRACT FROM FLOWERS OF PIPER CLAUSSENIANUM (MIQ.) IN STREPTOZOTOCIN-INDUCED DIABETES

Cavalcante, C. V. 1; Sudo, S. Z. 1; Pereira, S. L. 1; Marques, A. M. 2; Kaplan, M. A. C. 2; Sudo, R. T. 1; Zapata-sudo, G. 1

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2 Núcleo de Pesquisas de Produtos Naturais, UFRJ

Objectives:

Plants of the family Piperaceae can biosynthesize chalcones that have been responsible for different biological activities including antihyperglycemic activity. The purpose of this work was to investigate the pharmacological properties of methanolic extract from inflorescences of *Piper clausenianum* which is rich in natural chalcones.

Methods and Results:

The protocols were approved by Animal Care and Use Committee at Universidade Federal do Rio de Janeiro. Fourteen male Wistar rats (180-220 g) received a single streptozocin (STZ) intraperitoneal injection (45 mg/kg) for induction of type 1 diabetes. STZ-treated rats were randomly divided in two groups each one treated with either vehicle (DMSO) or the extract (75 mg/kg, i.p.). Seven days after the STZ-induced diabetes, rats with glucose levels above 200 mg/dL (Accu-Chek Performa) were treated with vehicle or extract for 14 days. During treatment period, glucose level was measured before and after 1, 3, 5 and 14 days of extract treatment. Glucose levels of both groups were also measured 7 days after interruption of treatment. Glucose levels in the STZ-treated rats in which vehicle was administered i.p. were 346.1 ± 41.6; 397.2 ± 60.0; 331.2 ± 52.0; 290.1 ± 32.6; 370.7 ± 77.8 mg/dL before and after 1, 3, 5 and 14 days of treatment, respectively indicating that STZ induced diabetes in all animals. In contrast, glycemia was significantly reduced to 165.4 ± 32.4; 112.1 ± 7.2; 122.7 ± 7.1; 137.5 ± 17.7 mg/dL (p< 0.05) in the group treated with vehicle and extract, respectively. These results indicate that the extract but not vehicle reduced the glucose level to control value 2 h after the glucose load.

Conclusions:

Methanolic extract of *Piper clausenianum* reduced blood glucose levels in rats with type 1 diabetes as well as prevented glucose intolerance in these animals.

Keywords: Diabetes , Hypoglycemic effect , *Piper clausenianum*
HYPOGLYCEMIC ACTIVITY OF HEXANIC AND METHANOLIC EXTRACTS FROM LEAVES OF EUGENIA ROTUNDIFOLIA IN STREPTOZOTOCIN-INDUCED DIABETES

Peixoto, M. V. C.; Sun, L. M. C.; Silva, J. S.; Castro-santos, J.; Kaplan, M. A. C.; Sudo, R. T.; Zapata-sudo, G.

1 Programa de Desenvolvimento de Fármaco, UFRJ
2 Núcleo de Pesquisas de Produtos Naturais, UFRJ

Objective:
Diabetes Mellitus (DM) is a common metabolic dysfunction that is characterized by an increase of the blood glucose levels and can cause severe vascular, cardiac and renal complications. The present work investigated the hypoglycemic activity of the methanolic and hexanic extracts from leaves of Eugenia rotundifolia, a plant which is popularly known to decrease the blood glucose levels.

Methods and Results:
The protocols were approved by Animal Care and Use Committee at Universidade Federal do Rio de Janeiro. Type 1 DM was induced in male Wistar rats (180 - 220 g) by intraperitoneal administration of streptozotocin (STZ, 45 mg/kg). One week after STZ injection, animals with blood glucose level higher than 200 mg/dL, measured by Accu-Chek Performa, were randomly divided in three groups: 1. intraperitoneal injection of vehicle (dimethyl sulphoxide); 2. intraperitoneal injection of methanolic extract (50 mg/kg); 3. intraperitoneal injection of hexanic extract (50 mg/kg) of Eugenia rotundifolia. Glucose levels were measured before and 1, 3, 5 and 7 days after treatment either with DMSO or extracts. At the end of treatment, rats were submitted to the oral glucose tolerance test. Rats were placed in fasting (12-18 h) and control glucose levels were obtained. Then, they received an oral administration of glucose (2 g/kg) and glucose level was measured after 15 min and 2 h. The blood glucose levels of the STZ-induced group treated with DMSO were 477.2 ± 54.9 mg/dL and 505.0 ± 95.0 mg/dL before and 7 days after injection, respectively. The animals treated with methanolic extract showed blood glucose levels of 135.7 ± 7.6 mg/dL and 131.2 ± 4.6 mg/dL after 1 and 7 days of treatment, respectively. These results indicate that methanolic extract significantly (P

Conclusions:
Both methanolic and hexanic extracts from Eugenia rotundifolia reduced the blood glucose levels in STZ-induced DM in rats. The administration of the hexanic extract for two weeks improved oral glucose tolerance in rats with type 1 diabetes.

Keywords: Diabetes, Eugenia rotundifolia, Hypoglycemic Activity, Streptozotocin-Induced

Financial Support: FAPERJ; CNPQ; PRONEX; INCT; PENSARIO; CAPES

EVALUATION OF THE POTENTIAL INSECTICIDE OF THE ANACARDIC ACID IN THE DIGESTIVE SYSTEM OF LARVAE OF Aedes aegypti (Diptera: Culicidae)

Financial Support: CNPq, FAPERJ, INCT, PRONEX, CAPES, PENSA RIO

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Resumo:26-242

HYPOGLYCEMIC ACTIVITY OF HEXANIC AND METHANOLIC EXTRACTS FROM LEAVES OF EUGENIA ROTUNDIFOLIA IN STREPTOZOTOCIN-INDUCED DIABETES

Peixoto, M. V. C.; Sun, L. M. C.; Silva, J. S.; Castro-santos, J.; Kaplan, M. A. C.; Sudo, R. T.; Zapata-sudo, G.

1 Programa de Desenvolvimento de Fármaco, UFRJ
2 Núcleo de Pesquisas de Produtos Naturais, UFRJ

Objective:
Diabetes Mellitus (DM) is a common metabolic dysfunction that is characterized by an increase of the blood glucose levels and can cause severe vascular, cardiac and renal complications. The present work investigated the hypoglycemic activity of the methanolic and hexanic extracts from leaves of Eugenia rotundifolia, a plant which is popularly known to decrease the blood glucose levels.

Methods and Results:
The protocols were approved by Animal Care and Use Committee at Universidade Federal do Rio de Janeiro. Type 1 DM was induced in male Wistar rats (180 - 220 g) by intraperitoneal administration of streptozotocin (STZ, 45 mg/kg). One week after STZ injection, animals with blood glucose level higher than 200 mg/dL, measured by Accu-Chek Performa, were randomly divided in three groups: 1. intraperitoneal injection of vehicle (dimethyl sulphoxide); 2. intraperitoneal injection of methanolic extract (50 mg/kg); 3. intraperitoneal injection of hexanic extract (50 mg/kg) of Eugenia rotundifolia. Glucose levels were measured before and 1, 3, 5 and 7 days after treatment either with DMSO or extracts. At the end of treatment, rats were submitted to the oral glucose tolerance test. Rats were placed in fasting (12-18 h) and control glucose levels were obtained. Then, they received an oral administration of glucose (2 g/kg) and glucose level was measured after 15 min and 2 h. The blood glucose levels of the STZ-induced group treated with DMSO were 477.2 ± 54.9 mg/dL and 505.0 ± 95.0 mg/dL before and 7 days after injection, respectively. The animals treated with methanolic extract showed blood glucose levels of 135.7 ± 7.6 mg/dL and 131.2 ± 4.6 mg/dL after 1 and 7 days of treatment, respectively. These results indicate that methanolic extract significantly (P

Conclusions:
Both methanolic and hexanic extracts from Eugenia rotundifolia reduced the blood glucose levels in STZ-induced DM in rats. The administration of the hexanic extract for two weeks improved oral glucose tolerance in rats with type 1 diabetes.

Keywords: Diabetes, Eugenia rotundifolia, Hypoglycemic Activity, Streptozotocin-Induced

Financial Support: FAPERJ; CNPQ; PRONEX; INCT; PENSARIO; CAPES

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Resumo:26-243

EVALUATION OF THE POTENTIAL INSECTICIDE OF THE ANACARDIC ACID IN THE DIGESTIVE SYSTEM OF LARVAE OF Aedes aegypti (Diptera: Culicidae)
Objectives:

This study analyzed the changes in the digestive system of larvae of *Aedes aegypti* caused by Anacardic acid (0.01 and 0.001%) by histopathological techniques.

Methods and Results:

Anacardic acid used in the experiments was obtained from the Kardol Ltda. Toxicity tests were performed for the bioassays, and so define the correct mortality concentrations of *A. aegypti* in 10%, 50% and 90% of the population (LD10, LD50 and LD90). At all stages, we used larvae of the end of the third instar in a total of 25 individuals per 25 mL solution in 200 mL glass container. The concentrations of the acid were diluted in DMSO to 1% (v/v), for a period of 24 hours, and four replicates made for each concentration. In parallel, the negative control was carried using only water and DMSO to 1%, the tests were conducted at the Entomology Laboratory of the hematophagous of Universidade Católica Dom Bosco (UCDB) in Campo Grande, MS. The histomorphology analyses were performed in the midgut of the larvae digestive system of *A. aegypti*. The histological processing was conducted in the Toxinology and Medicinal Plants Laboratory of Universidade Anhanguera-Uniderp. The results showed the anacardic acid as a substance with potential insecticidal presenting enough mortality profile to define the lethal dose, DL10 (0.001% v/v, confidence interval (CI) of 95% - 0.0006-0.009), LD50 (0.007% v/v, CI of 95% - 0.0003-0.015) and LD90 (0.013% v/v, CI of 95% - 0.005-0.02) were defined by Probit method as effective dosages. In the group treated with the concentration 0.01% and 0.001% morphological changes in the digestive system (estomodeu and midgut (anterior, middle and posterior) of the larvae in a longitudinal section were observed. Estomodeu region of cells in necrosis and loss portion of in the apical cytoplasm of the cell. In the gastric cecus integument, the necrotic cell and there was secretion granulous in the lumen. In the lumen of the digestive system was found cellular remains, showing the total destruction of cellular tissue. The peritrophic membrane was enlarged. Further tests will be conducted to assess the action of the substance at lower dosages at LD10 and then define the lowest concentration able to cause changes in the midgut.

Conclusions:

The anacardic acid caused effect on mortality of *Aedes aegypti* larvae (third instar) at all doses tested (0.001, 0.007 and 0.013% v/v). The histological analysis confirmed the evaluated substance larvicidal capacity.

Keywords: Anacardiaceae, biodegradable, bioinsecticide, dengue

Financial Support: MCT; CNPq; INAU; CPP; FUNDECT and the Universidade Anhanguera-Uniderp
Objectives:
Evaluated possible alterations on liver morphology of the fruit bat Artibeus lituratus submitted to cronic treatment with the insecticide endosulfan in two different concentrations.

Methods and Results:
28 male bats *Artibeus lituratus* were captured using mist nets in Forest fragments around Viçosa-MG, Brasil, between August 2010 and January 2011. Bats were kept in a 3x3x2 m enclosure at Zoology Museum of UFV. All animals were offered papaya because it has been well accepted for bats. All groups were treated for 35 days. Treated groups were offered fruit immersed on a endosulfan solution at 1.05 g/L (E1, n=7) or 2.1 g/L (E2, n=7) added to a 0.015g/L adhesive spreader solution and treated only with fruit immersed on 0.015g/L adhesive spreader solution (AS, n=7). Control group (CG, n=7) was fed non-exposed fruits. The concentrations used were those prescribed by the manufacturer for application in fruit cultures. The fruits were divided in half (~200 g) and offered to the animals with the skin side up so that during ingestion the bats would be in direct contact with endosulfan. The fruit was offered every night around 18hr, and water was available ad libitum. After treatment, animals were killed by decapitation and liver was rapidly removed, fixed on a Karnovsky solution and included in Historesina®. Cuts (3µm) were obtained and stained with toluidina-borax 1% and Periodic Acid-Schiff (PAS) and analyzed under light microscopy. Our results indicate no difference between groups in liver weight. The liver showed a tissue architecture similar to that described for mammals. We observed the presence of lymphocytes within the blood capillaries in all animals. Animals in the AS group showed hepatocytes containing abundant cytoplasmic circular vesicles of different sizes, suggesting accumulation of lipids. In this same group, the capillaries dilated sinusoids were shown compared to the CG. Cytoplasmic vesicles were also found in bats liver from E1 animals, but in smaller quantities. The PAS histochemistry showed the presence of abundant glycogen in the E1 treatment compared to CG. E2 animals showed accumulation of lipids in cytoplasm, but in smaller quantities compared to E1 treatment, and greater accumulation of glycogen (PAS +) when compared to other groups.

Conclusions:
Fruit-eating bats are important seed dispersers, being crucial to forests regeneration. However, individuals from this species are cronicly exposed to environmental concentrations of pesticide. Our work shows that organochlorine insecticides such as endosulfan may cause some histological damage to the liver, pointing one among several disorders that might be involved with wildlife cronic exposure to pesticides.

Keywords: Artibeus lituratus, endosulfan, hepatocytes, liver histopathological

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**Resumo:** 26-245

**ACTIVITY OF FUNGI EXTRACT BY FUNGI FROM AMAZON SOIL AGAINST KLEBSIELLA PNEUMONIAE**

Silva, P. V. 1,2; Silva, I. R. 1,2; Mendes, G. 3; Silva, J. C. 4; Carneiro, A. L. B. 5; Magalhães, J. T. 2; Fernandes, O. C. C. 4; Ferreira, J. M. S. 2; da Silva, S. L. 3; Soares, A. C. 1

1 Laboratory of Microbiology, LabMicro - UFSJ
2 Laboratory of Pharmacology, LabFarmaco - UFSJ
3 Laboratory of Protein Chemistry and Nanobiotechnology, LQPN - UFSJ
4 Research Center Leonidas and Maria Deane, CPqLMD-FIOCRUZ
5 Institute of Biological Sciences, ICB-UFAM
Objectives:

Many fungal produced antimicrobial substances were isolated and made into drugs, bringing about significant advances to both human and animal health. Considering the enormous potential of fungi in biotechnological and biochemical processes, as well as the increase of microbial resistance to the current antibiotics, isolation and production of new molecules and new classes of antibacterial substances has been sought. *Klebsiella pneumoniae* is emerging as an important nosocomial pathogen due to rapidly increasing resistance to all currently available antibiotics, in particular carbapenems. It is an opportunistic Gram-negative pathogen that may cause vary infections such as pneumonia, bacteremia and post-operative meningitis. Despite an increasing frequency and severity of antimicrobial resistance, the future development of new anti-infective agents is threatened by the cessation of research in this field by many major pharmaceutical companies. The aim of this study was to evaluate the in vitro antibacterial activity of eight fungi species of *Penicillium* and *Aspergillus* (CFAM – Coleção de Fungos da Amazônia de Centro de Pesquisas Lônidas e Maria Deane – FIOCRUZ), isolates from the Amazon region, to *Klebsiella pneumoniae* ATCC27736.

Methods and Results:

The eight fungi species (CFAM 01, CFAM 02, CFAM 03, CFAM 04, CFAM 05, CFAM 06, CFAM 07 and CFAM 08) were inoculated into three different medias, Malt Extract, Sabouraud and Czapeck, for seven days at 28 ºC and 150 rpm. A total of 24 different extracts were tested for the antimicrobial activity. It was used the disk diffusion method, in which the extracts (100 mg/mL) were dissolved in DMSO 20% and applied to Mac Conkey agar previously inoculated with *Klebsiella pneumoniae* (1,5 x 108 UFC/mL in 100µL). Streptomycin (1mg/mL) was used as a positive control and DMSO 20% as a negative control. The plates were incubated at 37°C and after 24h the zones of inhibition were evaluated. By the 24 samples tested only the extract from CFAM 02 fungi specie, growth in the Sabouraud media, showed inhibitory activity against *K. pneumoniae* with a zone of inhibition of the 1,2 cm. The positive control showed a zone of inhibition of the 1,8 cm and the DMSO 20% didn’t inhibit the bacterial growing. The CFAM species is in the process of morphological and molecular characterization.

Conclusions:

This study showed the potential of the CFAM 02 fungi specie, growth in the Sabouraud media, to produce antimicrobial substances. Besides, this active compound or compounds, produced by CFAM 02 fungi specie, are in the process of isolation and characterization, as well the in vivo antibacterial activity of this CFAM 02 extract will now be investigated in a model of pneumonia induced by *Klebsiella pneumoniae* in mice, in our laboratory.

Keywords: antimicrobial activity, fungi extracts, Klebsiella pneumoniae, pneumonia

Financial Support: Universidade Federal de São João del Rei

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**FLAVONOIDS AND SAPONINS ENRICHED FRACTIONS FROM PASSIFLORA ALATA PRESENT ANXIOLYTIC EFFECTS THAT SEEM TO BE INDEPENDENT ON A DIRECT BENZODIAZEPINE AND GABAA SITES ACTIVATION**

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Objectives:

Previous studies have been demonstrated anxiolytic and sedative properties of Passiflora alata crude extracts (Phytother. Res. 15:162, 2001; Lat. Am. J. Pharm. 27:845, 2008). In this study we evaluated the Central Nervous System activity of saponin (SAP) and flavonoid enriched fractions (FLA) obtained from P. alata hydroethanolic extract leaves.

Methods and Results:

P. alata air dried powdered leaves were extracted by reflux for 1h with ethanol 70% (plant:solvent, 1:10, w/v). The solvent was removed under vacuum at 40°C. This extract was submitted to Sephadex® LH 20 using H2O:EtOH gradient as eluent, which has furnished SAP and FLA. For behavioral tests male CF1 mice (20-35g) (n=8-10) were used. All experimental protocols were approved by CONEP-Brazil (National Commission of Research Ethics) (2005512/01-2006). The animals were divided in distinct groups and treated by gavage as follow: saline (SAL - 10 mL/kg), diazepam (2 mg/kg), SAP (900 mg/kg), FLA (300 mg/kg). Motor coordination and spontaneous locomotor activity were assessed by using rota-rod and open field. The anxiolytic effect was investigated in the elevated plus maze. In order to evaluate the involvement of benzodiazepine site, different groups of mice were treated with flumazenil (FMZ 10mg/kg i.p.) or saline (SAL) 30 min after SAP or FLA treatments. The hypnotic/sedative effects were evaluated by the potentiation of pentobarbital (40 mg/kg i.p.) induced-hypnoses. SAP and FLA were also tested on the pentilenotetrazol (PTZ - 80 mg/kg i.p.) induced-convulsions. One-way analysis of variance (ANOVA), followed by Student-Newman-Keuls, and Kruskal-Wallis test were performed depending on distribution data. Data from rota-rod test were analyzed by two-way ANOVA with repeated measures. Our results demonstrated that SAL and FLA did not cause locomotor and motor impairments, neither anticonvulsivant effects. SAL and FLA increased the mice time spent in the plus maze open arms (SAP+SAL 66.0; 25% 51.5; 75% 94.0 and (FLA+SAL 74.5; 25% 58.0; 75% 93.5) when compared to SAL+SAL (30.5; 25% 25.5; 75% 40.5); only SAP exhibited an increase in the number of entries in the open arms (SAP+SAL 6.0; 25% 5.0; 75% 7.25) when compared to SAL+SAL (4.0; 25% 3.0; 75% 6.0). Total arms entries were not altered. The administration of FMZ did not alter the effects of SAL and FLA on plus-maze. In addition to anxiolytic effect FLA also increased barbiturate sleeping time (86.0; 25% 32.0; 75% 121.0) when compared to SAL (25.0; 25% 17.0; 75% 44.0).

Conclusions:

Flavonoid and saponin enriched fractions from P. alata present anxiolytic effect characterized by an increase in the exploration of the plus-maze open arms, which was not inhibited by flumazenil indicating that this effect is not mediated by the benzodiazepine site. In addition, both fractions seem to be not active on GABAA site since they did not alter PTZ induced convulsions. FLA increased the barbiturate sleeping time, suggesting a hypnotic-sedative effect.

Keywords: Passiflora alata, anxiety, GABAA

Financial Support: CAPES/ PPGCF UFRGS.
Objectives:

Aim: The chemical composition of linseed oil of Linum usitatissimum L. (Linaceae) presents polyunsaturated fatty acids, linolenic (56.6%) and linoleic acid (13.2%), and the monounsaturated fatty acid oleic (17.8%) which are important for the maintenance of normal dermal structure (PLEFA. 74:17-21, 2006; Wound Rep. Reg. 18:629–636, 2010). Several studies have shown a role for ω-9, ω-6 and ω-3 in the process of tissue repair, to participate on inflammatory processes and increasing tissue granulation (Annu. Rev. Cell. Dev. Biol. 21:633–657, 2005). Therefore, contributing to a beneficial effect on wound healing. Our aim was evaluate the wound healing activity of a semi-solid formulation of Linseed oil (SSFLO) in albino rabbits by histological and morphometric analysis in skin wounds.

Methods and Results:

Methods: Two surgically standardized circular wounds (±78.5mm2) were made on the dorsum of albino rabbits (male and female; 500-900g). The animals were divided into five groups (n=4) and treated for 14 days with SSFLO (1%, 5% or 10%), petrolatum jelly (negative control) or commercial emulsion of sunflower oil - ESO (positive control). At the end of the experiment (14th day of treatment), the animals were euthanized in a CO2 chamber and the skin lesions collected for the histological and morphometric analysis of the material. The slides were examined under light microscope, five images per field (0.0018mm2 area) were captured with a digital camera (total magnification 400×) attached to the microscope. The images were stored and subjected to counting of inflammatory cells, fibroblast cells, number of blood vessels and evaluation of collagen density of all lesions with the aid of digital marking. The variables were expressed as mean ± standard error of the mean and subjected to one-way ANOVA followed by the Bonferroni’s multiple test comparing the SSFLO treatment groups (1%, 5% or 10%) to the petroleum jelly and ESO controls, considering significant values (p

Conclusions:

Conclusion: The results clearly demonstrated that topical administration of SSFLO 5% increased the maturity of the matrix tissue during the healing process, and may represent a novel therapeutic approach to skin wounds.

Keywords: Linum usitatissimum, skin wounds, histological, morphometric, albino rabbits

Financial Support: FACEPE, CAPES and UFPE

LOCAL ANESTHETIC ACTIVITY OF THE HEXANE (OAR-PH) AND METHANOL (OAR-MEOH) EXTRACTS AND MIXTURE OF AMIDES AMDI AND MIXAMD FROM ROOTS OF OTTONIA SP

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Objectives:
The family Piperaceae occurs in Brazil and some *Ottonia* species from this family have been used in folk medicine to treat many diseases for a long time, being a source for phytochemical and pharmacological research. The 23 species for the genus *Ottonia*, have described and they are mainly found in South and Southeast regions of Brazil. These plants, known as "anesthesia" and "false jaborandi" are popularly used as anesthetic, diuretic and sialogogue. The *Ottonia sp* is a promising source of secondary metabolites such as amides which are of great therapeutic interest. The purpose of this study was to evaluate the local anesthetic activity of the hexanic (OAR-PH) and methanolic (OAR-MeOH) extracts, and mixture of amides (AMD1 and MIXAMD) of *Ottonia sp* roots.

Methods and Results:

Sensory block was evaluated by intradermal administration of increasing concentration of OAR-MeOH (0.006 - 3 %) (n = 5), OAR-PH (0.006 - 0.994 %) (n = 5), AMD1 (0.006 - 0.187 %) (n = 5) and MIXAMD (0.006 - 0.094 %) (n = 4) in a volume of 0.1 ml in male guinea pigs (350 - 450 g). Lidocaine was used as a positive control of local anesthetic. Motor block was evaluated by perineural administration of increasing concentrations of OAR-MeOH (0.5 – 2 %) (n = 4) and OAR-PH (0.5 – 2 %) (n = 4) in a volume of 0.05 ml in the hindlimb of mice (25 - 30 g). Blockade of electrical conduction in motor fibers was quantified by decrease or loss of normal posture of the mice. The duration of sensory block increased in a concentration-dependent manner to lidocaine, OAR-MeOH, AOR-PH, AMD1 and MIXAMD. At the lowest concentration (0.006 %), lidocaine, OAR-MeOH, OAR-PH, AMD1 and MIXAMD caused sensory block of 15.2 ± 1.2, 10.8 ± 0.5, 14.5 ± 1.9, 9.3 ± 0.8, and 10.3 ± 0.9 min (P < 0.05, extracts, AMD1 and MIXAMD vs lidocaine), respectively. At 3 %, lidocaine and OAR-MeOH caused sensory block of 119.6 ± 3.2 and 67.8 ± 2.0 min, respectively (P < 0.01, lidocaine vs OAR-MeOH). Motor nerve fibers blockage was observed with lidocaine but not with OAR-MeOH.

Conclusions:

Sensory nerve fibers block activity was demonstrated with OAR-PH and OAR-MeOH extracts and mixtures of amides, AMD1 and MIXAMD from roots of *Ottonia sp*. However, motor nerve fibers blockage was not observed in this study.

Keywords: activity, amides, extracts , local anesthetic, ottonia

Financial Support: CAPES, CNPq, FAPERJ, INCT/INOFAR

QuebraPagina

Resumo:26-249

ALKALOIDS FROM CHONDRODENDRON SP INHIBIT EOSINOPHIL RECRUITMENT AND ACTIVATION IN ALLERGIC MODEL OF ASTHMA

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Objectives:

Since activated eosinophils play critical roles in the pathogenesis of allergic diseases, including asthma, the present work aimed to investigate the effect of the oral treatment (p.o.) with a fraction of alkaloids (IPB1) and the alkaloid IPB2 isolated from *Chondrodendron sp* (Menispermaceae) on leukocyte recruitment to the airways, eosinophil activation (lipid body formation) and cytokine production using an experimental model of allergic asthma.

Methods and Results:
Swiss male mice (CEUA 002/08-Fiocruz-RJ; n=6) were sensitized (i.p.) with ovalbumin (OVA) on days 1 and 10. From days 19 to 24, mice were challenged daily for 20 min with aerosol of OVA (5%) in PBS. Animals were treated (p.o.) with IPB1 (2.5 mg/kg) or IPB2 (2.5 mg/kg) 1h before each challenge. Dexamethasone (DEX 2 mg/kg, p.o.) was used as therapeutic control, 1h before each challenge. Twenty-four hours after the last challenge, animals were euthanized by exposure to CO2 atmosphere, the bronchoalveolar lavages (BAL) and lungs were collected for cell counts and cytokine quantification by ELISA. Total leukocyte counts were performed using a Neubauer chamber and differential counts were performed using cytofins stained by the May-Grunwald-Giemsa method. Cytospins stained with Osmium (OsO4) were used to enumerate eosinophil lipid bodies. The data were expressed as mean ± SEM. Differences were analyzed using the one-way ANOVA test and Tukey’s post-test. P6 ± 0.16 cells/BAL) and eosinophils (0.66 X 10⁶ ± 0.12) compared to the sham-challenged animals (0.17 X 10⁶ ± 0.02 and 0.01 X 10⁶ ± 0.03, respectively). The treatment with IPB1, IPB2 or DEX decreased significantly the number of total leukocytes (0.53 X 10⁶ ± 0.08; 0.41X 10⁶ ± 0.09; and 0.18 X 10⁶ ± 0.02, respectively) and eosinophils (0.25 X 10⁶± 0.06; 0.15 X 10⁶ ± 0.04 and 0.1 X 10⁶ ± 0.03, respectively) compared to the non-treated, OVA-challenged group. Also the treatments reduced significantly the number of lipid bodies/eosinophil (5.63 ± 0.32; 5.76 ± 0.17 and 7.20 ± 0.42, respectively compared to the non-treated, OVA-challenged group (12.10 ± 0.38), indicating that the alkaloids tested can inhibit eosinophil recruitment and activation. The animals treated with IPB1, IPB2 or DEX presented significantly reduced levels of IL-13 (85.94 ± 17.73; 130.90 ± 12.10 and 140.80 ± 10.13 pg/mg of protein, respectively) and eotaxin (0.13 ± 0.03; 0.17 ± 0.01 and 0.14 ± 0.01 pg/mg of protein, respectively) compared to the non-treated, OVA-challenged group (218.50 ± 8.41 and 0.28 ± 0.03 pg/mg of protein, respectively), demonstrating modulation of Th2 immune responses and eotaxin production.

Conclusions:

In conclusion, this study demonstrated that the alkaloids from Chondrodendron sp are molecules with anti-allergic properties and IPB2 is an active constituent with promising therapeutic potential.

Keywords: Alkaloids, Asthma, Chondrodendron sp, Eosinophil, Lipid Bodies

Financial Support: CNPq, CAPES, FAPERJ, PRONEX

QuebraPagina

Resumo:26-250

A NEW PLATELET AGGREGATION INHIBITOR FROM HAEMENTERIA DEPRESSA LEECH: CLONING, EXPRESSION, PURIFICATION AND CHARACTERIZATION

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Objectives:

The H.depressa leech salivary complex has been studied through biochemistry, transcriptomic and proteomic analysis. In this tissue were detected previously some clones similar to an inhibitor of platelet aggregation from H.officinalis leech named LAPP. The aims of this work are to clone, in an expression vector (pPIC9K) the transcript named H06A09 which presents 45% similarity with LAPP; to express in Pichia pastoris system; to purify the recombinant protein and start the characterization of the molecule activity.

Methods and Results:

The H06A09_pGEM11Zf from the H.depressa salivary complex cDNA library was elected for this study and after amplification by PCR with specific primers, the product was cloned in pPIC9K vector between EcoRI and NotI cloning sites. The H06A09_pPIC9K clone had its confirmed sequence and it was linearized using SacI digestion. The clone was transformed in Pichia pastoris (GS115) by electroporation and expressed in different conditions. The best expression method was standardized in BMGY culture medium at 28°C, 260 rpm using 96h of induction by 0.5% methanol feeding/24h. The recombinant protein
expressed was submitted to some different purification methods [ultra filtration (Amicon / Millipore 5 and 30kDa); gel filtration (Superdex 75 / GE); anionic exchange (Mono-Q / GE) and reverse phase (C18), affinity (Heparina-Sefarose)]. The different expression and purification steps were analyzed by SDS-PAGE. The best method of purification was to submit the culture supernatant initially to dialysis and concentration by ultra filtration (Amicon 5kDa) followed by the gel filtration in Superdex 75, and then the reverse phase (C18-column - HPLC system); the last chromatography method was able to purify the protein however the protein activity was lost. The N-terminal sequence of the purified protein was confirmed by Edman degradation. After the gel filtration the molecule was able to test on platelets activity. The inhibition platelet-aggregation assays were performed in PRP and whole blood, using initially collagen as agonist. The tests shown that the recombinant protein was able to inhibit the platelet aggregation induced by collagen both platelet aggregation in whole blood and in PRP. The same dose-dependently inhibited platelet aggregation by collagen in PRP and Washed Platelet with an IC(50) of 20ng and 712ng, respectively.

Conclusions:

The transcript H06A09 was successfully cloned and expressed in Pichia pastoris system and its N-terminal portion was confirmed by Edman sequencing method. The recombinant partially purified protein shown that it is a new potent molecule to antiplatelet studies.

Keywords: PLATELET AGGREGATION INHIBITOR, CLONING, EXPRESSION, PURIFICATION, LEECH

Financial Support: FAPESP/INCTTOX

QuebraPagina

Resumo: 26-251

EVALUATING PROTEOLYTIC ACTIVITY AND MORPHOLOGICAL ALTERATIONS INDUCED BY METALLOPROTEINASE BMOMMPα-II.

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Objectives:

The present study aimed to evaluate the proteolytic activity and morphological alterations induced by purified metalloproteinase from Bothrops moojeni venom.

Methods and Results:

The purification of enzyme, named BmooMPα-II, was carried out through two chromatographic steps (ion-exchange on DEAE-Sepharose and molecular exclusion on Sephacryl S300). BmooMPα-II is a monomeric protein with an apparent molecular mass of 27kDa by SDS-PAGE 14%. Fibrinogenolytic activity was evaluated by SDS-PAGE 14%. Fibrinogen bovine(3mg/mL) and BmooMPα-II(20µg) were mixed and incubated at 37°C for different time intervals (0-120min). Inhibition of fibrinogenolytic activity was determined by incubating BmooMPα-II for 15 min at 37°C with different inhibitors. Fibrinogenolytic activity was evaluated at different pHs (4-10) and temperatures (30-90°C). First, BmooMPα-II cleaves the Aα-chain of fibrinogen followed by the Bβ-chain, and shows no effects on the gamma-chain. Fibrinogenolytic activity is inhibited by β-mercaptoethanol, EDTA and 1,10-phenantroline. In contrast, leupeptin, aprotinin and benzamidine did not affect the activity of BmooMPα-II. Results showed that intervals of optimum temperature and pH for the fibrinogenolytic activity were 30-50°C and pH=8, respectively. The characterization of systemic morphological alterations induced by BmooMPα-II was analyzed in groups of four Swiss male mice (18-20g) which received i.p. injection of BmooMPα-II (50µg/100µL sterile saline solution). For histological examination of
myonecrosis, groups of mice received i.m. injection of BmooMPα-II (50µg/50µL sterile saline solution). Control animals received i.p. and i.m. injections of 100µL and 50µL saline, respectively. After 24h, mice were killed by overdose of ketamine/xylazine, and the heart, lung, liver, kidney and gastrocnemius muscle were dissected out. The tissues were then fixed in 10% formalin, dehydrated and included in paraffin. The resulting blocks were sliced in 5µm thick sections and stained with hematoxilin-eosin to be examined under a light microscope. BmooMPα-II caused morphological alterations in liver, lung, kidney and muscle of mice.

Conclusions:

We have purified a metalloproteinase from B. moojeni venom, BmooMPα-II, with fibrinogenolytic activities. Results indicate that this protein probably is a α-fibrinogease and belongs to class PI of SVMPs. Addition, BmooMPα-II induce relevant morphological alterations in vivo.

Keywords: Bothrops moojeni, metalloproteinase, histopathological

Financial Support: FAPEMIG, CNPq and MCT.

QuebraPagina

Resumo:26-252

REPRODUCTIVE PERFORMANCE OF FEMALE WISTAR RATS EXPOSED TO AQUEOUS EXTRAT OF HIMATANTHUS LANCIFOLIUS (MUELL.-ARG.)

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3 Departamento de Biologia Animal/Universidade Federal Viçosa, UFV

Objectives:

To evaluate the reproductive performance of Wistar’s female rats exposed to different concentrations of aqueous extract of Himatanthus lancifolius, popularly known as agonizing.

Methods and Results:

Specimens of Himatanthus lancifolius were collected at the Biodiversity Centre, Unileste-MG. The bark was removed and dried in an oven vent. After drying, they were pulverized and weighed for the preparation of the extract by infusion. The extract was prepared and delivered daily. Females were subjected to 5 mL of aqueous extract by gavage at different concentrations (0.13 g/mL, 0.09 g/mL, 0.06 g/mL e 0.0 g/mL), total of six replications. The experiment lasted 36 days. On the 15th day, the females were subjected to mating, 2 females per male. On day 20 the male were removed from the coexistence of the females and there was a break from treatment until the 27th day, returning until the 36th day. At the end of the experiment, all the females were weighed and euthanized, and his offspring counted and observed macroscopically. The ratio of offspring per mother was analyzed as mean ± standard error of mean. For the fertility rate, it was calculated the number of pregnant rats per number of mated females. The difference between the treated groups was assessed by analysis of variance (ANOVA) followed by Tukey test, with significance values p

Conclusions:

The cronic administration of high doses of Himatanthus lancifolius (Mueel-arg) Woodson extract interfered on some of the parameters of the reproductive performance of female wistar rats treated, obtaining the lowest fertility rate(33.33%), but did not
impaired the offspring development. Females also did not show any clinical sign of toxicity.

Keywords: Agonizing, Himatanthus lancifolius, fertility

Financial Support: FAPEMIG

THE DEPRESSIVE-LIKE BEHAVIOR INDUCED BY E. COLI LPS IS PREVENT BY ULIGINOSIN B

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Objectives:

In this study we investigated the antidepressant-like effect of uliginosin B (ULI), a dimeric phloroglucinol derivative obtained from Hypericum polyanthemum, in an animal model of depression induced by one session of swim stress followed by administration of lipopolysaccharide (LPS).

Methods and Results:

Male Swiss mice (25-30g) were subjected to forced swimming for 6 min as a pre-stressful stimulus. After 30 minutes, they were injected with lipopolysaccharide (LPS) from Escherichia coli serotype 0111:B4 (600 µg/kg, i.p.) or saline (SAL, negative control). The animals were assessed in behavioral paradigms, open field (OF) and tail suspension tests (TST) and sickness scores (0 = without sickness; 1 = with sickness), 6 and 24h after LPS administration. The treatment with ULI (15 mg/kg, p.o.) was performed in three different periods: T1 - 1h before the swimming session; T2 – 5 h and T3 – 23 h after LPS injection. Controls received saline (p.o). The LPS administration induced sickness scores (LPS: 1) while saline did not induced (SAL: 0). LPS reduced the number of crossings 6h (SAL: 182.53 ± 45.10; LPS: 55.41 ± 59.03) and 24h later (SAL: 166.90 ± 67.05; LPS: 102.66 ± 64.31) and increased the immobility on TST 24h after LPS injection (SAL: 136.66 ± 28.10; LPS: 189.09 ± 38.24), demonstrating its depressive-like effect. None of the treatments with uliginosin B prevented the sickness behavior, i.e. crossings increase or sickness scores. The treatments performed 5 and 23h after LPS injection, T2 and T3 respectively, decreased the immobility on TST when evaluated 24h after the stress plus LPS administration (T2 immobility – LPS+SAL: 189.09 ± 38.24; LPS+ULI: 136.55 ± 34.55; T3 immobility – LPS+SAL: 189.09 ± 38.24; LPS + ULI: 136.80 ± 45.88).

Conclusions:

In the depressive-like behavioral paradigm employed herein, we demonstrated that administration of LPS in mice previously submitted to a 6-min swimming session produced earlier signals of sickness behavior, 6 h post-LPS, which were followed by a later manifestation of depressive-like behavior, i.e. an increase of immobility time in the TST, 24 h post-LPS. This is in line with literature data indicating that sickness behavior represents a normal initial response to infectious stimuli although depressive-like states might persist after the initial sickness alterations had been solved. Besides that, in this work we confirmed the antidepressant-like effect of uliginosin B previously demonstrated in Porsolt’s forced swimming test (PCT/EP2010/051816). Interestingly, the treatment with ULI did not seem to affect the sickness behavior. Moreover, the uliginosin B treatment did not compromise the locomotion, according to results of open field test performed in animals that did not receive LPS administration.

Keywords: uliginosin, depression, LPS
PROTECTIVE EFFECTS OF BACCHARIS TRIMERA AND PTERODON PUBESCENS ETHYL ACETATE FRACTION ON THE IN VITRO CYTOTOXICITY OF DOXORUBICIN IN IMMUNE CELLS (SPLENOCYTES)

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Objectives:

The antitumoral drugs are widely used in cancer treatment by their intense cytotoxic effects on malignant cells. However, they also affect normal cells from different tissues, especially those with high proliferation rate, leading to various adverse effects. The immune cells also undergo cytotoxic effect of these drugs, reducing the body's defense against the tumor itself. The combination of antitumor drugs with herbal extracts aims to increase the treatment efficiency and/or protect the normal cells, allowing higher life quality for patients. Plant species Baccharis trimera (carqueja, Bt) and Pterodon pubescens (Sucupira, Pp) are popularly used to treat inflammatory diseases. The present work aims to study a possible protective action of the ethyl acetate fraction of both species on the cytotoxic effects of the antitumor drug doxorubicin in immune cells (splenocytes).

Methods and Results:

The in vitro antitumor effect of Doxorubina (DOX) was evaluated on the K562 myelogenous leukemia. The cytotoxic effect of DOX on splenocytes was also investigated. The murine splenocytes were isolated aseptically and incubated (1 x 10⁶ cells/ml) in RPMI 1640 with 5% FCS in the presence or absence of concanavalin (ConA, 5μg/ml), a known activator of T lymphocytes. The cells were also treated or not with DOX 0.5 μg/ml (IC50 for K562 cells) and/or with the ethyl acetate fraction of Bt (Bt-EAF) at 10, 50, 100 or 200 μg/ml and/or ethyl acetate fraction of Pp (Pp-EAF) at 5, 10, 50 and 100 μg/ml, for 48 h. Cytotoxicity was determined by the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, which determines the cell mitochondrial reduction activity (MRA), proportional to cell viability. After incubation of cultures with 10ìl MTT solution (5 mg/ml) for 2 h, at the end of culture, the formazan crystals were solubilized with 100 μL of sodium dodecyl sulfate 10% over night. The absorbance was determined at 570 nm in a microplate reader. DOX induced anti-leukemic effects (K562) in a concentration dependent manner, with IC50 of 0.5 μg/ml. The ConA stimulated murine splenocytes, increasing their MRA in 160 ± 18% (p

Conclusions:

This study demonstrated that certain concentrations of EtAc Fr of B. trimera or P. pubescens were able to protect immune cells (splenocytes) from the cytotoxic effects of the traditional chemotherapeutic doxorubicin.

Keywords: Baccharis trimera, células tumorais, efeito protetor, linfocitos , Pterodon Pubescens

Financial Support: CNPq, FAPERJ, UERJ.
Domiciano, T. P.; Ritter, A. M. V.; da Silva, L. G.; Ramos, F.; Estevão - Silva, C. F.; Arruda, L. L. M.; Caparroz-assef, S. M.; Silva, E. L.; Cuman, R. K. N.; Bersani-amado, C. A.

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Departamento de Química, UEM

Objectives:
The anethole (AN) (1-methoxy-4-(1 propenil) benzene) occurs naturally as a major component of essential oil from star anise (Illicium verum), accounting for more than 90% of the volatile components. The AN is widely used as a flavoring in bakery products, sweets and beverages. It also presents a variety of pharmacological activities such as antioxidant, fungicide, bactericide and insecticide. Thus, the purpose of this study was to evaluate the anti-inflammatory activity of AN on experimental acute inflammatory response.

Methods and Results:
The experimental protocol was approved by the Ethics Committee and Animal Experimentation (CEAE/UEM125/2010). It was used the model of ear edema induced by croton oil (CO) in mice and for evaluation of the enzyme's myeloperoxidase activity (MPO) to investigate the edema formation and leukocyte migration to the inflammation site. The edema was induced by applying 20μL of CO (200μg) diluted in vehicle (VE) of acetone/water (7:3) on the inner left ear surface of mice's. The right ear received only the VE (20μL). Immediately after the application of phlogistic agent, in the groups of treated animals were applied to the left ear 20μL of AN at concentrations of 2.5 or 5mg/ear, or indomethacin (ID) (1mg/ear). In the control group was applied 20μL of VE on the left ear. Other groups of animals were treated orally (gavage) with water, AN 250 or 500mg/kg or ID 10mg/kg one hour before the application of CO in the ear. After 6 hours, the animals were sacrificed, ears were sectioned into disks of 6.0mm diameter and weighed (mg) on an analytical balance. The activity of MPO was evaluated in supernatants of homogenates of ears sections. The ear tissue was homogenized, incubated for 1 hour at 60°C and centrifuged for 5 minutes at 2500rpm. At the obtained supernatant was added 200μL of a buffer solution containing O-dianisidine dihydrochloride, water, potassium phosphate buffer and H2O2. The enzyme activity was determined by measuring the absorbance in 460nm. The CO application in the left ear of mice has induced a quite evident inflammatory response in the 6th hour. The treatment with ID, topically (t) or oral (o), has reduced edema formation (IDt=80.3%; IDo=36.5% P<0.05).

Conclusions:
The data showed that in this model, the inhibitory effect of AN on the inflammatory response is dependent on the route of administration. Only the treatment with AN orally reduced the edema and the number of leukocytes migrated to the injury site. Moreover, the topical application of AN did not result in inhibitory effect. This can be explained by an unfavorable pharmacokinetics for topical absorption of the AN used, or alternatively, by the possibility that it's inhibitory effect is caused by metabolic products generated after hepatic biotransformation. This hypothesis is supported by previous studies which demonstrated that the metabolic products of AN had a greater inhibitory effect on vascular permeability when compared to own AN.

Keywords: ANETHOLE, ANTINFLAMMATORY, EAR EDEMA, MYELOPEROXIDASE

Financial Support: CNPq e Fundação Araucária/Pr.

QuebraPagina

Resumo:26-256

TWEEN 80 POTENTIATES RELAXANT EFFECT OF TRANS-CARYOPHYLLENE ON CONTRACTIONS INDUCED BY K+ 80 MM IN RAT TRACHEAL SMOOTH MUSCLE
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2 Universidade Federal de Minas Gerais, UFMG

Objectives:

Trans-caryophyllene (CRF) is an important constituent of essential oils in medicinal plants. Various effects have been attributed to CRF, such as myorelaxant activity in rat intestinal smooth muscle (Fundam Clin Pharmacol. 24:749, 2009). CRF is a hydrophobic molecule, then it is very common the use of Tween 80 (TW) as vehicle. Therefore, the aim of this study is to evaluate if TW enhances the effect of the tested constituents in tracheal smooth muscle.

Methods and Results:

Male Wistar rats (250-300 g) tracheal rings without epithelium were made to stabilize (tension 0.5 g) in a superfusion chamber for isolated organ containing Tyrode solution (pH 7.4; 37° C) and continuously aerated. The contractile response was measured isometrically by a force transducer connected to a computerized system for data acquisition and analysis. The tissue viability was determined by the contractile response to 60 mM of KCl. Contractions were performed with 80 mM KCl being CRF (10 mM) added, diluted or not in TW (0.03 %). The effect of TW on L-type calcium current was evaluated on isolated smooth muscle cells using the patch clamp technique. The results showed similarities in the responses of preparations with and without TW differing only in the strength of the effect of CRF. In preparations with TW the inhibitory effect of CRF occurred faster. Without TW the effect was similar but slower. In half of total time, preparations with TW inhibited 97 % of the initial contraction, while those without TW, only 50 %. TW was also able to amplify the inhibitory effect of CRF on L-type calcium current.

Conclusions:

TW potentiate the effect caused by CRF on contractions induced by 80 mM KCl and inhibited L-type calcium current. Therefore, caution should be taken when using TW as a vehicle.

Keywords: Trans-caryophyllene, Tween 80, Trachea, Smooth muscle

Financial Support: CNPq, FUNCAP

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2 Departamento de Ciências Veterinárias, UFPB

Objectives:

Distilled sugarcane spirit is a genuine Brazilian beverage produced by distillation after fermentation of sugarcane and is appreciated not only in Brazil but worldwide. Although sugarcane spirit consumption is an important drive to increase alcoholism in Brazil, little is known about its chronic consumption on anxiety/anxiolytic effects. In the present study, we investigated whether the chronic consumption of sugarcane spirit is responsible for inducing anxiolytic-like effects.

Resumo: 26-257

SUGARCANE SPIRIT CONSUMPTION INDUCES ANXIOLYTIC-LIKE EFFECTS IN MICE

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Objectives:

Distilled sugarcane spirit is a genuine Brazilian beverage produced by distillation after fermentation of sugarcane and is appreciated not only in Brazil but worldwide. Although sugarcane spirit consumption is an important drive to increase alcoholism in Brazil, little is known about its chronic consumption on anxiety/anxiolytic effects. In the present study, we investigated whether the chronic consumption of sugarcane spirit is responsible for inducing anxiolytic-like effects.
Methods and Results:

Male 3-3.5 month-old Swiss mice were exposed to the two-bottle free-choice paradigm for six weeks. Mice in group A were treated with sugarcane spirit + distilled water (n=16) and in group B (control) with distilled water + distilled water (n=14). The content of ethanol in beverages offered to groups B was 2% in the first week, 5% in the second week and 10% in the 4 remaining weeks. At the end of the treatments, animals were submitted to the elevated plus maze and the hole board test for assessment of anxiety-related behaviors. The concentrations of ethanol, volatile acidity, esters, aldehydes, furfural, higher alcohols (n-propylic, isobutylic and isoamylics), methanol, copper and lead of the sugar cane were also determined. Time spent in open arms was increased in mice exposed to chronic sugarcane spirit (36±9 vs 7±2 s, n=9, p

Conclusions:

Chronic consumption of sugarcane spirit elicits anxiolytic-like effects in mice.

Keywords: Sena, M.C.P, Salvadori, M.G.S.S, Nunes, F.C, Braga, V.A.

Financial Support: CNPq and Capes.
Keuls test. The minimum level of significance used was probability 95% (p

Conclusions:

The hydroalcoholic extract of pods from faveira (Parkia platycephala Benth) did not potentiate nor inhibit the estrogen effects in ovariectomized rats, showing lack of estrogenic and antiestrogenic activity.

Keywords: estrogen, faveira, antiestrogenic activity

Financial Support: Universidade Federal do Piauí

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ANTIINFLAMMATORY ACTIONS OF BARBATIMÃO (STRYPHNODENDRON ADSTRINGENS, MART.) AND ARNICA (ARNICA MONTANA, L,) EXTRACTS OF PAW EDEMA INDUCED BY CARRAGENIN IN RATS

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Objectives:
In the study, we compared the effects of extracts of Amburana-EB/Ac and Arnica montana L. (Arnica-EBAm) in a model with paw edema induced by intraplantar injection of Carageenan (Car).

Methods and Results:
Wistar rats (200-250g) were randomized into five groups of five animals per group: G1:control; G2:Car; G3:Car + Diclo; G4:Car + Arnica and G5:Car + Amburana. Saline (control) or Car (100 mg/kg/paw diluted in 0.1ml) were administered in the right hind paw by intraplantar way. The G1:control group received saline (0.1ml). The G2:Car received only Car and the other 3 groups received, respectively, Car + diclofenac (dose), Car + arnica and Car + amburana. Anti-inflammatory drugs were injected intraperitoneally 60 min. before the intraplantar injection of Car. Dehydrated extracts of Arnica and Amburana, were obtained by soaking in ethanol:water (1:1). They were filtered, dried in a water bath and diluted in saline solution for injection. The paw edema was measured, in mm, by a Mitutoyo thickness gauge before and after 1, 2, and 4h of injection of Car or saline, and was indicated by increased thickness of the paw compared to the control. The values are expressed as mean ± standard error of mean. The differences between the treated groups and control were significant (p

Conclusions:
The results suggest that extracts of Arnica and Amburana have been so effective in protecting mice against the edematogenic effects of Car as diclofenac, an commonly used anti-inflammatory therapy. However, in this assessment, the Amburana had a mild anti-inflammatory effect in this model. Both the studied plants showed antiiinflammatory (antiedematogenic) activity, justifying their jobs in herbal medicine.

Keywords: Inflammation, Paw edem, Carrageenan

Financial Support: FCFAr-UNESP e PIBIC/UNIFEB-Barretos/SP
ETHYL ACETATE AND HYDROMETHANOLIC FRACTION THAT FLOWERS OF HYPTIS FRUTICOSA REDUCES OROFACIAL NOCICEPTIVE BEHAVIOR IN MICE


DEPARTAMENTO DE FÍSIOLOGIA/UNIVERSIDADE FEDERAL DE SERGIPE, UFS

Objectives:

To evaluate the antinociceptive effect of the ethyl acetate fraction (FAE) and hydromethanolic fraction (FHM) flowers of Hyptis fruticosa in formalin, capsaicin and glutamate induced orofacial nociception on mice.

Methods and Results:

Male Swiss mice (25-30 g), were used throughout this study. Mice (n = 6/ per group) were pretreated with FAE or FHM at doses of 50, 100 and 200 mg/kg (v.o.), 60 min before of the tests. In all models, nociception was quantified at those periods by measuring the time (s) that the animals spent face-rubbing in the injected area with its fore- or hind paws. The experimental protocols were approved by the Ethics Committee on Animal Research at the Federal University of Sergipe (CEPA: 46/10). The results were analyzed using ANOVA followed by post Dunnet’s test, were considered significant at p < 0.05. In the formalin test, was found that both the FAE and the FHM reduced orofacial nociception in first phase of the test (39, 5%, 65, 1%, 84%) and (39, 8%, 45, 7%, 68, 6%), p < 0.001, respectively, at doses of 50, 100 and 200 mg/kg. In second phase, FAE and FHM were also effective to reduce orofacial nociception (66, 2%, 65, 8%, 84%; p < 0.001) and (73, 2%, 75, 1%, 76, 5%, p < 0.001), respectively, at doses of 50, 100 and 200 mg/kg. In glutamate-induced orofacial nociception, FAE and FHM produced a significant reduction in orofacial nociceptive behavior (48, 6%, 68, 4%, 78, 6%) and (45, 6%, 60, 4%, 72, 6%) with p < 0.001, respectively. In the test of nociception induced by capsaicin, FAE and FHM significantly reduced, painful response (24, 4%, 29, 6%, 33, 4%) and (23, 1%, 29, 2%, 33, 4%), p < 0.01, respectively, at all doses tested.

Conclusions:

Our results suggest fractions ethyl acetate and hydromethanolic of flowers of hyptis fruticosa has a significant effect in the treatment of orofacial pain.

Keywords: ETHYL ACETATE, Hyptis fruticosa, METHANOL, NOCICEPTION, OROFACIAL PAIN

Financial Support: CNPq

INOTROPIC EFFECT OF THE ETHANOLIC CHAMOMILLA RECUTITA (L.) RAUSCHERT EXTRACT ON THE GUINEA PIG ATRIUM.

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Departamento de Fisiologia/Universidade Federal de Sergipe, UFS
Objectives:

This work aimed to study the inotropic effects of the isolated left atrium produced by the ethanolic Chamomilla recutita extract.

Methods and Results:

The experiments were carried out on the guinea pig (Cavia porcellus) myocardium and were performed on isolated left atria mounted in an organ bath (4-5 ml, Tyrode, 29 ± 0.1 °C, 95 % O2, 5 % CO2), stretched to 1 gf, and artificially stimulated (400 V, 0.5 ms). The experimental procedures were previously approved by the Research Ethics Committee of the Federal University of Sergipe. The extracts were added cumulatively to the organ bath and the experiments were carried out using the following concentration range: 0.1 – 7.0 ug/mL. The force was recorded isometrically and stored in a computer. To determine the extract concentration required to produce 50% of the maximum extract effect (EC50), the experimental data were fitted by curves calculated by the Hill-Langmuir equation. The protocol of Dorigo et al (Cardiovasc. Drugs and Ther, 4:1477, 1990) were to used to determine the effect of the ethanolic Chamomilla recutita extract (2.0 ug/mL) on channel of the calcium in the guinea pig left atrium and to determine the involvement of the cholinergic receptors in the mechanism of action of the extract were carried out ethanolic Chamomilla recutita extract concentration-effect curves in the presence of atropine sulfate (1.5 uM). The force isometric from the left atrium was to reduced with relative efficacy of the 66.1% and EC50 de 1.89 ± 0.2 ug/mL in the presence of the ethanolic Chamomilla recutita extract. The effect was to returned on the washed. The ethanolic Chamomilla recutita extract reduced the sarcolema calcium current, shifting to the right the CaCl2 concentration-effect curve and changing the EC50 from 0.458±0.02 mM to 0.599 ± 0.04 mM. In the presence of the cholinergic antagonist, the extract to exhibited EC50 de 2.15 ± 0.3 ug/mL (p

Conclusions:

These data allowed to conclude that the myocardial effects promoted by active(s) compound(s) present in the ethanolic Chamomilla recutita extract can be to promoved by the sarcolemal L-type calcium channel block and to the activation of the potassium channels.

Keywords: Chamomilla recutita, guinea pig, negative inotropic

Financial Support: CNPq/UFS
Methods and Results:

Methods: Adult Swiss mice (n=24, 25-35g) were used for the assessment of acute toxicity. Animals of both sexes were randomly assigned to control and treated groups (6 males or females per group/cage). Treated groups received by gavage 2000mg/kg of stem bark ethanolic extract of Licania macrophylla. Control group received only the vehicle, an aqueous solution of DMSO. Mice were observed thoroughly during the first 24 hours for the onset of any immediate toxic signs and daily during a 14 days observation period to record any delayed acute effects. Body weight, food and water intake were recorded daily since the second day of treatment. All animals were killed by cervical dislocation after 14 days. Blood samples were collected and examined for hematological parameters; macroscopic analysis and weighing of organs (liver, heart and kidneys) were performed and compared with their respective control group. Numerical results were expressed as mean ± standard error of mean. Differences between groups were determined using analysis of variance (ANOVA) and method of Turkey. Hematological determinations were analyzed by the Student “t” test. All results were considered statistically significant when p

Conclusions:

Conclusion: Although the results showed stem bark ethanolic extract of Licania macrophylla has low acute toxicity, more detailed studies of toxicity are needed to design a more complete toxicity profile of the plant.

Keywords: ACUTE TOXICITY, ETHANOLIC EXTRACT, LICANIA MACROPHYLLA, SWISS MICE

Financial Support: CNPq / SETEC-AP / IEPA / UNIFAP / LACEN-AP

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Resumo:26-263

COPAIBA OIL-RESIN TREATMENT REDUCES NEUTROPHIL RECRUITMENT AND MICROGLIA/MACROPHAGE ACTIVATION AFTER MOTOR CORTEX EXCITOTOXIC INJURY

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3 Departamento de Neurociências, UFRN

Objectives:

The oil-resin of Copaifera reticulata Ducke is used in the Brazilian folk medicine as an anti-inflammatory and healing agent. There is experimental evidence suggesting that copaiba oil-resin (COR) possesses both anti-inflammatory and anti-oxidant effects after acute disorders in non-neural tissues in vivo and in vitro. However, there are no investigations on the possible anti-inflammatory and neuroprotective roles of COR in experimental models of acute neural disorders. In this study, we have investigated the anti-inflammatory and neuroprotective effects of COR following an excitotoxic damage to the motor cortex of adult rats.

Methods and Results:

Animals were injected with the neurotoxin N-Methyl-D-Aspartate (NMDA) (n=10) and treated with a single dose of COR (400 mg/kg, i.p) soon after surgery (Group 1) or with two daily doses (200 mg/kg, i.p) during 3 days (Group 2) postinjury. Control animals were treated with vehicle only. Histopathological analysis was performed using cresyl violet staining and immunohistochemistry against neutrophils and activated macrophages/microglia. COR treatment induced tissue preservation, a 40% decrease in the number of neutrophils (34.06 ± 3.5; 55.56 ± 4.55, p
Conclusions:

COR possesses both neuroprotective and anti-inflammatory effects following an acute damage to the central nervous system (CNS). This is the first experimental evidence suggesting that COR treatment can induce neuroprotection by modulating the inflammatory response following a CNS damage.

Keywords: Copaifera, Stroke, Neuroprotection, Microglia, Inflammation

Financial Support: Fundação de Amparo e Desenvolvimento a Pesquisa do Estado do Pará (FAPESPA)

Objectives:

The objective of this work was to verify a possible lipid-lowering action of the cinnamic acid esters (CAE) isolated from carnauba wax in dyslipidemia induced by Poloxamer-407 (PLX).

Methods and Results:

Swiss male mice (20-25g) had been divided in 6 groups (n=8): negative (NC) and positive (PLX) controls, GEMF (gemfibrozil 100mg/kg. v.o.), CAE 10 (CAE 10mg/kg, v.o); CAE 50 (CAE 50mg/kg, v.o.); CAE 100 (CAE 100mg/kg, v.o.). Dyslipidemia was induced by a single PLX (1000 mg/kg) intraperitoneal (i.p) injection in the groups: positive control, GEMF, CAE 10, CAE 50 and CAE 100. Animals were treated three times, 1h before, 22h and 46h after the PLX administration. After 24h and 48h of dyslipidemia induction blood samples were collected for determination of total cholesterol (TC) and triglycerides (TG). This protocol was submitted and approved (nº 90/10) by the Ethical Committee in Animal Research of the Federal University of Ceará, Fortaleza, Brazil. The results were expressed in mean ± E.P.M. and compared using ANOVA (Newman-Keuls post test) and p

Conclusions:

CAE isolated from carnauba wax had a lipid-lowering action effective in dyslipidemia induced by PLX but more studies are needed to give evidence to this effect and elucidate a possible mechanism of action of this compound.

Keywords: carnauba, cinnamic acid esters, poloxamer-407

Financial Support: Banco do Nordeste, CAPES
Resumo:26-265

SUSCEPTIBILITY TO TREATMENT COMPARED BETWEEN BENZONIDAZOL AND COUMARINS METALLIC COMPLEXES IN TWO STRAINS OF TRYpanosoma cruzi.

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Objectives:
Chagas disease, caused by the protozoan Trypanosoma cruzi, is widespread in rural areas of the Americas becoming a major public health problems. Benznidazole the drug, currently used in treatment has efficacy above 80% in the acute phase and decreased to 30% in chronic phase. The success of therapy touches on aspects such as prolonged treatment regimen, adverse reactions, genetic variability of parasite strains and naturally resistant to the drugs, indicating the necessity of developing new drugs more effective in the chronic phase. The coumarins are part of the human diet, being found in plants, grapefruit and blueberry fruits, green tea and cinnamon. Metallic complexes (Cu (II), Ni (II) and Zn (II) that mimic the effect of superoxide dismutase (SOD), a scavenger of the anion superoxide (O2-) have application to obtain the best antioxidants. Based on this we compare the activity of coumarins metallic complexes with Benznidazole and synergistic effect of compound associations aiming to obtain new molecules for the putative treatment.

Methods and Results:
We performed treatment with benznidazole, coumarin, 4-hydroxy-3-nitrocumarina and the latter complexed with copper, iron, zinc and nickel at different concentrations and combinations of compounds. In experiments in vitro we used cultures of epimastigote form parasites strains G and CL in LIT medium treated with compounds for 24 hours. For studies of intracellular form used 106 infected VERO cell culture incubated on coverslips in 24 well plates at 37 °C in 5% CO2, in RPMI medium or different concentrations of compounds for 24 hours. The tests were performed in triplicate and the results were determined by the rate of infection (IF) obtained from the total count of 200 cells, the percentage of infected and the number of intracellular amastigotes. The data in epimastigotes of two strains, showing that the CL strain is more susceptible to treatment with 4H3NC and with 4H3NC-Ni, 4H3NC-Cu and 4H3NC-Fe, while strain G are more susceptible to the compound 4H3NC-Zn. In amastigotes of strain G the 4H3NC-Cu, coumarin and 4H3NC-Fe, showed a decrease from 39 a 50% the IF versus 66% to 76% in the CL strain. We also studied the modulation of gene expression in the parasites of both strains treated by compounds selected. By analyzing the SDS-PAGE with electrophoretic profile of total proteins, we observed that the strain G did not change in the electrophoretic profile and the CL strain have a significant change in the electrophoretic pattern suggesting a modulation in the gene expression.

Conclusions:
Statistical analysis showed a lot of significance to the action of the compounds 4H3NC-Cu, 4H3NC-Fe and coumarin, in the G strain and extremely significant for the action of the compound 4H3NC Fe in CL strain. All the compounds showed antiparasitic action, however, the activity of the compound 4H3NC-Fe was highly significant on the CL strain when compared to control. Initial tests of associations of compounds showed better results for antiparasitic action in strain G. Theses studies with in vivo tests, may contribute to the use of these compounds as future drugs for Chagas disease.

Keywords: Trypanosoma cruzi, susceptibilidade, modulação da expressão, diferentes cepas, forma amastigota

Financial Support: Universidade Bandeirante de São Paulo, UNIBAN

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Resumo:26-266
EFFECTS OF COPAIFERA SP. OIL-RESIN (LEGUMINOSAE) ON GASTRIC EMPTYING IN RATTUS NORVEGICUS

Departamento de Biofísica e Fisiologia, UFPI

Objectives:
The Copaiba oil-resin (balsam) is widely used in folk medicine, especially as a healing, anti-inflammatory, antiseptic, antitumor agent and how to treat bronchitis, ulcers and skin diseases. This study evaluated the activity of the Copaifera sp oil-resin on gastric emptying.

Methods and Results:
Groups of 7 male rats (300-350 g) were fasted for 24 h and orally received 5 mL/kg 1% Tween 80 (Control Group - C), Copaiba oil-resin 200 and 400 mg/kg (COP200 and COP400) or atropine 3 mg/kg, i.p. (standard group - Atr). One hour later they all orally received phenol red (PR) 0.5 mg/mL in glucose 5 g% (1.5 mL/animal). After 20 minutes, the animals were euthanized with an overdose of sodium thiopental (100 mg/kg, i.p.) and the stomach and small intestine were removed. The small intestine was divided into proximal (P), medial (M) and distal (D) portions and each segment was homogenized in 100 mL of 0.1 N NaOH, and 10 mL the suspension centrifuged (10 min, 3000 rpm). Tissue proteins (in 5 mL homogenate) were precipitated with 0.5 mL of 20 g% trichloroacetic acid and centrifuged out (20 min, 3000 rpm). From the supernatant, an aliquot of 3 mL was added to 4 mL of 0.5 N NaOH. The concentration of PR was determined by absorbance at 560 nm and the content of the dye in each segment was expressed in % FV. Data were analyzed by ANOVA and Tukey’s post-test. Project was approved by the Ethics Committee for Animal Research at the Federal University of Piauí. The gastric retention of PR was higher (p

Conclusions:
The *copaifera sp* oil-resin at the 200 mg/kg dose but not at 400 mg/kg, produced an increase in gastric retention of phenol red. The *copaifera sp* oil-resin contains active (s) principle (s) that delays the gastric emptying. Additional studies are being conducted to evaluate the effects of other doses of this oil-resin on gastric emptying and intestinal transit.

Keywords: *Copaifera sp.*, gastric emptying, *Rattus norvegicus*

Financial Support: UFPI

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Resumo:26-267

FURTHER ANTINOCICEPTIVE PROPERTIES OF HYDROALCOHOLIC EXTRACT AND ISOLATED TERPENES FROM SALVIA OFFICINALIS IN MICE

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³ Universidade Federal de Santa Catarina - Química, UFSC
Objectives:

We sought to further investigate the antinociceptive effects of hydroalcoholic extract (HE) and isolated terpenes named carnosol (CA) and ursolic/oleanolic acid (UA/OA) obtained from Salvia officinalis leaves in models of chemical nociception in mice.

Methods and Results:

Adult Swiss mice (~30 g, n=4-7) received a single oral (p.o.) injection of vehicle (water plus 1% tween 80, 0.1 ml/10 g) or HE (3, 10, 30 and 100 mg/kg), CA (10 mg/kg) or UA/OA (30 mg/kg) 1 h before intraplantar injection (20 μl/paw) of 2.5% formalin, capsaicin (5.2 nmol) or cinnamaldehyde (100 nmol). We evaluated the nociceptive response (s), the paw oedema (I paw thickness mm) and mechanical allodynia (von Frey hairs, paw withdrawal frequency). All procedures were approved by the Ethics Committee on Animal Experiments of UFPR, number 443. HE (10, 30 and 100 mg/kg) reduced significantly both phases of formalin-induced nociceptive behaviors. The antinociceptive effect of HE (100 mg/kg) in both phases of formalin test was reversed by naloxone (1 mg/kg, i.p.) in 50 and 36%, respectively. In addition, CA and UA/OA reduced nociceptive responses by 56 and 48%, respectively, only in the second phase of the formalin test (118.8 ± 7.8 s). HE (10, 30 and 100 mg/kg) and ruthenium red (3 mg/kg, i.p.) reduced capsaicin induced-nociception in 42, 68, 39 and 76%, respectively (control group: 44.8 ± 6.7 s), but only the higher dose of HE reduced the paw oedema in 43% (control group: 1.25 ± 0.16 mm). On the other hand, both nociceptive behaviors and paw oedema induced by cinnamaldehyde were reduced by HE (10, 30 and 100 mg/kg) in 56, 42 and 32% (control group: 50.6 ± 4.3 s and 42, 51 and 45% (control group: 0.92 ± 0.06 mm). Camphor (7.6 mg/kg, i.p.) also reduced both parameters in 37 and 33%. Interestingly, CA and UA/OA also reduced cinnamaldehyde-induced nociception in 39 and 47%, respectively, but did not change the paw oedema. Furthermore, in cinnamaldehyde-induced mechanical allodynia, UA/OA showed an anti-allodynic effect lasting at 3 h (inhibition of 49%), whereas CA effect was observed only at 3 h (inhibition of 35%). Finally, it is important to mention that at all tested doses, HE did not change the locomotor activity of animals in the open field test.

Conclusions:

In summary, the present study clearly indicates that the isolated terpenes carnosol and ursolic acid/oleanolic acid might be responsible for the significant antinociceptive and anti-inflammatory effects promoted by HE.

Keywords: Antinociceptive, Salvia officinalis, Terpenes

Financial Support: CNPq

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Resumo:26-268

EFFECTS OF THE ETHYL ACETATE FRACTION OBTAINED FROM ERYTHRINA VELUTINA LEAF ON THE L-TYPE CALCIUM CURRENT ON MAMMALIAN CARDIOMYOCYTES

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Objectives:

Studies have shown that extracts from the leaves of Erythrina velutina (mulungu) have anxiolytic, analgesic, anti-inflammatory and anti-bacterial actions. This study aimed to characterize the effects of the ethyl acetate fraction (FAcEt) obtained from E. velutina leaf on the atrial contractility and calcium current in mice ventricular cardiomyocytes.
Methods and Results:

The effects of E. velutina on the atrial contractility were performed in guinea pig left atrium maintained in an organ chamber (5 mL, Tyrode, 27 ± 0.1°C; 2 Hz). The effects of the FAcoEt on the L-type calcium current were evaluated on ventricular myocytes isolated enzymatically. Shioya (J. Physiol. Sci, 57:327, 2007). Experimental procedures were approved by the Animal Research Ethics Committee of the Federal University of Sergipe (19/09). Whole-cell voltage-clamp recordings were obtained at room temperature (22 - 25 oC) using an EPC-9.2 patch clamp amplifier (HEKA Electronics, Rheinland-Pfalz, Germany). Upon attaining the whole-cell configuration, 3 to 5 minutes were allowed to the pipette solution equilibrate with intracellular milieu. For the time-course analysis of the effect of 80 μg/ml FAcoEt on the L-type Ca2+ channels, ICa,L was elicited every 10 s by test pulses starting from a holding potential of -80 mV and stepped to -40 mV for 50 ms in order to inactivate Na+ and T-type Ca2+ channels. Then, the membrane potential was raised to 0 mV for 300 ms. In the left atria, 800 mg/L FAcoEt increased the contraction force by 108 ± 29 %, what was accompanied by a slightly negative inotropic effect (14%). Propranolol (β-adrenergic antagonist) and nifedipine (L-type calcium channel blocker) abolished the positive inotropic effect induced by FAcoEt and maintained its negative inotropic effect. In ventricular cardiomyocyte, FAcoEt inhibited (25 ± 3%) the type-L calcium current. The effects were partially removed after washout.

Conclusions:

FAcoEt promotes a inotropic biphasic effect on the myocardium. Firstly, it was able to increase the contractile force by a mechanism that directly or indirectly involves activation of beta-adrenergic receptors and the opening of calcium channels. On the other hand, it also elicited a negative inotropic response that can be explained by a reduction of the L-type calcium current.

Keywords: Calcium current, Cardiomyocytes, Erythrina velutina

Financial Support: CAPES, FAPITEC/SE, UFS, ELETROBRÁS

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Resumo:26-269

TNF-Α INHIBITION AND HISTOPATHOLOGICAL DAMAGES PREVENTION AFTER ADMINISTRATION OF ETANOLIC EXTRACT FROM HYMENEA COURBARIL L. IN THE MODEL OF PAW EDEMA INDUCED BY CARRAGEENAN IN RATS

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2 Departamento de Ciências Fisiológicas, UECE

Objectives:

We previously demonstrated that Etanolic Extract from Hymenea courbaril L. (EEHC), popularly known as jatobá, has an efficient anti-inflammatory effect on pre-clinical animal models such as carrageenan (CARR) paw edema and formalin test (data not yet published). In order to evaluate a possible mechanism of action to explain EEHC pharmacological properties we investigated the tissue expression of TNF-α by immunohistochemistry after challenging with carrageenan. It was also observed the histopathological alterations by HE staining.

Methods and Results:

Paw edema was induced by CARR application to the right paw (Wistar Rats, 150-200g, n=6) and swelling was recorded by plethysmography (mL). Animals were treated orally with vehicle (distilled water) or EEHC (100 mg/Kg) 1 h before CARR injection. EEHC reduced (p < 0.001) the paw edema, mL, at the dose of 100 mg/Kg during the periods of measures: second hour (46%), third hour (60%) and fourth hour (44%) when compared to the groups that received only vehicle (ANOVA and Newman-
Keuls). After registration of paw edema samples from paw tissue were collected to perform immunohistochemistry assay to TNF-α and to make histopathological slides to analyze tissue alterations. Treatment with EEHC significantly reduces TNF-α expression in paw tissue. Considering a maximal score of 5 to the group treated with vehicle we assume a score of 1 to EEHC treated group. TNF-α expression can be noticed through the brown immunostaining both in leukocytes and macrophages. EEHC treatment also reduces neutrophils infiltration, edema formation and hemorrhage points as the HE staining slides could show. All slides were captured in 400x and processed by ImageJ® software.

Conclusions:
We conclude inferring that Etanolic Extract from Hymenea courbaril L. has an efficient pre-clinical anti-inflammatory effect and it is part a consequence of TNF-α down-expression at the inflammatory site. The EEHC reduces the number of inflammatory cells in the tissue as well. However, other tests must be performed to elucidate other pathways of action.

Keywords: carrageenan, inflammation, Hymenea courbaril, Natural products, TNF-alfa

Financial Support: FUNCAP, CNPq

CARDIOVASCULAR EFFECTS INDUCED BY THE BRAZILIAN MARINE ALGA DICTYOTA PULCHELLA (DICTYOTACEAE) IN RATS

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1 PgPNSB, UFPB
2 CFP-ETSC, UFCG
3 CCA, UFPB

Objectives:
The pharmacological effects induced by CH₂Cl₂/MeOH extract (EDP) and Hexane/EtOAc phase (FDP) from the Brazilian alga Dasyota pulchella were studied on the cardiovascular system of Wistar rats using a combined in vivo and in vitro approach.

Methods and Results:
All protocols in this study were approved by the CEPA/LTF (protocol n° 0208/10). In normotensive conscious male rats, EDP injections (5; 10; 20 and 40 mg/kg, i.v., randomly) produced hypotension (-4.1 ± 1.34; -7.0 ± 2.4; -46.9 ± 1.3 and -54.8 ± 4.3%; respectively) and bradycardia (-2.1 ± 1.6; -4.0 ± 2.3; -66.8 ± 5.2 and -74.7 ± 4.5%; respectively) (n=5). Isolated superior mesenteric artery rings (1-2 mm) were suspended by cotton threads for isometric tension recordings in a Tyrode’s solution at 37°C, gassed with a 95% O₂ and 5% CO₂, under a resting tension of 0.75g. In phenylephrine (Phe, 1µM)-pre-contracted rings, EDP (0.01 – 500 µg/mL) induced a concentration-dependent relaxation (Maximum Response = 101.4 ± 4.5%; EC₅₀ = 22.35 ± 5.09 µg/mL) and this effect was not modified by removal of the vascular endothelium (MR = 103.3 ± 8.3%; EC₅₀ = 21.43 ± 8.98 µg/mL, n=7). Similar results were found in the presence of Phe (0.01 – 500 µg/mL), FDP induced a concentration-dependent vasodilatation in both endothelium-intact (MR = 94.5 ± 7.5%; EC₅₀ = 24.1 ± 8.95 µg/mL, n=6) or endothelium-denuded mesenteric artery rings (MR = 95.6 ± 7.5%; EC₅₀ = 23.7 ± 5.65 µg/mL, n=6). Based on the preliminary results, the subsequent experiments were performed in rings without endothelium. To appreciate the involvement of potassium channels, the preparations were pre-incubated with Tyrode’s modified solution, KCl (20 mM) or with non-selective K⁺ channel blocker, tetraethylammonium (TEA, 3 mM). In both preparations the vasorelaxant activity was not changed. In the presence of a tromboxane A₂ agonist U-46619 (100 nM), EDP induced concentration-dependent vasodilatation (MR = 90.3 ± 7.8%; EC₅₀ = 24.63 ± 4.04 µg/mL, n=6) was similar to the response found under Phe-induced. After exposure to high concentrations of
extracellular K⁺ (KCl, 60 mM), the EDP induced concentration-dependent vasodilatation (MR = 97.7 ± 4.0%; EC₅₀ = 34.57 ± 5.11 mg/mL; n=6). In the same experimental condition, FDP induced concentration-dependent vasodilatation (MR = 113.5 ± 6.1%; EC₅₀ = 10.92 ± 2.81 µg/mL; n=6). This result indicates that both EDP and FDP act on voltage-operated calcium channel (Caᵥ). Furthermore, EDP and FDP (0.03; 0.3; 10; 30 e 100 µg/mL) antagonized CaCl₂-induced contractions. The extract also induced vasodilatation in the contraction evoked by L-type Ca²⁺ channel agonist (Bay K 8644, 200 nM) (MR = 113.3 ± 6.7%; EC₅₀ = 19.45 ± 6.66 µg/mL, n=7).

Conclusions:

These results suggest that EDP induces hypotension and bradycardia. Both EDP and FDP induce endothelium-independent vasodilatation that involves the inhibition of the Ca²⁺ influx through blockade of Caᵥ.

Keywords: Alga, Dictyota pulchella, mesenteric artery, vasorelaxant

Financial Support: CNPq and CAPES

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Resumo:26-271

RUTIN INHIBITS THE ACTIVITY OF ACETYLCHOLINESTERASE IN DIFFERENT BRAIN STRUCTURES IN VITRO

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Objectives:

Rutin (Rut) is a bioflavonoid compound with antioxidant, anti-inflammatory, antiviral and anti-carcinogenic properties and has been demonstrated to scavenge superoxide radicals. It protects the biomolecules in the brain from oxidative damage and have been shown good results in treatments of memory disfunction in animals. Considering that the mechanisms of memory formation, consolidation and evocation need the atuation of acetilcoline and your signaling, this work aims to investigate the effect of rutin in acetylcholinesterase (AChE) activity in different brain structures.

Methods and Results:

Twenty adult male Wistar rats (70–90 days; 220–300 g) were used. The animals were maintained at a constant temperature (23 ± 1oC) on a 12 h light/dark cycle with free access to food and water. The animals were submitted to euthanasia being previously anesthetized and brain structures were removed and separated into cerebral cortex, hippocampus and striatum. The brain structures were homogenized in Tris–HCl 10mMTris–HCl, pH 7.4, solution. Rutin was solubilized in DMSO at concentrations of 1, 5, 10, 25 and 50 µM. AChE enzymatic assay in synaptosomes was determined by method of Ellman et al (Biochem Pharmacol 7:88–95). The assay medium (2 ml final volume) contained 100 mM K+ phosphate buffer, pH 7.5, and 1 mM 5,5-dithiobisnitrobenzoic acid (DTNB). The enzyme was pre-incubated with different rutine concentrations for 2 min. The reaction was initiated by adding 0.8 mM acetylthiocholine iodide as the substrate and velocity was measured by increasing absorbance to 412 nm at 25oC. All samples were run in duplicate or triplicate and the enzyme activity were expressed in µmoles AcSCh/h/mg of protein. The data were analysed by one-way ANOVA followed by Duncan’s multiple range tests, P < 0.05 was considered to represent a significant difference. All data were expressed as means ± SEM. In the hippocampus and striatum, all doses significantly inhibited the activity AChE. There was no statistical difference in AChE activity in cerebral cortex.

Conclusions:
Compounds that can interact with the enzyme and cause an inhibition in its activity may be important in the treatment of pathologies related to loss of memory and the increased activity of AChE, such as Alzheimer's disease. Therefore, rutin shows a promising role in study of new approaches to treatment of some disorders of the cholinergic system.

Keywords: Rutin, Acetylcholinesterase, Flavonoids

Financial Support: CNPq,CAPES e FAPERGS

INVESTIGATION OF BIOLOGICAL EFFECTS OF OPERCULINA ALATA HAM.

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Objectives:

O. alata belongs to the family Convolvulaceae and is popularly known as Jalapa-do-Brasil. It is very common in the northeast of Brazil and is used as a laxative, purgative and to improve the digestive function. Despite the wide marketing in the form of hydroalcoholic extract (EHA) there are few studies about its safety in use. This study aimed to investigate the biological effects of the hydroalcoholic extract (EHA) of O. alata on prokaryotic and eukaryotic cells.

Methods and Results:

The antibacterial activity of EHA (0,1-1000 ug) was evaluated determining the minimum inhibitory concentration (MIC) against Gram negative and Gram positive bacteria (B. subtilis CCT0516, E. coli ATCC 2536 and ATCC 10536, P. aeruginosa ATCC 8027 and ATCC 25619, S. aureus ATCC 6538 and 2595 ). We investigated the hemolytic, hemagglutinating, oxidant and antioxidant potential of EHA (1, 10, 100 and 1000 ug) on human erythrocytes of types A, B and O. The human erythrocytes were from blood can not be used for transfusion (blood to be discarded) obtained from Transfusion Unit of the University Hospital Lauro Wanderley/UFPB. All experiments are performed in triplicate and the results were expressed as arithmetic mean. EHA was able to inhibit the growth of any strain tested and had no effect hemagglutinating. The hemolytic effect on human erythrocytes was dependent on concentration and type O erythrocytes were less susceptible. The hemolytic activity was low (less than 13%) on human erythrocytes of the three types until the concentration of 100 ug. At the concentration of 1000 ug EHA has presented high hemolytic activity (88,1% for type A, 85,2% for type B and 69,3 for type O). Oxidative effect has not occurred in any of the concentrations tested and the antioxidant effect was smaller (less 26%) than that of vitamin C (52%) a proven antioxidant.

Conclusions:

The EHA of O. alata in high concentrations can cause damage to the membrane of human erythrocytes, so further studies, are necessary to ensure its safe use.

Keywords: Operculina alata, antibacterial effect, hemolytic effect, antioxidant effect, hemagglutinating effect

Financial Support: CNPq and CAPES
UTEROTROPHIC EFFECT OF COPAIFERA LUETZELBURGII HARMS ETHANOLIC EXTRACT IN RATS.

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Objectives:
This study aims to investigate the possible estrogenic and/or anti-estrogenic action of ethanolic extract of Copaifera luetzelburgii Harms (EEtOH-CL), through the uterotrophic test.

Methods and Results:
To perform the uterotrophic assay, 90 immature female Wistar rats (21 ± 1 days postnatal, weighing about 65-75g) were used, divided into 9 groups of 10 animals, which were treated for three consecutive days with EEtOH-CL at doses of 125, 250 and 500mg/kg orally. Estradiol (5ìg/kg, intramuscular) and tamoxifen (4 mg/kg, orally) were used as estrogenic and anti-estrogenic positive control, respectively, and distilled water (1mL/100g, orally) was used as negative control. After treatment period, animals were weighed on an analytical balance and then were euthanized. The uterus and the ovaries were removed, after despising the liquid kept inside the uterus, the weight of these organs was checked in analytical scale (0,001g). The results were expressed as mean ± SEM and analyzed by ANOVA (One Way) followed by Bonferroni post-test. The relative mass of the uterus (determined and recorded on a percentage of body weight) for doses of 125, 250 and 500mg/kg of EEtOH-CL was 0.18 ± 0.05, 0.17 ± 0.04 and 0.25 ± 0.05, respectively, while for the negative control and estradiol were 0.40 ± 0.05 and 0.09 ± 0.05, respectively. These findings show that although the extract has shown estrogenic effects in relation to negative control, this was lower than the positive control, with no statistically significance (p>0,05). Regarding the anti-estrogenic activity, the percentages for EEtOH-CL associated to estradiol were 0.12 ± 0.02, 0.1 ± 0.02 and 0.10 ± 0.01, respectively, and 0.19 ± 0.07 for tamoxifen + estradiol. Comparing the treatments with estradiol, it was found that animals that received estradiol + tamoxifen (positive control for anti-estrogenic) showed a statistically significant (p

Conclusions:
It can be concluded that EEtOH-CL possesses anti-estrogenic activity in the the considered test.

Keywords: Copaifera luetzelburgii, uterotrophic effect, female, rats

Financial Support: Universidade Federal do Piauí

IN VITRO ANTIOXIDANT PROFILE OF CAESALPINIA PYRAMIDALIS TUL. (FABACEAE).

Santos, C. A. ; Santana, D. G. ; Estevam, C. S. ; Camargo, E. A. ; Thomazzi, S. M.
Objectives:

*Caesalpinia pyramidalis* Tul. is an endemic tree of the northeastern of Brazil and is one of the predominant species in the *caatinga* vegetation. In general, plants represent an important source of antioxidant products due to the large amount of metabolites presenting this activity. Preliminary data from our research group have shown that the ethanol extract of *Caesalpinia pyramidalis* possesses *in vivo* anti-inflammatory and antinociceptive activities. As these actions could be related to the antioxidant capability of this extract, the aim of this study is to evaluate the *in vitro* antioxidant activity of this extract.

Methods and Results:

Different *in vitro* free radical producing systems were used in this study and all experiments were performed in triplicate. The concentrations of the ethanolic extract of *Caesalpinia pyramidalis* used were 0.001, 0.01, 0.1, 1, 10, 100 and 1000 μg/mL. Lipid peroxidation was measured by thiobarbituric acid reactive substances (TBARS) determination. Superoxide dismutase (SOD)-like activity was determined by measuring the rate of inhibition of superoxide-mediated adrenaline auto-oxidation. Catalase (CAT)-like activity was measured through the decomposition of H₂O₂. Nitric oxide (NO) scavenging capacity was quantified by the generation of nitrite (measured by Griess reaction) from the spontaneous decomposition of sodium nitroprusside. All tests described above used vitamin C (1.67 μg/mL) as the antioxidant control. The total radical-trapping antioxidant parameter (TRAP) was assessed by chemiluminescent determination of 2,2'-azobis[2-methylpropionamidine] dihydrochloride (AAPH) decomposition and Trolox (2 μM) was used as the control. The data was analyzed by one-way ANOVA and Tukey's test *post hoc* and the difference was taken as significant when \( p \leq 0.05 \). *Caesalpinia pyramidalis* significantly decreased the lipid peroxidation of liposomes preparation at 100 and 1000 μg/mL \( (p \leq 0.05) \). *Caesalpinia pyramidalis* presented NO scavenger activity at all concentrations tested, like vitamin C. TRAP analysis showed that this extract possess scavenger activity at 1000 μg/mL \( (p \leq 0.05) \).

Conclusions:

Our results clearly show that the ethanol extract of inner bark of *Caesalpinia pyramidalis* presents *in vitro* antioxidant activity. We suggest that this activity may contribute to the anti-inflammatory and antinociceptive actions of this extract observed in our *in vivo* preliminary experiments.

Keywords: ANTIOXIDANT, CAESALPINIA PYRAMIDALIS, FREE RADICALS

Financial Support: CNPq
presents relaxant and antispasmodic effects on different types of smooth muscle, including those of airways (Phyt. Res 11, 299-304, 1997), we sought to investigate the action of estragole in the contractile parameters in the rat tracheal smooth muscle.

Methods and Results:

Tracheal rings of male Wistar rats (150-300 g), undergoing 1g tension, were placed to equilibrate in isolated organ bath with modified Tyrode solution (TM) (pH 7.4; 37 °C) and constant aeration. Changes in tension were measured isometrically by a force transducer connected to a system of computerized data acquisition. The tissue viability was determined by the contractile response to 60 mM potassium (K60). EOCz and estragole at 1000 μg/mL, abolished the contracturant effect of 80 mM potassium (EOCz IC50: 157.26 ± 16.32, n=4; estragole IC50: 335 ± 13.8 μg/mL, n=5) and relaxed tissues, in which the &beta2 adrenergic receptors had being saturated with adrenaline (EOCz 79.98%, n=8 and estragole 71.06%, n=5). EOCZ and estragole (1000 μg/mL) completely inhibited the contractions induced by [Ca2+] cumulative increase in absence of nifedipine in preparations maintained at 0 [Ca2+] solution. In presence of nifedipine, EOCz and estragole both at 1000 μg/mL elicited significant inhibitory effects. At 2000 μg/mL, only EOCz elicited complete inhibition.

Conclusions:

EOCz and estragole inhibit both the electromechanical and the pharmacomechanical couplings in the rat tracheal smooth muscle, indicating that estragole contributes in the mediation of EOCz relaxant effect. In the electromechanical coupling, EOCz was more potent compared to estragole

Keywords: OECz, estragole, smooth muscle, trachea

Financial Support: FUNCAP

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THE PUTATIVE HYPNOSEDATIVE ACTIVITY OF THE AQUEOUS EXTRACT FROM THE PERICARP OF P. ALATA.

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Objectives:

Passiflora alata Curtis is a Brazilian species from Passifloraceae family popularly known as sweet passionfruit. Some species of this genus are used in folk medicine due to their tranquilizing properties. Moreover, the leaves of this species were included in the fifth edition of the Brazilian Pharmacopoeia. Hence, the aim of this work was to evaluate the hypnosedative activity of the aqueous extract from leaves, pulp and pericarp of P. alata in the ethyl ether-induced hypnosis test.

Methods and Results:

The extracts from leaves and pericarp were obtained by infusion in water (1:10 and 1:3 w/v, respectively) and the pulp was crushed. Afterwards the extracts were filtered and lyophilized. Five groups of male Swiss mice (35-50g/3 months) were p.o. treated with the different extracts of P. alata (100, 300 and 600 mg/Kg) or vehicle (water) and, 1 h later, the animals were individually placed in an ethyl ether (6 mL during 13 min) saturated glass cage (20×15 cm). The latency to lose the righting reflex and the duration of sleep (in s) were recorded. Sleeping-time was measured by the loss of the righting reflex, with the recovery of this reflex. DZP (1 mg/kg, i.p.) was used as the positive control drug (standard anxiolytic/hypnosedative compound). The data were presented as mean ± S.E.M of the time (s) analyzed by one-way ANOVA followed by Dunnnett’s test.
the treatments with all extracts, only the highest dose (600 mg/kg, p.o.) of the aqueous extract obtained from the pericarp of *P. alata* significantly enhances the duration of sleep (p = 0.05), suggesting a possible hypnosedative activity.

Conclusions:

This study showed that the aqueous extract of the pericarp of *P. alata* can be effective as hypnosedative, an activity that deserves further investigation, but neither the extracts obtained from leaves or pulp presented this activity.

Keywords: hypnosedative, *Passiflora alata*, Passiflora, Passifloraceae, Pericarp

Financial Support: FAPESC, CAPES, CNPq

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**ANTI-ALLERGIC PROPERTIES OF THE NATURAL PRODUCT GEDUNIN: INHIBITION OF T LYMPHOCYTE FUNCTIONS VIA THE BLOCKADE OF NFAT AND NF&KAPPA;B SIGNALING PATHWAYS**

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Objectives:

T lymphocytes are crucial cells for the coordination and maintenance of the inflammatory response in allergic diseases. It has been proposed that a therapeutic strategy targeting T lymphocyte trafficking into the tissue could be effective to reduce allergic inflammation and control the disease. In the present study, we have evaluated the anti-allergic effects of gedunin, a tetranortriterpenoid derived from the seeds of *Carapa guianensis* Aublet, in a *in vivo* model of allergic pleurisy. In addition, we investigated the precise mechanisms underlying the suppressive activities of gedunin on T lymphocytes.

Methods and Results:

The intra-peritoneal (i.p.) pre-treatment of previously sensitized C57BL/6 mice with gedunin (0.5mg/kg) impaired total leukocyte (sal 2.7 ± 0.6 vs. ova 6.7 ± 1.5 vs. gedunin 3.8 ± 0.1 x 106 cells, n=7) and eosinophil influx (sal 0.4 ± 0.0 vs. ova 27.2 ± 0.8 vs. gedunin 3.4 ± 0.2 x 105 cells, n=7) into pleural cavities triggered by the intra-pleural (i.pl.) challenge with ovalbumin (OVA, 12.5 µg/cavity). In addition, flow cytometer analysis showed that gedunin pre-treatment inhibited the influx of T CD3+ cells (sal 8.6 ± 2.3 vs. ova 42.1 ± 2.3 vs. gedunin 18.6 ± 1.9 x 104 cells, n=7). In accordance, ELISA assays showed that chemokines which are involved in T lymphocyte recruitment were decreased in the pleural cavities of gedunin pre-treated mice (RANTES: sal 6.2 ± 0.6 vs. ova 90.7 ± 8.7 vs. gedunin 41.3 ± 5.3 pg/ml; MIP-1α: sal 16.2 ± 0.9 vs. ova 36.2 ± 2.7 vs. gedunin 12.0 ± 0.8 pg/ml; MCP-1: sal 20.6 ± 3.3 vs. ova 171.6 ± 7.5 vs. gedunin 16.6 ± 2.0 pg/ml and LTB4: sal 283.0 ± 8.2 vs. ova 383.5 ± 5.9 vs. gedunin 207.6 ± 14.8 ng/ml, n=4). *In vivo* pre-treatment with gedunin reduced the numbers of T CD3+/CD25+ cells in thoracic lymph nodes (sal 10.8 ± 1.3 % vs. ova 21.6 ± 1.1% vs. gedunin 9.2 ± 2.3% of CD3+/CD25+ cells, n=6). Likewise, gedunin pre-treatment (50 µg/ml, 1 h before stimulation) downregulated CD69 (sal 10.3 ± 0.3% vs. ova 88.9 ± 0.8% vs. gedunin 37.8 ± 1.9% of cells, n=4) and CD25 (sal 7.3 ± 0.2 % vs. ova 72.9 ± 2.4% vs. gedunin 10.2 ± 0.7% of cells, n=4) expression on cell surface of isolated T lymphocytes 24 hours after α-CD3 mAb (10µg/ml) stimulation *in vitro*. Moreover, gedunin impaired α-CD3 mAb-induced T lymphocyte proliferation (100% of inhibition, n=5) and IL-2 production (sal 13.6 ± 1.7 vs. ova 66.6 ± 2.8 vs. gedunin 3.6 ± 0.5 pg/ml, n=4). The *in vitro* pre-treatment of T lymphocytes with gedunin also impaired NFAT and NFKB nuclear translocation triggered by α-CD3 stimulation.

Conclusions:

Our results provide evidence that gedunin might contribute to the treatment of allergic inflammatory diseases by impairing T
lymphocyte functions.

Keywords: gedunin, natural product, lymphocyte, allergy

Financial Support: CNPq and FarManguinhos/FIOCRUZ

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Resumo:26-278

INSECTICIDAL ACTIVITY OF OPUNTIA FICUS INDICA LECTIN ON NASUTITERMES CORNIGER

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Objectives:
Biodegradation by termites is a serious problem for wood industry and responsible for crop losses of sugarcane, maize and rice, for example. Environmentally-friendly insecticides for termite control have been searched since the chemical insecticides usually used cause adverse effects in human and environment health. Plant lectins, carbohydrate-binding proteins, have showed insecticidal activity on Nasutitermes corniger, which stimulates the investigation of new plant lectins bioactive on this termite. This work reports the effects of lectin from Opuntia ficus indica (palma forrageira) cladodes (OfiL) on survival and behavior of N. corniger workers and soldiers.

Methods and Results:
Cladode powder was homogenized (16 h, 4 °C) in 0.15 M NaCl. After filtration through gauze followed by centrifugation (4000 g, 15 min), the crude extract was loaded onto a chitin column equilibrated with 0.15 M NaCl. The column was washed with the equilibrium solution. Afterwards, OfiL was eluted from the column with 1.0 M acetic acid and evaluated for protein concentration and HA. Termiticidal activity was evaluated using petri plates with the lower plate covered by filter paper. A filter paper disk impregnated with 200 μL of OfiL (0.1-1.5 mg/mL) was put in each plate. In negative control, papers were impregnated with 0.15 M NaCl. Twenty active termites (16 workers and 4 soldiers) were carefully transferred to each plate; the assay was kept in darkness at 28 ºC. Monitoring of assays was performed daily to detect the death of insects until all termites had died. Bioassay was achieved in quintuplicate. Survival rates (%) were obtained for each treatment. Effect of OfiL on termite behavior was evaluated in Petri plates filled up with 2% agar solution. After solidification, wells are made in agar by the removal of a central cylinder and 10 peripheral cylinders. Filter paper disks soaked with 15 μL of OfiL or 0.15 M NaCl (negative control) were placed in peripheral wells. Twenty termites were then transferred to the central well. Assays were made in triplicate and the following parameters were observed: absence or presence of termites in peripheral wells, construction standards of tunnels in agar and closing by insects of constructed galleries. OfiL (specific hemagglutinating activity of 40) showed stronger termiticidal activity on workers (LC50 of 0.116 mg/mL) and was toxic for soldiers only at 1.5 mg/mL. OfiL did not show repellent or attractive property since the insects built galleries randomly, with no preference or rejection for lectin and negative control. OfiL may promote termite mortality due to its chitin-binding ability.

Conclusions:
The results indicate that OfiL possesses biotechnological potential for use in control of pest termite since it was highly toxic for workers.

Keywords: Opuntia ficus indica, palma, Nasutitermes corniger, lectin

Financial Support: FACEPE, CNPq and CAPES
PHYTOCHEMICAL AND ANTIOXIDANT STUDY OF THE EXTRACTS OF THE STEAM BARK OF ANACRENANTHERA COLUBRINA (VELL.) BRENNAN

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Objectives:
This study examined the phytochemical profile, antioxidant activity and total phenolic content of the extract from the steam bark of Anadenanthera colubrina (Vell.) Brenan, a plant native of Brazil, distributed mainly in the Amazon Forest and Atlantic Rainforest, and popularly known as “angico-branco”. It belongs to the Leguminosae-Mimosoideae family and is used in folk medicine as astringent, purifying, hemostatic, and to treat leucorrhea and gonorrhea, cough, bronchitis and whooping cough, among other uses.

Methods and Results:
Hydroethanol extract and hexane, chloroform, ethyl acetate and hydromethanol fractions were subjected to phytochemical screening using colorimetric methods and the antioxidant activity was evaluated by decreasing the free radical DPPH (2,2-diphenyl-1-picyrylhydrazyl). The Folin-Ciocalteau method was used to quantify the total phenols and the results were expressed as milligram equivalents of gallic acid (mg EGA). The data are mean of three replicates (n = 3) ± standard deviation and ANOVA followed by Tukey’s test were used to determine statistical significance (p < 0.05). The phytochemical analysis detected the presence of relevant secondary metabolites such as alkaloids, catechins, tannins and flavonoids, among others. The highest content of total phenol was found for the ethyl acetate and hydromethanol fractions, with 610.52 ± 53.23 and 390.72 ± 36.82 mg EAG/g extract, respectively. Hydroethanol extract, as well as ethyl acetate and hydromethanol fractions, showed DPPH free radical scavenging activity, with inhibition percentage (IP) of 92.01, 92.09 and 92.44%, respectively, at 30 µg/mL and 60 min.

Conclusions:
The results in the present study shed light on the effectiveness of this specie in folk medicine because the plant extract showed the presence of classes of compounds of well-known biological effects. In addition, it presented antioxidant activity against the free radical DPPH, suggesting its activity against free radicals, which are related to several chronic diseases.

Keywords: Anadenanthera colubrina (Vell.) Brenan, antioxidant activity, total phenols

Financial Support: Capes

INHIBITORY EFFECT OF WATER-SOLUBLE LECTIN FROM MORINGA OLEIFERA SEEDS ON TRYSIN ACTIVITY FROM Aedes aegypti Larvae

Santos, N. D. L.; Pontual, E. V.; Napoleão, T. H.; Coelho, L. C. B. B.; Navarro, D. M. A. F.; Paiva, P. M. G.
Objectives:

Aedes aegypti is the vector of etiologic agents of dengue fever, which is re-emerging in tropical and sub-tropical regions. Since there are no effective vaccines, vector control remains as the sole form to minimize dengue incidence. Lectins are carbohydrate-recognizing proteins able to interact with glycoconjugates. A water-soluble and chitin-binding lectin (WSMoL) isolated from Moringa oleifera seeds showed larvicidal activity on A. aegypti fourth-stage (L4) larvae (Chemosphere 77; 934-938, 2009). Mechanisms of insecticidal effect of lectins may involve binding to digestive enzymes. This work evaluated the effect of WSMoL on trypsin-like activity from larvae gut.

Methods and Results:

M. oleifera powdered seeds were homogenized (16 h, 4 °C) with distilled water. After filtration and centrifugation (3000 g, 15 min), the extract was treated with a 60% saturated ammonium sulphate. The precipitated fraction was loaded onto a chitin column equilibrated with 0.15 M NaCl. After extensive washing with the equilibrating solution, WSMoL was eluted with 1.0 M acetic acid and dialyzed against distilled water. Groups of fifty A. aegypti L4 were collected and immobilized by cooling at 4 °C for 10 min. The gut of each larva was removed and immediately homogenized with Tris buffer (0.1 M Tris-HCl pH 8.0 containing 0.02 M CaCl2 and 0.15 M NaCl) using a 2 mL tissue grinder. The homogenate was centrifuged (9,000 g, 4 °C, 15 min) and the supernatant (L4 gut extract) was collected and evaluated for protein concentration. Trypsin-like activity was determined incubating L4 gut extract with 8 mM N-benzoyl-DL-arginyl-\(\rho\)-nitroanilide (BApNA) and followed by measurement of absorbance at 405 nm. One unit of trypsin activity was defined as the amount of enzyme that hydrolyzes 1 \(\mu\)mol of BApNA per minute. The activity of L4 gut trypsin was also determined after incubation (30 min, 37 °C) with WSMoL (25–50 \(\mu\)g) in Tris buffer. L4 gut extract showed trypsin-like activity (272 mU/mL) which was inhibited by WSMoL in a dose-response relationship. Highest inhibition was 51.7% (50 \(\mu\)g of WSMoL). Lectins are able to block enzyme by binding to the sugar moiety of glycosylated enzymes or binding other sites than the substrate binding site. It was previously reported that WSMoL was able to promote morphological changes in L4 like hypertrophy of the segments, increased gut volume and absence of epithelial layer delimiting the gut (Chemosphere 77; 934-938, 2009).

Conclusions:

The results indicate that the larvicidal activity of WSMoL may also be linked to inhibition of L4 gut trypsin. This work is part of a series of efforts to understand the larvicidal mechanism of WSMoL.

Keywords: Moringa oleifera, trypsin, Aedes aegypti, larvicide, protease inhibition

Financial Support: FACEPE, CNPq and CAPES
Objectives:

Since Pyrostegia venusta is used in folk medicine for treatment of pigmentation disorders (Quimica Nova, 23: 42, 2000), the objective of this work was to evaluate the effect of the hydroalcoholic extracts from the leaves and flowers of P. venusta on the melanogenesis process.

Methods and Results:

Powered dry leaves (100 g) and flowers (100 g) of P. venusta were extracted by turbolysis with EtOH/H2O (70:30 v/v) at room temperature. The hydroalcoholic extracts were concentrated under reduced pressure and then lyophilised, affording 9.7 (leaves) and 19.0 g (flowers).

Murine B16F10 melanoma cell line was cultured in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum and 1% penicillin/streptomycin (10000U/100 µg/mL) at 37 ºC with 5% CO2 in a humidified atmosphere. Briefly, 7 x 103 cells were added in each of a 96-well plate. After 24 hours cells were exposed to extracts of P. venusta at several concentrations (0.01 – 3.0 µg/mL) for 96 h and cell viability was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. All tested concentrations were not able to cause significant cell death. Then, melanin content was quantified to assess spontaneous melanogenesis. Cells were added into the wells of a 24-well plate (4x104 cells/well). After 24 h, extract samples in different concentrations and kojic acid (1 mM, as negative control) were added to the cells and the plates were incubated at 37 °C in 5% CO2, humidified atmosphere for 4 days. Cells were then lysed in 500 µL of a NaOH (1N) in 10% DMSO solution at 80 ºC for 1 h. Relative melanin content was determined by measuring absorbance at 470 nm in a microplate reader. Leaves extract increased the melanin content showing maximum effect of 33.3 ± 3% (3 µg/mL). Similar response was observed with flower extract, which enhanced the melanin content with maximum effect of 23.4 ± 3.6% (0.1 µg/mL). Since tyrosinase is the rate-limiting enzyme for melanin biosynthesis, the effect of the extracts of P. venusta on tyrosinase activity was determined according to a previously described method with slight modification (Biol. Pharm. Bull, 31:154, 2008). 10 μL of each sample solution in different concentrations and 20 µL of mushroom tyrosinase (500 U/mL in phosphate buffer, pH 6.5), were added to 170 µL of a mixture of L-tyrosine solution (1 mM), phosphate buffer (50 mM, pH 6.5), and distilled water (10:10:9, v/v) in a 96-well microplate. Plate was incubated at 37 °C for 40 min and the absorbance was measured at 490 nm on a microplate reader. Tyrosinase activity level was not affected by the presence of the extracts in the reaction mixture. This result suggests that P. venusta is increasing the melanin content not by interfering with tyrosinase activity.

Conclusions:

Tested concentrations of both extracts from Pyrostegia venusta were able to increase melanin content, without causing death or increase in cell proliferation. Our results support the folk use of the plant in the treatment of several hypopigmentation disorders. Further investigations are necessary in order to elucidate its mechanism of action.

Keywords: Melanogenesis, Pyrostegia venusta, Vitiligo

Financial Support: Grupo Herbarium/Farmoquimica, CAPES, CNPq, FINEP, UFPR.
Objectives:

The present study was designed to investigate the endothelium-dependent vasorelaxation effect of 6-[(E)-styryl]-pyron-2-one (Pyrone-198), a natural styrylpyrone isolated from ethanolic extracts of the green fruits of *Aniba panurensis*.

Methods and Results:

All protocols of this study were approved by the CEPA/LTF (protocol nº 0109/10). Isolated rat superior mesenteric rings (1-2 mm) were suspended by cotton threads for isometric tension recordings in a Tyrode’s solution at 37 ºC, gassed with a 95% O2 and 5% CO2, under a resting tension of 0.75g and the isometric tension changes were measured continuously by a sensitive myograph system. In rings pre-contracted with phenylephrine (Phe) 1 µM, Pyrone-198 (1nM - 1µM) caused concentration-dependent relaxation in presence of functional endothelium (pD2= 5.67 ± 0.14M, n=7), this effect was significantly inhibited after removal of the endothelium (pD2= 4.19 ± 0.06 M, n=7). The endothelium plays an important role in the control of vascular tone inducing vasorelaxation by muscarinic receptors activation, synthesis and release of EDRFs, including NO and PGI2. The vasorelaxant effect induced by pyrone-198 was significantly inhibited in the presence of eNOS inhibition (L-NAME 100µM; pD2= 4.25 ± 0.10M, n=6), in the presence of an inhibitor of the nitric oxide-sensitive guanylate cyclase (ODQ 10 µM; pD2= 3.97 ± 0.08M, n=6) and a scavenger of nitric oxide (PTIO 300µM; pD2= 4.80 ± 0.11M, n=6). The NO precursor, L-arginine (1mM), completely reversed the effect of L-NAME (pD2= 5.57 ± 0.14 M, n=6). Incubation with atropine (1nM) or indomethacin (1µM) not changed the vasorelaxant response induced by pyrone-198. In order to characterize the possible role of K+ channels in the relaxant effects of pyrone-198, we pre-incubated with 3mM TEA, a non-selective K+ channel blocker, in preparations with endothelium. In endothelium intact preparations the relaxant effect induced by pyrone-198 was not modified in rings with endothelium, suggesting that the K+ channels are not involved in the relaxant response elicited in these conditions.

Conclusions:

These results suggest that pyrone-198 induced endothelium-dependent vasorelaxation, is likely mediated by the NO/cGMP pathway.

Keywords: Pyrone-198, vasorelaxation effect, superior mesenteric artery rings, endothelium-dependent, *Aniba panurensis*

Financial Support: CNPq and CAPES

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**Resumo:**

ANTAGONISM OF THE CARDIOTOXIC EFFECT OF ISOLATED TOXINS FROM *B. JARARACUSSU* VENOM BY SURAMIN

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Objectives:

Snakebites caused by Bothrops species induce local damage with edema, hemorrhage and myonecrosis. These effects are not completely or poorly neutralized by antivenoms. In previous works we have investigated the *in vitro* ability of suramin, heparin...
or dextran sulfated to inhibit the cardiotoxic effect of *B. jararacussu* crude venom. In the present investigation we expanded this investigation by studying the ability of suramin to neutralize the cardiotoxicity of two isolated toxins from *B. jararacussu* venom named bothropstoxin I (BthTX I) and bothrops toxin II (BthTX II).

Methods and Results:

Adult male rats weighting 200-250g were anesthetized and euthanized in accord with animal care and of protocols of CEUA-UFRJ. Their hearts were isolated, placed in the Langendorff preparation, bathed and continuously perfused (2-5 mL/min) with a appropriated physiological saline solution at 37°C to evaluate the toxin effects or cardiotoxicity. The cardiac tension and the electrocardiogram (ECG) were continuously recorded and we evaluated the changes on QRS complex, cardiac frequency, perfusion pressure, PR interval. The rate of Creatine Kinase (CK) release was continuously determined The venom of *B. jararacussu* (10 &mu;g/mL) and the isolated toxins induced a gradual negative inotropic effect, decreased heart tension, and altered ECG waves, changing the others parameters markedly. The addition of suramin (10 &mu;M) diminished substantially the cardiotoxic effects of BthTX I, maintaining basal levels of CK release, whereas there was no effect against BthTX II. In relation to perfusion pressure, again, suramin inhibited 100% of BthTX I effect, but had no effect against BthTX II. On the other hand, the reduction in tension on the cardiac contraction was not completely prevented by the addition of suramin, although there has been approximately 40% of inhibition of BthTX I. Interestingly, whereas BthTX II alone caused a decrease of cardiac tension down to 0% of control values in less than five minutes of perfusion, when suramin was added to the bath solution the abolition of cardiac tension took approximately 60 minutes to occur. After perfusion the hearts were removed from the Langendorff preparation, the ventricles gently sliced and stained with triphenyl tetrazolium chloride (TTC) 1%. The area without injury was marked with intense red, while the infarcted areas remained unstained. TTC staining showed large infarcted areas following exposure to the venom, and protection by suramin.

Conclusions:

Our studies suggest that suramin, a polyanion, presents characteristics that can prevent some of BthTX I cardiotoxic effects, but shows almost no effect against BthTX II, showing the difference between the isolated toxins from *Bothrops jararacussu*.

Keywords: Cardiotoxicity, Langerdorff, Bothrops jararacussu, suramin, isolated toxins

Financial Support: PRONEX, CNPq, CAPES, FAPERJ

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Resumo:26-284

**HYPERTENSION AND METABOLIC CHANGES ASSOCIATED WITH DEVELOPMENTAL PROGRAMMING: EFFECTS OF VITIS VINIFERA GRAPE SKIN EXTRACT**

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Objectives:

Previous studies have demonstrated that a grape skin extract (GSE) of *Vitis vinifera* has antihypertensive, antioxidant and vasodilator effects. The aim of the present study is to evaluate the possible protective effect of GSE on the development of cardiovascular risk factors and insulin resistance (IR) in male offspring whose mothers were subjected to a high-fat diet during lactation.

Methods and Results:
We used adult offspring (male wistar rats) at 3 or 6 months whose mothers were fed a high fat diet (HF) during lactation. Four groups of female rats were fed: control diet (7% fat); GSE (7% fat plus 200 mg/Kg/day GSE orally); HF (24% fat); HF+GSE (24% fat plus 200 mg/Kg/day GSE orally) during lactation. From weaning onwards, all male offspring were fed a control diet and sacrificed at 3 or 6 months of age. Systolic blood pressure (SBP) was measured by plethysmography in adults (age 45 days to 180 days). The body weight (BW) and adiposity were measured from the age of 7 days until the ages of 90 or 180 days. We determined plasma total nitrite, cholesterol, triglycerides, glucose, insulin and insulin resistance (IR) was calculated by HOMA IR. The expressions of insulin cascade proteins IRS-1, GLUT-4 and AKT were determined in soleus muscle. There was no significant difference in BW (g) from HF group (298±30; 403±12; 3 and 6 months respectively), control (271±12; 375+11), GSE (270±7; 398±12.6) and HF+GSE (306±12; 402+9), however the adiposity of HF group (7.7±0.7; 13±0.9; respectively) was higher than the control (5.6±0.8; 9.7±0.8) GSE (6±0.9; 10±0.7) and significantly reduced in HF+GSE (6.9±0.5; 8±0.8). The SBP (mm Hg) of HF group (168±3; 150±1; 3 and 6 months respectively) was higher than the control (109±4; 136±4), GSE (125±3; 125±1) and significantly reduced in HF+GSE (122±4; 139±1.1). Nitrite levels (mMol/mg ptn) were decreased in HF group (0,06±0,05; 0,08±0,07) compared to control (0,22±0,03; 0,19±0,03), GSE (0,24±0,09; 0,19±0,02) and HF + GSE (0,14±0,01; 0,19±0,02). Increased plasma triglyceride (mg/dL) was observed in HF (50±2,7; 40±2,8; 3 and 6 months, respectively) compared to control (26±3,1; 33±5,8), GSE (29±6; 38±1) and was reduced in HF+GSE (40±2,0; 11±3,2). The glucose (mmol/dL) was significantly increased in HF (100±9; 88±2; 3 and 6 months respectively) compared to controls (77±5; 76±1), GSE (82±3; 78±5) and HF+GSE (83±5; 82±1). Insulin levels (µU/mL) were increased in HF group (11±1; 45±3.4; 3 and 6 months, respectively) compared to control (9.3±1; 31±3), GSE (9±1; 20±3) and HF+GSE (7±0.1; 32±3). The IR was increased in HF (3±0.2; 12±0.1; 3 and 6 months, respectively) compared to control (1.8±0.1; 6±0.2), GSE (1.8±0.08; 4±0.1) and HF+GSE (1.4±0.1; 6.5±0.1). Finally, the increased expressions of IRS-1, AKT and GLUT-4 in HF group were significantly reduced by treatment with GSE (P< 0.05).

Conclusions:

The results suggest that GSE appears to protect adult offspring, whose mothers were subjected to high-fat diet during lactation, against the development of hypertension, elevated glucose levels and IR, possibly by increasing NO and insulin sensitivity.

Keywords: GRAPE SKIN EXTRACT, HYPERTENSION, INSULIN RESISTANCE, OXIDATIVE STRESS

Financial Support: FAPERJ and CNPq

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Resumo:26-285

EFFECTS OF ESSENTIAL OIL FROM CROTON ZEHNTNERI PAX AT HOFFM. AND ITS CONSTITUENT ANETOL AT THE LOCAL INFLAMMATORY REACTION ON HEALING OF EXPERIMENTAL WOUNDS

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Objectives:

Croton zehntneri is a plant with its origins at northeast of Brazil and it is found in great amounts at the state of Ceará. Its leaves and stalks are popularly used to treat gastrointestinal disorders and scientific studies are being developed in order to prove the therapeutical properties of the essential oil extracted from Croton zehntneri (OECZ). According to previous studies on antinociceptive actions of OECZ it is clear that this compound exerts your actions through different pathways: the essential oil showed anti-inflammatory effects via CNS and antinociceptive actions mediated by prostaglandins. Such results motivate us to research the mechanisms of action associated to OECZ and its anti-inflammatory properties and also to investigate how this essential oil behaves at the tissue healing process.
Methods and Results:

Swiss mice, male, 25-30 g, 12 to 16 weeks-age, were anesthetized by intramuscular injection of ketamin and xilasin 0.2 ml/100 g of weight (60 mg/kg + 10 mg/kg) to each animal. A cutaneous bilateral incision of 1 cm² were made approximately 0.5 cm distant from the medial dorsal line in order to expose the muscular fascia. The region was treated during 5 days with vehicle PF127 10% (w/w) incorporated with the following concentrations of 2%, 10% and 20% of OECZ and anetol in different groups (n = 12). The following control groups were designed in order to evaluate the progression of the healing: pluronic/saline; positive control with fibrase 5 mg; negative control with dexamethasone. Animal were treated twice daily with 0.1 ml of the cited doses. The clinical signs of inflammatory reaction were observed: edema, exsudate, crust and granulation tissue. After that a criteria were assumed to evaluate the wounds by giving scores. At the third day it was observed that 33,3% of the wounds treated with the concentration of 20% of OECZ showed a discrete edema such result being similar to the groups treated with dexamethasone. Anetol at the concentration of 20% showed in 100% of the wounds a intense edema when compared to control group (p < 0.001) (Student Newman-Keuls). The groups treated with fibrase, OECZ 2%, OECZ 10% and the group saline showed lesion with moderate exsudation in comparison to anetol 20% which demonstrated intense exsudate. The groups anetol 20% and OECZ 20% presented intense granulation tissue (p < 0.001) (Student Newman-Keuls).

Conclusions:

The essential oil from C. zehntneri 20% was capable to promote an anti-inflammatory effect when administered topically to open wounds in mice.

Keywords: anetol, croton zehntneri, healing, wounds

Financial Support: FUNCAP

QuebraPagina

Resumo:26-286

I.CINNAMOMEA OIL PROMOTES THE HEALING ACTIVITY IN SKIN PRESSURE ULCER

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Objectives:

Many popular medicinal plants have been used indiscriminately without a solid scientific basis for their efficacy, as is the case of I.cinnamomea oil (ICO). This plant is used to treat inflammation. In this work we examined the effects of ICO on the healing of a skin pressure ulcer induced in Wistar rats.

Methods and Results:

The ulcer was induced by cycles of ischemia-reperfusion by applying and removing a rectangular permanent magnet (40 x 25 x 10 mm) to a dorsal region of rat skin under which a ferromagnetic steel plate was implanted. Seven male Wistar rats (6-8 weeks) were randomly allocated to 3 groups: control, ICO (4mg) and Diclofenac (0.04mg) treatment. To clarify the mechanism of the effect of ICO, area of the lesion, exudates volume, number of migratory cells and nitrite level were investigated. The ischemia-reperfusion promoted a lesion area of 87.33 ± 15.32%, while the lesion area of the ulcers treated with Diclofenac and ICO were decreased in 22 ± 2.7% and 42 ± 0.98%, respectively. The volume of exudates decreased (6.5 ± 3.5; 1.83 ± 0.23; 1.25 ± 0.35, respectively to control, Diclofenac and ICO). The ICO oil markedly inhibited the cellular migration to lesion area (6.5 ± 3.5; 1.83 ± 0.23; 1.25 ± 0.35, respectively to control, ICO and Diclofenac). Therefore, the level of nitrite was also significantly decreased by ICO (10.45 ± 0.91; 3.02 ± 3.14; 3.13 ± 1.66, respectively to control, ICO and Diclofenac). All procedures involving animal care and experimentation were performed in accordance with guidelines of the Ethical Committee for Research with Experimental Animals of the UFPA (BIO001-09).
Conclusions:

These data suggest that ICO is a powerful healing to skin pressure ulcer methods due to inhibit the first inflammatory phase of healing process.

Keywords: Healing, inflammation, skin pressure, ulcer pressure, ischemia-reperfusion

Financial Support: CNPq, CAPES, UFPA

CELLULAR ASPECTS OF SKIN-HEALING ACTIVITY INDUCED BY A PROTEINASE FRACTION FROM VASCONCELLEA CUNDINAMARCENSIS (CARICA CANDAMARCENSIS)

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3 Bioquímica e Imunologia / Instituto de Ciências Biológicas, UFMG

Objectives:

Prior studies demonstrated that proteins from latex of C. candamarcensis, syn V. cundinamarcensis (Voucher # 15063, University of La Serena, Chile) act as mitogen on fibroblasts and epithelial cells (Planta Med. 69:926, 2003; Planta Med. 71:1, 2005). P1G10, a fraction containing cysteine proteases recovered after Sephadex G10 chromatography of the latex, demonstrated angiogenic effect and healing activity on scarified skin at 0.1% concentration, without adverse local irritation or systemic toxicological action after a prolonged period of use (unpublished). In this study, we present results to better characterize the pathway of this protein mixture as a healing wound skin.

Methods and Results:

Hairless mice of both sexes (8 weeks) were divided in 2 groups (n=10). Two dermal abrasions were induced by rubbing a nail file on the cervical and caudal dorsal region (2 cm²) per animal. Each area was treated with 0.1% P1G10 or the fraction without proteolytic activity (inhibited by Iodoacetamide ligation - P1G10-IAA), both incorporated into a hydrophilic vehicle (Polawax®). The wounded area was covered with gauze and adhesive hypoallergenic tape (Higipore®) for 4 h. The treatment was applied at 48 h intervals until one of the affected sites was healed. The number of healed areas measured at different intervals was compared to the total number of wounded area at day 0 and expressed as % healing. A tissue section was removed at the end or 10 days after of healing wound. Histological analysis, after hematoxylin-eosin and Gomori thricrome stained, were performed to evaluate the new organization of tissue treated. Cellular proliferation was determined by immunohistochemical method. Samples of affected tissue were collected after 2 and 4 days of treatment, prepared and incubated with antibody anti-CDC47. The proliferative index was calculated by counting the positive nuclei for CDC47 staining (500 cells/lesion). As described before, 0.1% P1G10 promotes faster healing (3 fold, at 8th day of treatment) compared to a control containing vehicle alone. The same level of healing was observed with 0.1% P1G10-IAA (p

Conclusions:

Thus, we demonstrated that the wound healing activity of P1G10 in scarified lesions is independent of it proteolytic activity and, probably, explained by the epithelial cell proliferation stimulus. Moreover, the scars showed better organization, corresponding to more advanced levels of healing.
**Resumo:**

**PHYTOCHEMICAL PROFILE AND ANTIOXIDANT ACTIVITY OF **Caryocar coriaceum** Wittm. HYDROETHANOLIC AND METHANOLIC EXTRACTS

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2 Departamento de Química Biológica, URCA

**Objectives:**

*Caryocar coriaceum* Wittm. (pequi) fruits and leaves have been secularly employed by traditional medicine from Northeastern Brazil in the treatment of pain, skin inflammation and respiratory affections. In line with this, we aimed to examine the presence of secondary metabolites (tannins, alkaloids, flavonoids and phenolic compounds) in extracts from pequi leaves as well as their potential antioxidant activity, in order to evaluate their therapeutic potential.

**Methods and Results:**

*C. coriaceum* hydroethanolic (CCHE) and methanolic fraction (CCME) extracts were obtained from pequi tree leaves collected in Araripe plateau, Ceará State, Brazil. Qualitative phytochemical analysis from both extracts was performed according to Matos' method (Introdução à Fitoquímica Experimental, Ed. UFC, 1997). The quantification of phenolic and flavonoid compounds was performed by HPLC-DAD. Antioxidant activity from vehicle ethanol, CCHE and CCME (concentrations ranging from 6.25 to 50 μg/mL, n = 4) was evaluated by DPPH free radical scavenging method (Plant. Sci. 153:1161, 2002), using ascorbic acid (AA) as positive control (n = 4). The protective effect of FeSO4-induced lipid peroxidation in rat brain in the absence or presence of vehicle ethanol, CCHE and CCME (10 and 100 μg/mL, n = 3) was evaluated using TBARS method (Anal. Biochem. 95: 351, 1979). For *in vitro* studies, statistical analysis were performed by one-way ANOVA followed by Student-Newman-Keul test, when appropriated. Phytochemical analysis revealed the presence of pyrogallic (CCHE) and hydrolisable (CCME) tannins, phenolic compounds, flavonoids (flavones, flavonols and xanthones) and phenolic compounds. Alkaloids were not detected. HPLC analysis showed a variety of compounds in both extracts, including gallic acid, chlorogenic acid (majority for CCME), caffeic acid, rutin (majority for CCHE) and quercetin, important antioxidant molecules. According to in vitro studies, the extracts exhibited strong DPPH scavenging activity in all concentrations tested (P < 0.001). The IC50 estimated for CCME, CCHE and AA were, respectively, 7.65 ± 1.053, 11.90 ± 3.883 and 1.163 ± 0.085 μg/mL. Both CCHE and CCME at concentration 100 μg/mL were also capable to reduce FeSO4-induced lipid peroxidation in rat brain when compared to non-treated group (124.4 ± 19.2 and 382.9 ± 143.2 nmol MDA/g tissue, respectively, vs 1256 ± 131.9 nmol MDA/g tissue, P < 0.01).

**Conclusions:**

These findings demonstrated that both CCME and CCHE are important sources of antioxidant molecules and, in comparison with data from literature, their flavonoid and phenolic compounds may be involved in the antioxidant effect observed in *in vitro* assays.

**Keywords:** Antioxidant activity, DPPH, Caryocar coriaceum, Flavonoids, Phenolic compounds

**Financial Support:** CNPq; FUNCAP
CHEMICAL COMPOSITION AND ANTI-INFLAMMATORY ACTIVITY OF THE ESSENTIAL OIL OF SCHINUS TEREBINTHIFOLIUS RADDI (ANACARDIACEAE) FRUITS COLLECTED IN MATO GROSSO DO SUL.

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Objectives:

Schinus terebinthifolius Raddi (Anacardiaceae), popular known as “Brazilian pepper” or “pink-pepper”, exhibit a large spectrum of biological and pharmacological properties, such as for treatment of uterine inflammations (Rev. Bras. Ginec. Obstetr; 25, 95, 2003) and antioxidant activity (Fitoterapia; 74, 91, 2003). The essential oil of S. terebinthifolius was available commercially and these plants were included in Brazilian Pharmacopoeia (Rev. Bras. Farmacog.; 16, 408, 2006). Previous preclinical studies confirmed the anti-inflammatory, anti-allergic, antiulcer and wound healing effects (Int. Immunopharmacol; 8(11), 1552, 2008) from plants collected in different places of the world. The aim of the present study was to investigate the chemical composition and the effects of oral treatment with essential oil from S. terebinthifolius fruits (EOST) (collected in Mato Grosso do Sul) in experimental models of inflammation in rats.

Methods and Results:

The essential oil (EOST) from fruits was extracted by hydrodistillation in Clevenger and analyses by CG/MS. Male Wistar rats (n=6 per group) received EOST (3-200 mg/kg, p.o.), DEXA (0.5 mg/kg, s.c.) or vehicle (10 ml/kg, p.o.) and 1h after an injection of carrageenan (300 µg/in the right paw) or Complete freund adjuvant (CFA, 20 µl/in the right paw). The oedema were measured before and 0.5-4h or every day for 7 days after induction of inflammation using a digital micrometer. In air pouch model, rats were injected with 4 ml of sterile air (s.c.) and 2 mL on the back on day 0 and on day 3, respectively. On day 6, mice were treated with vehicle, EOST (100 mg/kg, p.o.), DEXA (0.5 mg/kg, s.c.) one hour prior to Cg injection (0.1%, 0.25 ml). Four hours after Cg, pouch lavage was performed with 2 ml of heparinized (10 UI/ml) PBS. Leukocytes that migrated were counted on Neubauer chamber and protein levels determined in the lavage fluid. All procedures were approved by the Institutional Ethics Committee (number 05/2010). The analysis by GC/MS showed 18 identified compounds with a predominance of α-pinene (22.56%), sabinene (15.78%), z-salven (10.69%), α-pinene (10.52%), α-funebrene (8.82%) and limonene (5.52%). The oral administration of EOST significantly inhibited, in dose dependent manner, the carrageenan (Cg) induced rat paw oedema. The observed inhibitions after two hours of Cg-injection were 57±8% (100 mg/kg) for EOST and 66±6 % for dexamethasone (DEXA). In another model of inflammation, the oral administration of EOST (100 mg/kg) reduced 56±11 % while DEXA inhibited about 91±5 % the Cg-induced leukocyte migration in the air pouch model. Finally, in the persistent inflammation induced by CFA, the daily oral treatment with EOST (200 mg kg-1) inhibited 33±6 % in 4 days after injection of CFA.

Conclusions:

The essential oil (EOST) exhibited a marked anti-inflammatory activity and studies will be performed to elucidate the active compound and mechanism by which exerts this effect.

Keywords: anti-inflammatory activity, essential oil, Schinus terebinthifolius

Financial Support: Fundect, CNpq and FCA, FCS – UFGD
EFFECT OF *MELISSA OFFICINALIS* EXTRACT ON IN VITRO CEREBRAL ACETYLCHOLINESTERASE ACTIVITY OF RATS

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Objectives:

Alzheimer’s disease (AD) is an age-related neurodegenerative disease. Brain aging is known to be related to excessive neuronal loss, decrease in ACh level, increase in inflammation and oxidative stress (OS). A number of drugs used to treat AD has been shown to produce several side effects and yield relatively modest benefits. So, extensive researches are in progress to identify new effective drugs that are free of undesirable side effects. Considering the important role of OS in several neurological diseases such as AD, and the innumerable benefits described for *M. officinalis* in previous studies, we determine the chemical composition and potential inhibitory effect of *M. officinalis* crude extract (MOCE), ethyl acetate fraction (MOEA) and purified compounds (gallic acid and quercetin) on the AChE activity.

Methods and Results:

Chemical composition of MOEA from *M. officinalis* extract was analyzed by HPLC. For the AChE assay, rats were killed and the cerebral tissue was rapidly dissected and placed on ice. Tissues were homogenized in cold 50 mM Tris–HCl, pH 7.5 (1/10, w/v). The homogenate was centrifuged for 10 min at 4,000 X g to yield a pellet that was discarded and a low-speed supernatant (S1) that was used for the AChE assay. The reaction mixture (2 ml final volume) was composed of 100 mM phosphate buffer pH 7.5, 1 mM 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB). The method based on the formation of yellow anion 4,4'-dithio-bis-2-nitrobenzoic was measured by absorbance at 412 nm during 5 min at 25°C. An aliquot of 100 μL of S1 was pre-incubated in the presence or absence of plant extracts (0, 1, 10, 100 and 1000 μg/mL) or compounds (quercetin or gallic acid 0, 0.1, 1 and 10 μg/mL) for 20 min. The reaction was initiated by adding 0.8 mM acetylthiocholine iodide. The enzyme activity was estimated in terms of percentage change in absorbance compared to the control. Physostigmine (Eserine) 1 μM was used as the reference standard. According to HPLC analysis, we demonstrated the presence of rutin (16.81 ± 0.03%), quercetin (19.22 ± 0.01%), gallic acid (4.91 ± 0.04%), chlorogenic acid (2.63 ± 0.10%) and caffeic acid (5.32 ± 0.09%). MOAE did not change AChE activity. Conversely, MOEA significantly inhibited this enzyme when compared with control in a concentration dependent manner. Quercetin or gallic acid did not cause any AChE inhibition.

Conclusions:

Although the inhibitory effect of a minority compound can not be ruled out, the effect observed for MOEA could be attributed to the synergistic effect or interaction between different constituents of the fraction. This data indicate that *M. officinalis* can be considered as potential alternative medicine for the AD treatment. Moreover, this result is in accordance with literature data that also demonstrate AChE inhibition by crude extracts from *M. officinalis*.

Keywords: Alzheimer's Disease, Acetylcholinesterase, *Melissa officinalis*, Oxidative Stress

Financial Support: The financial support CAPES and CNPq is gratefully acknowledged.

EVALUATION OF LEAVES CRUDE EXTRACT OF *RUDGEA VIBURNOIDES* (CHAM.) BENTH. (CONGONHA-DE-BUGRE) ON RENAL FUNCTION IN RATS WITH NEPHROTOXICITY INDUCED FOR GENTAMICIN

Financial Support: The financial support CAPES and CNPq is gratefully acknowledged.
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Objectives:

*Rudgea viburnoides* is a cerrado plant used in Brazilian folk medicine as antihypertensive, antirheumatic, diuretic and blood depurative. In this study, we have investigated the effect of leaves ethanol crude extract of *Rudgea viburnoides* (EBRv) on renal function in rats with nephrotoxicity induced by gentamicin (GT).

Methods and Results:

Male Wistar rats (200-280 g) were kept in metabolic cages for 8 days and received GT (Gentatec®, 80 mg/kg/day, sc) or NaCl 0.9% (control, CTL; 1ml/kg/day, sc) for 5 days. The EBRv (50 mg/kg or 200 mg/kg) or saline (NaCl 0.9%, CTL) were administered by gavage for 7 days at 9 am and 4 pm. The animals were divided into 6 experimental groups: a) CTL CTL (n=6); b) GT CTL (n=6); c) EBRv 50 CTL (n=8); d) EBRv 50 GT (n=6); e) EBRv 200 CTL (n=7); f) EBRv 200 GT (n=7). Samples of urine and of plasma were collected. The parameters evaluated were body weight, proteinuria, glomerular filtration rate (GFR), plasma and urinary creatininna and urinary flow. As expected, GT induced renal injury was characterized by GFR decrease (0.38 ± 0.04 vs 0.92 ± 0.3 ml/min in control group), plasma creatinine increase (1.32 ± 0.1 vs 0.69 ± 0.3 mg/24 h in control group), polyuria (15.3 ± 2.4 vs 6.3 ± 1.3 ml/24h in control group) and proteinuria (33.9 ± 7.1 vs 13.9 ± 3.3 mg/24 h in control group). In the rats with acute tubular necrosis, EBRv (50 mg/kg or 200 mg/kg) kept the body weight (0.2 ± 13.3 g; 17.7 ± 5.7 g), urinary flow (4.7 ± 1.0 ml/24 h; 7.0 ± 1.9 ml/24 h), proteinuria (8.2 ± 1.4 mg/24 h; 10.7 ± 2.2 mg/24 h) and GFR (1.1 ± 0.4 ml/min; 0.64 ± 0.2 ml/min) similar as in the control group (11.7 ± 3.3 g; 6.3 ± 1.3 ml/24 h; 13.9 ± 3.3 mg/24 h; 0.92 ± 0.3 ml/min; respectively), at the doses used. However, in contrast to the literature (Lat Am J Pharm 29: 30, 2010), the EBRv administration did not produce diuresis in normal rats.

Conclusions:

Our results indicate that EBRv may prevent acute necrosis tubular installation induced by gentamicin but would not greatly affect renal parameters.

Keywords: congonha-de-bugre, gentamicin, renal function

Financial Support: Support: CNPq, UFG

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Resumo:26-292

**FREE RADICAL SCAVENGING AND ANTI-INFLAMMATORY ACTIVITIES OF ESSENTIAL OIL OBTAINED FROM TRICHILIA SILVATICA DC. (MELIACEAE)**

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³ Faculdade de Ciências Agrárias, UFGD
Objectives:

Trichilia silvatica DC. (Meliaceae), commonly known as “cachúa”, “cachúa-blank”, is a small evergreen tree which grows in Mato Grosso do Sul, Brazil. Some plants from this genus are used in Brazilian folk medicine for the treatment of rheumatism and fever, as well as emetic and purgative agents. Among other, antiviral, analgesic and insecticidal activities have been described with extract of genus Trichilia (Phytotherapy Research; 6, 38, 1992; American Chemical Society; 8, 95, 1989). The aim of this study was free radical scavenging and anti-inflammatory activities evaluation of the essential oil of T. silvatica leaves.

Methods and Results:

The essential oil (EOTS) from the leaves of T. silvatica was extracted by hydrodistillation in Clevenger and analyses by CG/MS. Free radical scavenging activity of the essential oil were determined by a spectrophotometric method based on the reduction of a methanol solution of 2, 2-diphenyl-1-picrylhydrazyl free radical (DPPH) (Blois, 1958). Male Wistar rats received EOTS (100 mg/kg, p.o.), DEXA (0.5 mg/kg, s.c.) or vehicle (10 ml/kg, p.o.) and 1h after an injection of carrageenan (300 µg/in the right paw). The oedema were measured before and 0.5-4h after induction of inflammation using a digital micrometer. All procedures were approved by the Institutional Ethics Committee (number 05/2010). Main compounds identified of essential oil were sesquiterpenes such as α-Selinene (15.21%), viridiflorene (8.90%), espatulenol (8.40%), β-caryophyllene (8.01%), δ-cadinene (7.39%), γ-cadinene (6.25%) and β-copaene (5.21%) were the main components, comprising 59.37% of the essential oil. The essential oil showed moderate free radical scavenging activity with IC50 of 174.8 µg mL-1, compared with the BHT (16.8 µg mL-1). The oral administration of essential oil (100 mg kg-1), significantly inhibited the carrageenan (Cg) induced rat paw oedema. The observed inhibitions were 54±7% and 49±6% (100 mg kg-1) for EOTS and 68±6% and 66±11% for DEXA after two and four hours (respectively) after Cg-injection.

Conclusions:

The anti-inflammatory effects of T. silvatica essential oil seem to be mainly associated with the high levels of sesquiterpene in the leaves of this plant. Studies will be performed to elucidate the compound responsible by the observed effects.

Keywords: Anti-inflammatory activity, Essential oil, Free radical scavenging activity, Trichilia silvatica

Financial Support: Fundect, CNpq and FCA, FCS – UFGD.

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Resumo: 26-293

EFFECT OF CALEA SERRATA ON GLUTATHIONE-S-TRANSFERASE ACTIVITY OF LARVAE FROM RHIPICEPHALUS (BOOPHILUS) MICROPLUS

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Objectives:

The tick Rhipicephalus (Boophilus) microplus, important cattle ectoparasites, causes great economic losses. Extensive use of insecticide has been induced resistance, which can be related to elevated Glutathione-S-Transferase (GST) activities. It has been suggested that the pesticide may conjugate to glutathione by GST, a detoxification mechanism in arthropods. Recently, n-hexane
extract of *Calea serrata* Less. (Asteraceae) showed an acaricidal activity on larvae of this species. Natural products for GST inhibition have not been explored extensively. Our aim was to investigate the effect of *Calea serrata* on GST activity of larvae from *Rhipicephalus* (Boophilus) microplus.

Methods and Results:

Plant material of *Calea serrata* (leaves and stems) was collected in Porto Alegre, Rio Grande do Sul, Brazil. Air dried and powdered plant material was extracted by maceration with hexane. This extract was evaporated under reduce pressure and treated with acetone, and subsequently filtered and evaporated. *Rhipicephalus* microplus tick larvae were homogenized (1:10) in 100 mM Tris–HCl buffer (5 mM EDTA, pH 8.0) and centrifuged at 15,000×g for 5 min. The supernatant was used for determination of GST activity. The GST activity of the extracts was measured using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. Conjugation of the thiol group of glutathione (6.2 mM) to the CDNB was measured at 340 nm during 5 minutes. Various concentrations of extract *Calea serrata* (0, 0.5, 1.0, 1.5 and 3.0 mg/ml) were incubated with homogenized larvae for 30 min at 25° C. The effect of plant extract on GST enzyme activity was determined. One way ANOVA test were used for the comparison of enzyme activities. *Calea serrata* inhibited significantly GST activity at 3.0 mg/ml (51.34±25.73) about 50% compared with the control (F(8,35)= 7.045, p

Conclusions:

Present findings suggest that compounds of hexane extract from *Calea serrata* may be potential inhibitors of Glutathione-S-Transferase. Considering that higher GST activities would reduce the efficiency of the pesticides, we can suppose that *Calea serrata* may be an adjuvant in tick control.

Keywords: Calea serrata, Rhipicephalus microplus, Glutathione-S-transferase

Financial Support: CNPq, CAPES

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PROPOLIS MODULATES THE ANGIOREGENIC, INFLAMMATORY AND FIBROGENIC COMPONENTS OF INTRAPERITONEAL ADHESION IN MICE.

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Objectives:

Adhesion formation develops in 90% of patients who have undergone abdominal surgery and constitutes a major source of postoperative morbidity and mortality. Attenuation of components of these lesions (angiogenesis, inflammation and fibrosis) represents to prevent or treat adhesion. Propolis, a sticky material produced by honeybees, has been reported to possess multiple biological action including antiinflammatory and anti-angiogenic. Our aim was to study the effects of aqueous extract of green propolis on intraperitoneal adhesion formation in mice.

Methods and Results:

Male Swiss mice were implanted intraperitoneally with polyesther-polyurethane sponge discs to induce adhesion. The animals received daily oral doses (50 or 500 mg/kg). At day 5 postimplantation the animals were killed with an over dose of the anesthetic (ketamin-xylazin) and the implants removed and processed. Hemoglobin content was used as vascularization index, the inflammatory enzymes, myeloperoxidase (MPO) and n-acetyl-β-glucosaminidase (NAG) was the parameter of the
inflammatory response and the level of collagen was used to determine fibrosis. The results showed that the small dose increased Hb (CT 3.476±0.218 n=21; vs PR 4.485±0.482 n=11) and collagen content (CT 1.336±0.035 n=17; vs PR 1.477±0.036 n=5) and decreased the levels of the inflammatory enzymes (CT 10.23±0.508 n=19; vs PR 8.257±0.658 n=13) compared with the control group. Conversely, the high dose decreased the Hb content (CT 3.476±0.0218 n=21; vs PR 2.481±0.242 n=16) and collagen deposition (CT 1.336±0.035 n=17; vs PR 1.196±0.054 n=15) but increased the activity of the inflammatory enzymes (CT 10.23±0.508 n=19; vs PR 12.16±0.638 n=13).

Conclusions:

Our results reveal that propolis extract presents a biphasic effect on the modulation of angiogenic, inflammatory and fibrogenic components of intraperitoneal adhesion induced by sponge implantation.

Keywords: Propolis, Intraperitoneal adhesion, Angiogenesis, Inflammation, Fibrosis

Financial Support: FAPEMIG/CNPq

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**Resumo:**

THE IMMUNE MODULATOR EFFECTS AGAINST EHRLICH SOLID TUMOR OF TREATMENT WITH *TURNERA ULMIFOLIA L.* HYDRO-ALCOHOLIC EXTRACT

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Objectives:

The *Turnera ulmifolia* L. is a species popularly known in Brazil as Chanana. This plant grows spontaneously and it can be found from north to southeastern Brazil (CORREA, 1984 apud GRACIOSO, 2002). The *Turnera ulmifolia* L. decoction has several popular applications such as amenorrhea, pelvic pain and inflammatory processes (AYENSU, 1978 apud GRACIOSO, 2002). Recently, it was reported that the *Turnera ulmifolia* L. treatment induced the immunological response and increasing the immunity cells in blood (RÊGO, 2008). Thus, this study evaluated the potential of *Turnera ulmifolia* L. prophylactic treatment to inhibit the growth of Ehrlich solid tumor in mice.

Methods and Results:

Swiss mice (n=28) were separated into four groups: the animals treated with extract and inoculated with Ehrlich tumor (Extract+tumor group), the animals treated with extract without Ehrlich tumor (Extract group), the animals treated with PBS and inoculated with Ehrlich tumor (Tumor Group) and the animals treated with PBS without Ehrlich tumor (Control Group). The extract was obtained from the dry hydroalcoholic infusion from leaves of *Turnera ulmifolia* L.. The animals were treated daily, by oral administration with PBS or extract (1000 mg/kg) for 10 days, before the Ehrlich tumor implantation, and maintained to 20 days. The tumor cells (1x10³x10³) were implanted on the left footpad and was measured every 2 days tumor-bearing mice. At the end of the *Turnera ulmifolia* L. treatment, the total cell number in lymphoid organs was quantified. The results showed that the *Turnera ulmifolia* L. treatment induced to decrease the tumor (Extract+tumor group: 2.7mm ± 0.3 and Tumor group: 3.0mm ± 0.5), but not significance. However, the tumor footpad weight was lower in the group treated with extract as compared with the tumor group (0.2g ± 0.07 vs 0.4g ± 0.1). The analysis of lymphoid organs showed that animals inoculated with tumor and treated with extract induced the increased of spleen cells when compared with other groups (Extract+tumor group: 27500x10³ ± 3,1; Extract group: 15800x10³ ± 5,0; Tumor group: 19000x10³ ± 4,8 and Control group: 18100x10³ ± 2,0). However, there was not differences in the number of bone marrow and inguinal lymphnodes cells in different groups.

Conclusions:
The results showed that the *Turnera ulmifolia* L. extract treatment induced a reduction of solid tumor in mouse footpad and an increase the spleen cellularity, suggesting an antitumor effect of *Turnera ulmifolia* L. extract with a probable activation of immune response.

Keywords: *Turnera ulmifolia* L., inflammatory, treatment, immunological, Ehrlich

Financial Support: PIBIC/FAPEMA N. and Immunophisiology Laboratory/UFMA.

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Resumo:26-296

COUMARIN TREATMENT DECREASES LUNG INFLAMMATION AND IGE-PRODUCTION IN OVALBUMIN-SENSITIZED MICE.

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Objectives:

Many people have allergic lung diseases. Lung inflammation, mucus production, increase of Th2-cytokines and IgE production are characteristic these diseases. One strategy for the treatment of allergic diseases is the development of new drugs. Coumarin is a compound derived from plants and is known to has antioxidant, anti-inflammatory and immunomodulatory properties. Here we investigated whether coumarin oral treatment could modulate allergic lung disease in ovalbumin-sensitized mice.

Methods and Results:

This model was used to evaluate the effectiveness of the coumarin (3-Chlorocoumarin 97%, MW = 180.59, source: Sigma-Aldrich) treatment in the modulation of IgE production, cell migration in the bronchoalveolar lavage fluid (BAL), mucus production and lung morphological. We used four groups: Saline group (n=10), OVA group (n=10), Dexamethasone group (n=10), Coumarin group (n=10). At weekly intervals female swiss mice (25g) were subcutaneously sensitized twice with OVA/Alum and challenged twice with OVA given intranasally mice. Coumarin treatment (30 mg/kg) was performed 1 h before each OVA-sensitization and OVA challenge. Animals treated with coumarin decreased in the number of total cells (157±23) in BAL when compared with OVA group (860±240). In the analysis of the lungs, we observed that peribronchovascular inflammation HE stained as the mucus production, PAS stained, reduced in coumarin group when compared to animals in the OVA group, where we could observe a large inflammatory infiltrate, as well as a hypertrophy bronchial mucosa and the presence of the mucus. Also the coumarin treatment inhibited in 5 fold dilutions OVA-specific IgE titers (1: 16) as compared with OVA group (1: 512). In animals treated with the standard drug, dexamethasone, was also observed a significant reduction in the inflammatory BAL (64 ± 16), OVA-specific IgE titers (1: 16) and a restoration of the morphology-related pulmonary inflammation and mucus production.

Conclusions:

The present data demonstrated the coumarin downregulates the airway allergic inflammation and IgE production suggesting an immunomodulatory effect in ovalbumin-sensitized mice.

Keywords: coumarin, ovalbumin, lung inflammation, IgE

Financial Support: Propesq/UFRN
CITOTOXICITY AND MELANOGENSES EVALUATION OF CAESALPINIA FERREA EXTRACT ON MELANOCYTES

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Objectives:

Caesalpinia ferrea Mart. commonly known as “juca” or “pau-ferro” is a species from the Leguminosae family widely distributed in northern and northeastern Brazil. This species has several therapeutic properties, which include: antiinflammatory, analgesic, antipyretic and antimicrobial properties that may indicate the presence of pharmacological composites of interest. These properties lead to popular use (folk medicine) of this specie to treat several illnesses. Previously a screen from several extracts made by our group revealed that C. ferrea might interfere in melanogenesis. Melanin is synthesized via an enzymatic cascade controlled by tyrosinase. The aims of this work were to evaluate tyrosinase inhibition by stem bark of C. ferrea and, also, to investigate the citotoxicity and melanogenesis inhibition in Melan-A cells (normal murine melanocytes).

Methods and Results:

Aqueous extract from the bark of C. ferrea was evaluated for their inhibitory effect in vitro on the tyrosinase, using a colorimetric procedure. Citotoxicity studies were performed by the Alamar Blue assay, using a fluorescent procedure. Extract was also investigated for their inhibitory effect on melanogenesis in cultured Melan-A cells. Kojic acid was used as positive control. The melanin dosage was determined using a standard curve made from known concentrations of synthetic melanin, and the absorbance of the samples was determined by spectrophotometer at 405 nm. Extract of the stem bark of C. ferrea showed significant inhibition of tyrosinase, showing IC50 of 65.02 µg/mL. Kojic acid showed IC50 of 5.78 µg/mL. Cell based studies demonstrated a reduction of 59.2 % in melanin content without displaying cell toxicity at 25 µg/mL after 72 hours. Kojic Acid 12.5 µg/mL decreased in 60% the melanin content on Melan-A cells.

Conclusions:

The bark extract of C. ferrea is a promising skin-depigmenting agent, however further investigating in clinical studies should be considered.

Keywords: Melan-A, cytotoxicity, melanin content

Financial Support: CNPq, CAPES and FAPEAM
Objectives:

Evidences suggest that flavonoids have key functions in the regulation of crucial cellular processes, however, their effects have been poorly examined in human pluripotent stem cells. We have tested the hypothesis that neurogenesis induced by all-trans retinoic acid (RA), one of the most powerful morphogens regulating neuronal cell fate commitment, is induced and/or potentiated by flavonoids.

Methods and Results:

In the current study, human embryonic stem (hES) cells and induced pluripotent stem cells (iPS) were used. Neural induction was performed using RA (4μM), the apigenin (API 10 μM), agathisflavone (FAB 10μM), and association of RA + API or FAB for 6 days. Proliferation, cell death and neuronal differentiation were revealed by immunocytochemistry. The flavonoid FAB did not interfere in mitotic rate as evidenced by the similar percentages of cells labeled with the mitotic-specific marker Ki67 in control (2.8±0.4%) and FAB-treated groups (2.9±1.3%). FAB and API were also able, by themselves, to induce neuronal differentiation in hES and iPS cells. On the other hand, FAB reduced cell death in both controls and RA treated groups by 1.8 and 1.7 times, respectively. Furthermore, FAB enhanced neuronal differentiation induced by RA in hES as revealed by the increase in nestin (86±3.5%) and βIII-tubulin (74.2±3.3%) labeled cells when compared to RA treatment only. FAB and API increased the expression of RA receptors α and β in hES and iPS cells, suggesting that the availability of RA receptors is limiting RA-induced neurogenesis in pluripotent stem cells.

Conclusions:

To our knowledge, this is the first report to describe that naturally occurring flavonoids regulate cellular processes such as apoptosis and neuronal differentiation in human pluripotent stem cells.

Keywords: Human pluripotent stem cell, Flavonoid, Differentiation

Financial Support: CNPq, CAPES, Ministério da Saúde/BNDES
Methods and Results:

It was made the expression and extraction of H-Ras protein in cultures of *E. coli*, which is a carrier of plasmids encoding H-Ras mutated or wild. Then, there was the gel electrophoresis in SDS-PAGE 12.5% with the samples. The gel electrophoresis analysis of protein corresponding to the wild protein suggests that the crude extract of *C. longa* promoted a positive modulation of protein expression in the absence of IPTG or in the presence of IPTG (induced by 2 hours) associated with the concentration of 25 μg/mL of the crude extract. When the induction was held for three hours it wasn't found a significant modulation in comparison with the absence or presence of the extract in the concentrations of 25 μg/mL or 500 μg/mL. In the test performed with the mutated protein, only the presence of crude extract at a concentration of 500 μg/mL promoted upregulation in protein expression as well as the presence of IPTG (induced by 2 hours) with the same extract concentration. Results obtained by induction with IPTG for 3 hours were not significant.

Conclusions:

The addition of crude extract in the concentration of 25 μg/mL was able to enhance the positive modulating effect on wild protein in the presence and absence of IPTG. In the test with the mutated protein the crude extract, in the concentration of 500 μg/mL, was able to enhance the positive modulating effect on protein expression in the presence and absence of IPTG. The mechanism that triggered this increase in the protein expression is not known, however it is known that the number of substances present in crude extract of the plant is large and that they possess relevant biological activities.

Keywords: *Curcuma longa*, H-Ras, Protein expression

Financial Support: CNPq

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Resumo: 26-300

GASTROPROTECTION OF DEHYDRODIEUGENOL (DDE) ON ETHANOL-INDUCED GASTRIC MUCOSAL LESION: THE POSSIBLE INVOLVED PARMACOLOGICAL MECHANISMS

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Objectives:

The objectives of the present study is to investigate the mechanisms of action of the gastroprotective effect presented by dehydrolieugenol (DDE) in mice using the ethanol-induced ulcer model.

Methods and Results:

For this male swiss mice were used 20-30 g each group containing 8 animals. The ulcer was induced in all animals by the administration of 0.2 mL of absolute ethanol p.o., animals were pre-treated with Saline (control), DDE 50 mg/Kg, L-NAME 10 mg/Kg (an inhibitor of the NO synthase activity), Glibenclamide 10 mg/Kg (K+ channels blocker) p.o thirty minutes before the induction of ulcer and Indomethacin 10 mg/Kg (an inhibitor of cyclooxygenase) were treated two hours before the induction of ulcer. For the investigation of the mechanisms of action animals were pre-treated with L-NAME, Glibenclamide 15 minutes after the treatment with DDE and thirty minutes after the ulcer was induced, indomethacin was given two hours before the administration of DDE. Thirty minutes after the ethanol administration the animals were sacrificed and the stomachs removed, opened by the greater curvature, and squeezed between two watch glasses to get a better view of the damaged area of the stomach. The total areas of injured and stomachs (glandular face) were determined by planimetry, the stomach being drawn, and the count of the ulcerated area made with the program ImageJ. The damaged area was expressed as a percentage relative to total.
area of the gastric body. The analysis was performed using the One way ANOVA followed by Student Newman Keuls post hoc. Results showed that the administration of ethanol was able to promote lesions in the stomach mucosa (15.61±0.49) and the administration of DDE 50 was able to revert those lesions (4.48±0.36 p

Conclusions:

Accordingly to the results showed the gastroprotection promoted by DDE is not linked to Nitric Oxide or K+-Channels but it’s probably due to the increase of prostaglandins content.

Keywords: Dehydrodieugenol, Ethanol-induced ulcer, L-name, Glibenclamide, indomethacin

Financial Support: Capes and CNPq

LOOKING FOR FUNCTIONAL DOMAINS INVOLVED IN PSD1 DEFENSIN ANTIFUNGAL ACTIVITY

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Objectives:

Psd1 is a pea defensin which exhibits antifungal activity by a not well-known mechanism of action. It exhibits a cysteine-stabilized α/β motif as determined by NMR spectroscopy. The analysis of CSP and 15N relaxation showed its interaction with the fungal component monohexosyl ceramide (CMH), markedly involving some aminoacid residues present in the loop1 (Ala7-Asn17) and turn3 (His36-Trp38) regions. In this work, we evaluated the strength of its interaction by Surface Plasmon Resonance (SPR) and by growth inhibition tests of Candida albicans deficient in the glucosylceramide synthase enzyme (ΔGCS1).

Methods and Results:

Firstly, we realized the expressions of Psd1 and two different site-directed mutants (Psd1Gly12Glu and Psd1His36Lys) in methylotrophic yeast Pichia pastoris, and target genes were under control of AOX1 promoter, strongly induced by methanol addition to the growth medium. Subsequently, the presence of the proteins of interest was confirmed by SDS-PAGE 18%, and expected size bands (5.4 kDa) were observed after Comassie Blue staining. Psd1, Psd1Gly12Glu and Psd1His36Lys were then applied into a Sephadex G-50 Fine column (gel filtration), using 25 mM Tris-Cl pH 7.5 as the elution buffer. Fractions collected were then analyzed by RP-HPLC on a semi quantitative I VYDAK-C8 column, equilibrated with 0.1% TFA and 90% acetonitrile. Proteins were then used in bioassays against WT Candida albicans and in a strain lacking GCS1 (ΔGCS1), the glucosylceramide synthase enzyme. To measure the interaction of Psd1 with CMH, found in the membrane and cell wall of fungi vesicles, we used the technique of Surface Plasmon Resonance (SPR) with the purified proteins (Psd1, Psd1Gly12Glu and Psd1His36Lys). These assays were performed to evaluate the importance of the amino acids Gly12 and His36 (present in a loop1 and turn3 respectively). Psd1 was able to bind to CMH doped Small Unilamelar Vesicles (SUV) with a 60 times higher affinity than for PC-SUV. Site-directed mutants (Psd1Gly12Glu and Psd1His36Lys) were designed and both presented a lower CMH affinity when compared to the wild type Psd1 (Reqs = 65RU and 160RU against 260RU). Psd1-biological activity showed also a CMH dependency since the wild type C.albicans was more susceptible to 10 µM of Psd1 than the ΔGCS1 strain (Psd1Gly12Glu showed only a growth inhibition less than 10% for both strains while Psd1His36Lys provoked growth inhibitions of 25% and 40%, respectively.

Conclusions:

These results give substantial support to the fact that loop1 and turn3 are crucial for CMH binding and biological activity of Psd1.
EFFECTS OF PECAN NUT SHELLS INFUSION ON OXIDATIVE STATUS IN LIVER, KIDNEY AND BRAIN CORTEX OF RATS.

Rech, V. C. ; Zago, A. M. ; do Carmo, G. M. ; Prevedello, C. P. ; de Franceschi, I. D. ; Mott, M. P. ; Mezzomo, N. J. ; Fratton, P. ; Ferigolo, P. C. ; Wannmacher, C. M. D.  
Área de Ciências da Saúde/Curso de Biomedicina, UNIFRA

Objectives:
Phytochemical investigations have shown that pecan nut shells are rich in polyphenols. This by-product of the pecan industry has been used by some people to prevent or even cure diseases. The present study evaluated the role of pecan nut (Carya illinoensis) shells infusion on some oxidative stress parameters in liver, kidney and brain of rats.

Methods and Results:
In this experiment, 24 adult male Wistar rats (200g) were randomly assigned to three groups and treated for 2 months. Control group: the animals drank water; 5% Infusion Group (T5%): animals drank 5% infusion of pecan nut shells; 10% infusion group (T10%): the animals drank 10% infusion of pecan nut shells 10%. All animals drank and had access to standard chow ad libitum. There were no differences between groups in relation to the amount consumed daily. Rats were fasted for twelve hours, weighted and killed by decapitation without anesthesia. Cerebral cortex, kidneys and liver were rapidly excised and homogenized in appropriate buffer, pH 7.4. The homogenates were centrifuged at 800 x g for 10 min at 4 °C and the supernatants were used to measure thiobarbituric acid-reactive substances (TBA-RS) (nmol of MDA per mg of protein), carbonyls content (nmol of carbonyls per mg of protein) and oxidation of 2′, 7′-dihydrodichlorofluorescein (DCFH) (nmol of DCF per mg of protein). Data were expressed as mean ± standard deviation. Comparison between means was performed by one-way ANOVA followed by the Tukey test when the F value was significant (p < 0.05). In the kidney, the effect of infusions significantly decreased the carbonyl content: Control= 2.71 ± 0.29; T5%= 1.78 ± 0.10 (p

Conclusions:
These results suggest that ingestion of pecan nut shell infusion may have partial antioxidant effect mainly in the liver. It is possible that the antioxidant substances, mainly polyphenols, are metabolized in the liver before reaching kidneys and brain.

Keywords: Carbonyl, 2′, 7′-dihydrodichlorofluorescein, oxidative stress , PECAN NUT SHELLS , thiobarbituric acid-reactive substances

Financial Support: Sources of research support: PROBIC/UNIFRA, PROPESQ/UFRGS, FAPERGS.

CHRONIC ORAL TOXICITY OF AQUEOUS EXTRACT OF ARTEMISIA ABSINTHIUM L.
Objectives:
The Aim of this work was to evaluate the chronic oral toxicity of the aqueous extract of *A. absinthium* L. leaves, which is used in folk medicine against digestive problems.

Methods and Results:
*A. absinthium* L. aqueous extract was administered orally, in male and female adult Wistar rats daily. Control group was treated with water and the test group was treated with aqueous extract of plant at doses 1, 10 and 100 mg/kg for 12 consecutive weeks. We did not observe differences in mass gain in all groups neither behavioral changes. After the experimental time, the animals were euthanized by thiopental injection and the blood and stomach, liver and kidneys were collected. To Biochemical analyses were used commercial kits. It was observed that there was variability between the sex and the effect of the plant is not dose-dependent. The lower values of LDL-cholesterol and triglycerides occurred only in females. It could be a specific hormonal response. In the group of males was observed an increase in total cholesterol, HDL-cholesterol, LDL-cholesterol and a decrease of AST (aspartate aminotransferase) at doses of 10 and 100mg/kg. For histological analyses, the organs were fixed in 10% formalin and the histological slides were prepared. In histological sections of stomach, was not observed any lesions, to any group, demonstrating that neither the statement nor the gavage procedure were nocive to this organ. In the females liver in groups at 1 to 10 mg/kg was observed necrosis occurred in isolated cells and at 100 mg/kg, also demonstrate a mild biliary retention. The group of male and females of 100 mg/kg was showed necrosis in hepatocytes located near the portal vein, indicating increased of liver detoxified activity, probably due to the metabolites present in the extract. Renal histological evaluation showed tumefaction and protein in the tubular lumen in males and females, it was more evident at 10 and 100 mg/kg. These results indicate epithelial reactive to toxic substances.

Conclusions:
Biochemical analyses showed that aqueous extract of *Artemisia absinthium* L. not cause intensive or extensive hepatotoxicity and nephrotoxicity in any of the concentrations, even after daily administration for 12 weeks. However, the histopathological results observed in all concentrations demonstrate the potential toxicity of the plant with its constant use. Therefore, the population should be cautious in using this extract daily, and should be avoided by people with liver or kidney disease.

Keywords: losna, medicinal plants, toxicity

Financial Support: PUCPR

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Resumo:26-304

**ABSCISIC ACID, A VEGETAL HORMONE, EXERTS METABOLIC EFFECTS TO PREVENT DIABETES AND INFLAMMATION**

Lellis-santos, C. 1; Gonçalves, A. E. D. S. S. 1; Garofolo, I. C. 1; Santos, L. R. B. D. 1; Pilon, G. 2; Desjardins, Y. 2; Abrams, S. 2; Bordin, S. 1; Marette, A. 2; Curi, R. 1

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2 Université Laval, Université Laval
Objectives:

Abscisic acid (ABA) plays essential role as responsive signaling molecule to abiotic stress in vegetal. Studies have pointed out ABA as a possible molecule to treat metabolic diseases due to its effects on insulin secretion, glucose tolerance and macrophages infiltration. However the mechanism underlying these effects is not completely understood. We looked further into the mechanism of ABA effects in myocytes, macrophages, hepatocytes and pancreatic beta cells.

Methods and Results:

We investigated the anti-inflammatory effects of ABA by measuring iNOS expression and nitric oxide (NO) production in L6 muscle cells, FaO hepatocytes and J774 macrophages. ABA induction of glucose uptake and AMPK pathway signaling activation in L6 cells were measured as well. ABA effects on hepatic glucose production. Additionally, protective effects of ABA on palmitate-induced apoptosis were evaluated in INS1e pancreatic beta cell. We found that ABA (25 µM) inhibit LPS/cytokine-induced (TNFá, IFNá) NO production in L6 muscle cells and FaO hepatocytes by 30% and 80%, respectively (P

Conclusions:

These results provide further scientific validation for the beneficial effects of ABA in key metabolic, endocrine and inflammatory cell types that are known to be affected in obesity-linked type 2 diabetes. Thus the phytohormone should be considered for the development of new nutraceuticals or functional foods for improved management of type 2 diabetic patients.

Keywords: Abscisic acid, diabetes, inflammation

Financial Support: Quebec MDEIE, FAPESP, Canadian Institutes of Health Research and CDA

 resin:26-305

TOXICOLOGY AND SAFETY OF AMBURANA CEARENSIS SYRUP IN PATIENTS WITH ASTHMA.

Dept. de Fisiologia e Farmacologia-NPDM, UFC

Objectives:

Amburana cearensis A. C. Smith syrup is used in Northeast Brazil in the treatment of respiratory tract diseases. The aim is evaluating the toxicology and safety of cumaru syrup in patients with mild persistent asthma.

Methods and Results:

This study that consisted of 3 phases, pre-treatment, treatment and post-treatment. It was also a randomized, double-blind, placebo-controlled study. Patients in the cumaru and placebo groups were asked to record any adverse events during the treatment. Hematological and serum chemistry tests were evaluated in pre-treatment and post-treatment in the Amburana cearensis (cumaru) and placebo groups. Tests were performed for determination of hemoglobin, red blood cells, hematocrit, platelets, leukocytes, lymphocytes, basophils, eosinophils, monocytes, neutrophils, prothrombin time (PT), activated partial thromboplastin time (APTT), glucose, creatinine, urea, serum glutamic oxaloacetic transaminase (aspartate aminotransferase – AST), and serum glutamic pyruvic transaminase (alanine aminotransferase – ALT), as well as HCG β-subunit in female patients of child-bearing potential. Analyses were performed at a local accredited laboratory. The research project, with the experimental
protocol and the term of free and informed consent, were submitted to the Research Ethics Committee of the Federal University of Ceará, which approved the protocol of nº 169/07. Forty seven met the inclusion criteria. Twenty-five patients were randomized to receive cumaru syrup and 22 to receive placebo. Five patients were withdrawn from the trial (04 from cumaru and 01 from the placebo group) due to a deviation from the experimental protocol. The hematological and serum chemistry tests performed in pre-treatment and post-treatment showed no statistically significant differences (P>0.05). Adverse events were reported by three patients (14.29%) in the cumaru group and three patients (14.29%) in the placebo group. The adverse events were dizziness, headache and nausea.

Conclusions:
This clinical study showed the safety of the Amburana cearensis syrup treatment in patients with asthma.

Keywords: Amburana cearensis, Clinical Toxicology, Safety, Natural Product, Asthma

Financial Support: CAPES, CNPq, MS-RNPC-UNIFAC-HM, INCB and NPDM.

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Resumo:26-306

HISTOLOGICAL AND MORPHOMETRIC ANALYSIS OF CUTANEOUS WOUNDS RATS TREATED WITH A SEMI-SOLID FORMULATION OF AVOCADO (PERSEA AMERICANA) OIL.

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2 Department of Pathology/CSS, UFPE
3 Department of Histology and Embryology/CCB, UFPE
4 Department of Antibiotics/CCB, UFPE

Objectives:
Avocado (Persea americana Mill) oil is rich in oleic (Ô-9), linoleic (Ô-6) and fatty acids linolenic (Ô-3), as well as proteins, minerals, vitamins A, D e E (Ciência. Tecnol. Aliment. 28:20-26, 2008; Rev. Bras. Fruit. 26:17-23, 2004; JWC. 17:123-125, 2008). Several studies have shown a role for Ô-9, Ô-6 and Ô-3 in the process of tissue repair (Rev. Bras. Enferm. 61:620-629, 2008; Rev. Bras. Farm. 88:53-58, 2007; Wound. Rep. Reg. 12:235-243, 2004). The aim of this study was to evaluate the wound healing activity of a semi-solid formulation of avocado oil (SSFAO) on cutaneous wound healing of rats by histological and morphometric analysis.

Methods and Results:
Methods: Wistar rats (200-250g) were anesthetized with intraperitoneal injection of ketamine (75 mg/kg) plus xylazine (15 mg/kg) followed by shaving of the skin at wounding site (dorsal region), and an circular area (78.5mm²) of skin was surgically removed from of the animals. After surgery, the animals were divided in groups (n=5) and treated with topical application of SSFAO (1%, 5% or 10%), CuratecAge® (positive control) and petroleum jelly (negative control) once daily for 14 days. At the end of the experiment, the animals were euthanized in a CO2 chamber and collected the material for the histological and morphometric analysis of the skin lesions. The slides were examined under light microscope, five images per field (0.0018mm² area) were captured with a digital camera (total magnification 400x) attached to the microscope. The images were stored and subjected to counting of inflammatory cells, fibroblast cells, number of blood vessels and evaluation of collagen density of all lesions with the aid of digital marking. The variables were expressed as mean ± standard error of the mean and subjected to one-way ANOVA followed by the Bonferroni's multiple test comparing the SSFAO treatment groups (1%, 5% or 10%) to the Curatec Age® and petroleum jelly controls, considering significant values (p
Conclusions:

In conclusion, the results clearly demonstrated that topical administration of SSFAO (5%) promoted increase in migration inflammatory cells and collagen synthesis during the healing process, and may represent a novel therapeutic approach to skin wounds.

Keywords: PERSEA AMERICANA, CUTANEOUS WOUNDS, WOUND HEALING, HISTOLOGICAL, MORPHOMETRIC

QuebraPagina

Resumo:26-307

CARDIOVASCULAR EFFECTS INDUCED BY ESSENTIAL OIL OF LIPPIA MICROPHYLLA CHAM. IN RATS

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1 PgPNSB, UFPB
2 ICS, UFBA
3 DFP, UFPB
4 CCA, UFPB

Objectives:

The genus Lippia (Verbenaceae) has yielded a great number of medicinal and economically important species that are frequently used in folk medicine for treatment of several diseases, such as: coughs, bronchitis, liver disorders and hypertension. L. microphylla Cham. is a plant of the genus Lippia found in the Northeast of Brazil, and there is no data in the literature concerning its cardiovascular effects. Therefore, this study aimed to evaluate the cardiovascular effects of essential oil of L. microphylla Cham. (EOLM) in rats.

Methods and Results:

Male Wistar rats (250-300 g) had polyethylene catheters implanted into the abdominal aorta and inferior vena cava to data recordings and administration of drugs, respectively. Experiments were performed 24 hours after the surgery. Rat superior mesenteric rings (1-2 mm) were suspended by cotton threads for isometric tension recordings in Tyrode’s solution, 37 °C, gassed with 95% O2 and 5% CO2, resting tension 0.75 g. Calcium current was recorded using the whole-cell configuration of the patch-clamp technique in freshly dissociated vascular myocytes isolated from rat superior mesenteric arteries. In normotensive non-anaesthetized rats, EOLM injections produced hypotension (ED50=5.5 (4.1-7.3) mg/Kg, n=5) and bradycardia (ED50=5.2 (4.3-6.2) mg/Kg, n=5). Isometric tension recordings revealed that EOLM (1-300 µg/mL) caused concentration-dependent relaxation in isolated mesenteric rings, with functional endothelium, pre-contracted with phenylephrine (10 μM) (EC50=28.2 (25.3-31.4) µg/mL, n=5) and this effect was not attenuated by removal of the vascular endothelial layer. In preparations without endothelium, pre-incubated with KCl 20 mM, the relaxation was not changed. Furthermore, EOLM caused relaxation in mesenteric rings pre-contracted with KCl 60 mM (EC50=23.7 (18.3-30.6 CI) µg/mL, n=7) and antagonized Ca2+-induced vasoconstriction in a concentration-dependent manner. In addition, EOLM antagonized the contractions elicited by the L-type Ca2+ channel activator, S(-)-Bay K 8644 (EC50=36.7(31.6-42.5) µg/mL, n=5), indicating that the vasodilatation is related to the inhibition of Ca2+ influx through L-type Ca2+ channels. To confirm this hypothesis, whole-cell L-type Ca2+ currents were recorded in freshly dispersed rat mesenteric artery myocytes and characterized using 20 mM Ba2+ ions as charge carrier. EOLM (1-100 µg/mL) significantly inhibited Ba2+ currents in a concentration-dependent manner (EC50=11.9 (9.4-15.0) µg/mL, n=4).

Conclusions:
Taken together, these data suggest that the relaxant activity induced by EOLM is probably due to the inhibition of Ca\(^{2+}\) influx through L-type Ca\(^{2+}\) channels in vascular smooth muscle cells. This effect could account for the hypotensive response induced by EOLM in rats.

Keywords: Calcium channel blocker, Essential oil, Hypotension, Lippia microphylla Cham, Vasorelaxant action

Financial Support: CNPq/CAPES/ FAPEMIG.

**ETHANOL EXTRACT OF CAESALPINIA PYRAMIDALIS ATTENUATES INFLAMMATORY RESPONSE DURING CYCLOPHOSPHAMIDE-INDUCED CYSTITIS IN RATS**

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Objectives:

Hemorrhagic cystitis is a major complication of the treatment of cancer with cyclophosphamide (CP). New drugs or approaches that reduce the unwilling effects of CP are of great value for the adjuvant therapy of cancer. This study was undertaken to investigate the effect of ethanol extract (EE) of the inner bark of *Caesalpinia pyramidalis* Tul. (Fabaceae) on the cystitis induced by CP in rats. The rational for that is based on our preliminary results that show the anti-inflammatory and antioxidant activities of this extract.

Methods and Results:

Male Wistar rats (180 - 250g) were orally treated with vehicle (tween 80, 0.2%) or EE (100-400 mg/kg). One hour thereafter animals were submitted to the i.p. injection of CP (200 mg/kg) or saline. After 24 h of CP injection, rats were euthanized and samples of blood or tissues (urinary bladder, and lung) were collected. Bladder weight and tissue myeloperoxidase (MPO) activity and malondialdehyde (MDA) determination were measured, as well as the leukocyte counts were quantified in blood. Values are shown as mean ± SEM. All experimental procedures were approved by the institution’s Ethic Committee (CEPA/UFS no 59/2009). We observed that the administration of CP increased the urinary bladder weight (p<0.05).

Conclusions:

Our data lead us to conclude that EE of *Caesalpinia pyramidalis* is able to prevent some inflammatory parameters of cystitis induced by CP, but does not affect the edematogenic response in bladder, suggesting that it is of limited use as an adjuvant for the treatment of cystitis.

Keywords: Hemorrhagic cystitis, Cyclophosphamide, *Caesalpinia pyramidalis*

Financial Support: CNPq and FAPITEC/SE
MYOTOXICITY OF BOTHROPSTOXINS I AND II IN MOUSE FAST- AND SLOW-TWITCH MUSCLES

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² Departamento de Bioquímica e Imunologia/USP, USP

Objectives:

Previous works have shown that mouse muscles, specially isolated extensor digitorum longus (EDL) and soleus (SOL), are suitable for studying myotoxic effects of snake venoms and their myotoxins. However, EDL and SOL muscles have different biochemical and functional properties. EDL, a fast-twitch muscle, is composed mainly by white glycolytic fibers, while SOL, a slow-twitch muscle, comprises principally red oxidative fibers. It was previously shown, by using a protocol measuring the rate of creatine kinase (CK) release from isolated mouse EDL and SOL in vitro, that EDL is more sensitive than SOL to the myotoxic components of some snake venoms or isolated toxins (Toxicon 34, 653, 1996; Toxicon 43, 111, 2004; Toxicon 52, 551, 2008). The aim of the present study was to analyse the effect of bothropstoxins I and II, isolated from Bothrops jararacussu, on mouse EDL and SOL muscles, and to investigate the effect of the potassium channel agonist diazoxide on the myotoxicity of the toxins.

Methods and Results:

We used Adult Swiss mouse which were fed ad libitum until the day of the experiments, when they were sacrificed by cervical dislocation following anesthesia with diethyl ether, according to the Guide for the Care and Use of Laboratory Animals from our University. Mouse isolated EDL and SOL muscles were removed and mounted in perfusion batches continuously perfused with Ringer solution. During perfusion, two groups of four muscles each were exposed to each of the toxins (10 mcg/mL), and other two groups were pretreated with diazoxide and then exposed to the toxins. CK release was measured every 30 minutes. EDL muscles presented higher rates of CK release than SOL for both BthTx I (14,1 ± 2,3 U/g/h for EDL and 5,9 ± 1,5 U/g/h for SOL) and BthTx II (11,1 ± 2,4 U/g/h for EDL and 2,7 ± 1,0 U/g/h for SOL), confirming data observed with the crude venom. Interestingly, treatment with diazoxide (100 mcM) prevented a marked increase in the rate of CK release, so that these rates were smaller than with toxins alone, but yet EDL has shown higher rates.

Conclusions:

Some possibilities were raised to explain the difference in response to the myotoxic agents: (1) EDL has electrophysiological and/or biochemical properties that enhance the effects of the toxins; or (2) EDL has more binding or acceptor sites for the toxins than SOL, as has been suggested early works (FEBS 293, 29, 1991; Toxicon 43, 111, 2004). Alternatively, the particular kinds of innervations of each muscle will result in different profiles of ion channels distribution in the sarcolemma, consequently altering the dynamics of depolarization and calcium influx, which are important steps in damage process.

Keywords: Bothropstoxin, Extensor digitorum longus, Myotoxicity, Soleus

Financial Support: FAPERJ, CAPES, CNPq

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Resumo:26-310

EVALUATION OF ANTI-INFLAMMATORY PROPERTY OF MORINDA CITRIFOLIA IN A PLEURISY MODEL

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² Universidade Federal de Alagoas, UFAL
Objectives:

The present study evaluated the anti-inflammatory properties of aqueous extracts from M. citrifolia leaves (AEMC).

Methods and Results:

Non-fasted adult Swiss mice of both sexes (18–25 g), aged 2 months, were used throughout the experiments. The animals were kept in a room at constant temperature (22±2°C), with alternating 12 h periods of light and darkness and fed on food and water ad libitum. The number of animals was n=6 by group in study of carrageenan induced pleurisy. M. citrifolia leaves were collected in São Cristóvão, Sergipe, Brazil. Herbarium voucher specimens (registry number 13503) were prepared and deposited at the Department of Biology of the Universidade Federal de Sergipe. The experimental protocols were approved by the institution’s Ethic Committee (CEPA/UFS # 27/09). Data were evaluated by ANOVA followed by Tukey’s test. The AEMC was prepared by boiling in distilled water (7.5%; w/v) for 15 minutes; the solvent evaporated under reduced pressure and lyophilized. To the anti-inflammatory effect test, pleurisy was induced by the intrathoracic injection (i.t) of 0.1 ml of a carrageenan suspension (300 µg/cavity) diluted in sterile saline (NaCl 0.9%). Animals were pretreated with AEMC (100, 200 or 400 mg/kg orally), vehicle (saline) or indomethacine (10 mg/kg, i.p.) 1 h before pleurisy induction. Four hours after carrageenan injection, mice were killed by excess carbon dioxide and the pleural exudate was collected by pleural cavity lavage with 1 ml of PBS solution containing EDTA (10 mM). Several samples of the pleural fluid were collected for further determination TNF-α levels by ELISA or cells in a Neubauer chamber and cytocentrifuged. The results demonstrated that M. citrifolia was more effective (100 mg/kg) (p < 0.01) than indomethacine in inhibiting the levels of proinflammatory cytokines in the pleural fluid leakage. The administration of the AEMC (400 mg/kg) significantly reduced the total leukocyte (p

Conclusions:

M. citrifolia may be a source of new therapeutic candidates with a spectrum of activity similar to the current anti-inflammatory non-steroids such as indomethacine. Further studies are underway to investigate which compounds in the extract are responsible for the anti-inflammatory activity and the precise mechanism and site of action.

Keywords: Morinda citrifolia, anti-inflammatory, pleurisy

Financial Support: CNPq, CAPES, FAPITEC/SE
Methods and Results:

Fish were transported at a load density of 169.2 g L\(^{-1}\) for 4 h in fifteen plastic bags with 7 L of water and 8 L of pure oxygen, and they were divided into five treatments (three replicates each). These treatments were as follows: control; 1.5 or 3.0 µl L\(^{-1}\) of eugenol (Odontofarma®, Porto Alegre, Brazil, equivalent to 1.5 or 3.0 mg L\(^{-1}\), respectively, because the density of this anesthetic is about 1.06) and 10 or 20 µl L\(^{-1}\) of the EO of L. alba (equivalent to 8 or 16 mg L\(^{-1}\), respectively, because the density of this EO is about 0.80) (both first diluted in ethanol: 1:10). Before the transport, it was evaluated the ventilatory frequency (VF) of the fish exposed to all treatments. The times chosen to evaluate the VF were 0, 0.5, 1, 2, 3 and 4 h. On the other hand, after transport, blood gases also were determined: pH, P\(_{vO2}\), P\(_{vCO2}\), hematocrit (Hct) and HCO\(_3^-\). All data are expressed as mean ±S.E.M. and the analysis were performed using the software Statistica (PL. alba. After 1 h of exposure, there was no significant difference among treatments, but at 2, 3 and 4 h, the VF was significantly lower in all treatments with anesthetics compared to the control treatment. The P\(_{vO2}\), P\(_{vCO2}\) and HCO\(_3^-\) values increased and Hct decreased significantly in all of the treatments after transporting, but blood pH did not change.

Conclusions:

In conclusion, the results confirmed the effect of the anesthetics on ventilatory frequency and blood gases of silver catfish. Moreover, additional experiments using higher load densities and different body size would be of interest in order to assess the importance of these anesthetics in more stressful situations.

Keywords: Anesthesia, fish transport, blood parameters, opercular or buccal movements

Financial Support: CNPq (process 470964/2009-0) and (FAPERGS/PRONEX, process 10/0016-8)

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Resumo:26-312

EVALUATION OF THE HEALING EFFECT OF LATEX VASCONCELLEA CUNDINAMARCENSIS IN EXCISIONAL SKIN WOUNDS IN MICE

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Objectives:

The study of biological activities and pharmacological properties of latex Vasconcellea cundinamarcensis (P1G10) was developed by our research group. The fraction resulting from the latex of this fruit is rich in cysteine proteases with high proteolytic activity, and demonstrates angiogenic activity, mitogenic and wound healing effect. (Phytomedicine, 15(4); 237, 2008) The aim of this study was to expand the study of the healing action of P1G10 to identify possible pathways involved in its mode of action.

Methods and Results:

Excisional wounds (5 mm Ø) were made in the dorsal region of C57Bl/6 mice (n=24), with the aid of a circular punch, followed by local treatment with Polawax® with or without the P1G10 fraction (0.01% or 0.05%) or, 0.05% P1G10 fraction inhibited with iodoacetamide (IAA). On days 0, 3, 7 and 14 of treatment the wound area was measured by a digital caliper. On days 3rd and 7th of treatment, animals in the 0.05% P1G10 group attained 40% and 80%, wound closure, while the control groups accomplished 20% and 70% closure, respectively. (p

Conclusions:

Thus, our results suggest that P1G10 0.05% accelerates the healing of excisional skin wounds by stimulating collagen deposition,
increasing levels of VEGF and protecting against apoptosis. This effect appears to be dependent on the fraction proteolytic action.

Keywords: cicatrização, mecanismo, proteases

Financial Support: CNPq, FAPEMIG and CAPES

**EFFECTS OF MATERNAL METHIONINE INTAKE ON FETAL PROGRAMMING OF HYPOTHALAMIC GENE EXPRESSION RELATED TO ALZHEIMER DISEASE**

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2 Department of Psychobiology, UNIFESP
3 Department of Biological Sciences, UPE

Objectives:

Fetal antecedents have been associated with increased offspring disease risk through maternal environment, placental changes, and epigenetic programming. Alterations in the methionine-homocysteine cycle are described in Alzheimer disease (AD). It has already been shown that DNA methylation is involved in amyloid precursor protein (APP) processing and β-amyloid (βA) production through the regulation of Presenilin1 (PS1) expression and that exogenous S-adenosylmethionine can silence the gene reducing Aβ production in cell lines. Since DNA methylation is one of several epigenetic mechanisms that regulate genomic programming and imprinting during embryogenesis, the aim of this study was to investigate the effects of methionine supplementation, during gestation and lactation, in the maternal programming of hypothalamic gene expression of the offspring. We analyzed APP, PS1, PS2, ADAM10, TACE, BACE and p53 that are important in the AD pathogenesis.

Methods and Results:

One month before pregnancy, 13 Swiss female mice were distributed into 2 groups: control (CT=6) group and methionine supplemented (MS=7) group (1% of methionine in water ad libitum). After 20 days, plasma homocysteine levels from MS group were approximately 50% higher than CT group (MS= 8.71 µmol/L; CT= 4.45 µmol/L, p=0.0001). Three months old offspring (female: CT=8, MS=7; Male: CT=8, MS=6) was euthanized by decapitation, the hypothalamus was harvested and mRNA isolated by Trizol® method. Gene expression was quantified by real time PCR using GAPDH as the housekeeping gene. Data were analyzed by 2-ΔΔCT method, using Mann-Whitney U test for nonparametrics and T-test for parametrics data, considering significant p

Conclusions:

Considering these results, we can speculate that the organism develops compensatory mechanisms during high methionine intake which prevents alterations in the methylation of these genes, although the hyperhomocysteinemia presented by the dams.

Keywords: MATERNAL PROGRAMMING, METHIONINE, ALZHEIMER DISEASE, GENE EXPRESSION

Financial Support: FAPESP, CNPq and AFIP
THE EFFECT OF SIMVASTATIN AND PRAVASTATIN IN OXIDATIVE PARAMETER ON A STREPTOZOTOCIN-INDUCED MODEL OF DEMENTIA IN RATS.

Universidade do Rio Grande do Sul, UFRGS

Objectives:

Alzheimer’s disease (AD) is the most common form of dementia. The exact cause of AD remains elusive, however is known that oxidative stress is a major factor implicated in the degeneration of neurons in Alzheimer's disease. Statins are a class of cholesterol-lowering drugs and also presents anti-inflammatory and immunomodulatory properties, and some studies support the idea that statins can act reducing the oxidative damage. The aim of this study was to evaluate the effect of simvastatin and pravastatin in hippocampal oxidative stress parameters in an Alzheimer’s disease model induced in rats.

Methods and Results:

Dementia was induced by a single intracerebrovascular injection of streptozotocin (stz) (3mg/kg). Statins treatment initiated in day 2 after the surgery, and the animals received 5mg/kg p.o. of simvastatin or pravastatin. Nitric oxide was determined by measurement of nitrite, based on the Griess reaction. Intracellular production of reactive oxygen species (ROS) was detected using the non-fluorescent cell permeating compound, 2′-7′-dichlorofluorescein diacetate. Glutathione content was measured by a fluorimetric assay with o-phthaldialdehyde, and lipid damage was evaluated by TBARS. Statistical analysis was performed by two-way ANOVA followed by Tukey’s pos-hoc. STZ increased the total content of nitrites, the production of ROS and lipid damage. simvastatin and pravastatin prevented this effect. Glutathione content was reduced by STZ injection, and the statins also prevented this effect.

Conclusions:

Streptozotocin increased the total content of ROS and NO, as well as the lipid damage, also reducing the GSH content, what leads to the development of oxidative stress in rats. Statins exhibited a neuroprotective effect on these parameters. These results support the idea that these drugs could be effective in the prevention of alterations observed in the STZ dementia model, and may contribute to reduce cognitive impairment and brain damage in AD patients.

Keywords: Statins, Altzheimer, Oxidative Stress

Financial Support: CNPq, CAPES, FAPERGS, Rede IBN-Net.

SEPARATION FROM PUPS IN THE NEONATAL PERIOD: DEPRESSIVE-LIKE EFFECTS ON DAMS

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Objectives:
This study was carried out to ascertain the effects of repeated separation (using different times) of mothers from their pups in the neonatal period on parameters related to a depressive-like state in dams.

Methods and Results:

30 Pregnant Wistar rats were divided into 3 groups: control, brief separation (10 min/day) and long separation (3h/day). The neonatal interventions were done on postpartum days 1-10. After weaning, the dams were subjected to the elevated plus maze and predator odor tests to evaluate risk-taking behavior. Taste reactivity patterns to a sweet and to a bitter solutions were also evaluated. The levels of adenosine A2A and dopamine D2 receptors in the dorsoventral striatum and hippocampus were measured using immunohistochemistry. We observed an increase in the risk-taking behavior by the 3h separated group, shown by a diminished time in the closed arms of the elevated plus-maze [t(6) = 2.72, p < 0.05] and by absence of effect of a predator odor when compared to the control and the brief separated group (these groups showed increased time hiding, which was not observed in the long separated group). This data is consistent with reports from the literature that show an increase in this type of behavior in depressed patients and in a few animal models of depression. The 3h separated dams also had diminished expression of liking with a sucrose solution [F (2,23) = 5.8; p < 0.01; F (2,23) = 6.4; p < 0.01 for lower and higher concentrations of sucrose, respectively] and an increased sensitivity to an aversive solution [F (2,23) = 5.85, p < 0.01], which is congruent with a depressive like state profile. Furthermore, we found a decrease in the dopamine D2 receptor quantity in the striatum of the 3 h separated mothers [F (2,11) = 4.79, p < 0.05], which could be related to anhedonia, experienced in depression.

Conclusions:

It is concluded that the withdrawal of pups from their dams for long periods make the dams more susceptible to the development of depressive-like features. The depressive-like effects demonstrated in this study can also be useful to the development of a model to study the consequences of depression in the post-partum period.

Keywords: adenosine, depressive-like, dopamine, maternal separation

Financial Support: CNPq, FAPERGS-PRONEX, CAPES

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Resumo:27-072

ENRICHED ENVIRONMENT PREVENTS MEMORY DEFICITS BUT NOT MOTOR ACTIVITY IMPAIRMENT IN TYPE 1 DIABETIC RATS.

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Depto Ciências Morfológicas/ICBS, UFRGS

Objectives:

Recent studies have shown that the patients with diabetes mellitus (D) have increased risk of cognitive and psychomotor impairment, dementia, and neurodegeneration. These deficits are possibly related to decrease hippocampal neurogenesis and neuronal death or reduce dopamine production in the substantia nigra pars compacta and ventral tegmental area. Enriched environmental (EE), however, provides social, sensory, cognitive and motor stimulation, enhances hippocampal neurogenesis and dendritic branching in rodents, improving the performance in learning and memory task. Thus, our objective was to investigate the influence of EE on the memory and motor activity decline of diabetic rats.

Methods and Results:

41 male Wistar rats, 21 days old, were assigned to two experimental groups: (1) control (C), two rats housed in standard housing,
and (2) exposed to an enriched environment (EE; 7 animals per cage), both for 3 months. The EE protocols employed consisted of a large home cage with 3 different floors connected by ramps, which contained various objects with different textures like tunnels, running wheels, pots, cubes and balls. At adulthood, control and EE groups were divided and half of them induced to diabetes by a single injection of streptozotocin, 50 mg/kg, via i.v. Non-diabetic rats (ND) were injected only with citrate buffer, pH 4.3, 1 ml/kg, i.v. Memory deficit was evaluated in these groups in the novel object-placement recognition task, for 5 min, 41 days after diabetes induction, and the motor activity was evaluated in the open field, for 3 min, one day before memory task. All the procedures were approved by the Ethics Committee of Universidade Federal do Rio Grande do Sul (18434/10). The data were analyzed using two-way ANOVA and Student-Newman-Keuls post hoc test. Data were expressed as mean ± S.E.M. The results showed that EE prevented/attenuated memory deficits in diabetic-induced rats (percentage of preference for exploring the relocated object: NDC 64.97 ± 4.19%; DC 48.84 ± 4.90%; NDEE 67.06 ± 3.69%; DEE 77.71 ± 6.52%; p

Conclusions:

These findings suggest that enriched environment is able to prevent or delay the development of memory deficits caused by diabetes in rats but not motor activity impairment and could contribute with the quality of life of diabetic patients.

Keywords: Diabetes, Enriched environment, Memory deficits, Motor activity impairment

Financial Support: CNPq and UFRGS

CHRONIC METHYLPHENIDATE ADMINISTRATION ALTERS ANTIOXIDANT DEFENSES AND BUTYRYLCHOLINESTERASE ACTIVITY IN BLOOD OF JUVENILE RATS

Schmitz, F. 1; Scherer, E. B. 1; da Cunha, M. J. 1; da Cunha, A. A. 1; Lima, D. D. 2; Delwing, D. 3; Netto, C. A. 1; Wyse, A. T. 1

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2 DEPARTAMENTO DE MEDICINA, UNIVILLE
3 DEPARTAMENTO DE CIÊNCIAS NATURAIS, FURB

Objectives:

Methylphenidate (MPH) is a psychostimulant used to treat Attention Deficit Disorder/Hyperactivity Disorder (ADHD), is a neurobehavioral disorder that affects children of school age and often persists into adulthood. Long-term stimulant treatment in children and adolescents has been shown to affect the development of neurotransmitter systems, which could lead to structural changes in the brain and long-term behavioral changes in adulthood. On the other hand, although the main target of action of MPH is the central nervous system (CNS), this drug can also act in the peripheral system, causing adverse effects such as peripheral vasculopathies, which has been associated with free radicals production. Considering that many children are treated with MPH, but the consequences of its long-term use on the periphery are poorly investigated, the objectives of this study were to evaluate the effects of chronic treatment with MPH on oxidative stress parameters in plasma and erythrocytes, as well as on butyrylcholinesterase (BuChE) activity in serum in young rats.

Methods and Results:

Wistar rats (n = 6, for all parameters) were treated for 30 days from the 15th to the 45th days-of-age with an intraperitoneal (i.p.) injection of saline (0.9% NaCl) or MPH (2.0 mg/kg) once a day. Animals were sacrificed and blood was collected. The following parameters of oxidative stress in blood were evaluated: dichlorofluorescein formed (DCF); total non enzymatic antioxidant potential (TRAP); Enzymatic antioxidant system (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) activities); index of lipid peroxidation - thiobarbituric acid reactive substances (TBARS) and nitrite levels (one of nitric oxide
metabolites). In addition we also investigated BuChE activity in serum of rats. Statistical analysis was performed using Student’s t test using SPSS 15.0 for all techniques. Values are expressed as mean ± standard deviation and p

Conclusions:

Taken together, our results show that MPH altered antioxidant defenses and increased BuChE activity, suggesting that this psychostimulant drug may promote peripheral oxidative adaptations and cholinergic hypofunction.

Keywords: ANTIOXIDANT DEFENSES, BLOOD, BUTYRYLCHOLINESTERASE, METHYLPHENIDATE

Financial Support: CNPq and FAPERGS
inoculations (IA, n = 14; IY, n = 17; EY, n = 20) it was detected a significant decrease in borrowed food only in EY subjects (one-way ANOVA, Bonferroni a priori test, p < 0.05). In the open field tests at the same temporal window, all antibody-enhanced infected subjects from all experimental groups (EY, n = 16; EA, n = 10; IY, n = 18), revealed a significant increase (one-way ANOVA, Bonferroni a priori test, p<0.05).

Conclusions:

We report for the first time that an enriched environment after an antibody-enhanced dengue disease induces more intense behavioral changes, and lower disease resolution when compared to animals exposed to impoverished housing. These results are in line with previous findings of our laboratory that environmental enrichment induces more severe disease progression.

Keywords: dengue, infection, behavior, environment, age


QuebraPagina

Resumo:27-075

GRAPH ANALYSIS OF PSYCHOTIC SPEECH: DIFFERENTIAL DIAGNOSIS BETWEEN SCHIZOPHRENIA AND MANIA

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4 Instituto do Cérebro, IC  
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Objectives:

The differential diagnosis between maniac and schizophrenic patients presents a substantial challenge during acute psychotic crises. Successful diagnosis requires long-term training in the identification of symptoms assessed by a qualitative analysis of speech. Here we sought to quantify differences in the graph structure of speech of schizophrenic and maniac psychotic subjects.

Methods and Results:

We recorded oral interviews with 24 subjects (8 schizophrenics, 8 manics and 8 controls) and applied the SCID DSM IV, PANSS and BPRS scales to identify psychotic symptoms. Patients were asked to report on recent dreams. The reports were transcribed, parsed into semantics units (SU) and represented by a directed graph in which each node corresponded to a SU and each edge represented the link between consecutive SU. Nine graph attributes were calculated (nodes, edges, self-loops, parallels edges, largest connected component, largest strongly connected component, average total degree, wake nodes and wake edges), and non-parametric statistical tests were used to assess significant differences. Manic reports contained more words than schizophrenic group (p = 0.0067) and almost all attributes were higher. When the data were normalized by the total number of words in each report, graphs from the manic group still displayed more parallel edges (p = 0.0050) than graphs from the schizophrenia group, reflecting the “logorrhea” symptom typical of manics. Conversely, schizophrenic reports presented more words than manic group (p = 0.0067) and almost all attributes were higher. Then the data were normalized by the total number of words (p = 0.0050) than graphs from the schizophrenic group, reflecting the “logorrhea” symptom typical of manics. Conversely, schizophrenic reports presented more nodes (p = 0.0114) and a higher average degree (p = 0.0074) than graphs from manics, reflecting “poor speech”. Manic patients had a significantly higher rate of interruptions of the dream report to comment on unrelated waking events. This effect persisted when the data were normalized by the total number of words (p = 0.0087), and seems to reflect the symptom of “flight of thoughts”. An interview classifier based on graph attributes sorted schizophrenic from manic group with 93% of sensitivity and
specificity, and kappa = 0.88 and AUC = 0.88 indicating great agreement and good quality on classification. None of these graph attributes were correlated with BPRS and PANSS total score, which indicates that our approach is not redundant with psychiatric scales, but rather measures complementary features such as structural speech symptoms.

Conclusions:

Altogether, the analyses reveal quantitative differences between graph representations of speech from schizophrenic and maniac psychotic patients, which may reflect classical symptoms not well grasped by psychiatric scales. Quantitative speech analysis can therefore help the differential diagnosis of psychosis.

Keywords: psychosis, schizophrenia, mania, diagnosis, graph

Financial Support: AASDAP

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Resumo:27-076

DEFAULT MODE NETWORK CHANGES INDUCED BY AYAHUASCA

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2 Departamento de neurociências e comportamento, FMRP, USP

Objectives:

Ayahuasca is a potion that has been used for ages by indigenous populations in South America, notably in the Amazon region, for religious and medicinal purposes. The drink is used ritually by modern Brazilian syncretic religions in urban centers since the first half of the 20th century. In 1987 it received recognition and authorization by the Brazilian government that considers legal its use in religious context. The tea is obtained by the decoction of leaves from Psychotria viridis with the bark and stalk of a shrub, Banisteriopsis caapi. The first is rich in N-dimethyltryptamine (DMT), which has an important and well-known hallucinogenic effect due to its agonistic effects in serotonin receptors, specifically 5-HT2A. On the other hand, β-carbolines present in B. caapi, particularly harmine and harmaline, are potent monoamine oxidase inhibitors (MAOi). In addition, the tetrahydroharmine (THH), also present in B. caapi, acts as mild selective serotonin reuptake inhibitors and a weak MAOi. DMT in itself it is not orally active since it is inactivated by MAO. However, the presence of MAOi in the drink allows DMT to be psychoactive when ingested. The access of DMT to the systemic circulation and the central nervous system causes a number of affective, perceptual and cognitive changes. Moreover, its effects have been linked to increased interoceptive attention. On the other hand, there is a growing interest in the default-mode network (DMN), which has been widely detected in functional neuroimaging studies and has been associated with introspective mental activity. Therefore, in the present functional magnetic resonance imaging (fMRI) study we set out to investigate changes in the DMN during the resting state (lying quietly with closed eyes), elicited by the ingestion of ayahuasca.

Methods and Results:

10 volunteers, five male and five female, with prior experience in the use of Ayahuasca from the Santo Daime church completed two fMRI scanning sessions: the first one before taking the tea, and the second one after taking it. During each fMRI session, subjects completed a typical verbal fluency task using a block paradigm. A general linear model (GLM) was used to analyze the fMRI data. Group maps of the default-mode network (DMN) were generated and compared between the two sessions. Moreover, a post-hoc region of interest (ROI) analysis was conducted. Ayahuasca produced significant decreases of the BOLD signal in some of the regions that comprise the DMN, notably in the anterior cingulate gyrus (two-tailed paired t test, p=0.019), frontal medial cortex (p=0.0043) and posterior supramarginal gyrus (p=0.0037).
Conclusions:

The results of this study are consistent with the hypothesis that Ayahuasca produces acute alterations in the DMN as assessed by fMRI, during periods in which the subjects are under effect of the tea. Moreover, the observation of a reduction in BOLD signal amplitude in DMN structures would be in line with the concept of an increased interoceptive attention induced by Ayahuasca.

Keywords: Ayahuasca, DMN, fMRI

QuebraPagina

Resumo:27-077

THE INTERFERENCE OF NEGATIVE AND AROUSING EMOTIONAL PICTURES ON AN ATTENTIONAL TASK

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Objectives:

It is argued that emotional stimuli, specially negative and arousing ones, can capture attention automatically, although some studies show that a high demanding attention condition could modulate this effect. This study aimed to investigate whether an attentional demanding task would modulate the interference of negative and arousing stimuli, by comparing two different attentional conditions – low and high demand – for neutral and emotional pictures.

Methods and Results:

Ninety seven college students took part in this experiment, of whom 54.5% were female, with ages between 19 and 37 (M=24.6, SD=4.31). We used a task where 120 neutral and 120 emotional – negative and arousing – pictures were equally distributed and displayed among three blocks, one with low attentional demand (LA) and two with high attentional demand (HA). The pictures (9º x 12º) were flanked by two peripheral bars (0.3º x 0.3º), placed at the same distance from the centre of the picture (9º). Participants were instructed to respond with their index finger by pressing one of two keys (Q,P) to indicate whether the bars had the same orientation or not. In the low and high demanding conditions, respectively, the bars differed with 90º and 6º in half of the trials. Blocks and pictures were randomly presented in a 17 inch computer screen. Each trial started with the presentation of a fixation cross which remained on the screen for 1500ms and was followed by the pictures and the bars presented for 200ms. After that, a chess board remained on the screen until a response was given or for 2000ms. E-Prime software was used to program the task. The two HA conditions were collapsed and for each condition – LA and HA – an effect index for the emotional interference was calculated by subtracting the mean latency of emotional pictures from the mean latency of neutral ones. Error rates were also investigated. Analyses were conducted with one-sample t-test, paired t-test and ANOVA for repeated measures. The project was approved by the Federal University of Rio Grande do Sul Ethics Committee, and participants signed an informed consent prior to experiment. There was an emotional interference in both LA (M=14.1, SD=39.9) and HA (M=21.5, SD=7.8) conditions. These effects differed significantly from zero, t(96)=3.49, p=.001; t(96)=2.75, p=.007, and did not differ between conditions, t(96)=1.02, p=.309. There was no difference in the interference considering gender as a between participants factor, F(1,91)=.321, p=.572. The difficulty of the HA condition was demonstrated by a significant higher error rate (M=43.8, SD=10.5), compared to LA (M=10.8, SD=10.28, F(1,92)=791.19, p

Conclusions:

In accordance with previous studies, for some people emotion seems to capture attention despite the effort to try to avoid the processing of such stimuli. We plan to further investigate whether this automaticity varies as a result of anxiety levels and if it can change after participants have undergone a focused attention meditation training.
PARAMETRIC EVALUATION OF PRE-PULSE INHIBITION TEST.

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Objectives:
Prepulse inhibition (PPI) is widely used model to study psychiatric disorders, like schizophrenia. PPI is the reduction in amplitude of startle response (ASR) to an intense auditory stimulus (pulse, P) when it is preceded by a stimulus of low intensity (pre-pulse, PP). There is great variability in the PPI according to the parameters and configuration of equipment used in tests on rodents and humans. Among the factors that influence PPI are: the ratio of background noise/ stimuli (stimulus salience), the interval between stimuli presentations and the intensity and duration of stimuli. This study evaluated the following hypotheses: i) the pulse intensity influences the percentage of PPI, ii) the intensity of background noise influences the percentage of PPI, iii) the stimuli interval between influences the percentage of PPI.

Methods and Results:
Male Wistar rats (180-400g) were tested individually in each startle boxes (INSIGHT) with the following protocol: five minutes of acclimatization (background noise only – BN), then they received 10 presentations of P (white noise, 40 ms) and the PPI test consisting of pseudorandom presentation of 64 stimuli: P, PP (pure tone, 3 kHz, 69, 73 and 81dB, 20 ms), PP + P (100 ms between stimuli) and null (no stimuli). Experiment 1: Pulse intensity varied on 100, 110 and 120 dB with BN of 60 dB and interval of 15 s; Experiment 2: BN varied on 57, 60 and 65 dB and P of 110 dB; Experiment 3: intervals varied from 15 and 30 s with BN of 65 dB and pulse 100 dB or and BN of 60 dB and P of 110 dB. The percentage of PPI was calculated as follows: % PPI = 100-(100*PP+P/P) in three levels of PP. In the experiment 1, repeated measures ANOVA of %PPI with pulse and intensity of PP factors showed significant overall effects of pulse (P

Conclusions:
Results demonstrate the importance of setting parameters for a better %PPI. This study showed that pulse and background noise influences the PPI, however the stimuli interval does not seem to influence the %PPI. Further studies are necessary to demonstrate the influence of other variables involved in this pre-attentional test.

Keywords: sensory-motor filter, prepulse inhibition, pulse, background noise, amplitude of startle response

Financial Support: UFABC

EFFECT OF CHRONIC ADMINISTRATION OF TAMOXIFEN AND/OR ESTRADIOL ON BEHAVIORAL PARAMETERS IN OVARIECTOMIZED RATS.
Lampert, C. 1; Pettenuzzo, L. 1; Diehl, L. 1; Laureano, D. P. 1; Toigo, E. V. P. 1; Mota, C. 1; Lima, I. 1; Bairros, D. M. 1; Dalmaz, C. 1,2; Vendite, D. 1
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Objectives:

There are evidences that activation of estradiol receptor (E) reduces anxiety and depressive behavior, body weight and stimulates the intake of palatable food in ovariectomized (OVX) rats. Tamoxifen (TAM) is a selective estrogen receptor modulator, widely used in the treatment of breast cancer and may have estrogenic or antiestrogenic activity depending on the target tissue. The aim of this study is to investigate the possible effects of chronic treatment with E, TAM and E + TAM in rats OVX under feeding behavior (palatable food), exploratory behavior / anxiety (open field), depressive behavior (Forced Swim Test) and contextual fear (contextual memory). Parameters of body weight, adrenal and uterine weight was also measured.

Methods and Results:

Female Wistar rats (n=50) 60-75 days old, were OVX and after 14 days were injected (ip.) for 25-30 days with: E (100μg/kg), TAM (2 mg/kg), E + TAM (same dose). Control groups (OVX or SHAM) were injected with vehicle (V) (5% DMSO +10% EtOH + H2O). After 25 days of treatment were initiated behavioral tests. The treatment lasted until the sacrifice of the rats, after performing all the behavioral tasks. Adrenal gland and uterus were removed and weighed. The data were expressed as media ± S.E.M. Statistical analysis were evaluated by a one-way analysis of variance (ANOVA) followed by Duncan’s post hoc test. Differences were considered significant if p

Conclusions:

These results suggest that TAM presented estrogenic activity in most analyzed parameters, because TAM affected uterus and body weight in the same way as estradiol, also showed the same memory profile in contextual fear and feeding behavior that E and E + TAM groups. Moreover, in the forced swim test, estradiol and tamoxifen acted synergistically to decrease depressive behavior.

Keywords: anxiety, behavior, depression, estradiol, tamoxifen

Financial Support: CNPq

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QuebraPagina

Resumo: 27-080

MALE URINE EXPOSURE REVERSES THE EFFECT OF NEONATAL HANDLING IN THE SEXUAL RECEPTIVENESS OF FEMALE RATS.

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1 Departamento de Fisiologia, UFRGS
3 Faculdades Integradas de Taquara, FACCAT

Objectives:
Repeated handling during the first 10 postnatal days change the hypothalamic-pituitary-gonadal axis of female rats, causing long-lasting changes in central nervous system (CNS), anovulatory cycles, and reduction of sexual behavior. Studies show that male urine pheromones modulate reproductive physiology of female rats. Pheromonal stimulation made through male’s urine presentation active neural circuits related to facilitation of lordosis behavior. The present study aimed to evaluate the effects of olfactory stimulation by male urine pheromones in sexual behavior of the neonatally handled female rats.

Methods and Results:

Methods: Neonatal handling procedure consisted of handling the pups for 1 min/day in the first 10 days postnatally. At the age of 80 days, we verified the estrous cycle of nonhandled female rats (NH- without intervention in the neonatal period) and handled female rats (H). On the evening of proestrus the female rats receiving pheromonal stimulation. The pheromonal stimulation was done by spraying saline solution or male urine near the females rats nostrils every 10 min for 1 h. Seventy-five minutes after spray application, sexual behavior was recorded. Immediately after sexual behavior female blood was collected in order to measure estradiol and progesterone levels. Results: Handled female rats that were exposed to saline (H-S) before of sexual behavior show a decrease in lordosis quotient (lordosis/mounts), compared to the nonhandled ones. However, handled female rats that were stimulated with urine (H-U), the lordosis quotient was elevated in relation to H-S and NH-S rats (n = 13-14; NH-S = 0.94 ± 0.02; NH-U = 0.90 ± 0.03, H-S = 0.79 ± 0.02; H-U = 0.89 ± 0.02). There was no significant changes in estradiol levels and only a tendency to reduction in the progesterone levels (p = 0.06) after sexual behavior in H-S (n = 6; ng / ml - NH-S = 6.15 ± 0, 38, NH-U = 6.27 ± 0.30, H-S = 4.88 ± 0.54, H-U = 5.68 ± 0.59).

Conclusions:

Long-lasting changes in the HPG axis reduce the reproductive capacity of handled females. However, in this study we show that at least the effect of handling in the female sexual behavior is reversible. The reduction in the lordosis quotient observed in the handled females was reversed by a previous sensitization performed by spraying urine before the sexual behavior on the evening of proestrus. This pheromonal stimulation effect may be acting through stimulation of regions related to lordosis reflex facilitation, such as the VMH. Elevation in progesterone levels found after sexual behavior in handled female rats may be involved in mediating of lordosis reflex facilitation. We intent to analyze parameters related to reproductive success, such as hormone patterns restoration on the afternoon of proestrus day with pheromonal stimulation, as well as ovulatory cycles promotion in handled females to fully understand the process.

Keywords: Neonatal handling, Sexual behavior, Urine pheromones, Female rats, Olfactory stimulation

Financial Support: CNPq, CAPES, FAPESP and FAPERGS.
Methods and Results:

Four different age groups, with 12 subjects each, were studied: 20-25, 40-45, 60-65 and 70-75 years old. The experiment was approved by the ICB-USP’s Research Ethics Committee. Volunteers participated in 4 different experimental blocks with 300 trials each. On each trial, the task was to choose between two gray squares on each side of the monitor’s screen, one of which was hiding a black ball (target). Each correct choice of the target’s location was rewarded with R$ 0.05. The target appeared either on the right or on the left side with probabilities of 70% and 30% in Markov chain sequences of order 0 and with probabilities of 50% in Markov generated sequences of order 1 to 3, respectively. To see what strategies were employed by different subjects, the accuracy and the entropy rate were analyzed (higher entropy rates are related to less predictable sequences). Age had no influence on accuracy for 0th-order sequences (p>0.10), but significantly influenced accuracy when subjects were to predict 1st-, 2nd- or 3rd-order sequences (p

Conclusions:

Our results show that aging changes the strategy people use to make decisions in random binary sequences, which also depends on the degree of predictability (Markov chain’s order) of the sequence. These changes may originate from a global and progressive decrease in cognitive capabilities, such as those observed in the aging process, or from specific modifications in neuronal modules involved in decision-making.

Keywords: ontogeny, strategy, decision making, binary sequence, markov chain

Financial Support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (Cnpq))
Conclusions:

These results indicate that atorvastatin could have a role in increasing cognition in animals infused with A&beta, probably due to the neuroprotection exerted by the drug, but not directly related to glutamatergic uptake and GS activity. The mechanisms related to the neuroprotective and cognition-enhancement effect of statins must be better investigated.

Keywords: ATORVASTATIN, GLUTAMINE SYNTHETASE, GLUTAMATE UPTAKE, COGNITIVE MEMORY

Financial Support: CNPq, CAPES, INCT, FAPESC

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Resumo: 27-083

NEUROPROTECTOR EFFECT OF ETHANOLIC EXTRACT FROM THE RAPANEA FERRUGINEA IN A MODEL OF SPORADIC DEMENTIA OF ALZHEIMER'S TYPE IN MICE.

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Objectives:

Alzheimer disease, a form of dementia in which loss of memory is the first and the most characteristic symptom, especially the deficit of memory. Results obtained in our laboratories showed that the ethanolic extract of R. ferruginea (EERF) facilitates the acquisition, consolidation and retrieval of inhibitory avoidance. In addition, the extract also shows a significant effect on the cholinesterase enzyme, inhibiting its activity. Objectives: The present study was designed to investigate the possible protective effect of EERF in a model of sporadic dementia of Alzheimer's type induced by intracerebroventricular (i.c.v.) injection of streptozotocin (STZ) in mice.

Methods and Results:

Methods and results: Swiss Mice female were divided into five groups (N=10-13, 25-30g): (I) control (vehicle+STZ), (II) (EERF-50 mg/kg.v.o.+STZ), (III) (EERF-150 mg/kg.v.o.+STZ), (IV) (EERF-300 mg/kg.v.o.+STZ), and (V) (Galantamine, 5 mg/kg.i. p+ STZ). An additional group of animals without induction of Alzheimer by STZ (Naive group) was also evaluated in the memory tests. Mice were treated with STZ or vehicle [2µl of 2.5 mg/ml solution; intracerebroventricularly (i.c.v.)] twice, 48h apart. (EERF or vehicle was orally administered 60min prior to each STZ treatment. Neuroprotector effect of EERF on the behavioral performance of mice on memory consolidation was investigated in the Inhibitory avoidance test 24 hours after training of inhibitory avoidance task, followed by treatments. Our results confirmed that i.c.v. STZ caused memory deficits in mice [VEC = (10.0 s (6.0 – 19.0 s)] compared with NAIVE (50.0 s (22.0 – 60.0 s) which were reversed by treatment with EERF 50 (125.5 s (65.0 – 180.0 s), 150 (145.0 s (84.5 – 180 s) and 300mg/kg (180.0 s (130.0 – 180 s) and Galantamine (133.0 s (60.0 – 180 s).

Conclusions:

Conclusions: The most important findings of the present study are that EERF was able to reverse the learning and memory impairments induced by STZ, and this effect appears to involve the activity of AChE increasing of activity. All these findings support the neuroprotective role of (EERF) in a mice model of SDAT induced by i.c.v. STZ.

Keywords: RAPANEA FERRUGINEA, DEMENTIA OF ALZHEIMER, STREPTOZOTOCIN, MEMORY

Financial Support: CNPq- PROPEC- UNIVALI
NOOTROPIC AND ANTIOXIDANT PROPERTIES OF A GLYCERIDE OBTAINED FROM RAPANEA FERRUGINEA IN A MODEL OF SPORADIC DEMENTIA OF ALZHEIMER'S TYPE IN MICE

Bürger, C. ; Tridapalli, B. A. ; Wolff, F. ; Costa, P. ; Silva, B. D. S. ; Malheiros, A. ; De- Souza, M. M.
PMCF/CCS, UNIVALI

Objectives:

This study aimed to investigate the nootropic and antioxidant properties of a glyceride (GLY) obtained from fruits of Rapanea ferruginea in a model of sporadic dementia of Alzheimer's type induced by intracerebroventricular (i.c.v.) injection of streptozotocin (STZ) in mice.

Methods and Results:

Swiss Mice female were divided into five groups (n = 10-13, 25-30g): i) control (vehicle 3 mL/kg+STZ); ii) GLY (5 mg/kg+STZ), iii) GLY (15 mg/kg+STZ), iv) GLY (30 mg/kg+STZ), and (v) (Galantamine, 0.5 mg/kg+ STZ). An additional group of animals without induction of AD by STZ (Naive group) was also evaluated in the memory tests. Mice were treated with STZ 2.5 mg/mL (2µL, i.c.v.) twice, 48h apart. GLY or vehicle was orally administered 60 min prior to memory tests. Neuroprotector effect of GLY on the behavioral performance of mice on memory consolidation was investigated in the Inhibitory avoidance test 24 hours after training of inhibitory avoidance task, followed by treatments. After the tests, the animals were sacrificed and the brains were removed for the measurements of antioxidative systems and oxidative products: catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and malondaldehyde (MDA) levels. Our results confirmed that i.c.v. STZ caused memory deficits in mice [VEIC = (10,0 s (8,5 – 16,0 s)] compared with NAÏVE (50,0 s (22,0 – 60,0 s) which were reversed by treatment with GLY 5 mg/kg (165 s (85 – 180 s), 15 mg/kg (130 s (40 – 180 s) and 30 mg/kg (76 s (62 – 175 s) and Galantamine (133 s (60 – 180 s). GLY 5 mg/kg induced significant increase (p < 0.01) in the activity of the CAT (763.98 ± 6.66 µmol H2O2/h/mg protein); SOD (1.62 ± 0.016 µM O2-./h/mg protein); GPx (30.18 ± 0.83 µM NAD+/h/mg protein) and GR (11.19 ± 0.07 µmol NADPH/h/mg protein) in brain when compared to control group (CAT: 556.97 ± 8.0 µmol H2O2/h/mg protein; SOD: 0.96 ± 0.02 µM O2-./h/mg protein; GPx: 23.64 ± 0.14 µM NAD+/h/mg protein; GR: 8.98 ± 0.05 µmol NADPH/h/mg protein). The level of MDA in the brain of STZ treated mice (0.27 ± 0.0024 µM) was higher than that in the GLY 5 mg/kg (0.08 ± 0.0009 µM) [p < 0.01].

Conclusions:

Our study demonstrated the antioxidant potential of GLY obtained from R. ferruginea and that GLY was able to reverse the learning and memory impairments induced by STZ. All these findings support the neuroprotective role of GLY in a mice model of AD induced by i.c.v. STZ.

Keywords: Alzheimer Disease, Rapanea ferruginea, Glyceride, antioxidant enzymes, nootropic effect

Financial Support: CNPq, ProPEC/UNIVALI

CHARACTERIZATION OF PRIMARY AND SECONDARY INJURY IN EXPERIMENTAL COMPRESSIVE ENCEPHALIC TRAUMA

QuebraPagina

Resumo:27-085
Objectives:

Aim: Traumatic brain injury (TBI) is the leading cause of death among young individuals. Extradural haemorrhage may be the consequence of brain trauma and has been related to high rates of mortality. Thus, such lesion is considered the main neurosurgical emergency. The aim of this study is to characterize the primary and secondary injury on a new model of compressive encephalic trauma.

Methods and Results:

Methods: Wistar male adult rats were submitted to experimental extradural brain compression (simulating an extradural haematoma) and followed postoperatively. One group of animals were submitted to long term memory analysis at the fifth day after surgery and sacrificed at the end of the second week. Their brains were removed and tissue was stained with DAPI, Fluorojade C and TUNEL to detect neurodegeneration and apoptotic process. Another group was sacrificed on the immediate postoperative time and the brain tissue was analyzed by electron microscopy to detect primary brain lesions. The protocol was approved by the ethical committee under the number IBQM 039. Results: Data we obtained indicated that traumatized rats had significant long term memory deficit in comparison with controls (sham-traumatized rats) (p

Conclusions:

Conclusion: The presented model of compressive encephalic trauma is reproducible and reliable, allowing further studies for clarify the pathophysiological process that underlies both primary and secondary injuries after tissue damage. Elucidation of the entire neurodegenerative process in this specific model of brain trauma is an important step for development of new therapeutical strategies aiming the blockade of neuronal death in TBI.

Keywords: BRAIN TRAUMA , EXTRADURAL, EXPERIMENTAL MODEL, PRIMARY INJURY, SECONDARY ONJURY

Financial Support: CNPQ/FAPERJ

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DISTRIBUTION OF AUTOMATIC ATTENTION IN A VISUAL HEMIFIELD IN A SHAPE DISCRIMINATION TASK

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Objectives:

Some models have been proposed to represent the distribution of attention in space. The multifocal model considers that attention can form independent foci. The present study tested this hypothesis. It looked for evidence that two spatially separate attentional foci can be formed at the same time.
Methods and Results:

Thirteen young adult voluntarily participated in the experiment. All were right handed (as evaluated by the Edinburgh Inventory), presented normal or corrected-to-normal vision and normal color vision. Each volunteer participated in two testing sessions on separate days, not more than 7 days apart. Each trial began with the appearance of the Fixation Point and three light gray rings on each side, at an eccentricity of 9 degrees. The prime stimulus (S1) was of four types, represented by the darkening of the superior, middle or inferior ring or both the superior and inferior rings on one side. Fifty milliseconds after the offset of the S1 (and thus, 100 ms after its onset), a target stimulus (S2) appeared equally and randomly in any one of the marked locations, with the constraint of always appearing on the same horizontal level as the single S1. This target stimulus could be either a vertical line or a small ring. The volunteer was instructed to respond as fast as possible to the vertical line with the left index finger and with the right index finger to the small ring. Reaction time was shorter when the S2 appeared on the same side as the S1 than when it appeared on the opposite side to the S1 (469 ± 13 ms and 484 ± 13 ms, p = 0.009). The attentional effect was longer for the single S1 condition than the double S1 condition (24 ± 5 ms and 8 ± 7 ms, respectively, p = 0.017). The interaction between the type of the S1 and position of the S2 was not significant (p = 0.268), indicating that the attentional effects did not differ between the superior, middle and inferior condition, with the single S1 (18 ± 9, 24 ± 6 and 31 ± 7 ms, respectively) and the double S1 (13 ± 9, 0 ± 7 and 10 ± 9 ms, respectively).

Conclusions:

These results suggest that automatic visual attention can not focus on two separate locations simultaneously. Financial Support:

CAPES

Keywords: automatic attention, attention division, visual stimuli, reaction time, spatial attention

QuebraPagina

Resumo:27-087

MICROGLIAL ACTIVATION INDUCED BY TRAUMATIC BRAIN INJURY IS SUPPRESSED BY DELAYED ADMINISTRATION OF A PARP INHIBITOR

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Objectives:

Microglia are the resident macrophage population of the central nervous system (CNS). Microglia activation is essential to brain innate immunity. Once activated, microglia switch from a benign phenotype to a pro-inflammatory state. Although microglia activation is crucial for clearing debris, it can also cause neurodegeneration and impair recovery after brain injury. Many aspects of the inflammatory response are regulated by the transcription factor NF-κB. The nuclear enzyme poly (ADPribose) polymerase-1 (PARP-1) is a coactivating factor for NF-κB and is required for microglia activation and neurotoxicity (Neurosci. 145; 1267, 2007). In the present study we tested the effect of delayed administration of the PARP inhibitor INO1001 on microglial activation and neuronal survival in a rat model of traumatic brain injury (TBI).

Methods and Results:

Male Sprague-Dawley rats (250-300g) were anesthetized with intraperitoneal injections of ketamine (80mg/Kg) plus xylazine (8mg/Kg) and maintained at 37°C ± 0.5°C throughout the surgical procedure. TBI was produced by controlled cortical impact to the rat parietal cortex. Microglial activation was assessed by evaluating the morphology of microglia immunostained for
Microglial activation peaked at 5 - 7 days after injury in both the perilesional cortex and the underlying hippocampal dentate gyrus. To assess the anti-inflammatory effect of the PARP inhibitor INO1001 on this inflammatory response, we treated rats daily with 10mg/kg INO1001 or saline intraperitoneally for 7 days, with the first dose given 6 hours after TBI. Rats treated with INO1001 for 7 days showed a 40% reduction in microglia activation score in the perilesional cortex and an 80% reduction in microglia activation score the hippocampus dentate gyrus, compared to the vehicle group. To determine whether this anti-inflammatory effect of INO1001 would persist after stopping the treatment, we treated the rats with 10mg/kg INO1001 for 12 days, with the first dose 20h after TBI, and euthanatized the rats at either day 12 or 4 days after the last INO1001 dose. We found that in this regimen INO1001 reduced in microglia activation score in both cortex and hippocampus with no rebound effect after 4 days off treatment. Neuronal survival was increased in the perilesional cortex of rats treated with INO1001.

Conclusions:

PARP inhibition can suppress microglial activation in vivo, even when administered many hours after TBI, and increase neuronal survival after TBI.

Keywords: brain, inflammation, microglia, PARP

Financial Support: NIH

QuebraPagina

Resumo:27-088

EARLY SIZE-DISTANCE PROCESSING AND EYE MOVEMENTS

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Objectives:

Texture gradient is a reliable depth cue and has a strong effect in size perception. Size and distance are closely related, because perceived size of an object is the product of the visual angle and its distance, as predicted by Size-Distance Invariance Hypothesis (SDIH). Although recent studies suggest that the size and distance processing occurs in early stages of visual system little is still known about the time required for size and distance integration. Furthermore, most studies were accomplished with line-drawing backgrounds, e.g. perspective, and there are few experiments that used texture elements photographed, which might ensure more ecological validity. Based on this, the present study aimed to investigate how the exposure time of the texture gradients (line-drawing perspective gradient and photograph of texture gradient) affects size comparisons. Beside this, we verified the occurrence of eye movements in this task.

Methods and Results:

In the first experiment, 96 volunteers compared two circles presented in the vertical meridian and the task was to report which one was perceived as bigger. Using a double random staircase procedure, these circles were presented simultaneously for 50, 100, 150 or 200 ms in three background conditions: without depth cues, line-drawing perspective gradient and texture gradient photographed. The Points of Subjective Equality (PSE) were calculated in pixels (px) and a simple effects analysis for background conditions showed that PSE for exposure time were equal in control [F(3,84)=0.186, p=0.905] and significantly different in perspective [F(3,84)=3.405, p=0.021] and in photographed backgrounds [F(3,84)=5.653, p=0.001]. The Bonferroni post hoc analysis (p

Conclusions:

We concluded that size and distance to be integrated in a coherent manner, as indicated by SDIH, take longer on photographed
background than on line-drawings perspective. The results of the second experiment ruled out the explanation that eye movements play an important role in size and distance perception in brief exposures. These evidences argue in favor of a bottom-up processing of information of size and distance. That is, top-down mechanisms, e.g. attention, are not needed in early integration of size and distance information even with peripheral stimuli presentation.

Keywords: brief exposure time, eye movements, photograph, size perception, texture gradient

Financial Support: Capes and CNPq

QuebraPagina

Resumo:27-089

IN INVOLVEMENT OF MUSCARINIC M1 RECEPTOR IN THE EXTINCTION OF CONTEXTUAL FEAR CONDITIONING IN RATS.

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Objectives:
The extinction memory is a process in which the exposure to the learned stimulus without reinforcement that leads a decrease in the conditioned response. This process is thought to be based on new learning and that may depend basic properties of memory formation. Because, previous studies in our laboratory found that administration of dicyclomine, a M1 muscarinic antagonist, impaired the acquisition and retrieval of contextual fear conditioning, it is possible suppose that the administration of dicyclomine may be affect the extinction of contextual fear conditioning. Thus, the purpose of this study was to evaluate the effect of dicyclomine after re-expose session, without foot-shock, on the contextual fear conditioning test.

Methods and Results:
In the conditioning trial, forty male Wistar rats (age 3 month, weight 300-400g) were placed in the conditioning chamber for 3 min and received two footshocks 0.7 mA, 2-s separated by a 30 sec interval. Before returning to the home cages, animals were kept in the conditioning environment for an additional minute. Reexposition to the context were performed 48 h later, animals were re-exposed to the same context for 3 min, without receiving a foot-shock and immediately after 2 groups received ip injection of saline (n=11) or dicyclomine (32mg/kg; n=10). Two control groups were submitted to the same treatment, but without the reexposition to the context, home cage saline (n=9) and home cage dicyclomine (n=10). Twenty-four hours later, all animals were tested for 5 min in the same context and freezing time was registered and used as a memory index. One-way analysis of variance (ANOVA) did not detect a significant difference in the freezing time among both groups, saline and dicyclomine in the reexposition to the context [F(1,19)=0.32; p=0.57]. One-way analysis of variance (ANOVA) detected a significant effect for the factor groups [F(3,36)=3.51; p=0.024]. Duncan’s test indicated that the saline group showed freezing time lower that dicyclomine (p=0.014), home cage saline (p=0.0079) and home cage dicyclomine groups (p=0.04) on the contextual fear conditioning test (mean saline group= 29.54; SEM= 3.71; mean dicyclomine group=43.56; SEM=4.31; mean saline home cage=45.11; SEM= 3.38; mean dicyclomine home cage=40.13; SEM=3.37). Two-way ANOVA detected a significant difference in freezing time between the reexposition to the context and contextual fear conditioning test [F(1,19)=7.13; p=0.015]. Duncan’s test indicated that the saline group displayed freezing time on the contextual fear conditioning test lower that on the reexposition to the context (p=0.003) (meanA saline group=29.54; SEM= 3.71, meanB saline group= 39.60; SEM= 9.13). The freezing time of dicyclomine group was similar between the reexposition to the context and contextual fear conditioning test (p=0.73); (meanC dicyclomine group= 42.56; SEM= 4.54, meanD dicyclomine group=43.56; SEM=4.31).

Conclusions:
The results showed that a reexposition to the context induced an extinction of contextual fear conditioning. In addition, administration of dicyclomine, after the reexposition to the context, impaired the extinction process. indicating that muscarinic
M1 receptor may be involved with associative learning process underlying extinction after reexposition to the context.

Keywords: contextual fear conditioning, memory, extinction, dicyclomine, rats

Financial Support: AFIP

MATERNAL DEPRIVATION AFFECTS LONG-TERM EMOTIONAL REACTIVITY TO TRAUMATIC STRESS

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Objectives:

Adequate emotional reactivity to stressful situations in rats depends on efficient maturation of all components of the HPA axis and of brain structures involved with emotion. A body of evidence has shown that disruption of the mother-infant relationship has a robust impact on adulthood emotional features, indicating that presence of the mother and specific aspects of maternal behavior are crucial for development of the stress system. Here we investigated the effects of 24 h maternal deprivation at different stages of development on long-term consequences of a traumatic stress.

Methods and Results:

Eighty-eight male Wistar rats (8-12 per group), originally from 30 different litters, were either submitted to 24 h maternal deprivation [3rd (DEP3) or 11th (DEP11) day of life] or left undisturbed with their dams (CTL) until weaning. All rats were raised under standard animal facility conditions and behavioral tests were performed starting on the 90th day of life. We exposed adults to a model of traumatic stress (footshock, 2 mA, 1 s) that enables to distinguish between different degrees of fear conditioning and then we evaluated fear upon reexposure to trauma context and long-term effects on the elevated plus maze. All numeric results are expressed as mean ± standard deviation. Maternal deprivation did not alter fear conditioning (CTL = 82.96 ± 15.3; DEP3 = 84.2 ± 16.5; DEP11 = 74.4 ± 13.8) but significantly enhanced fear sensitization in the DEP11 group (CTL = 22.19 ± 10.8; DEP3 = 32.0 ± 22.65; DEP11 = 45.5 ± 25.1). The overall effects of maternal deprivation on the elevated plus maze comprised of augmented locomotion on both open (CTL = 7.2 ± 2.1; DEP3 = 10.3 ± 3.8; DEP11 = 10.2 ± 2.9) and closed (CTL = 8.1 ± 2.1; DEP3 = 9.4 ± 1.4; DEP11 = 12.0 ± 1.6) arms, while anxiety-like behavior, measured as percentage of entries (CTL = 46.7 ± 8.2; DEP3 = 51.1 ± 9.5; DEP11 = 45.5 ± 6.1) and of time (CTL = 24.7 ± 10.6; DEP3 = 36.6 ± 10.6; DEP11 = 31.9 ± 4.6) on open arm entries, was not affected. Long-term effects of footshock were only seen in DEP groups; expressed as impaired exploration: less open (CTL-CI = 5.1 ± 2.3; CTL-CC = 6.0 ± 2.7; DEP3-CI = 6.1 ± 1.4; DEP3-CC = 7.7 ± 2.5; DEP11-CI = 5.7 ± 2.6; DEP11-CC = 7.5 ± 2.1) and closed (CTL-CI = 7.8 ± 2.9; CTL-CC = 7.7 ± 2.1; DEP3-CI = 6.8 ± 1.3; DEP3-CC = 6.8 ± 2.4; DEP11-CI = 7.62 ± 1.8; DEP11-CC = 9.6 ± 2.3) arm entries. Ethological evaluation on the elevated plus maze showed enhanced risk assessment behavior (DEP11-SC = 13.4 ± 6.1; DEP11-CI = 25.8 ± 13.3; DEP11-CC = 18.6 ± 11.0) and impaired rearing behavior (DEP11-SC = 58.9 ± 22.6; DEP11-CI = 31.9 ± 9.6; DEP11-CC = 57.6 ± 18.2) caused by footshock only in DEP11 sensitized rats.

Conclusions:

Our data suggests that maternal care plays a role in the development of adequate emotional reactivity to aversive events. While exploratory behavior was not affected by traumatic stress in non-deprived rats, maternally-deprived rats showed enhanced fear sensitization and exploratory behavioral inhibition as a consequence of footshock, in an age-dependent fashion.
EFFECT OF AYAHUASCA BEVERAGE ON THE LOCOMOTOR AND EXPLORATORY ACTIVITIES AND ON THE FEAR AND ANXIETY BEHAVIORS IN RATS.

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Objectives:

The consumption of Ayahuasca beverage is usual in religious ceremonies like “Santo Daime”. The drug has been discussed to have antidepressive effect and to result in behavioural alterations. This work aimed at investigating whether treatment with Ayahuasca might induces alterations on the locomotor and exploratory activities and on the fear and anxiety behaviors in rats.

Methods and Results:

Male Wistar rats (230-250g) received 200ml kg-1 of Ayahuasca beverage (n=12) or water (control group, n=13) orally. After twenty minutes the animals were submitted to behavioral tests: 1- circular open arena: They were put into the middle of the arena and the responses scored every minute for 5 minutes: Number of crossings (horizontal exploration) and number of rearing (vertical exploration). Then, after five minutes the levels of anxiety and the spatial memory were analyzed on the elevated plus-maze (EPM) where the responses were scored for 5min: number of entries and time on the open arms, number of entries and time on the closed arms, number of stretched attend postures (SAPs). Animals were retested after 24 hours. Groups were compared by Student t-tests and the level of significance was set at p0.05). However, a significant reduction was found in all score for control and treated groups when they were re-exposed to EPM 24 hours later. In addition, the number of SAPs was significantly reduced in both groups.

Conclusions:

The consumption of Ayahuasca may induce alterations in specific behaviors like locomotor and exploratory activities. The effect of Ayahuasca on the fear and anxiety behaviors may be related to the dose and frequency of ingestion.

Financial Support: CNPq and FPA.
AUTOMATIC MOTOR CONTROL

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2 NEUROCIENCIAS E COMPORTAMENTO, IPUSP

Objectives:
Several evidences from animal and human studies demonstrated that the depletion of dopamine, associated with basal ganglia lesion, impairs the implicit learning, particularly in late stages, in which the automatic control is consolidated. The deficiency in automatic motor control compromise the ability to execute secondary tasks in parallel the motor tasks. Recent studies with Parkinson's disease patients (PD) showed that is possible to recover the automatic motor partially, after training extensively. Virtual environments, in which motor responses are triggered by visual stimuli simulating complex situations, stimulate automatic motor control while attention is dedicated to the visual task. Thus, the aim this study was verify the ability of the PD patients to improve their performance in virtual tasks provided by Nintendo Wii Fit Plus®, after extensively training.

Methods and Results:
The performance in 10 different virtual tasks (games) provided by Nintendo Wii Fit Plus®, of 12 PD patients, with a mean age of 66.4 years (S.D. = 7.14), 6 men and 6 women, at stages 1 and 2 of disease evolution according to the Hoehn and Yahr Classification, asymptomatic for depression and dementia, and 10 healthy elderly with mean age of 65.3 years (S.D. = 6.46), was compared before and after 14 training session, two session per week. The games were selected on the Nintendo Wii Fit Plus® which elicited slow and fast movements from the center of gravity, associated or otherwise with upper limb movement, and step alternation under different conditions. Games were performed on a 60X40cm platform and guided by visual stimuli projected onto a 150X 100cm screen placed 150cm away from the patient. The PD patients were trained in on period of dopamine medication. All participants signed the informed consent term. Performance on each of the games during the 14 sessions was analyzed using 2x7 repeated measures ANOVA, with group (CG and PD) as a factor, and sessions (S1, S2, S3, S4, S5, S6, S7) as repeated independent variables. Tukey's post hoc test was applied for all interactions reaching a level of significance. The results evidenced a significant interaction between group and sessions (p<0.0001).

Conclusions:
PD patients were able to improve their performance on visual-motor tasks, trained in a virtual environment. This finding suggests that, given suitable training, impairment of the automatic motor control in PD patients can be minimized.

Keywords: MOTOR LEARNING, PARKINSON'S DISEASE, EXECUTIVE FUNCTION, ATTENTION, DUAL-TASK

Financial Support: CAPES
Objectives:

The large scale commercialization of personal Brain Computer Interfaces (pBCIs) led to technical improvements that made them very compact, friendly and low price. Accurate pBCI devices can then be used as affordable academic tools. Aim: Test the performance of a commercial low price pBCI compared with a clinical electroencephalography (EEG) device.

Methods and Results:

We compared the signals acquired with the wireless pBCI ‘emotiv EPOC’ (www.emotiv.com) with the ‘EMSA-BNT36’ (www.emsamed.com.br) on a P300 paradigm. The EPOC acquire (at 128Hz) only from 14 channels (AF3, F7, F3, FC5, T7, P7, O1, O2, P8, T8, FC6, F4, F8, AF4) a subset of the 20 channels (10-20 system) acquired by the BNT36 (at 600Hz). We used a P300 paradigm that consists of detecting the orientation change of a small bar (0.1 degree) embedded on a noise background (moving stimuli). The correct detection was accessed by 2 methods: 1) the counting of orientation switches; 2) pressing a key to each switch. The switch frequency was random (average of 12 per minute). Each subject performed around 100 detections per condition in 5 blocks of 3 minutes each. With the BNT36 we found a clear P300 component in channel Pz, decaying progressively moving away from it. Nevertheless the P300 component was clearly seen in the T7, P7, P8 and T8 channels. In the EPOC setup, we also found similar P300 components in the T7, P7, P8 and T8 channels. We are still analyzing the comparative quality of the signals in the frequency domain. The subject’s freedom and comfort acquired with the wireless EPOC system has to be taken care as it generates many electromyogram artifacts.

Conclusions:

So far it seems that low price pBCIs can be used to acquire electrophysiological data for scientific purposes.

Keywords: BCI, P300, EEG

Financial Support: PIBIC/UFRJ
age average of 9.9±0.3 years old, among them 5 boys and 10 girls and the no-training group (NP), composed with 18 children of 9.9±0.4 years old, 10 boys and 8 girls, all dexterous. The PP and MP groups were submitted to one session of training with 2400 repetitions with the dominant hand; they were evaluated, as well as group NP, before the repetitions, immediately after, and in the next 4, 7, 14 and 28 days after the training. The motor performance with the non-dominant hand was evaluated before the training, immediately after, and in the next 7 and 28 days after the training in all groups. The results have shown that regardless the form of training the children were capable to improve their speed performance throughout the training. Regarding acquisition, although being slower, children of MP group have reached the same performance as PP group in the end of the reevaluations. For generalization, groups PP and MP presented improvement in performance of the reversal finger opposition sequence (RS), but both slower in comparison to the trained finger opposition sequence (TS), with a better performance of MP group in the last reevaluation (28 days after the training) compared with the initial evaluation. Regarding to the other hand, the PP and MP groups improved the performance at the end of reevaluations.

Conclusions:

The obtained results in this study show that 10 year old children submitted to the mental training, in spite of presenting a process of slower learning, are equally efficient regarding the real practice, however with a formation larger capacity of the internal model and, consequently, larger capacity for task generalization for a reverse sequence and transferring for opposite hand.

Keywords: aprendizado motor, prática mental, imaginação motora, crianças

QuebraPagina

Resumo:27-095

LONG TERM MEMORY DEFICIT IN C57BL/6 AND TNFRI KNOCKOUT MICE SUBMITTED TO SUBLETHAL POLYMICROBIAL SEPSIS INDUCED BY CECUM LIGATURE AND PUNCTURE

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Objectives:

Aim: Sepsis is a systemic inflammatory response to a pathogen and is responsible for the main cause of delirium in intensive care unit patients. Memory and learning deficits, anxiety and depression are among the neuropsychiatric symptoms that have been described as long term consequences of sepsis. The goal of this study was to investigate the role of TNF-α receptor type 1 (TNFRI) in the short and long term memory after sublethal sepsis induced by cecal ligature and puncture (CLP).

Methods and Results:

Methods: CLP was induced by perforation and semi-obstruction of cecum of mice. C57BL/6 (WT) and TNFRI knockout (KO) male mice aged 8-12 weeks were evaluated for object recognition memory before and ten days after sublethal sepsis induced by CLP. Results: Before CLP, there was an increase in the recognition index of the new object 24h after training session both in WT (training: 0.40±0.18 and 24h later: 0.55±0.10, p<0.05, n=7) and KO mice (training: 0.53±0.06 and 24h later: 0.63±0.14, p<0.05, n=6). This latter result suggests the development of learning deficit after CLP. The results express the recognition index and were represented as media ± SEM.

Conclusions:

Conclusion: Sublethal sepsis induced by CLP can lead to long term memory deficit in mice. Signaling through TNFRI
does not seem to play a relevant role in this phenomenon.

Keywords: sublethal sepsis, cecum ligature and puncture, TNFR1 knockout mice, memory deficit

Financial Support: CNPq and FAPEMIG.

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Resumo:27-096

EFFECTS OF MATERNAL ETHANOL INTAKE DURING PREGNANCY AND LACTATION ON THE LOCOMOTOR AND EXPLORATORY ACTIVITIES IN PUPS AT DIFFERENT STAGES OF BRAIN DEVELOPMENT

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Objectives:

This work aimed at investigating whether ethanol consumption during the pregnancy and lactation might induces changes on the locomotor and exploratory activities in pups at different stages of brain development.

Methods and Results:

Female Wistar rats (150g) were exposed to 5%-10% of ethanol, increasing 5% per week (habituation), and 10% maintained for 60 days (chronic ingestion), corresponding to the period before mating (8 days), mating (10 days), pregnancy (21 days) and lactation (21 days). The control group was exposed to water. After weaning the pups from control (n=8) and alcoholic mothers (n=13-16) were left undisturbed in their cages (4/cage) until PND30, 60 or 90 with food and water ad libitum. Then, the animals were tested in a circular open arena. The animals were put into the middle of the arena and the behavioral responses scored every minute for 5 minutes: Number of crossings (horizontal exploration) and number of rearings (vertical exploration). Control and alcoholic groups were compared by Student t-tests and the level of significance was set at p

Conclusions:

The results from PND60 suggest that maternal alcohol intake during the pregnancy and lactation induces long term changes on the pup brains leading to behavioral alterations such as locomotor hyperactivity in adulthood. The absence of alterations on PND90 suggests that neuronal plasticity mechanisms may compensate the deficits induced by maternal alcohol intake. The opposite changes observed on PND30 may be due to the immature brains.

Keywords: Arena, Ethanol, Exploratory Activity, FAS, Locomotor Hiperactivity

Financial Support: Fundação Padre Albino

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DEPRESSIVE-LIKE PARAMETERS IN RATS SURVIVOR BY PNEUMOCOCCAL MENINGITIS
Objectives:

Aim: Pneumococcal meningitis is a severe infectious disease of the central nervous system, associated with acute inflammation and might cause damage to the host, such as deafness, blindness, seizure, learning deficits. However, infectious diseases can play a significant role in the etiology of neuropsychiatric disturbances. In this context, we evaluated depressive-like parameters; corticosterone and ACTH levels in pneumococcal meningitis survivor rats.

Methods and Results:

Methods and Results: Wistar rats, were underwent a magna cistern tap receiving either 10µL sterile saline or a S. pneumoniae suspension at the concentration 5x10⁹ cfu/mL. After 3 days of the induction of the meningitis procedure, the animals were treated with imipramine at 10 mg/kg or saline during 14 days (days 3-17). The consumption of sweet food was measured for 7 days (days 10-17). The meningitis group decreased the sucrose intake and increased the levels of corticosterone and ACTH, however the treatment with imipramine reverted the reduction of sweet food consumption and normalized hormonal levels.

Conclusions:

Conclusion: Our results supported the hypothesis that rats survivor of the pneumococcal meningitis show depressive-like behavior and alterations in the hypothalamus-pituitary-adrenal axis.

Keywords: anhedonia, ACTH., Corticosterone, Meningitis, Streptococcus pneumoniae

Financial Support: CNPq, FAPESC, UNESC and INCT-TM
their first pregnancy. 42% of the women had finished high school and 26.6% had finished primary school (9.3% did not finish high school, 15.1% did not finish college and 5.8% had a degree). About half of the women (53.2%) were working and 41.9% belonged to the economic class C1. On LSSI, 66.3% of these women showed stress symptoms. 3.5% were in the alert stage, 68.5% were in the resistance phase, 17.5% were in near-exhaustion and 10.5% were in exhaustion; 22.8% presented mainly physical stress. 73.2% had psychological stress and 3.5% presented both forms of stress. Several sociodemographic characteristics were assessed in their relation with the presence of stress. The majority (79%) of participants with stress presented indicators of mental disorder in SRQ-20. Pregnant women with stress showed more mental disorders than those who did not stress [chi-square (1, N = 86) = 40.26, p

Conclusions:

Although stress symptoms can be confused with symptoms of pregnancy itself, the results obtained in LSSI were close to or higher than those obtained with LSSI in the general population of São Paulo, regarding the general level of stress, the tendency to psychological stress and the predominant phase of resistance (Estud Psicol.Campinas. 22:53, 2005). It is also important to point out that 28% of the pregnant women were at the stage of almost-exhaustion and exhaustion, similar to the 29% of the general population. Pregnant women with stress were better identified by SRQ20 than by the sociodemographic variables. Considering the problems that stress can cause, maybe the use of SRQ20 or LSSI in medical treatment would help to identify risks to the health of mother and child. The research was approved by the Ethics Committees of the Instituto de Psicologia of the Universidade Federal do Rio Grande do Sul and of the Secretaria Municipal de Saúde de porto Alegre and all pregnant women authorized the publication of the collected data through a Letter of Consent.

Keywords: pregnancy, stress, mental disorders

Financial Support: CNPq PIBIC (S.A.N.D.) and PQ (L.B.)

QuebraPagina

Resumo:29-078

INVOLVEMENT OF P2X AND P2Y RECEPTORS ON REGULATION OF ATRIAL FUNCTION IN NORMOTENSIVE AND HYPERTENSIVE ANIMALS

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Objectives:

The receptors P1 and P2 (P2X and P2Y) are expressed in cardiac tissue and are activated by purines (ATP) and pyrimidines (UTP), released by sympathetic neurons, endothelial cells and other tissues such as cardiomyocytes (Burnstock, G 2008). However, the role of these receptors in the heart in physiological and pathological situations is still unclear. Therefore, this study proposed to investigate the role of P2X and P2Y on the inotropy effect of the left atria (LA) isolated from normotensive Wistar rats (NWR) and spontaneously hypertensive rats (SHR).

Methods and Results:

LA of NWR and SHR animals (4-6 months) were isolated and mounted in isolated organ bath and submitted to transmural electrical stimulation (2 Hz, 5 ms and 8-12 V) to study the effect of ATP (10-4 M) and UTP (10-3.5 M) on atrial inotropism, in the absence and presence of the P2x desensitization agonist α, β-methylene ATP (10-5 M / incubated for 30 min.). The results were analyzed by unpaired t test and one-way ANOVA. ATP (10-4 M) produced an initial 60-70s negative inotropic effect (NIE), with an amplitude of 30.0 ± 2.1% for NWR and 33.9 ± 2.3% for SHR in relation to the baseline value. After this time, atrial inotropism gradually increased, showing a positive inotropic effect (PIE) reaching the plateau at 180-200s with a value of 22.7 ± 1.1% for NWR and 36.5 ± 1.9% for SHR in relation to the final value of the NIE. In the presence of α-β-Methylene-ATP the PIE effect induced by ATP was reduced by 54.7% (22.7 ± 1.1% to 10.3 ± 0.6% (p
Conclusions:

The results suggest that P2X and P2Y receptors participate in the regulation of contractile function of LA in normotensive and hypertensive animals. Furthermore, the positive inotropic response of these receptors is increased in SHR.

Keywords: Hypertension, Heart, Purinergic P1 and P2, ATP, UTP

Financial Support: CAPES and FAPESP

QUEBRAPEGINA

Resumo: 29-079

STUDY OF PERIPHERAL ADRENOCEPTORS IN THE SMOOTH MUSCLE OF YOUNG DESCENDANTS OF RATS TREATED WITH SIBUTRAMINE DURING PREGNANCY AND NURSERY

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Objectives:

The sibutramine hydrochloride monohydrate is a drug used in the treatment of obesity working through a dual mechanism of action: the increase of energy expenditure and the decrease of appetite by inhibition of noradrenaline and serotonin reuptake, respectively. The use of medicines during pregnancy deserves a special attention due to its potential hazard to the fetus development and should, on principle, be avoided. Therefore, the aim of this work was to investigate the alterations in the autonomic nervous system (adrenergic) of descendants of female rats treated with sibutramine during pregnancy and nursery.

Methods and Results:

In order to investigate this alterations, we chose two different experimental models: the vas deferens, which has a wide α-adrenergic innervation; and the trachea, which has a wide β-adrenergic innervation. It was established 14 female rats, divided in two groups: a control group treated with saline solution (NaCl 0.9%), and a group treated with sibutramine 6mg/Kg/day by oral administration. The macroscopic parameters of the mothers, male descendants and its organs were analyzed. The male descendents were used for experiments between the 40th and 50th day of life, the vas deferens and trachea were used in cumulative concentration-response curves of adrenoceptors agonists in the presence or absence of reuptake I and II inhibitors, and others specific adrenergic antangonists. Functional pharmacological parameters as pD2, DR, pA2 and its respective plots de Schild were calculated from the cumulative concentration-response curves. The results showed a tendency of the sibutramine to detain the weight gain of the mothers, when compared to the control group, as a tendency to the descendants of rats treated with sibutramine, to have a lower body weight comparing to control rat descendants. In relation to the responsiveness of α-adrenergic receptors we verified a significant decrease in the pA2 value for α1B-receptor subtype. We verified a significant increase in the pA2 value for β-adrenergic receptors.

Conclusions:

Therefore, the sibutramine administered in female rats during pregnancy and nursery showed effects on its offspring interfering in some adrenergic receptors.

Keywords: peripheral adrenoceptors, sibutramine, smooth muscle, young descendants

Financial Support: CAPES, CNPq e FAPESP
REDUCTION IN P2X RECEPTOR FUNCTION IN ENDOTHELIAL CELLS AND MACROPHAGES FROM SCHISTOSOMA MANSONI-INFECTED MICE

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2 Instituto de Ciências Biomédicas, UFRJ

Objectives:

Aim: The objective of this study was to evaluate the P2X7 purinergic receptor activation in endothelial cells and macrophages from Schistosoma mansoni-infected mice, an intravascular parasite that causes chronic inflammation related with physiological vascular alterations (Comp. Biochem. Physiol. 120:417, 1998; Vasc. Pharmacol. 46:122, 2007).

Methods and Results:

Methods: Cells: Endothelial cells and macrophages were obtained from control and S. mansoni-infected mice. Primary cultures of mesenteric endothelial cells were obtained and characterized as previously described (Br. J. Pharmacol. 151:195, 2007). Peritoneal macrophages were identified as F4/80+ cells by flow cytometry analysis. Fluorescent dye uptake assay was used to determine the function of P2X7 receptors: Concentration-response curves to (0.5, 1 and 3 mM) ATP were performed with endothelial cells and F4/80+ macrophages (105 cells/well) using 2.5 µM ethidium bromide (BE) as tracer (15 min; 37°C) and analyzed by flow cytometry. Confluent endothelial cells were treated with ATP in the presence of 5 µM BE (37°C) and the P2X7 function was also measured by fluorescence microscopy. Apoptosis assay: Confluent endothelial cells were firstly incubated with 5 mM ATP (3 hs), treated with 10 µM BE, removed from the plate and the percentage of hypo diploid cells was measured by flow cytometry. Immunocytochemistry: Endothelial cells (104 cells/well) were fixed with 4% PFA 10 min. The non specific bind sites were blocked with 10% FBS/ 10% BSA (30 min) followed by the sequential incubation with (1:100 overnight) an anti-P2X7 primary antibody and (1:300) an anti-rabbit biotinylated secondary antibody and fluorophore-conjugated streptavidin (texas red, 1:100). P2X7 expression was observed by fluorescence microscopy. Results: Dye uptake in macrophages from infected mice was smaller than in control mice (95.36 ± 1.55 %; 53.91 ± 6.02 %; respectively, n=12, P < 0.05, Student’s t test). Similarly, endothelial cells from infected mice showed a reduction of permeabilization induced by 3 mM ATP (22.16 ± 7.34 %, n = 4) when compared to endothelial cells from the control group (45.35 ± 11.25 %, n = 4). P2X7 receptor activation can result in apoptosis (Biochem. Biophys. Res. Comm. 248: 822–829, 1998). However, our preliminary data showed no difference of ATP-induced apoptosis between endothelial cells from infected (25.11 ± 8.07 %, n = 3) and control mice (32.99 ± 9.74 %, n = 3). We also evaluated the endothelial P2X7 receptors expression and we observed the presence of P2X7 receptors in both groups.

Conclusions:

Conclusion: Altogether, our data show that macrophages and endothelial cells Schistosoma mansoni-infected mice exhibit a reduction in the response mediated by the P2X7 purinergic receptor that could be related with modifications in the receptor function or with receptor-independent mechanisms as increased of the ATP cleavage mediated by ectonucleotidases.

Keywords: ENDOTHELIAL CELLS , MACROPHAGES, P2X RECEPTOR , SCHISTOSOMA MANSONI

Financial Support: CNPq, FAPERJ-PRONEX, FAPERJ
THE SYMPATHETIC NERVOUS SYSTEM REGULATES BONE GROWTH AND BODY COMPOSITION VIA ADRENERGIC α2A RECEPTOR.

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Objectives:

One of the most important findings of the recent years is that bone remodeling is subjected to the control of the central nervous system (CNS), with the sympathetic nervous system (SNS) acting as a peripheral effector. A number of studies suggest that the SNS negatively regulates bone mass, acting only via β2-adrenergic receptor (β2-AR), which is expressed in osteoblasts. However, a recent study of our laboratory showed that mice with double inactivation of α2A and α2C adrenergic receptor genes (α2A/α2C-AR−/−) have a high bone mass phenotype (HBM), in spite of showing chronic hyperactivity and intact α2-AR. It was also observed that the mRNA of both receptors (α2A and α2C-AR) are expressed in bone of wild mice and that α2A-AR is expressed more than α2C-AR (J Bone Miner Res, 26:591, 2011). These findings strongly suggest that the β2-AR is not the only adrenoceptor involved in the control of bone metabolism. The aim of this study is to evaluate whether the isolated inactivation of α2A-AR affects bone structure and physiology.

Methods and Results:

Thirty day old female C57BL/6J mice (wild-type; n = 6) and α2A-AR knockout mice (α2A-AR−/−; n = 6) were weighed weekly and sacrificed when they were 60 days old. The heart, the axillary and retroperitoneal fat pads and the extensor digitorum longus (EDL), gastrocnemius and rectus femoris muscles were collected weighted for body composition analysis. The femur and tibia were also collected for the evaluation of bone length. We found that α2A-AR−/− animals, compared to wild animals, present a 9% increase in heart weight.

Conclusions:

This initial analysis suggests that there is an involvement of α2A-adrenergic receptors in the control of body composition and bone growth.

Keywords: Bone, Sympathetic Nervous System, Bone Growth, Body Composition

QuebraPagina

Resumo: 29-082

DUAL MODULATORY ROLE OF PURINERGIC RECEPTOR STIMULATION ON PINEAL GLAND ACTIVITY

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Objectives:

Noradrenaline and ATP are cotransmitters involved in the control of the pineal melatonin synthesis which begins with the conversion of serotonin to N-acetylserotonin (NAS) by the enzyme arylalkylamine N-acetyltransferase (AA-NAT). β1-adrenoceptor activation is an essential step and activates the Aa-nat promoter via phosphorylation of the CREB transcription factor and formation of the AP1. α1-adrenoceptor or P2Y1 receptors activation have no effect alone, but potentiate β-adrenergic
response through an increase in intracellular calcium. Methylation of NAS by hydroxy-indole-O-methyltransferase (HIOMT) is the last step in melatonin synthesis (see Simonneaux and Ribelayga, Pharmacol Rev. 55:325,2003). Besides this photo-neural stimulation systemic circulating molecules reach the pineal and modulate melatonin production. Corticosterone potentiates the noradrenaline-induced effects on Aa-nat transcription and NAS and melatonin production through glucocorticoid receptors (GR) by a mechanism mediated by NFKB inhibition (Ferreira et al. J Pineal Res. 38:182,2005), an effect reversed in the presence of concomitant stimulation of α1/β adrenergic receptor subtypes (Yuwiler, J Neurochem. 52:6,1989). Here we investigate the role of purinergic receptor stimulation in modulating pineal gland activity in the presence of glucocorticoids.

Methods and Results:

Ethical committee (CEA/IB 106/2010). Rat pineal glands were cultivated for 48h and then stimulated with the β-adrenergic agonist isoproterenol (0.1M, 5h) in the absence or presence of the P2Y1 receptor selective agonist ADP (1mM). When indicated corticosterone (1μM, 48h) was added. NAS and melatonin content was measured in the medium by HPLC. NFKB and AP1 nuclear translocation were assayed in the pineal nuclear extracts stimulated with ADP (1mM, 30sec - 5min) by EMSA. Treatment of cultured pineal glands with the isoproterenol led to an accumulation of NAS (34.93 ± 0.67 ng/200μl, n=4) and melatonin (46.17 ± 2.35 ng/200μl, n=3) in the incubation media. P2Y1 receptor stimulation by ADP potentiated isoproterenol-induced NAS production (75.23 ± 11.18 ng/200μl, n=3, p

Conclusions:

Purinergic stimulation has a dual effect on the melatonin biosynthetic pathway with participation of the transcription factors AP1 and NFKB. Multiple neurotransmitters and receptors subtypes on membrane pinealocytes suggest a complex and dynamic cross-talks and cooperation networks on intracellular regulatory mechanisms. The data presented here point to a purinergic negative regulation of the enzyme HIOMT unraveling the relevance of purinergic neurotransmission to pineal activity.

Keywords: ATP, melatonin, pineal gland, purinergic receptor

Financial Support: FAPESP, CAPES, CNPq

QuebraPagina

Resumo:29-083

ACTIVATION OF JNK1/2 PROTEIN BY THE ANTICANCER DRUG PERILLYL ALCOHOL IN A CULTURED HUMAN Glioblastoma cell line (U87).

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Objectives:

The monoterpene perillyl alcohol (POH) is used in phase II clinical trials as a chemotherapeutic agent for several tumors, including gliomas. In our recent studies, POH showed a significant inhibitory effect on Na/K-ATPase activity. Na/K-ATPase is an enzyme involved in physiological functions and in signal transduction mechanisms. Aims: The present work was undertaken to determine the ability of POH in modulating the activity of mitogen activated protein kinase (MAPK) – in particular, JNK1/2 - in culture of human glioblastoma cell line (U87).

Methods and Results:
Methods: Cells were exposed to POH 0.1; 0.5 and 1.5 mM for 30 minutes. JNK1/2 activation was analyzed by western blotting. Additionally, cell viability (POH 0.1; 0.5; 1.5; 2 and 4 mM for 0.5; 1; 2 and 4 h) was analyzed by measurement of lactate dehydrogenase (LDH) activity in cell supernatants. Results: We showed that 1.5 mM POH treatment for 30 minutes induced a significant stimulation in JNK1/2 phosphorylation. Moreover, our data indicated that a 2 h treatment even with 1.5 mM POH can be cytotoxic, causing impairment on cell viability, as measured by LDH leakage.

Conclusions:

Conclusion: The present study showed an initial result of activation of intracellular signaling pathways in human glioblastoma cell line (U87) by POH. Since ouabain, a specific Na/K-ATPase inhibitor, also activates this same protein, we are now comparing other members of the signaling cascade triggered by this glycoside in order to compare with the response obtained when POH is used in place of ouabain.

Keywords: Na/K-ATPase, perillyl alcohol, anticancer drugs, U87 glioma cells , JNK1/2

Financial Support: FAPERJ; CNPq; FOPESQ-UFF; CAPES.

QuebraPagina

Resumo:29-084

ANTIESTROGEN FULVESTRANT REGULATES THE EXPRESSION OF ANDROGEN RECEPTOR IN RAT EPIDIDYMIS.

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Objectives:
The epididymis is a highly specialized tissue of the male excurrent duct system, which plays an important role in sperm maturation, and can be functionally divided into four distinct regions: the initial segment, caput, corpus and cauda. The functional segmentation of the epididymis is regulated by androgens and growth factors (J Endocrinol 190:779, 2006), and is reflected at molecular level by complex region-specific gene expression (Biol Reprod 65:696, 2001). Although estrogen and the classic estrogen receptors (ESR1 and ESR2) are also present in the epididymis (reviewed in Arq Bras Endocrinol Metab 53:923, 2009), the role of estrogen in epididymal function is still not completely understood. The antiestrogen fulvestrant (ICI 182,780), which does not modify testosterone and 17α-estradiol plasma levels in male rats (Biol Reprod 79:432, 2008) but impairs estrogen action on ESR1 and ESR2, can regulate the expression of AR (Mol Cancer Ther 5:1539, 2006), and the expression of ESR1 (Reprod Biol Endocrinol 10:1, 2003; Reprod Biol Endocrinol 2:3, 2005) within the male reproductive tract. Thus, this study was proposed to further investigate the role of estrogens during pubertal development of rat epididymis, by analyzing the effect of ICI 182,780 treatment on the expression of steroid receptors (AR, ESR1 and ESR2) in the caput, corpus and cauda of the epididymis.

Methods and Results:
Thirty-day-old rats were treated once a week for 2 months with corn oil (control group) or fulvestrant (ICI 182,780) (10 mg/rat, s.c.). Testosterone and estradiol levels were measured by radioimmunoassay. Expression of the steroid receptors AR, ESR1 and ESR2 were measured by real time PCR and Western blot. Fulvestrant did not modify testosterone and estradiol levels in the caput of the epididymis. In the corpus and cauda, fulvestrant decreased the testosterone levels by 35% and 47%, respectively, and increased the estradiol levels by 178% and 100%, respectively. Treatment with fulvestrant did not affect the mRNA levels for AR, but markedly increased the AR protein levels in caput (3.5-fold), and to a lesser extent in corpus (1.3-fold) and cauda (1.6-fold). The mRNA levels for ESR1 were lower in corpus with the treatment, but the protein did not change in any epididymal region. Fulvestrant did not modify the mRNA and protein levels for ESR2.

Conclusions:
Fulvestrant up-regulates AR expression in the epididymis through post-transcriptional mechanisms. Furthermore, the results suggest the involvement of differential signaling mechanisms in regulating and/or mediating the actions of estrogen in the different regions of the epididymis. This regulatory diversity is probably important to control region-specific sperm-related functions.

Keywords: Androgen receptor, Antiestrogen, Epididymis, Fulvestrant, Rat

Financial Support: FAPESP, CNPq

QuebraPagina

Resumo:29-085

DESENSITIZATION AND INTERNALIZATION OF RECOMBINANT α1A-ADRENOCEPTORS INDUCED BY OXYMETAZOLINE

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Objectives:
Oxymethazoline (OXY) is an imidazoline derivative largely used in nasal decongestant formulas. This compound is known as an α1A-adrenoceptor (α1A-AR) agonist, and in previous experiments performed in our laboratory, OXY induced tachyphylaxis in tissues whose contractions result from α1A-AR activation, despite the weak partial agonism of OXY. It is interesting that full agonists like norepinephrine (NE) do not induce the same tachyphylaxis that OXY does. The aim of this study was to investigate if OXY induces desensitization and internalization of human recombinant α1A-AR.

Methods and Results:
HEK293 cells were transfected with the cDNA encoding human recombinant α1A-AR using Lipofectamine®. The intracellular calcium was detected using the fluorescence dye FLUO4-NW. The concentration of IP3 was measured by fluorescence polarization and was based in a standard curve of IP3. The IP3 and calcium experiments were performed in a black walled 96-wells plates, using bandpass 428/20 filter for excitation and bandpass 585/20 filter for emission. For fluorescence polarization, a dichroic mirror was used. Concentration-response curves were analyzed in the Prism 5 software. In both experiments the curves for NE after pre-treatment with NE or OXY (10µM) were compared with a control curve of NE. The internalization was measured by whole cell binding assay using [3H]Prazosin. The non-specific binding was determined with 100µM of phentolamine. Data are presented as mean±SEM of 3 to 5 experiments. Data from calcium assay are presented as percentage of control, data from IP3 measurements as concentration (nM) and data from binding assay as fmol/mg of protein. The pre-treatment with NE 10µM/5 min did not change the increase in intracellular calcium (control: 100.0±4.33 vs 114.8±6.90) neither the IP3 formation (control: 25.25±1.89 vs 22.87±2.39) induced by NE, indicating that this pre-treatment do not desensitize the α1A-AR. The pre-treatment with OXY 10µM/5 min desensitized the α1A-AR, as NE increased the intracellular calcium by only 20.18±4.40 and the IP3 formation by only 2.17±1.07. OXY for 5 minutes induces small internalization of α1A-AR as the maximal binding capacity of [3H]Prazosin to the cell membrane of intact HEK 293 cells decreased from 248.4±10.1 to 222.7 ±14.2 without affecting the affinity of the radioligand.

Conclusions:
Albeit being only a weak partial agonist, OXY desensitizes but not internalizes the α1A-AR expressed in HEK293 cells. The receptor desensitization may explain the tachyphylaxis observed in the responses to OXY.

Keywords: DESENSITIZATION, OXYMETAZOLINE, ALPHA1-ADRENOCEPTOR
Objectives:
The pineal gland, due to the nocturnal secretion of melatonin, synchronizes the organisms to the environmental light/dark cycle. Besides its well-known chronobiologic effects, melatonin has properties of an immune modulator, influencing the activation of T, B and NK cells and the release of several cytokines (Int J Biochem Cell Biol. 38; 313, 2006). Now it has been demonstrated that this is a two-way pathway, since the melatonin production by the pineal gland is modulated in response to inflammatory mediators (Neuroimmunomodulation 14; 126, 2007). In fact, pinealocytes express receptors for pathogen- and danger-associated molecular patterns (PAMPs/DAMPs) such as toll-like receptor 4 (TLR4), and for pro-inflammatory cytokines such as tumor necrosis factor (TNFR1), whose signalizes through the nuclear transcription factor kappa B (NFKB) (J Pineal Res. 49; 183, 2010). Taking into account the relevance of central inflammatory processes in neurological diseases, the aim of this study was to investigate whether the amyloid-beta peptide (Ab), a hallmark in Alzheimer’s disease (AD) that may act through TLR4 receptors, can affect the physiology of the pineal gland.

Methods and Results:
Pineal glands were obtained from male Wistar rats (Animal Committee Care IB-USP: 111/2010), 2 month-old, kept under 12h:12h light/dark cycle. The glands were immediately placed in 24-wells plate with BGJb medium and maintained at 37°C, 95% O2, 5% CO2 for 48h. The glands were then incubated with Ab1-40 fragment (1 µM, 0.5h to 24h), and melatonin production was induced by norepinephrine (100 nM, 5h). Melatonin in the medium was determined by high performance liquid chromatography (HPLC) and pineal nuclear NFKB content were assessed by electromobility shift assay (EMSA). Cultured glands showed a transient increase in nuclear translocation of NFKB (peak at 2h), followed by a 43.33% inhibition of norepinephrine-induced melatonin (basal = 45.88 ± 8.7 ng/well; n = 3-5). Along with the increase in NFKB nuclear content, it was observed a significant increase in TNF levels in the medium (control: 20.65 ± 5.56 versus Ab: 74.45 ± 19.27 pg/mL; n = 4), determined by ELISA commercial kit. In addition, pineal glands obtained from animals submitted to Ab1-40 in vivo infusion along 35 days in the lateral ventricle presented a marked increase in the expression of TLR4 in pinealocytes compared with animals that received vehicle solution, analyzed qualitatively by confocal microscopy.

Conclusions:
The data presented here indicates that the pineal gland is capable of responding not only to classical inflammatory mediators but also to DAMP molecules such as Ab. Moreover, Ab peptide activates a signaling cascade that culminates in the activation of NFKB, leading to inhibition of melatonin synthesis and production of TNF, which can further amplify this signal. Taking into account the well documented reduction in circulating melatonin in AD patients and also in animal models for AD, the investigation of the pineal response to Ab will contribute to a better understanding of the first processes evoked by Ab.

Keywords: Alzheimer, immune-pineal axis, Melatonin, NFKB, pineal

Financial Support: FAPESP, CNPq
PHARMACOLOGIC EVALUATION OF NEW MULTI-TARGET ALPHA1A/D-ADRENOCEPTORS AND 5-HT1A ANTAGONISTS CANDIDATES TO LEAD COMPOUNDS FOR THE TREATMENT OF BENIGN PROSTATIC HYPERPLASIA


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Objectives:

Previously, we described that new alpha1A/D adrenoceptor (a1A/D-AR) antagonists (LASSBio-772 – USPTO 20070219213 - and LDT series) (Romeiro et al, Eur. J. Med. Chem., accepted) also bind to 5-HT1A receptors in the nM range. Both a1A/D-AR and 5-HT1A receptors are implicated in the physiopathology of benign prostatic hyperplasia (BPH), therefore these new multi-target compounds could be considered as hit compounds in the search of drugs for BPH treatment. The objectives of this study were to i) assess the affinity of these compounds for other G-protein coupled receptors of biogenic amines as they share some structural features at the ligand binding region and ii) to estimate their intrinsic activity for the 5-HT1A receptors.

Methods and Results:

All protocols were approved by the ethics committee of UFRJ (CAUAP; DFBC-ICB011). Binding assays: 150 µg of protein obtained from rat cortex (α2-AR), striatum (D2-like) and hippocampus (5-HT1A) were incubated with 0.5 nM [3H]RX-821002, 0.1 nM [3H]-YM091512 or 0.5 nM [3H]-pMPPF, respectively, for 45-60 min at 25-37°C in the absence or presence of LDT65–LDT68 and LASSBio-772 (0.001-50 uM). The reaction was stopped by the addition of Tris-HCl buffer (pH = 7.4), followed by filtration under vacuum. The radioactivity was quantified in a liquid scintillation counter. The data were analyzed by non-linear regression using GraphPad Prism 4.0 (USA) to yield the IC50 values that were converted to Ki values using the Cheng-Prusoff equation (Biochem. Pharmacol. 22: 3099-108, 1973). The competition curves indicated a concentration-dependent inhibition with all tested substances. The mean Ki values (µM) were: 1.8, 0.9, 0.6, 0.7, 0.1 for the α2-AR, and 0.04, 0.04, 0.009, 0.008, 0.02 for the D2-like receptors, respectively. Preliminary results with an antagonist radioligand ([3H]-pMPPF) indicated Ki values of 25, 3.3, 1.3, 3.2, 0.7 nM for 5-HT1A receptors. As a whole, the compounds had the following sequence of affinity: 5-HT1A > D2 >> α2. Binding assays for 5-HT1A receptors with an agonist radioligand (8-OH-DPAT) and Ki calculation were previously performed (data not shown). The Ki ratio (Ki when the radioligand was antagonist/Ki when the radioligand was agonist) (Eur. J. Pharmacol. 386: 97-103, 1999) were closed to unity, indicating that our compounds are 5-HT1A receptors antagonists.

Conclusions:

The compounds have a lower affinity for α2-AR than for D2-like receptors. On the other hand, these compounds show relatively high affinity for a1A/D-AR and 5-HT1A receptors acting as an antagonist of receptors involved in the BPH physiopathology, being potential lead compounds in the BPH treatment.

Keywords: alpha1-adrenoceptors, antagonist, benign prostatic hyperplasia, multi-target, 5-HT1A

Financial Support: FAPERJ, CNPq, CAPES
**PROTEIN EXPRESSION OF ESTROGEN RECEPTORS (ERα AND ERβ) AND AROMATASE IN MYOMETRIUM AND UTERINE LEIOMYOMA**

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**Objectives:**

Leiomyomas are common benign tumors of the female reproductive tract. They are a major public health problem since nowadays surgical treatment is the most effective. Evidence suggests that estrogens regulate cell proliferation and myoma growth. This estrogen-mediated effect might be due to different amounts of estrogen receptors (ERα and ERβ) in normal and myoma tissues and overexpression of aromatase P450 in myomas, which is responsible for conversion of androgens to estrogens in situ.

**Methods and Results:**

Samples were collected from 12 premenopausal women admitted for abdominal hysterectomy due to fibroids. Inclusion criteria were hysterectomy due to menorrhagia, compressive symptoms and rapid tumor growth. Exclusion criteria were adenomyosis, malignancy or use of hormonal therapy in the previous 3 months before surgery. The fragments of leiomyoma and adjacent myometrial tissue collected were immediately frozen in liquid nitrogen and stored at -80°C for subsequent extraction of total RNA, by RT-PCR, and proteins, by Western blot. The diagnosis of leiomyoma was confirmed by histopathologic examination. The protein expression of ERα, ERβ and aromatase was similar in leiomyoma and normal myometrium. The protein expression of ERα was similar in leiomyoma [0.782 (0.468 - 1.727)] and normal myometrium [1.137 (0.559 – 2.456)] (Wilcoxon T test P = 0.239). In the protein expression of ERβ there was no statistical difference between normal myometrium (0.560 ±0.125) and leiomyoma (0.583 ± 0.158) (Student’s t test for paired samples P = 0.695). The analysis of cytochrome p450 aromatase also showed no statistical difference in protein levels of myoma (3.964 ± 0.929) and adjacent myometrium (2.678 ± 0.463) (Student's' t test for paired samples P = 0.203).

**Conclusions:**

In this analysis of 12 matched leiomyoma and myometrial samples, the data do not support the theory that overexpression ERα, ERβ and aromatase in uterine leiomyomas compared to adjacent myometrium. The estrogenes may exert their growth-stimulatory effects on leiomyomas intermediated by other elements, such as cytokines and growth or apoptosis factors. The effect of estrogen on the growth and development of fibroids is complex and far from being completely understood.

**Keywords:** Leiomyoma, Myometrium, ERα, ERβ, Aromatase

**Financial Support:** FIPE/HCPA, UFRGS and CNPq

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**ADENINE EFFECTS ON KINASE PROTEINS AND APOPTOSIS IN LLC-PK1 CELLS**

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3 Universidade Federal Fluminense, UFF
Objectives:

Extracellular adenine, an adenosine metabolite derived by hydrolysis, has been related to different physiological properties. Therefore, reversible interconversion reactions of adenine nucleobase into adenine nucleoside and nucleotides, together with nucleoside transporters, nucleotide channels and purine receptors suggest a molecular network responsible for the great diversity of biological effects induced by these purine derivatives. During ischemic condition occurs breakdown of high energy compounds, the loss of cell integrity and the activation of degenerative molecular pathways. We observed in our laboratory that during ischemia metabolic protein kinase A and C (PKA and PKC) were activated, the intra- and extracellular ATP levels were reduced, and the extracellular adenine level was increased. However, nothing is known about the effects of high adenine concentration during ischemia. Therefore, the aim of this study was to investigate the effect of adenine on protein kinase activities and apoptosis.

Methods and Results:

Methods and results: PKC and PKA activities were measured by histone phosphorylation. Initially, the PKA and PKC activities were measured in normoxic condition in the presence of increasing doses of adenine. We observed that increasing adenine concentration from 10-9 to 10-7M stimulated PKA and PKC activities in a dose dependent manner reaching the maximum effect at 10-7 M (raised from 3.4±0.3 to 15.8±2 pmol histone-P.mg-1.min-1 for PKA and raised from 4.3±0.2 to 23±2,1 pmol histone-P.mg-1.min-1 for PKC) (n=12). On the other hand, higher concentrations of adenine (10-6 to 10-4 M) reversed the kinase activities to control level. It is well known that in ischemic condition, where we observed an increase in adenine level, cells undergo apoptosis (ref). To verify the possible apoptotic effect of adenine, LLC-PK1 cells were labeled with annexin V-FITC, in combination with propidium iodide (PI), to quantitatively determine the percentage of cells undergoing apoptosis by flow cytometry. It was observed that the increase in adenine concentration from 10-9 to 10-6 M increased the percentage of annexinV-FITC+ cells in a biphasic mode with maximum effect in 10-8 M, reaching 86%, while higher concentrations reversed the percentage of annexinV-FITC+ cells to control levels (n=5).

Conclusions:

Together, these results indicate that in normoxic condition PKA and PKC are stimulated and apoptosis was induced by increasing adenine concentration. These data suggest that high extracellular adenine during ischemic condition could be responsible, at least in part, for the stimulation of PKA and PKC activities together with early apoptosis events. Therefore our data suggest a potential role for adenine during ischemic condition.

Keywords: kidney, metabolism, purine

Financial Support: FAPERJ, CAPES CNPq and INCT-INBEB/CNPq/MCT

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Resumo:29-090

TACHYPHYLAXIS IN RESPONSES MEDIATED BY THE LOW EFFICACY AGONIST OXYMETAZOLINE AT NATIVE AND RECOMBINANT ALPHA1B-ADRENOCEPTORS.

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Objectives:
To investigate the ability of the full agonist noradrenaline and the partial agonist oxymetazoline to induce tachyphylaxis in responses mediated by α1B-adrenoceptor activation in human recombinant and rat native receptor systems.

Methods and Results:

For the studies of rat native α1B-adrenoceptors, the contractions of the rat spleen were used as models of actions of noradrenaline and oxymetazoline mediated by α1B-adrenoceptors. HEK293 cells expressing recombinant α1B-adrenoceptors tagged with the FLAG epitope at the N-terminus were used to detect intracellular calcium increases with FLUO-4 NW and cell surface receptors in an intact cell type ELISA employing anti-FLAG monoclonal antibodies. In the rat spleen there was no tachyphylaxis in the contractions induced by noradrenaline as three consecutive cumulative concentration-response yielded similar maximal effects (Emax) and potencies (pD2). On the other hand, albeit oxymetazoline was a weak partial agonist inducing approximately 30% of the contraction induced by noradrenaline, there was intense tachyphylaxis as the rat spleen did not contracted in response to consecutive exposures to oxymetazoline. In addition, after treating the rat spleen with oxymetazoline 100 µM for 15 minutes there was a shift to the right in the concentration-response curve to noradrenaline (pD2=4.6) associated with a reduction in the Emax (0.77). In HEK293 cells expressing FLAG-tagged α1B-adrenoceptors and loaded with the fluorescent intracellular calcium indicator FLUO-4 NW, oxymetazoline was virtually ineffective in inducing intracellular calcium increases, whereas noradrenaline was a potent (pD2=6.7) and full agonist in relation to adrenaline. In cell-based Elisa, both noradrenaline and oxymetazoline were able to induce loss of cell surface α1B-adrenoceptors with similar potencies (pD2=6.8), although whereas noradrenaline internalized approximately 85% of the receptors, oxymetazoline reduced the cell surface receptors by approximately 55%. In order to investigate if the α1B-receptors were capable to recycle, we conducted an experiment allowing the cells to recover up to 150 minutes recover after treatment with maximal concentrations of noradrenaline and oxymetazoline. The surface expression of α1B-adrenoceptors was fully recovered after 15 minutes of treatment with oxymetazoline, whereas there was no recovery of cell surface receptors cells after treatment with noradrenaline.

Conclusions:

The low efficacy agonist oxymetazoline induces tachyphylaxis in responses mediated by human recombinant and rat native α1B-adrenoceptors, whereas there is no tachyphylaxis in the responses induced by the full agonist noradrenaline. There is agonist trafficking or biased agonism for oxymetazoline at recombinant α1B-adrenoceptors as this agonist was virtually unable to increase intracellular calcium in HEK293 cells, but was a potent agonist in inducing α1B-adrenoceptor internalization. However, the tachyphylaxis in the responses induced by oxymetazoline is not completely explained by the ability of this weak partial agonist to induce receptor internalization as receptor recycling was faster indicating that other factors are involved.

Keywords: alpha1B-adrenoceptors, oxymetazoline, tachyphylaxis

Financial Support: CAPES

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Resumo:29-091

MODULATION OF THE PLASMODIUM FALCIPARUM ERYTHROCYTIC STAGE BY CAMP-DEPENDENT PROTEIN KINASE

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Objectives:
**Plasmodium falciparum** is the etiologic agent of the most severe form of malaria. Drug-resistant parasite strains associated with insecticide resistance in mosquitoes and the absence of an effective vaccine turn malaria a serious public health problem worldwide, and strategies are required for the identification of potential therapeutic targets. Previous studies have revealed that several homologues of eukaryotic signaling proteins are conserved in *P. falciparum* and there are evidences that protein kinases are essential for the control of the parasite life cycle so that inhibition of such activities can have anti-malarial effects. cAMP-dependent protein kinase (PKA) is an important signaling transduction element in mammalian cells, and the regulatory mechanisms of the cAMP pathway are well known. We recently demonstrated that Angiotensin II (Ang II), the main effector molecule of the rennin-angiotensin system (RAS), when converted into Ang(1-7) controls the erythrocytic cycle of the malaria parasite in a MAS-mediated PKA inhibition (PLos One; 6, e17174, 2011). There are reports of PKA homologues (PfPKA) in the *P. falciparum* genome. However, components of the cAMP pathway and the precise function of PKA, have not been clearly defined. In this work, we report a pivotal role of the cAMP pathway in the host cell invasion process and a possible cross talk with other signaling pathways.

**Methods and Results:**

*P. falciparum* (W2 strain) erythrocytic cycle was maintained in RPMI 1640 medium in the presence of A+ type human blood, 10% of A+ human serum and 50μg/ml gentamicin at 5% hematocrit. After synchronization in the schizont form, the cultures were treated or not with different agents, as described subsequently. After 24h of treatment, the parasitemia was measured by counting the ring forms in a thick blood smear. Dibutyryl-cAMP 10⁻⁶ M, a permeable cAMP, was able to stimulate erythrocyte invasion by parasite in 44%. The pre-treatment of erythrocytes with 10⁻⁶ M dibutyryl-cAMP, for 3 consecutive days, stimulated invasion even more, in 66% in relation to the control. In addition, we tested the effect of 10⁻⁶ M calfostin C, a protein kinase C (PKC) inhibitor and 10⁻¹² M phorbol myristate acetate (PMA), a well-known PKC activator. PMA treatment did not have any effect in the *P. falciparum* erythrocytic cycle, however 10⁻⁶ M calfostin C inhibited invasion in 50% when compared to the control. Immunodetection of PKA catalytic and regulatory subunits in erythrocytes ghosts revealed a displacement of both subunits when treated with 10⁻⁶ M dibutyryl-AMPc.

**Conclusions:**

Taken together, the results presented in this study provide evidence of potential roles for the host cAMP and PKC pathways in controlling the *Plasmodium falciparum* erythrocytic cycle.

**Keywords:** Plasmodium falciparum, Red blood cells, cAMP-Dependent protein kinase

**Financial Support:** FAPERJ, CAPES, INBEB, INPETAm and CNPq

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**Resumo:**

A PHOSPHOLIPASE A2 ISOLATED FROM LACHESIS MUTA SNAKE VENOM INCREASES THE SURVIVAL OF RETINAL GANGLION CELLS IN VITRO: AN EFFECT MEDIATED BY LYSOPHOSPHATIDYLCHOLINE (LPC) AND EGF RECEPTOR.

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**Objectives:**

Snake venoms consist of a complex mixture of proteins that are responsible for a wide range of pharmacological activities. Among these proteins, we may highlight the phospholipase A2 enzymes. Phospholipase A2 (PLA2) is a member of growing family of enzymes (E.C. 3.1.1.4.) that catalyzes the hydrolysis 2-acylester bond in 3-sn-phosphoglycerides leading to the production of two active products: free fatty acids and lysophospholipids also called lysophosphatidylcholine or lysolecithin.
Previous data from our group demonstrate that PLA2 [CT48h=53.1% ± 2.6%; PLA2 2.5μg= 55.5% ± 3.7%; PLA2 5.0μg= 84.4%±2.5%; PLA2 10μg=70.3% ± 2.8%; PLA2 5μg=45.5 ±3.3%; n=7] and its product LPC [CT48h=53.6% ± 4.0%; LPC 1μM= 53.5% ± 1.9%; LPC 5μM= 61.9%±2.7%; LPC 10μM =90.9% ± 1.8%; LPC 25μM=47.4 ±3.2%; LPC 50μM= 29.1%±4.1%; n=7] induced an increase in retinal ganglion cells (RGC) survival. The aim of this work was to evaluate the signaling pathways involved in LPC effect on RGC survival.

Methods and Results:

To identify the RGC, neonatal rats received injections of horseradish peroxidase (HRP) into both superior colliculi. After 16h the animals were sacrificed and their retinas dissected, dissociated and plated (10^5 cells /cm²) in the culture medium (CCM). The cultures were maintained for 4 hours at MCC and then some coverslips were fixed in aldehydes (100 % of the population of RGC). Other cultures were treated and maintained for more 48 hours. After this time the cultures were fixed in aldehydes. The presence of HRP was revealed by histochemistry using tetramethylbenzidine as chromogen. The number of RGC was obtained by counting in brightfield microscopy. Our previous results show that the effect of LM-PLA2 on RGC survival was dependent on the enzyme activity and the same effect was obtained with commercial LPC treatment. The data present here show LPC effect was not blockade by: P38 inhibitor [CT48h=53.9% ±3.1%; LPC 10μM=87.1%±0.5%; SB202190 20μM=53.0%±3.0%; LPC +SB=95.4 ±5.6% n=5]; and PKA inhibitor [CT48h=53.8%±2.4%; LPC10μM=88.0%±0.8%; H89 1μM=48.6%±0.9%; LPC +H89=88.1% ±3.5% n=5. However, the Src inhibitor abolished the LPC effect [CT48h=52.3%±1.4%; LPC 10μM=90.9%±3.1%; PP1 1μM=50.12%±1.4%; LPC +PP1=44.83 ±1.6% n=6]. We also observed an involvement of EGF receptor and JNK pathway in the LPC effect [CT48h=51.5%±1.3%; LPC 10μM=91.9%±3.7%; AG1478 2.5μM=48.8%±1.7%; LPC +AG1478 =46.50 ±2.3% n=6] [CT48h=48.5% ±1.2%; LPC10μM=90.80% ±3.7%; iJNK 1μM=50.01 % ±3.2%; LPC +iJNK=47.9% ±2.9%, n=6].

Conclusions:

Our results indicate that the trophic effect of PLA2 in the population of RGC is mediated by its hydrolysis product LPC. Interestingly, the effect also involves the activation of EGF receptor. Taken together our results show an important role for PLA2 and LPC in controlling the survival of axotomized neurons.

Keywords: Phospholipase A2, Lysophosphatidylcholine, retinal ganglion cells, Survival

Financial Support: FAPERJ, CNPq e PRONEX-MCT.
Methods and Results:

Hippocampal neurons are exposed to 0.2 mM of sodium palmitate and the levels of IRS-1 serine phosphorylation are analyzed by immunocytochemistry. The serine residues of IRS-1 analyzed were Ser616, Scr307 and Ser636. These serine residues are known to be involved in mechanisms of insulin resistance. The results show an increase in IRS-1 serine phosphorylation, similar to what we observe in hippocampal neurons exposed to Aβ oligomers, considered the proximal toxins in AD. In addition, adult rats that received intracerebroventricular (i.c.v.) injections of sodium palmitate were used and hippocampal levels of pSerIRS-1 are currently being analyzed. We will further test if AD-like pathologies can be driven by i.c.v. injections of palmitic acid.

Conclusions:

Our results show that palmitic acid and Aβ oligomers impair neuronal insulin signaling by inhibiting IRS-1 signaling. Results further suggest common mechanisms accounting for peripheral insulin resistance may be at play across the blood-brain barrier, instigating the initial steps of AD.

Keywords: Aβ oligomers, Alzheimer's disease, Insulin signaling, lipotoxicity, Palmitic acid

Financial Support: CNPq, FAPERJ, HFSP, John Simon Guggenheim Memorial Foundation.

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Resumo:29-094

EXPRESSION AND PHARMACOLOGICAL CHARACTERIZATION OF ALPHA-1 ADRENOCEPTOR SUBTYPES IN THE RAT DISTAL CAUDA EPIDIDYMIS

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Objectives:

The aim of this study is to identify the mRNA expression of α1-adrenoceptor subtypes in the rat distal cauda epididymis and to characterize its pharmacological properties using radioligand binding and functional assays.

Methods and Results:

Methods: Tissue isolation. Adult male Wistar (12-16 weeks / 300-350 g) rats were killed by decapitation and the distal cauda epididymis was isolated and cleaned. Total RNA extraction and Real-time RT-PCR. Total RNA was extracted using the Trizol reagent and Real-time PCR was performed using specific primers for each receptor subtype. Tissue segment binding. The distal cauda epididymis was cut into small segments (2 mm length) and used for radioligand binding saturation or competition assays. In saturation experiments, increasing concentrations of [3H]Prazosin (20 to 2000 pM) were used. In binding-competition experiments, a concentration of 350 pM [3H]Prazosin was used and the ability of increasing concentrations of non-radioactive competitors to displace the binding of the radioligand was determined. Each segment was incubated with [3H]Prazosin for 16h at 4°C. Non-specific binding was defined in presence of phentolamine 100 µM. In vitro contraction studies. Segments of distal cauda epididymis were suspended in organ baths and the isometric contractions were recorded. Concentration-response curves to noradrenaline were obtained in the absence and presence of selective α-adrenoceptor antagonists. Results: mRNA for all three α1-adrenoceptor subtypes was found in the rat distal cauda epididymis at different levels. The mRNA levels for α1A-adrenoceptor (9e-06 ± 3e-06 ng.DNA) and α1D-adrenoceptor (3e-06 ± 1e-06 ng.DNA) were similar, but higher than that for the α1B-adrenoceptor mRNA level (1.5e-07 ± 2.9e-8, P
Conclusions:

The three α1-adrenoceptor subtypes are expressed in the rat cauda epididymis, however, only the α1A-adrenoceptors mediated the contractile responses to noradrenaline. The binding studies confirm the presence of α1A-adrenoceptor and α1D-adrenoceptors, but the function of the α1D-adrenoceptors remain unclear.

Keywords: alpha-1 adrenoceptor subtype, cauda epididymis, smooth muscle, tissue segment binding assay

Financial Support: CAPES and Fapesp
plays an important role during the development and adult life of retinal cells.

Keywords: IL-6, BDNF, CYTOKINE, GANGLION CELL, RETINAL

Financial Support: CAPES, CNPq, FAPERJ e PRONEX.

ROLE OF ADENOSINE A2A RECEPTOR AND CHRONIC STRESS ON ADRENAL CATECHOLAMINE REGULATION.

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Objectives:
Stress related disorders are one of the greatest burdens of disease worldwide. Antagonists of adenosine A2A receptors (A2ARs) afford neuroprotection against some of the modifications induced by chronic stress in the central nervous system. In the peripheral nervous system the stress response activates the sympathetic nervous system and the synthesis and release of catecholamines. The aim of our work was to study the role adenosine A2AR in the modulation of peripheral modifications such as catecholamine release both in vitro (in adrenal chromaffin cell culture) and in vivo (in mice subject to chronic stress).

Methods and Results:
Primary cultures of C57B6 mouse adrenal chromaffin cells were obtained as previously described (Proc. Natl. Acad. Sci USA, 103:10497–10502, 2006). Cells were incubated for 5 min either with Krebs (basal) with Krebs containing the compounds tested. The levels of norepinephrine (NE) and epinephrine (EP) in the medium were determined by HPLC with electrochemical detection. Alternatively, C57B6 mice were submitted to chronic unpredictable stress (CUS, a protocol including uncontrollable daily stressors such as tilted cage, food/water deprivation, foot shock, paired caging, continuous light, wet bedding, for 21 days). Some mice were exposed to caffeine (1g/L, a non-selective A2AR antagonist) or KW6002 (3 mg/kg, a selective A2AR antagonist) administered in the drinking water 4 weeks before the beginning of CUS and during all the protocol. In mouse chromaffin cells in culture, CGS21680 (30 nM, an A2A receptor agonist), but not CPA (100 nM, an A1 receptor agonist) stimulated NE and EP release (p

Conclusions:
In conclusion, our results showed that A2AR can modulate catecholamine release both in vitro and in vivo, further supporting the potential of A2AR antagonists to manage modifications induced by chronic stress.

Keywords: A2A receptors, catecholamines, stress

Financial Support: FCT-Portugal
ANALYSIS OF THE HTR1A SEROTONIN RECEPTOR EXPRESSION IN NEURAL STRUCTURES RELATED TO LEARNING AND MEMORY IN RATS TREATED WITH STANDARDIZED EXTRACT OF GINKGO BILOBA (EGB 761)

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Objectives:
The standardized extract of Ginkgo biloba (EGb761) is currently used to improve cognitive decline that occurs during aging or in treatment of symptoms of Alzheimer's diseases. But its effects on the central nervous system (CNS) are not restricted to memory; EGb761 is also neuroprotective and anxiolytic. To investigate the underlying molecular mechanisms in the process of memory formation of conditioned fear might contribute to better understand the effects of EGb761 on the CNS. Serotonin receptors (5-HT) has been associated with anxiety and memory. Among the subtypes of 5-HT receptors, the 5-HT1A receptor is differently expressed in neural structures such as the prefrontal cortex (PFC) and amygdaloid complex (AC), both involved in fear conditioning. Numerous signaling cascades that include extracellular-signal-regulated kinase 2 (Erk2) have also been demonstrated to be involved in the acquisition of conditioned fear and to be modulated to EGb761.

Methods and Results:
To elucidate the effects of EGb761 in CNS, we investigate whether the expression of Htr1a and Erk2 in the PFC and AC of rats Wistar, males, was modulated following acute, subacute or chronic treatment with EGb761 (n=3/group). The off-baseline CER procedure was used to evaluate fear memory and was calculated for each rat, in 6 trials. Additionally, we investigate relative 5-HT1A and Erk2 mRNA using quantitative PCR (qPCR). Expression of Htr1a was reduced in PFC of rats treated with 1.0 EGb761 g.Kg-1 compared to groups handled, 12% Tween 80 and 0.5 EGb761 g.Kg-1 (p

Conclusions:
Our findings suggest that the effects observed in the previous study in our laboratory may be mediated by serotonin and corroborate with the evidence that the reduced suppression of conditioned fear in animals submitted to treatment with EGb761 may be correlated to the anxiolytic effect of the extract

Keywords: Gingko biloba, fear memory, 5-HT1a, Erk2

Financial Support: FAPESP
Considering that the mineralocorticoid receptor (MR) is the receptor for aldosterone and that this steroid can bind to glucocorticoid receptor (GR), the objective of this study is verify the participation of these receptors in the effects of aldosterone on H⁺-ATPase in the proximal tubule.

Methods and Results:

The genomic and nongenomic effects of aldosterone on the intracellular pH recovery rate (pHirr) via H⁺-ATPase and on cytosolic free calcium concentration ([Ca²⁺]i) were investigated in isolated proximal S3 segment of rat (Wistar, male, 90g), during superfusion with Na⁺-free solution, by using the fluorescent probes BCECF-AM and FLUO-4-AM, respectively. The pHirr, after cellular acidification with an NH₄Cl pulse, was 0.064 +/- 0.003 pH units/min (n = 74) and was abolished by concanamycin. Aldosterone [10⁻¹², 10⁻¹⁰, 10⁻⁸ or 10⁻⁶M with 1h or 15 or 2 min preincubation (pi)] increased the pHirr. The baseline [Ca²⁺]i was 103 +/- 2nM (n = 58). After 1 min of aldosterone addition (10⁻¹² or 10⁻⁶M) to the bath there was a transient and dose-dependent increase of [Ca²⁺]i and after 6 min pi there was a new increase of [Ca²⁺]i that persisted after 1h. Spironolactone (MR antagonist), actinomycin D or cycloheximide did not affect the effects of aldosterone (15 or 2 min pi) on pHirr and on [Ca²⁺]i, but inhibited the effects of aldosterone (1 h pi) on these parameters. RU 486 (GR antagonist) prevented the effect of aldosterone (10⁻¹² or 10⁻⁶M, 15 or 2 min pi) on both parameters.

Conclusions:

The data indicate a hormonal genomic (1h, via MR) and a nongenomic action (15 or 2 min, probably via GR) on the H⁺-ATPase and on [Ca²⁺]i and are in accordance with our finding showing expression of these receptors in the proximal S3 segment. The results are compatible with stimulation of the H⁺-ATPase by increases in [Ca²⁺]i (at 10⁻¹² or 10⁻⁶M aldosterone) and inhibition of the H⁺-ATPase by decreases in [Ca²⁺]i (at 10⁻¹² or 10⁻⁶M aldosterone plus RU 486). These aldosterone effects may represent physiologically relevant regulation of proximal tubular acidification in the intact animal.

Keywords: aldosterone, calcium, GR, MR

Financial Support: FAPESP, CNPq

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Resumo:29-099

CHARACTERIZATION OF THE ACTIVITY OF PROMOTER REGIONS OF CD36 GENE IN THE NERVOUS SYSTEM

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Objectives:

CD36 (Cluster of Differentiation) is a multifunctional glycoprotein expressed in many cells and have been associated with numerous biological processes that define it as a multiligand scavenger receptor. Recent studies indicate a possible relationship between CD36 and its Drosophila homologue expressed in the responses to pheromones and reproductive behavior. The objective of this study was to characterize the active promoters regions of CD36 in NS. These sequences will be used as reporter gene to trace neuronal projections in vivo by expression of Tau-EGFP fusion.

Methods and Results:

Two putative promoter regions of mouse Cd36 gene or the CMV promoter were cloned in pGL3 basic plasmid modified in phase with TauEGFP. To verify the behavior of these regions, fluorescence levels were analyzed after transfections of the constructs in
the following cell lines: HepG2, which naturally express the \textit{Cd36} gene, Raw 264.7 cells, macrophage lineage that also express the gene, and Neuro 2A (N2A) which do not express \textit{Cd36} (confirmed by RT-PCR). The buildings without the promoter and the CMV promoter served as negative and positive controls, respectively. N2A cells was not observed activity of both promoters, as well as RAW 264.7 cells. In contrast, the distal promoter showed activity in HepG2 cells. Assays of 5' -RLM RACE suggest that the distal region is also active in brain regions that express \textit{Cd36}. These constructs are subcloned into lentiviral expression plasmids by recombination in vectors clonase pEntr4 and pLenti6-Blockit.

Conclusions:

The results may imply that the putative distal promoter region (~ 20kb amontante) is adequate for the purpose exposed. This is the first study of regulatory regions that control the expression of this gene in NS.

Keywords: Cd36, promoter, nervous system, receptor, gene

Financial Support: FAPESP

MASPIN IS PHOSPHORYLATED IN MCF10A MAMMARY EPITHELIAL CELLS

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Objectives:

Post-translational modifications are critical to regulate several protein functions, determining activity, subcellular localization and dynamic interaction with other proteins. Maspin is a tumor suppressor gene originally described as a serpin (serine protease inhibitor) due to sequence homology, however it doesn’t inhibit proteases. A single gene has been described for maspin, despite its great variety of functions, ligands and subcellular localizations. Maspin biological functions include modulation of cell adhesion, inhibition of tumor growth, invasion and angiogenesis, pro-apoptotic effect and control of oxidative stress response. This function diversity cannot be justified only by its primary structure, thus we hypothesize post-translational modifications are responsible for diversity of maspin function. Phosphorylation is the most ubiquitous PTM and is involved in numerous function in cellular processes as proliferation, differentiation, migration, cell cycle and metabolism control. The objectives of this project are to characterize maspin phosphorylation, to investigate a possible role of phosphorylation in maspin subcellular localization and to investigate the intracellular signals involved.

Methods and Results:

Total cell lysate and subcellular fractions of MCF10A mammary epithelial cells were analyzed by 2D-SDS-PAGE followed by immunoblot with anti-maspin. Phosphorylation was investigated by lysate treatment with acid phosphatase and by immunoprecipitation followed by western blot with anti-phospho-tyrosine and anti-phospho-serine. Maspin appears phosphorylated even in serum-starved MCF10A cells. EGF treatment resulted in increased maspin phosphorylation. Pervanadate treatment, a potent tyrosine phosphatase inhibitor, resulted in increased maspin phosphorylation and maspin accumulation in the cytoplasm fraction.

Conclusions:

Phospho-maspin are differently distributed in nucleus and cytoplasm. Maspin is phosphorylated in tyrosine and serine residues in MCF10A cells. Tyrosine phosphorylation is related to subcellular localization of maspin. EGF signalling pathway seems to be
involved in maspin phosphorylation through activation of a phosphatase or inhibition of a kinase.

Keywords: maspin, phosphorylation, MCF10A, Tyrosine, EGF

Financial Support: CNPq and Fapesp

QuebraPagina

Resumo:29-101

STUDY OF MASPIN ROLE ON THE BETA-CATENIN PATHWAY

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Objectives:

Beta-catenin is a protein that has dual-function: is part of adherent junctions with E-cadherin in the plasma membrane and is a central molecule on the Wnt/Wingless signaling pathway. Wnts are secreted proteins that regulate multiple processes in embryogenesis and in the adult. When this pathway is activated, beta-catenin is translocated to the nucleus, interacting with TCF/LEF family transcription factors regulating genes involved in polarization, differentiation and proliferation. Maspin belongs to the serpin family (serine protease inhibitors), that in contrast with to the others, it does not inhibit serine proteases. It has been described as a tumor suppressor gene because it inhibits invasion and tumor growth, angiogenesis, it has a pro-apoptotic effect, it modulates adhesion and it controls oxidative stress response. In spite of efforts, molecular mechanisms that regulate these many functions are still poorly understood. Our group uses a normal human mammary epithelial cell line model (MCF10A cells) which expresses high levels of maspin. Preliminary results show that maspin co-immunoprecipitate with beta-catenin. Knowing that, the objective of this project is to study the role of maspin in the Wnt/beta-catenin pathway.

Methods and Results:

Total protein extracts of MCF10A were immunoprecipitated with mouse polyclonal anti-maspin (BD) or rabbit, and rabbit anti-β-catenin (Abcam). Immunoblot showed co-immunoprecipitation of β-catenin and maspin.

Conclusions:

An interaction of maspin with β-catenin can begin to unveil the mechanism of maspin in the cell. But more experiments are needed to better understand this interaction.

Keywords: maspin, beta-catenin, pathway

Financial Support: CNPq and Fapesp

QuebraPagina

Resumo:29-102

UREASE OF HELICOBACTER PYLORI AND ITS INTERACTION WITH PLATELET MEMBRANE RECEPTORS

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Objectives:

Ureases (EC 3.5.1.5) are nickel-dependent enzymes that hydrolyze urea into ammonia and carbon dioxide. *Helicobacter pylori* is a gram-negative bacterium that colonizes the human gastric epithelium, and is considered a risk factor associated to gastric and duodenal ulcers through mechanisms not yet fully understood. Recent studies show a positive correlation between the infection with *H. pylori* and cardiovascular diseases. Urease produced by *H. pylori* (HPU) is considered a virulence factor since its ureolytic activity enables the bacterium to survive in the acidic medium of the stomach. Our group has shown that HPU induces platelet aggregation independent of its ureolytic activity, requiring ADP secretion through the 12-lipoxygenase pathway, a signaling cascade also triggered by collagen. Here our aim is to investigate the interaction of *Helicobacter pylori* urease with known platelet membrane receptors in rabbit platelets.

Methods and Results:

A recombinant HPU produced in *Escherichia coli* and purified by ion exchange and gel filtration chromatographies was used for the experiments. His-tagged recombinant UreA e UreB chains produced in *E. coli* were purified by Ni affinity chromatography. rHPU and its isolated chains were tested in rabbit platelet aggregation assay in a Lumi-aggregometer and in a SpectraMax in 96 well plates. Our results show that: 1) HPU-induced platelet aggregation is inhibited in the presence of antibodies against glycoprotein VI (GPVI), a collagen receptor in platelets; 2) rUreB alone has no effect on platelets but it inhibited collagen-induced platelet aggregation, in dose-dependent manner; 3) rUreA caused partial inhibition of collagen-induced aggregation; 4) rUreB also interferes with ADP-induced platelet aggregation.

Conclusions:

Our data show that activation of platelets by HPU shares at least partially the signaling cascade triggered by collagen, a platelet physiological agonist. This newly described pharmacological property of HPU reinforces the hypothesis that this protein could play an important role in the pathogenesis of the cardiovascular diseases indirectly caused by *H. pylori*.

Keywords: Helicobacter Pylori, Platelet, Urease

Financial Support: CAPES, CNPq and FAPERGS

QuebraPagina

Resumo:30-031

EFFECTS OF BONE MARROW-DERIVED MONONUCLEAR CELLS IN THE INFLAMMATORY PROCESS AND RENAL FUNCTION AFTER ISCHEMIA AND REPERFUSION INJURY

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Objectives:

Among the etiologies of acute renal failure 62% are due to acute tubular necrosis consequent to ischemic causes. In this study, we investigated the effect of bone marrow derived-mononuclear cells (BMDMC) therapy on kidney function parameters and inflammatory mechanisms of rat model of renal ischemia-reperfusion injury (I/R).
Methods and Results:

Animal protocols were previously approved by Animal Ethics Committee. Female Wistar rats weighing (250-300 g) were divided into five groups: control (Ct), sham + saline (S-S), sham + BMDMC (S-C) ischemia / reperfusion + saline (I/R-S) and ischemia / reperfusion + BMDMC (I/R-C). The rats (n = 5–8/each group) were anaesthetized via intraperitoneal (i.p.) injection of 100 mg/kg ketamine, 12.5 mg/kg xylazine and were subjected to bilateral renal pedicles clamping for 1 hour followed by renal reperfusion. One million (10^6) of BMDMCs were injected intra jugular vein 1 hour after onset of reperfusion. Urine volume was collected 24h after the onset of reperfusion and both kidneys were removed and frozen in liquid nitrogen, serum samples were taken, and the animals were subsequently euthanized. Urinary flow (µL/min): I/R-C group has no significant difference in urinary flow (4.11 ± 0.01) compared to Ct group (4.14 ± 0.14), S-S (4 ± 0.4) and S-C (4.25 ± 0.33), however I/R-S group (2.5 ± 0.68) was decreased in this parameter (n=8, p

Conclusions:

BMDMCs therapy restored glomerular and tubular function as well as inflammatory cytokines levels to control levels protection kidneys under renal ischemia-reperfusion injury.

Keywords: BONE MARROW-DERIVED CELLS, INFLAMMATORY CYTOKINES, ISCHEMIA-REPERFUSION INJURY, RENAL FUNCTION, CELL THERAPY

Financial Support: FAPERJ, CNPq, CAPES.

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Resumo:30-032

INDUCTION OF FUNCTIONAL RECOVERY BY BONE MARROW-DERIVED MESENCHYMAL STEM CELLS AFTER UNILATERAL FOCAL ABLATION OF THE CEREBRAL CORTEX IN RATS

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Objectives:

Lesions of the Central Nervous System are diseases recognized as a leading cause of disability in humans. Different types of lesions in the cerebral cortex induce different results in loss of function and plasticity of connections. In opposition to ischemia, the ablation lesion does not induce significant adaptive plasticity of corticocortical and corticostriatal connections and leads to different functional changes than from observed in ischemia. The induction of functional recovery by treatment with mesenchymal stem cells (MSCs) derived from bone marrow in models of focal cerebral ischemia has been demonstrated. In this study, the objective was to evaluate the therapeutic effect of these cells in the treatment of injury in motor and somesthetic cerebral cortices made by ablation, by assessing the recovery of sensory-motor functions.

Methods and Results:

Male Wistar rats (2-3 months, 240-380g) were subjected to a focal brain lesion induced by the removal by suction of most of the primary motor cortex and part of the somesthetic cortex of the left hemisphere. A day after the animals were treated with MSCs (previously cultivated from bone marrow) (n=8) or PBS (n=8). The route of administration was intravenous (jugular vein). Functional tests (cylinder and adhesive) were performed before and after injury. The monitoring of functional recovery for the two tests was performed weekly for a period of three months post-ablation. In the adhesive test, an adhesive was pasted on each forepaw of the animal. The calculation of percentage of first removal with the impaired (right) forelimb was based on direct observation of the animal paw that first removed the adhesive. In the cylinder test, each animal was placed inside a transparent glass cylinder and filmed. In the analysis of the videos was quantified the number of support with its forepaws on the cylinder wall, measuring their level of use and generating an asymmetry rate. Data were statistically evaluated by analysis of repeated
measures. By statistical analysis, our results showed significant recovery of sensorimotor function in the treated group with MSCs from the beginning of the second month post-ablation.

Conclusions:

Thus, our results suggest that MSCs present therapeutic effect in a model of brain injury that, unlike ischemia, does not induce significant plasticity of the cortical connections. Further studies are necessary to investigate the mechanism of action of the MSCs in this model of cortical lesion, as for example, a possible induction of plastic changes in the cortical connections by these cells.

Keywords: brain lesions, cell therapy, neuroplasticity

Financial Support: UENF, FAPERJ, CNPq

Resumo:30-033

BIOLOGICAL POTENCY ASSESSMENT OF RECOMBINANT HUMAN INTERLEUKIN-11 IN PHARMACEUTICAL FORMULATIONS

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Objectives:

Interleukin 11 (IL-11) is a multifunctional cytokine of the IL-6 type subfamily of long-chain helical cytokines, which modulates the proliferation, differentiation and maturation of various types of hematopoietic cells. Recombinant human interleukin-11 (rhIL-11) produced by DNA technology in Escherichia coli, consists of a 177 amino acids with a molecular mass of 19 kDa. It is currently being used worldwide for the prevention of severe chemotherapy-induced thrombocytopenia and to reduce the need for platelet transfusions in patients with non-myeloid malignancies. The aim of this work was to assess the biological potency and the cytotoxicity of the degraded forms and of the intact biomolecule in biopharmaceutical formulations.

Methods and Results:

The biological potency of biopharmaceutical formulations of rhIL-11 was assessed by the TF-1 in vitro cell proliferation assay against the reference reagent interleukin-11, human rDNA derived, (WHO 92/788). The in vivo bioassay was based on the increase of the platelets number of mytomicin-treated mice. The mean potencies obtained were within 93.46% and 97.28%, respectively, with the results of the independent assays 3.82% higher for the in vitro TF-1 cell proliferation assay, but with non-significant difference, as calculated by the student’s t-test (p>0.05). The samples were also artificially degraded and subjected to in vitro and to the in vivo bioassays to evaluate the bioactivity of the sulfoxides / deamidated forms, non-detecting significant changes related of the intact rhIL-11. The cytotoxicity test was performed on degraded forms versus the intact molecule, in order to detect possible effects resulting from the instability of samples during storage. However this showed non-significant differences (p> 0.05) of 35.42 micrograms/ml.

Conclusions:

The results obtained demonstrated the bioactivity and cytotoxicity of the intact biomolecule and of the degraded samples. Besides, the assays can be applied for the studies of alternative physico-chemical methods, to improve the characterization of rhIL-11 by monitoring its instability during the biotechnology process and, through subsequent purification steps, and to assure the batch-to-batch consistency of the bulk and finished biological products.

Keywords: INTERLEUKIN-11, BIOASSAY, TF-1 IN VITRO, IN VIVO BIOASSAY, DNA TECHNOLOGY
Objectives:
During ischemic injury the proximal tubule cells are particularly affected, thus impairing kidney function. Bone marrow-derived cells (BMDC) and, more specifically, mesenchymal stem cells (MSC) can be mobilized to sites of injury where they play an important role in tissue recovery. In this study we aimed to investigate the interaction between BMDC/MSC and tubular cells, by comparing their paracrine potential to protect and to stimulate renal cells proliferation.

Methods and Results:
Rat BMDC/MSC were co-cultured with LLC-PK1 renal epithelial cells using a porous membrane insert (4 µm pore diameter) to separate the two cell populations, thus allowing communication only by secreted factors. Renal cells proliferation was evaluated by counting viable cells or by PCNA immunofluorescence analysis. Cell death was determined by picnotic nuclei quantification, activated caspase-3 immunofluorescence or propidium iodide staining. Conditioned media were obtained by culturing BMDC/MSC for 72 h in serum free. The co-cultured medium was obtained by co-culturing BMDC/MSC and renal cells for 72 h. The results showed that renal cells proliferated more rapidly when co-cultured with increasing concentrations of BMDC (100% increased). MSC are more effective in stimulating renal cells proliferation (270% increased). The renoprotective effect was observed by the reduction of apoptosis (30%) in the presence of BMDC or MSC (50%). Again, increasing BMDC amounts decreases cell death, and MSC leads to a more marked reduction in cell death, indicating their higher paracrine potential. Proliferative and protective effects were not observed when renal cells were cultured with BMDC/MSC conditioned media. However co-cultured medium presented both proliferative (100% increase) and protective (reduction of 50%) effects. These results indicate that beneficial effects mediated by BMDC/MSC secreted factors require a previous stimulation from renal cells.

Conclusions:
The results indicate that the crosstalk between BMDSC/MSC and renal cells is mediated by a paracrine mechanism that leads to protection and proliferation of renal cells.

Keywords: mesenchymal cells, paracrine secretion, renal injury, co-culture, ischemia-reperfusion

Financial Support: CNPq; DECIT-MS; CAPES; FAPERJ
COMPARATIVE ASSESSMENT OF STEM CELL THERAPY IN A RAT MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Objectives:

Multifactorial etiology, onset of symptoms in the adult life and progression of the disease over time are common characteristics to many neurodegenerative diseases, including Amyotrophic Lateral Sclerosis (ALS). Due to a lack of efficient therapies that can interfere in degeneration progression, stem cells (SC) arise as a versatile treatment for these patients. But a rational use of SC-based therapy should consider the diversity of ALS patients, including distinct stages of this syndrome that continually modifies the spinal cord microenvironment. Besides that, many sources of SC are currently available, with distinct characteristics between them. In this context we investigated whether transplantation of mesenchymal stem cells (MSC) or neural stem cells (NSC) or the association of both — allowing MSC and NSC to interact and may obtain a third tissue response — could change the degeneration course in pre-symptomatic and symptomatic transgenic rats that develop a condition very similar to human ALS.

Methods and Results:

MSC and NSC (obtained from rats expressing GFP) were expanded in culture and transplanted in the lumbar spinal cord. Transplantation (n=8-12 for each group) was taken in pre-symptomatic (100 days after birth) and symptomatic (130 days after birth) transgenic rats that superexpress the mutated human SOD1 gene (G93A) and in non-transgenic Sprague-Dawley rats (115 days old). The disease progression was followed weekly for one month using the Basso Beattie and Breshnahan locomotor scale and besides transplantation (MSC, NSC or MSC+NSC) limb paralysis developed in the same way as for control groups (saline or naïve) in both pre-symptomatic (Kruskal-Wallis, p=0.17) and symptomatic (Kruskal-Wallis, p=0.79) stages of the disease. SC (GFP+) kept on lumbar region of spinal cord and some NSC presented astrocyte (GFAP+) and neural precursor (Nestin+) markers, but we did not observe any co-localization for neurons (MAP2) or motor neurons (SMI32) markers. In order to investigate how SC transplantation modifies the spinal cord microenvironment during degeneration progression in SOD1 rats we analyzed specific cells populations in ventral horn and the modulation of immune response. MSC promoted an increase in the astrocyte (GFAP+) and neural precursor cells (Nestin+) populations, while NSC reduced the mobilization of Nestin+ cells in symptomatic rats. We evaluated the cytokines and chemokines profile expression in the spinal cord by Bioplex. The chemokines GM-CSF, GRO-KC, RANTES, MCP1 were differentially expressed in transplanted animals (ANOVA, p<0.05).

Conclusions:

Our data points towards cellular and immune effects of SC transplantation that depend on disease stage and cell type or combination. However no functional improvement was observed in paralysis progression so far. According to our results, adult spinal cord is not instructive for neuronal differentiation of these cells. Even though SC can play a role in the degeneration process through the modulation of the microenvironment, that comprises cellular activation and mobilization and also secretion of proteins that may benefit motoneurons.

Keywords: amyotrophic lateral sclerosis, mesenchymal stem cell, neural stem cell, neurodegeneration, spinal cord

Financial Support: FAPESP, CNPq

QuebraPagina

Resumo:30-036
LIPID-CORE NANOCAPSULES CONTAINING RESVERATROL REDUCES Aβ-INDUCED TOXICITY IN RATS

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Objectives:

Alzheimer's disease (AD) is a progressive neurodegenerative disorder marked by accumulation of extracellular deposits of amyloid-β (Aβ) peptide in brain regions that are important for memory and cognition. The buildup of Aβ aggregates in the AD is followed by the formation of intracellular neurofibrillary tangles and activation of neuroinflammatory reactions. Despite of the advances in understanding this pathology, there are currently no pharmacological treatments that have proven to be effective in preventing or modifying the course of the disease. Several studies have considered the resveratrol a promising molecule in protecting against AD; however, its use is limited because it is easily oxidized and presents a very low bioavailability. Considering that nanoparticles have become an important component of therapeutic researches because they have the ability to deliver a wide range of drugs to the body for a sustained period of time, the aim of this study was to evaluate the effects of treatment with nanocapsules against Aβ1-42-induced damage in rats.

Methods and Results:

Nanocapsule suspensions containing resveratrol (1 mg/ml) were prepared by interfacial deposition of the polymer. The pH values of the suspensions were determined and mean diameters (z-average), polydispersity and zeta potentials were measured using a Zetasizer® nano-ZS ZEN 3600 model. In an attempt to induce brain damage like that observed in AD, Aβ1-42 peptide (2 nmol) was stereotaxically injected bilaterally into lateral ventricles of male Wistar rats weighting 300-350g. Behavioral analysis was performed 14 days after the Aβ1-42 intracerebroventricle injection through Object Recognition and Spontaneous Alternation. The treatment with free resveratrol (RSV) or resveratrol-loaded lipid-core nanocapsules (RSV-NC) (5 mg/Kg i.p. every 12h) started 24h after Aβ1-42 injection and was maintained for 14 days. In an attempt to evaluate if nanocapsules could improve the bioavailability of resveratrol, the amount of resveratrol in the brain was analyzed by high-performance liquid chromatography (HPLC). The lipid-core nanocapsules had a mean diameter of 258±5 nm, a polydispersity index of 0.1±0.001, a zeta potential of -15±1.5 mV, a pH of 5±0.4 as well as a high entrapment of resveratrol (98±2%) (Mean±S.D., n=20, p>0.05). Memory impairment induced by Aβ1-42 was significantly reduced only by the treatment with resveratrol-loaded lipid-core nanocapsules (Results from Control, Aβ1-42, RSV, RSV-NC, respectively. Mean±S.D., n=9: Recognition index: 0.78±0.07; 0.48±0.08; 0.47±0.12; 0.64±0.08; p

Conclusions:

Taken together, lipid-core nanocapsules exhibited great resveratrol encapsulation efficiency and increased the concentration of this polyphenol in the brain tissue. The present observations that resveratrol-loaded lipid-core nanocapsules reduce the memory impairment induced by Aβ1-42 provide interesting perspectives on the use of this formulation for future therapeutics strategies for Alzheimer’s disease.

Keywords: Nanocapsules, Resveratrol, Alzheimer's disease

Financial Support: CNPq/Brazil, FINEP, Capes
Objectives:
The accumulation of mineral composed mainly by calcium and phosphate ions that form hydroxyapatite crystals is known as biomineralization process. The discovery of carbon nanotubes (CNT) in the past decade has opened new frontiers in the field of nanotechnology and nanoscience. However, it is necessary a thorough evaluation about possible damages that a new material might have on living organisms before it can be incorporated into biomedical applications, being extremely necessary to study its toxicity and biocompatibility in a biological environment. Thus, we investigated the effects of carbon nanostructures deposited on the surface of titanium used in implants on the biostimulation of osteoblasts grown on this surface.

Methods and Results:
Cell viability of osteoblasts grown on titanium disks covered with different CNT was analyzed using a colorimetric assay known as MTT. In this methodology, the tetrazolium salt [3-(4,5-dimethyl-2-y1)-2,5-diphenyl tetrazolium bromide] produces the highly colored formazan dye on reduction of NADH dehydrogenase, which reflects a living cell. The test is finalized by spectrophotometric measurements of absorbance using an ELISA system, at a wavelength of 560 nm, allowing for the background absorbance at 690 nm. Cell viability is expressed as a percentage relative to control cells cultured without the nanostructured surface. The coil-type CNT with hydrophilic properties were synthesized directly on the titanium surface via the chemical vapor deposition method. The different surfaces were analyzed by scanning electron microscopy (SEM). The viability of osteoblasts grown on these structures varied considerably, from 78.8 up to 40.9%, depending on the CNT employed, when compared to the control cultures grown on the plate surface. The best result was observed when we used titanium disks with a less thick CNT coverage, where titanium had a bigger participation during cells growth.

Conclusions:
Cell viability tests are extremely important to choose the ideal CNT to proceed with studies to further investigate how these nanotubes interfere with the biomineralization process, with the ultimate goal of finding a CNT that present low toxicity and the ability to stimulate cell growth on titanium surfaces, i.e., a fully biocompatible system able to assist the events leading to the calcification process.

Keywords: osteoblasts, carbon nanotubes, cell viability

Financial Support: CAPES, CNPq and FAPESP

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Resumo:30-038

EFFECT OF THE TREATMENT WITH PREDNISOLONE IN FIBROSE FORMATION ON ALGINATE MICROCAPSULES

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Centro de Terapia Gênica/ Hospital de Clínicas, CTG-HCPA
Objectives:

Cell encapsulation is a promising strategy to control, locate and maintain the delivery of therapeutic products in vivo. This strategy consists in encapsulating cells in semipermeable spheres able to prevent the access of the immune system. It also allows the exchange of metabolites and nutrients between internal and external environment. Previous studies from our group observed the formation of fibrosis around microcapsules implanted in the peritoneum of mice deficient in alpha-L-iduronidase (IDUA), an animal model of Mucopolysaccharidosis type I. Therefore, our aim is to assess the effect of the treatment with prednisolone on the enzyme released by modified microencapsulated cells implanted in mice peritoneum.

Methods and Results:

Baby Hamster Kidney (BHK) cells over expressing IDUA were encapsulated in a number of 1x106 cells/g of mice weight and implanted in the intraperitoneal cavity of C57-BL6 wild type mice (n=7). The animals were subdivided in two groups, one received 1.5 mg/kg of prednisolone by gavage during five consecutive days and two more doses in alternate days (n=3). The other group did not receive any treatment other than the microcapsules (n=4). After fifteen days, the mice were sacrificed; the microcapsules were recovered and divided into two groups. Part of the microcapsules was maintained intact in culture while others were dissolved in Trypsin and the free cells were cultured. After 24 hours, the medium was collected and IDUA activity was measured. Comparisons between groups were performed by using a non-parametric Mann Whitney Test. Results: The microcapsules removed from control mice and maintained intact in culture showed an activity of 9.9±12.1 nmol/h/mL of medium, and the released cells of 110.4±187.4 nmol/h/mL of medium (p=0.248). In mice treated with prednisolone, intact microencapsulated cells showed an activity of 93.7±46.1 nmol/h/mL, whereas released cells presented an activity of 288.2±48.0 nmol/h/mL of medium (p=0.05).

Conclusions:

In the group not treated with prednisolone, enzyme activity increased six fold after the microcapsules were dissolved (p=0.034) suggesting that fibrosis could be preventing the enzyme release to the extracapsular medium. The use of an anti-inflammatory drug seemed to decrease fibrosis formation, as shown by the higher enzyme release in the intact group. It is also possible that cell viability could also be affected as in the prednisolone treated animals enzyme activity is higher than in controls even in free cells. These data suggest that prednisolone could be used in combination with the microcapsules to improving this treatment system.

Keywords: Alginate microcapsules, Cell therapy, Fibrosis, Prednisolone

Financial Support: FIPE–HCPA, PROBIC-FAPERGS, CNPq

QuebraPagina

Resumo:30-039

EFFECT OF MURINE EMBRYONIC STEM CELLS IN THE RETINA RETINOTECTAL PLASTICITY FOLLOWING A RETINAL LESION

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Objectives:

The development of central nervous system (CNS) is completed during the postnatal period, when it is extremely susceptible to environmental stimulation. Neurotransmitters, cytokines and neuromodulators are essential for correct development of synaptic connections. Also normal development and the reorganization followed injury in the CNS induces organization of extracellular matrix molecules, release of neurotrophins and pro-inflammatory cytokines, and expression of molecules involved in neurodegenerative processes. In vertebrates, the zygote is the totipotent stem cell which is able to generate all body cells. Four or five days after fertilization, the blastocyst is formed, composed of trophoblast cells and cellular inner mass. From the inner mass
are obtained the embryonic stem cells (ES), with pluripotent character capable to generate cells of the three germ layers - endoderm, mesoderm and ectoderm. ES culture when maintained in the presence of certain factors and conditions can generate cell lines, dividing indefinitely in vitro. Retinotectal pathways have been used as a model of the development of connections in CNS, particularly during critical periods. The use of embryonic stem cells has a huge potential for functional repair because involves replacement tissue and also provide trophic factors and substrates necessary for regeneration of CNS. Thus, this project aims to examine the behavior of murine embryonic stem cells (mES) implanted into rodent models of CNS injury, contributing to the understanding the mechanism of interaction between the host environment followed by a trauma and transplanted mES, and also expanding knowledge about cell therapy.

Methods and Results:

Lister hooded rats of 21 day-old were submitted a temporal retina injury as described in Brain Res. Bulletin 128:66, 2005. Cultures of mES were prepared over a carpet of inactivated murine embryonic fibroblast and then injected in the injured rat eye 24 hours after lesion. The injury in the temporal retina correlated with the injured area at superior colliculus. We injected HRP enzyme in the injured eye after 7 days to label ganglion cells axons, revealed the correspondent colliculus projections by histochemistry for TMB and removed the eyes in order to analyze the effect of transplanted mES cells. During culture, mEs were kept pluripotent confirmed by immunofluorescence of specific markers like OCT-4 and SSEA-1. We observed that after one week of transplantation there is no signal of teratogenesis in both normal and injured eye. The lesion caused a denervation at t he superior colliculus and transplanted mES caused changes in pattern of reorganization of retinal projections.

Conclusions:

Keeping in mind that the mES can produce and release several factors that could protect from insults caused by the injury, more study about the role of the mES in the CNS can be helpful for future cell therapy.

Keywords: central nervous system development, murine embryonic stem cells, retinotectal plasticity

Financial Support: FAPERJ, Proppi-UFF

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Resumo:30-040

NEONATAL VERSUS ADULT ENZYME REPLACEMENT THERAPY IN MUCOPOLYSACCHARIDOSIS TYPE I MICE

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2 Centro de Terapia Gênica, CTG - HCPA
3 Hospital de Clínicas de Porto Alegre, HCPA

Objectives:

Since we have recently shown that storage of glycosaminoglycans (GAGs) occurs from birth in mucopolysaccharidosis (MPS) patients, this work aimed to test if there were benefits in starting the enzyme replacement (ERT) from birth compared to the adult period in MPS I mice.

Methods and Results:

We compared four groups of male mice: in the first group, MPS I mice (knockout for the alpha-l-iduronidase gene) received ERT (Laronidase®, Genzyme) from birth (Neo-ERT, n=8) at 1.2 mg/kg intravenously every two weeks. The second group received
the same treatment but started at 60 days of age (Ad-ERT, n=6). Those groups were compared to untreated MPS I (MPS, n=13) and normal mice (NI, n=10). All animals were sacrificed at 6 months of age. No obvious adverse reactions were observed in Neo-ERT or Ad-ERT. Using the dymethyl-blue test we verified that both Neo-ERT and Ad-ERT were equally able to restore normal GAG levels in the liver, spleen, lung, kidney and heart (p

Conclusions:

Neo-ERT and Ad-ERT showed similar benefits in the aspects studied. More important, the ERT dose and regimen studied (chosen based on a trial published by our group - Mol Genet Metab 96:13-9, 2009) seem to cross the BBB in small quantities, which acts in favor of this regimen for patients instead of the regimen currently used (0.6 mg/kg, weekly). Poorly-vascularized organs such as joints still prove to be hard to correct, which indicates the need of ancillary therapies. Additional experiments will verify if formation of antibodies against the enzyme occurs in Ad-ERT and neo-ERT.

Keywords: enzyme replacement therapy, mucopolysaccharidosis type I, alpha-l-iduronidase

Financial Support: FIPE-HCPA, CNPq, FAPERGS

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Resumo:30-041

COMPARATIVE ANALYSIS OF MINOCYCLINE AND MONONUCLEAR BONE MARROW CELL TREATMENT AFTER STRIATAL FOCAL ISCHEMIA

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2 Federal University of Pará/Institute of Health Science, UFPA/ ICS

Objectives:

To comparatively investigate the anti-inflammatory, neuroprotective and functional effects of microglial inhibition with minocycline and bone marrow mononuclear cells (BMMCs) transplantation in the acute phase of experimental striatal stroke caused by microinjections of endothelin-1 (ET-1).

Methods and Results:

Male adult Wistar rats weighing 250-350g, four months old, were divided in four experimental groups: saline-treated (N=4), minocycline-treated (N=4) and BMMC-treated (N=4). Behavioral tests were performed at 1, 3 and 7 days post-ischemia to evaluate functional recovery between groups. Animals treated with minocycline received four 50mg/kg (i.p.) doses in the first two days plus five single 25 mg/kg (i.p.) daily doses up to the sixth day post-ischemia. 1x106 BMMCs were obtained from Wistar rats and directly transplanted into the striatum at 24h post-ischemia. Animals were perfused at 7 days after ischemia onset. Coronal sections were stained with cresyl violet for gross histopathological analysis and immunolabeled for identification of neuronal bodies (NeuN), activated microglia/macrophages (ED1) and apoptotic cells (active caspase-3). Gross histopathological analysis revealed pallor, tissue loss and intense microglial/macrophage activation in ischemic animals treated with sterile saline. BMMC transplantation induced a higher reduction (p<0.05). Both treatments afforded comparable levels (p>0.05) of neuronal preservation compared to control (61.3± 1.5; 86.8± 3.4; 81±3.4). BMMC treatment induced a higher decrease in the number of apoptotic cells compared to control and minocycline treatment (26, 5± 1.6; 13.1± 0.7; 19.7± 1.1). Both therapeutic approaches improved functional recovery in the ischemic animals.

Conclusions:

BMMC transplantation is more effective in modulating microglial activation and reducing apoptotic cell death than minocycline,
although both treatments are equally efficacious on improving neuronal preservation. Future studies should investigate whether minocycline treatment concomitant with BMCC transplantation produces synergistic effects, which might improve neuroprotection and functional recovery.

Keywords: Bone Marrow Mononuclear Cells, Inflammation, Microglia, Minocycline, Stroke

Financial Support: CAPES/ CNPq

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Resumo: 30-042

INTRACELLULAR LABELING AND QUANTIFICATION PROCESS BY MRI USING NANOPARTICLES OF IRON OXIDE INTO RAT GLIOMA CELL LINES C6 FOR APPLICATION IN MAGNETOHYPERTERMIA

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Objectives:
The Magnetohyperthermia (MHT) is based on increasing the temperature in a region of the body that is affected by a neoplasm to lyse tumorigenic cells, previously labeled with magnetic nanoparticles (MNP), when labeled cells are subjected to the action of an alternating magnetic field. Cell labeling is performed by three ways: (i) Intravenous administration of MNP, (ii) direct injection into the tumor tissue and (iii) marking the cells administered to induce tumor tissue. The aim of this study was the cell labeling of rat glioma cell lines C6 with MNP for later use in tumor induction. After tumor development will be possible to apply the technique of MHT without the need for direct application of MNP in tumor tissue. The present study also aims at the development or establishment of the quantification process by Magnetic Resonance Imaging (MRI).

Methods and Results:
Magnetic nanoparticles (MNP) based on iron oxide were used as markers for rat glioma cell lines C6. The MNP that were used had a size of magnetic core and a hydrodynamic size of about 10 nm and 50 nm, respectively, which were conjugated to the transfection agent PLL which aims to ensure the internalization of MNP in cells. The capture of MNP by the cells was visualized by light microscopy performed after staining of the Prussian blue. Quantification of MNP per cell was performed by the measures of relaxometry using a phantom of 24 wells which varied the number of labeled cells ($10^3$, $10^4$, $10^5$ and $10^6$) as well as the concentration used in each well (200, 400, 500 and 750 μg Fe/mL). This quantification allows first determine the threshold of detectability of MNP by MRI. Subsequently it was shown by measurements that the cell had a good uptake of MNP ($6.08x10^5$ pg of Fe). The study was performed with a 3.0 T MRI scanner. Characterization of MNP was performed in order to control the physical and chemical characteristics appropriate (size and size distribution, morphology, crystal structure, magnetic features, among others) that allow its application in intracellular labeling and quantification by MRI. The results showed a distribution polydisperse with spherical morphology, crystalline phase corresponds to magnetite with superparamagnetic characteristics. The intracellular labeling with MNP showed a cell viability of 90%. We conducted an evaluation of cell proliferation through the actin cytoskeleton architecture of cells, indicating that the proliferative capacity was maintained in comparison with the control sample.

Conclusions:
Implementation of the process of intracellular labeling of rat glioma cell lines C6 and the process of quantification by MRI in vitro studies contribute as an efficient tool for future studies in the application of the technique of Magnetohyperthermia, these studies lay the groundwork and provide proof of principle applications of MNP used as markers of cells in vitro and detection by MRI in providing guidance for clinical applications.
Resumo: 30-043

TREATMENT OF SKELETAL MUSCLE INJURY WITH BONE MARROW CELLS-DERIVED SOLUBLE FACTORS

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Biociências da Atividade Física/EEFD, UFRJ

Objectives:

Muscle injuries are the most common injuries in sports. Unfortunately, there is no approach that is totally effective in the treatment of severe muscle damage. The aim of this study was to evaluate the effects of the use of conditioned medium of bone marrow cells in the recovery from skeletal muscle injury.

Methods and Results:

Bone marrow cells were isolated from male rats (12 to 15 weeks of age, 300 to 400 g body weight), and after separation of mononuclear cells by Ficoll density gradient, adherent cells were expanded until third passage, called multipotent mesenchymal cells. Culture medium was conditioned for 72 hours at 37°C in hypoxic condition (1% O2). Hypoxic-conditioned medium (HCM) were concentrated by ultra filtration technique, using ultrafiltration devices (centripep Y-10, Millipore ®). Thirty six male rats were submitted to muscle injury by laceration of right soleous muscles. Sham operated animals were submitted to the same procedures except by the muscle laceration. Twenty-four, 48 and 72 hours after injury, 30 µL of HCM were injected directly into muscle, around the injured area. Seven and 14 days after injury, muscles were harvested to functional and histological analysis.

Maximal tetanic force production was performed through 50Hz stimulation (Grass S88X) and normalized by contra lateral non-injured muscle. Muscle force was recorded and analyzed by Powerlab apparatus and Chart7.0 software (ADinstruments®). After functional analysis, muscles cross sections were stained with hematoxylin and eosin to assess the number of centronucleated fibers and quantification of muscle cross-sectional area. One way analysis of variance was performed as statistical technique and significance was set at p < 0.05. Animals treated with concentrated hypoxic conditioned medium (HCM) developed a greater tetanic force when compared to the group that received DMEM, 7 days after injury (68.9 ± 10.4% vs 51.78 ± 15.24%, respectively; p

Conclusions:

We conclude that the use of conditioned medium from bone marrow cells, contributed to accelerate skeletal muscle regeneration and muscle force recovery.

Keywords: Muscle injury, Conditioned medium, Muscle regeneration

Financial Support: CAPES, FAPERJ, CNPq, Vital Brasil

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Resumo: 30-044

Keywords: Magnetohyperthermia, MRI, nanoparticles, iron oxide, rat glioma cell lines C6

Financial Support: IIEPAE, CNPq, FINEP/CAPES
BONE MARROW CELL THERAPY IN A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Objectives:

Amyotrophic lateral sclerosis (ALS) is a progressive neurological disease that affects selectively the motor neurons. The loss of the motor neurons from the brain and spinal cord leads to a progressive muscle dysfunction and death within 3-5 years of onset. The detailed mechanisms of selective motor neuron death remain to be unknown and no effective therapy has been developed. Recent studies showed that transplantation of bone marrow cells (BMC) have neuroprotective effects in rodents after brain injuries such as cerebral ischemia or traumatic brain injury. The aim of this work is to study the therapy with BMC in a mouse model of ALS (SOD1-G93A mice).

Methods and Results:

In this work, we isolated 106 BMC (mononuclear fraction) from wild type mice and inject them in the lumbar portion of the spinal cord of the SOD1-G93A mice. We injected the cells at two time points: 9 weeks (pre-symptomatic) and 14 weeks (post-symptomatic). In each condition, we analyzed the progression of disease and the lifespan of animals. We observed a delay in approximately 3 weeks in the disease onset when we injected the cells (n=12) at 9 weeks of life compared with the animals that we injected saline (n=11), but we did not observe an increase in the lifespan of the SOD1-G93A animals. When we injected the BMC at 14 weeks (n=13), we observed only a slightly increase one week after injection, but this increase was not statistically significant, in the animals performance at the rotarod test when compared with the animals that receive saline injection (n=10). In this protocol, we also did not observe difference in the animal’s lifespan.

Conclusions:

The treatment with BMC injected in the spinal cord of a mouse model of ALS, delayed the onset of the symptoms, but did not increase the lifespan of the animals, indicating that is necessary more study to find an efficient treatment for this disease.

Keywords: amyotrophic lateral sclerosis, cell therapy, bone marrow cells

Financial Support: Inbeb, Protecel Cnpq, Capes, Faperj

CHARACTERIZATION OF CELLS FROM HUMAN AMNIOTIC FLUID AND EVALUATING THEIR POTENTIAL TO DIFFERENTIATE INTO CARDIOMYOCYTES

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2 Instituto Nacional de Cardiologia, INC
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Objectives:
The aim of this study was to characterize mesenchymal stem cells derived from human amniotic fluid (LA-MSC) and their differentiation into cardiomyocytes.

Methods and Results:

The LA-MSC were obtained from discarded amniotic fluid in cases of amniocentesis performed between 13-20 weeks of pregnancy to test for prenatal diagnosis. The LA-MSC were adherent to plastic, with a fibroblastoid morphology (n = 16). Functional test was performed for colony-forming units fibroblasts (CFU-F) to check clonogenicity potential. This test was conducted on cells freshly isolated and generated an average of three colonies for each 104 cells (n = 5) plated. All other tests were performed after the third passage. Surface antigens were analyzed by flow cytometry. It was observed that all cell types of mesenchymal cells expressed antigens (CD90 and CD73) and CD105 expression was variable. Furthermore, all cells were negative for hematopoietic antigens (CD34, CD45 and HLA-DR) for endothelial antigens (CD31 and CD133) and for factor receptor CD117 stem cells (c-kit) (n = 5). The LA-MSC also expressed adhesion molecules (CD54, CD44 and CD166). When subjected to protocols of osteogenic differentiation and adipogenic, the LA-MSCs formed aggregates calcium and lipid vacuoles, respectively (n = 3). The characterization of pluripotency of these cells, we observed by RT-PCR amplification of transcripts for Rex, and Dnmt3b Nodal. At the protein level was the expression of SSEA-4 and TRA1-60 by immunofluorescence and the absence of expression of Oct-4. It was considered the time that these cells leads to double its original number (PDT), we observed that the cells took 1.7 days ± 0.26 to double its population (n = 3). It was also made to assess chromosome number and found that over 80% have euploidia (n = 5). For cardiogenic induction, the LA-MSC were subjected to two protocols for co-cultivation. The first protocol, cells were co-cultured with neonatal rat cardiomyocytes through a transwell membrane without cell-cell contact for 72 hours (n = 3). After this period, the LA-MSC expressed scn5a e actc. In the second case, the cells were co-cultured directly with neonatal cardiomyocytes obtained from mice with the GFP gene under the control of α actin promoter, allowing the contact of the cell (n = 3). With this protocol, LA-MSCs expressed MYHCB cTnT ACTC and MYL2A the mRNA level. In the induction protocol used chemical DMSO or 5-azacytidine for 48 hours after induction cells were maintained 21 days in culture. After 21 days, cells induced with DMSO became to express myhcb, scn5a ctn actc and myl2a with 5-azacitidine scn5a, ctn, actc e myl2a.

Conclusions:

In conclusion, the LA-MSC can be easily isolated and expanded in vitro without alteration and phenotypic genotípica. They were able to generate CFU-Fs, showed the expression of surface antigen similar to bone marrow stromal cells and mesenchymal differentiation potential of osteogenic and adipogenic. Although it was observed the expression of pluripotency markers in the levels of mRNA and protein, the properties of pluripotent LA-MSC are still controversial. The induction protocols, we can conclude that the LA-MSCs can be differentiated in the cardiac lineage very efficiently through several different protocols, suggesting that they may be used as an alternative source for cell therapy in cardiovascular disease, but we need to do more studies on this differentiation.

Keywords: Amniotic fluid, stem cell, mesenchymal stem cells, cardiomyocyte, cell therapy

Financial Support: CNPQ, FAPERJ, CAPES, MINISTERIO DA SAÚDE

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Resumo:30-046

PRELIMINARY STUDY OF MOUSE EMBRYONIC STEM CELL TRANSPLANTATION IN MICE EXPERIMENTALLY INFECTED WITH T. CRUZI.

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2  Dep. Fisiologia e Farmacologia-INSTITUTO BIOMÉDICO, UFF
3  Instituto Nacional de Cardiologia, INC
Objectives:

Our aim was to study the impact of undifferentiated DsRed mouse embryonic stem cell (mESC) transplant in mice infected with Trypanosoma cruzi (T. cruzi) since studies of alternative therapies are needed for Chagas disease which lacks effective treatments.

Methods and Results:

CD1 mice (n=28), 9 weeks-old, were divided in the following groups: uninfected (G1, n=4), infected and placebo-treated (G2, n=15), infected and cell-treated (G3, n=9). Mice were infected intraperitoneally (IP) with 30000 trypomastigotes of the Brazil strain of T. cruzi. Survival and parasitemia were evaluated until 50 days post-infection (dpi). DsRed mESC were cultured on feeder layers and the undifferentiated state was confirmed by Oct4 and SSEA-1 expression. Cells (500000) were injected IP at 13 dpi. Electrocardiography and right ventricular area (RVa) measurements by echocardiography were performed before infection and 30 dpi. Peripheral blood flow cytometry was performed at 28 dpi. Parasitemia was statistically similar in infected groups (G2 and G3), peaking on 20 dpi. The survival rate in G2 and G3 was approximately 34%, differing significantly from G1 (P< 0.0001) when compared to G1. The values of citotoxic T lymphocytes (P=0.0311), helper T lymphocytes (P=0.0311) were also significantly different between groups. T Lymphocytes did not differ between groups. In general the parameters did not differ between infected groups.

Conclusions:

These data suggested that 500000 mESC were not sufficient to improve health in infected cell-treated animals 30 dpi and perhaps it would be necessary to increase the number of injected cells. However, 30000 trypomastigotes of the Brazil strain of T. cruzi were able to produce the acute mice model of Chagas disease with impaired immunity of T and B cells. Institutional Animal Ethical Committee License number: IBCCF-027. Acknowledgements: We thank Dr José Xavier Neto and Dr Debora Schechtman for kindly providing us the aliquotes of DsRed mESC to start our culture experiments related to cellular therapy in experimental chagasic cardiomyopathy.

Keywords: mESC, stem cell, T. cruzi, Chagas disease, DsRed

Financial Support: FAPERJ, CNPq, CAPES, MS, MCT

Resumo:30-047

TRANSGENIC BACTERIA DEVELOPMENT FOR MINICIRCLE PRODUCTION USING PHIC31 RECOMBINASE SYSTEM

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2 Universidade de Santo Amaro, UNISA

Objectives:

A major difficult to the implementation of Gene Therapy (GT) in medical practice is the construction of suitable vectors that are able to ensure patient safety with high transfection rate and a sustainable level of gene expression for therapeutic treatment of disease. The minicircles vectors (MC) plasmid have reduced size, they are devoid of bacterial sequences as the origin of replication and antibiotic resistance gene, allowing a prolonged transgene expression and low immunogenicity. These vectors are produced through the process of site-specific recombination mediated by integrases that recognize certain sequences for
integration, inversion or excision depending on the position and orientation of sites of recombination. One of the difficulties in the production of MC is contamination with the parental bacterial DNA. As a way of improving the production of these vectors the objective of this work was to create a bacterial strain derived from E. coli DH1 expressing integrase under the control of ΦC31 BAD promoter.

Methods and Results:

In order to reduce the size of the parental plasmid and increasing the efficiency of MC production, was developed the DH1ΦC31 bacteria using the homologous recombination method. The plasmid pBADΦC31 was constructed and the integrase gene was inserted into the site between the LacZ gene, the vector was transformed into E. coli DH1 (has the LacZ gene in its genome) and subjected to the protocol of recombination. The product was plated on agar plates containing LB medium X-Gal and IPTG for growth and differentiation. The white colonies were analyzed by PCR sequencing using specific primers to integrase ΦC31, the DH1ΦC31 bacteria was amplified by the protocol for the production of plasmid vectors.

Conclusions:

With the construction of DH1ΦC31 will be possible produce MC efficiently, quickly and free of contamination. Moreover, the bacteria may decrease the production cost of future drug that is already being successfully tested in preclinical treatment of various diseases.

Keywords: Gene therapy, Minicircle vectors, Recombinase phiC31, Homologous recombination, Non-viral vectors

Financial Support: FAPESP

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EFFECTS OF THE INJECTION OF BONE MARROW-DERIVED MESENCHYMAL STEM CELLS INTO HIPPOCAMPUS OF WISTAR RATS

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2 Laboratório de Histologia/Instituto de Ciências Biológicas, FURG

Objectives:

Cell therapy using bone marrow-derived mesenchymal stem cells (MSC) seems to be a new alternative for the treatment of neurodegenerative diseases. However the consequences of the interaction from MSC with the nervous tissue are still unclear. Previous data from our group showed that the conditioned medium from MSC induce selective cell death and neuroinflammation in organotypic hippocampal slice cultures. In this context, the aim of the present study was to investigate the effects of stereotaxic intra-hippocampal injection of MSC in rats.

Methods and Results:

For this purpose, 30-day female Wistar rats received through stereotaxic surgery 100,000 cells in 3µL of HBSS in the right hippocampus (ipsilateral). Control group receieved only HBSS. After 3 or 7 days of recovery animals were sacrificed and both hippocampi (ipsi and contralateral of injection) were dissected, homogenized in lysis buffer, the proteins were resolved (50 &mi;g per lane) on 10% SDS-PAGE and submitted to Western Blotting analysis (for α-tubulin 3, Neu N, sinapsin and GFAP). Furthermore, 7 days after the surgery the animals were submitted to object recognition comportamental test to evaluate a possible deficit on their memory. Western blotting data (expressed as % of control±SD, n=6, p

Conclusions:
Our results suggest that the injection of MSC in the hippocampus could induce changes in neuronal cytoskeleton and astrogliosis. The transplantation of MSC in hippocampus caused comportamental changes in the condition and model used. More studies are necessary to evaluate possible side effects of MSC transplantation directly in the nervous tissue.

Keywords: astrogliosis, hippocampus, mesenchymal stem cells

Financial Support: FAPERGS, CNPq, INCT-EN
COTIARA VENOM IN MICE

Pontifícia Universidade Católica do Paraná, PUCPR

Objectives:
To describe the kinetics of the experimental local damage induced by intradermic injection of Bothrops cotiara venom in mice.

Methods and Results:
Local damage was evaluated by analysis of morphological inflammatory alterations, mast cells counting, and analyses of collagen deposition. Bleeding was evident four hours after inoculation. After 24 h, a large area of injury was apparent presenting disorganized tissues, large hemorrhage and acute inflammation. After 3 days the area of injury was extensive, with large amount of inflammatory cells and the presence of crust. In seven days lesion area showed the beginning of healing and re-epitization and after 21 days the epithelium showed a less infiltration and no skin appendages. The number of mast cells was similar to controls after 4 hours, with a decrease of 50% at 24 hours, followed by an increase until the 21st day. No differences of collagen deposition were observed among experimental groups.

Conclusions:
Wound healing follows similar parameters to the wounds caused by other bothropic venoms.

Keywords: Bothrops venom, Wound healing, Inflammation, Local damages

Financial Support: PIBIC/PUCPR

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ACTION OF SEBASTIANIA HISPIDA (EUPHORBIACEAE) EXTRACT IN THE LIVER OF RATS TREATED WITH BROTHOPS MOOJENI POISON: ENZYMATIC LIVER AND HISTOLOGICAL EVALUATION.

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laboratório de toxinologia e plantas medicinais, Anhanguera-Uniderp

Objectives:
To evaluate the actions of Sebastiana hispida extract in the liver of rats poisoned with the venom of Bothrops moojeni through assessment of liver enzymes AST, ALT and ALP and histology.

Methods and Results:
The aqueous extract of dried leaves of S. hispida collected at Fazenda Saint Emilia, at Pantanal do Negro -MS, was prepared via maceration and diluted to µ/ml. The venom collected from snakes of laboratory of Zoology, Anhanguera-Uniderp University was lyophilized and refrigerator stored. In this study 36 adult male Wistar rats (300g) were divided into three groups: SS group:
injected with sterile saline 0.9%, Vb Group: injected with venom; ExtSh vg Group: injected with crude venom in times of 3h, 3 and 7 days and treated with aqueous extract daily by gavage, per group in the times had an n=4. The venom route of inoculation (40µ/mL) or saline (1 ml) was intramuscularly (im), the aqueous extract of *S. hispida* was via gavage (vg) in a dose of 1 mL for each rat. The animals were anesthetized with Zoletil ® (ip) 0.1 mL/100 g to collect the liver tissue and blood samples. Liver was removed, weighed and measured and after immersed in 4% paraformaldehyde solution for 24 hours, processed (alcohol and xylene), embedded in paraffin, cut in microtome (5µm) and stained with hematoxylin and eosin (HE). The liver enzyme data in the time of 3h, 3 and 7 days were compared using ANOVA (P28th day was observed mild mononuclear inflammatory infiltrate in the spaces between the lobes; and at 7th day, the liver tissue showed a better tissue architecture in relation to previous periods. Regarding markers of liver enzymes AST, ALT and ALP was no changes observed (P >0.05) between groups. Rats from Vb Group had the AST peak at 3 hours (149.3 ± 34.1), ALT (53.9 ± 3.1) and AF (280.3 ± 64.7) in 7th day. It was observed a significant decrease in activity of the enzyme AST of ExtSh vg group in related to group Vb in time 3h and 7 days

Conclusions:

It can suggest that the aqueous extract of *Sebastiana hispida*, taken orally could have a protective effect against inflammatory actions of *Bothrops moojeni* venom in the liver. More study on the subject are being developed to better understand the actions of the plant against *Bothrops moojeni* poisoning.

Keywords: Bothrops, ENZYMATIC, Liver

Financial Support: MCT, CNPq; INAU, CPP, FUNDECT and the University Anhanguera-Uniderp.

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CHARACTERIZATION OF A FIBRINOGENOLITIC METALLOPROTEINASE FROM BOTHROPS LEUCURUS SNAKE VENOM.

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Objectives:

The venom produced by these snakes include a truly biochemical arsenal, consisting of several enzymes with activity that have aroused the curiosity of researchers for centuries, in an attempt to understand its systemic action in order to get a pharmacologically application (Toxicon 45, 1021-1039, 2005; Comp. Biochem. Physiol. C 142, 328-346, 2006; Journal of Proteomics 72, 200-209, 2009). In this work, we purify a new metalloproteinase from snake venom of Bothrops leucurus and try to assess its systemic action in search of a clinical application.

Methods and Results:

The fibrinogenolytic nonhemorrhagic metalloproteinase (BleucMP) was purified by two chromatographic step. Initially of B. leucurus venom by ion exchange chromatography on DEAE-Sephadex A-25 column produced five major protein peaks. All fractions obtained throughout the purification process were assayed with fibrinogenolytic activity and SDS-PAGE. The fraction D1, which showed fibrinogenolytic activity was further fractionated on CM-Sepharose Fast Flow. In this second chromatographic
step we obtained a metalloproteinase named BleucMP (fraction CM3). This sample was analyzed by RP-HPLC C18 and by MALDI TOF/TOF, showed a molecular mass of 23057.54 Da and when alkylated and reduced, the mass is at 23830.40 Da. Their peptides analyzed in MS/MS (MALDI TOF/TOF) showed significant score when compared with those of other metalloproteinases by NCBI-BLAST2 alignment display. The enzymatic characterization of BleucMP was carried out by using different assays. The proteolytic activity of BleucMP was assessed upon azocasein, fibrinogen, fibrin and with protease inhibitors. BleucMP was strongly active on the Aα and Bβ chains of fibrinogen, was inhibited by EDTA and 1.10-phenanthroline and had activity on azocasein and fibrin. This enzyme was also able to decrease significantly the plasma fibrinogen level provoking blood incoagulability, however was devoid of hemorrhagic activity and did not induce relevant biochemical hematological. BleucMP showed a low edema-inducing effect compared with the venom and histopathological alterations were analyzed in muscle, liver, kidneys, heart and lung. Which revealed a low toxicity of BleucMP.

Conclusions:

In summary, our data showed that BlecMP, class P-I metalloproteinase, has an efficient proteolytic action over fibrinogen and showed a low toxicity. Future studies with BleucMP can reveal structural details which could lead to its use in part as a molecular model for clinical purposes mainly in the treatment and prevention of cardiovascular disorders.

Keywords: Bothrops leucurus, fibrin(ogen)olitic, metalloproteinase

Financial Support: FAPEMIG, CNPq
micronuclei frequency (48 and 96h – 0.285 ± 0.041; 0.406±0.066) in relation to control (48 and 96h – 0.135 ± 0.019 ; 0.125 ± 0.014). Furthermore, δ-ALA-D activity was decreased only when diphenyl ditelluride was exposed to 48 hours (6.94±1.084) when compared to control group (12.90 ± 1.412).

Conclusions:

Our results indicate that diphenyl ditelluride can be toxic in mice. These effects may be linked to the pro-oxidant activity exhibited by high doses of organotellurium compounds. This data is supported by many studies that have been published about organochalcogen compounds and its farmacological, toxicological and/or biological effects. Additional studies will be needed to elucidate the mechanism(s) that diphenyl ditelluride mediates its toxicity.

Keywords: Selenium, Organochalcogens, Comet Assay, Micronucleus, δ-ALA-D

Financial Support: CNPq

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Resumo:31-096

FULLERENE (C60) ACTIONS UPON SPATIAL MEMORY, OXIDATIVE STRESS AND BDNF LEVELS: EFFECTS RELATED TO PARTICLES SIZE


Objectives:

Few studies have shown the interaction of Fullerene C60 (nC60) with biological systems correlating biochemical responses concomitantly with behavior and memory. Thus, the aim of this work was to evaluate the action of the water-soluble nC60 in hippocampus of adult rats, through the analysis of behavioral parameters, brain-derived neurotrophic factor (BDNF) levels and oxidative stress.

Methods and Results:

In this study were used two different fullerene suspensions, with 0.2 μm and 0.45 μm particle size. The suspensions carbon concentration was estimated by TOC-V CPH total carbon analyzer and adjusted to 10 mg/ml. Later, the new suspensions were characterized by transmission electronic microscopy. For suspensions administration, male Wistar rats of 2-3 months of age were anesthetized and bilaterally implanted cannulae above the hippocampus CA1 area. After 48 h recovery from the surgery, these animals were trained in Water Maze (WM) task by five days and tested in sixth day. Infusions of 1 μl saline or nC60 0.20 μm or nC60 0.45 μm were given 0 min after each training session. After the WM test, the rats were killed and the hippocampus was removed for biochemistry analyses. The BDNF levels are measured by ELISA method; reactive oxygen species (ROS), determination of antioxidant capacities against peroxyl radicals, lipid peroxides content and determination of reduced glutathione (GSH) concentration and glutamate cysteine ligase (GCL) activity are perform by fluorimetric methods. The WM task results were compared by Repeated Measures ANOVA followed by Tukey's Test and the results of oxidative stress parameters and BDNF determination were analyzed by One-way analysis of variance followed by Newman-Keuls Test. Statistical significance were considered when ρ value was lower than 0.05. In the start of WM task, all groups (n=9-10) showed the same escape latency to found the platform and presented a decrease escape latency at second day. From the third day on, was observed a raise in the escape latency to find the hidden platform in the 0.45 μm group, with significant difference when compared with the group with 0.20 μm sized particles and the control group. The same condition was observed on the testing day, when the 0.45 μm group did not perform well concerning their orientation in the dark tank. The BDNF levels was significant diminished in the hippocampus in the group exposed to 0.45 μm particles size of nC60 (n=4-5). There was a significant decrease in ROS concentration (n=4) in the group exposed to 0.45 μm particles size when compared with other groups. Total antioxidant capacity against peroxyl radicals was augmented (n=4) in both treatment groups when compared to control group. Any significant changes in terms of TBARS or
GCL activity and GSH was observed (n=4) in all groups submitted to experimental conditions.

Conclusions:

The bigger particle size impaired spatial memory with significant decrease on BDNF neurotrophin, but, showed high antioxidant capacity and no injured membrane lipid. This deleterious action on memory that may be attributed to high antioxidant capacity and a decrease in BDNF levels, since an amount of ROS is necessary for cell signalization and thereby memory consolidation. These results are very interesting, because, the same nanomaterial in different particle size demonstrated different action on brain tissue.

Keywords: Fullerene C60, spatial memory, BDNF, oxidative stress, rat hippocampus

THE EFFECT OF ALCOHOL ON RAT FETAL OSTEOBLASTS SUBMITTED TO ETHANOL CHRONIC CONSUMPTION DURING PREGNANCY.


Objectives:

The alcohol, besides affecting the organism causing various diseases, acts through direct action on bone metabolism causing an antiproliferative effect on osteoblasts. The purpose of this paper was to evaluate the effects of alcohol 20% chronic consumption in osteoblasts obtained from the calvaria of newborn rats.

Methods and Results:

The alcohol was administrated to pregnant rats throughout the entire pregnancy. For that purpose, 18 rats were used, divided in groups according to the diet: 6 receiving alcohol 20%, 6 belonging to the isocaloric group and 6 receiving water and ration at will. At three days of life, the newborns were euthanized so as to remove the calvaria and start the cell culture procedures. The osteoblastic lineage cells were isolated by sequential enzymatic digestion and the osteoblasts were cultivated for periods of until 14 days. Tests were performed on the culture slides to evaluate the effect of alcohol based on adhesion, proliferation and cellular viability on total protein content, alkaline phosphatase activity and nodule formation of mineralized matrix. The results have shown that the alcohol group presented significant increase in proliferation, except for the period of one day, and in nodule formation. A significant increase in the alcohol group concerning cellular viability was only observed in the period of 3 days and there was no statistic difference in adhesion. The total protein content was higher in the control group in 7 days, and the average higher in the isocaloric and alcohol groups in 14 days, according to the periods of time studied. An increase in the activity of the alkaline phosphatase was observed in the isocaloric and alcohol groups in all periods of evaluation.

Conclusions:

By this methodology, we have concluded that the alcohol has not presented any deleterious effect on osteoblasts, possibly due to the short period of administration.

Keywords: ethanol, osteoblast, gestation

Financial Support: FAPESP and CAPES

DETERMINATION OF BEHAVIOR OF ADULT FEMALE RATS EXPOSED TO ENVIRONMENTAL POLLUTION OF PORTO ALEGRE.

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2 Colégio Militar de Porto Alegre, CMPA
Objectives:

Studies with rats show that inhalation of particulate matter (PM) from the burning of fossil fuels causes their motor activity decrease in the open field. Changes in the cerebellum and striatum can promote changes in motor functions, and may be related to inhalation of particulate matter, which generates oxidative stress. The pollution is the environmental factor that leads the ranking as the most prevalent source that induces inflammation and oxidative stress. The aim of this study is to investigate the damage of air pollution on the central nervous system and behavior of female rats and to analyze the influence of different phases of the estrous cycle on oxidative stress parameters.

Methods and Results:

In this study, were used 32 female Wistar adults rats divided into four groups, accordingly the air chamber and ovarectomized procedure: Air filtered chamber (CAF) and Non-ovarectomized (Sham) female (G1); CAF and Ovarectomized (OV) female (G2); Non-filtered air chamber (NFAC) and Sham female (G3); NFAC and OV female (G4). We conducted the following procedures: Analysis of feeding behavior, open field test habituation, and weighing of the animals. In all evaluations, both groups of non-ovarectomized female rats were tested in estrous cycle pro-estrus. As results, we have: in terms of weights starting the experiment and day of euthanasia, it was found that all groups in the final weight was statistically significant compared to the initial weights (P

Conclusions:

Our results demonstrate that the test of sweet food and number of fecal bollus during the open-field tests showed to be not changed. The weights, however, increased with age of rats. However, there was an increase in the weights of ovariectomized rats of both chambers when compared to sham rats of the corresponding chambers. The ovariectomized rats remain in a cycle phase, known as anestrus. Thus, the cyclicity of hormones affect the body weight.

Keywords: Air pollution, Oxidative stress, Estrou cycle

Financial Support: UFCSPA and CNPq.

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Resumo:31-100

QUERCETIN INHIBITS IN VITRO ACTIVITY OF ACETYLCHOLINESTERASE IN FRACTIONS OF RAT SYNAPTOSOMES

Università Federal de Santa Maria, UFSM

Objectives:

In the present study we investigated the in vitro effect of a flavonoid compound, quercetin, on acetylcholinesterase (AChE) activity from cerebral cortex synaptosomes.

Methods and Results:

Quercetin was obtained purified (Sigma Chemical Co./St. Louis, MO, USA) and diluted with methanol as vehicle. Solutions were added in concentrations of 1, 5, 10, 25, 50, 100 and 200μM. To evaluate the activity of AChE were used adult male Wistar rats
submitted to euthanasia. The cerebral cortex was removed and homogenized in 10 volumes of Medium I (pH 7.5). The synaptosomes were isolated (Journal of Neurochemical 43; 1984) using a discontinuous Percoll gradient. The sediment was suspended in an isosmotic solution and the final protein concentration was adjusted between 0.4 and 0.6 mg/ml. AChE activity was determined (Pharmacology 7; 1961). Data were analyzed by one-way ANOVA followed by Duncan test (p

Conclusions:

Quercetin was effective in the reduction of AChE activity only in the highest concentration tested. From these results it is concluded that quercetin can be considered a promising compound in regard to natural therapies against hypocholinergic diseases and can thus be applied after pre-clinical studies for the treatment of neurodegenerative diseases such as behavioral and functional therapeutic inhibitor of AChE.

Keywords: Flavonoid, Quercetina, ACETYLCHELINESTERASE, SYNAPTOSOMES, cortex

Financial Support: CAPES, CNPq, FAPERGS.

Objetivos:

Sinteticos moléculas de organoselênio, incluindo ebselen e diseleneto de fenil (PhSe)2, são reconhecidas por suas propriedades antioxidantes associadas com atividade de peróxido de glutatióna, associado ao potencial biológico do selênio e incluem ant depressantes, anti inflamatórios e neuroprotectivos. Essas moléculas apresentaram baixos efeitos tóxicos quando administrados em modelos in vivo e in vitro, demonstrando a importância de estudos que analisem os efeitos biológicos de moléculas de organoselênio. An antitumoral effect associated with selenium supplementation in human was previously demonstrated. In this regard, some works pointed to citotoxic effects of selenium compounds in cell cultures associated with modulation of several protein kinases. Considering the importance of biological effects and very low toxicity already demonstrated for (PhSe)2, in this study we aimed to analyze the effects of prolonged treatment with (PhSe)2 on cell viability and modulation of signaling pathways, including MAPKs and PKC phosphorylation in human neuroblastoma lineage SH-SY5Y.

Métodos e Resultados:

Cultures of SH-SY5Y cells were maintained for 24 hours in serum free media in the presence of crescent concentrations of (PhSe)2 (0.3-30 μM). For some experiments, U0126, a pharmacological ERK1/2 inhibitor was added toward the diiselenide in the media. Finished the treatments, cells were used for analysis of cell viability and presence of apoptotic nucleus. The study of PARP cleavage, ERK1/2, p38 and PKC substrates phosphorylation was performed by technique of western blotting, through specific antibodies. Prolonged treatment of SH-SY5Y lineage with (PhSe)2 (from 10 μM) decreased cell viability in 30% (SD 3.7, N=16). This effect was followed by PARP cleavage associated with presence of apoptotic nucleus and inhibition of p38, ERK1/2 and PKC substrates phosphorylation. At low concentration (3 μM) the diselenide increased ERK1/2 phosphorylation in 20% (SD 6.2, N=4) without affecting cell viability. In the presence of U0126, an ERK1/2 inhibitor, the selenium compound (3 μM) caused a decrease of 37% in cell viability (SD 1.82, N=4) and improved PARP cleavage.

Conclusões:
The treatment with (PhSe)$_2$ caused an antitumoral effect in human neuroblastoma cell lineage SH-SY5Y characterized by apoptotic cell death in higher concentrations, followed by inhibition of protein kinases. Moreover, the results pointed to a citoprotective role for ERK1/2, since the inhibition of this enzyme improved apoptotic effects of the compound. These results contributed to clarify molecular targets of (PhSe)$_2$ in the cells and pointed to a potential antitumoral effect for this selenium compound.

Keywords: diphenyl diselenide, neuroblastoma, MAPK, ERK1/2, PHOSPHORYLATION

Financial Support: This work was supported by CNPq. MT were recipient of CNPq Scholarship.

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Resumo:31-102

EVALUATION OF HEPATIC OXIDATIVE STRESS IN MICE SUBMITTED TO ALCOHOL AND BACLOFEN TREATMENT


Farmacologia - Setor de Ciências Biológicas, UFPR

Objectives:

Alcoholic liver disease (ALD) is a common consequence of long-term alcohol abuse. Oxidative mechanisms participate in the ALD pathogenesis, contributing to alcohol-induced liver damage. Baclofen, a GABAb receptor agonist, was used for reducing alcohol consumption. Our aim was to evaluate hepatic oxidative stress in mice submitted to long-term alcohol diet under baclofen or saline treatment.

Methods and Results:

Swiss mice (n = 60) had choice among ethanol (5 and 10%) and water during 16 weeks in a four-phase paradigm: acquisition, withdrawal, re-exposure and quinine-adulteration (AD). Each individual intake was characterized as: addicted (ethanol preference, no reduction in AD), heavy drinker (reduction in AD) or light drinker (water preference all time). After the classification, the three groups were divided into two subgroups because in the last 4 weeks the mice received randomly baclofen (1.25, 2.5, 5.0 mg/kg, i.p.) or saline (i.p.) in 2 consecutive days followed by 4 abstinence days. In the end of treatment mice were submitted to euthanasia and liver samples were collected and kept at –70°C for further analysis: activity of catalase (CAT), superoxide dismutase (SOD) and glutathione-S-transferase (GST), and levels of glutathione (GSH) and lipoperoxidation (LPO). All values were correlated with the amount of protein measured in each sample by Bradford method. The data were analyzed by one-way or two-way ANOVA, followed by Kruskal-Wallis or Newman-Keuls as a post-hoc test. The data revealed that GSH level and GST activity were significantly reduced in heavy drinker mice. We also observed that baclofen reduced statistically SOD and CAT activities and LPO level.

Conclusions:

Oxidative stress in alcoholic liver disease is known as a consequence of increased production of oxidants and decreased antioxidant defenses in the liver. Present results show that high ethanol consumption disrupts the redox equilibrium. Despite the interference in antioxidants enzymes, baclofen can reduce significantly the peroxidation in lipids induced by long-term alcohol intake. These effects of baclofen should be detailed investigated because of its potential use as therapy for alcohol abuse.

Keywords: alcohol, baclofen, oxidative stress, liver
EVALUATION OF THE EFFECTS OF AN AQUEOUS EXTRACT OF SCHINUS TEREBINTHIFOLIUS INVOLVING IN DIFERENT WILD BACTERIAL AND PROTOZOA CULTURE.

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2 Microbiologia Geral Universidade Federal do Rio de Janeiro, UFRJ

Objectives:

Separately assess the bacterial and protozoal survival, subjected to treatment with Schinus terebinthifolius aqueous extract. And assess in bacterial model, if this extract can protect the cells from damaging power related to stannous chloride (SnCl2).

Methods and Results:

In the present study we used two different bacteria to test the survival of bacteria on solid medium: Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus). The evaluation consists of inoculate these different strains in solid Luria Broth culture medium during liquid state, and then administering aliquots of test solutions and control solutions to assess the presence of bacterial growth inhibition zones. Different concentrations of aqueous extract of Schinus terebinthifolius (10, 20, 30 and 40mg/mL) were used as test solutions, stannous chloride (SnCl2 25 mg/mL) were used as experimental solution of free radicals harmful action, known as toxic to these strains, and another experimental solution to evaluate the growth inhibition, the present extract was also associated with SnCl2. As control solution was used Sodium Chloride 0,9% (NaCl 0,9%) (negative) and Amoxicilline + Acid Clavulanic (50 ug/ml + 12,5 ug/mL) (positive). The plates were incubated at 37°C for 24 hours, and were evaluated after this period. To test de protozoal survival, the methodology is conducted in the minimum inhibitory concentration (MIC), using different parasites: Leishmania amazonensis (L. Amazonensis), Trypanosoma cruzi Y (T. cruzi Y) and Trypanosoma cruzi DM (T. cruzi DM) using BHI medium to L. Amazonensis and PBHIL medium to T. cruzi, both supplemented with 10% Fetal Bovine Serum and the extract of aqueous solution at a concentration of 500ul/ml. The procedure involves exposing the cells to different concentrations of the extract from a serial dilution 500ul/ml considering as initial concentration, and was diluted in until four times, assessing whether the extract is able to inhibit the growth of these cells or kill them. The plates used were incubated at 28°C for 72 hours, then added 25ul of resazurin to each well, and returned to incubation for two hours at 28°C, the was used to evidence the toxic action in protozoa. Have been used three controls: medium, medium associated with the extract and culture medium associated with the protozoa.

Conclusions:

Based on experiments involving bacteria is permissible to speculate that the aqueous extract of S. terebinthifolius is not toxic in their concentrations, it was not observed inhibition zones indicate that have been cell death compared with SnCl2 and positive control of Amoxicilline + Acid Clavulanic, and don't demonstration inhibitory action as stannous chloride and the association of the extract with SnCl2 inhibits your harmful action, in concentrations of extract of 30 and 40mg/mL in E. coli and in 20,30 and 40mg/mL in S. aureus. In experiments involving protozoa, the extract did not presented any significant toxic activity at the concentration used, the controls cited also did not show any toxic activity.

Keywords: AQUEOUS EXTRACT, EFFECTS, Schinus terebinthifolius

Financial Support: FAPERJ
THE EFFECTS OF DELTAMETHRIN (BUTOX® CE25) IN THE CARDIORESPIRATORY FUNCTION OF NILE TILAPIA, OREOCHROMIS NILOTICUS.

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Objectives:

Deltamethrin is a synthetic pyrethroid pesticide type II, considered of low persistence in the environment, widely utilized in agriculture, on the control of aquatic parasites and plagues. In spite of its low toxicity in mammals, this pyrethroid can be very toxic for fish. In the present study we evaluated the effects of deltamethrin (Butox® CE25) on the cardiorespiratory function of Nile tilapia, Oreochromis niloticus (Wt ~ 170 g).

Methods and Results:

The cardiorespiratory variables (oxygen uptake - VO$_2$, respiratory frequency - f$_R$, ventilatory tidal volume - V$_T$, gill ventilation - V$_G$, oxygen extraction from the ventilatory current – EO$_2$, and heart rate – f$_H$) were measured during 24 h (control) in a flow-through respirometry system. After this period, the water of the experimental system was contaminated with of a sub-lethal concentration of deltamethrin (7.3 µg L$^{-1}$, 50% of the CL50-96 h) while the cardiorespiratory variables were continuously measured. Oxygen uptake (VO$_2$ - mLO$_2$.kg$^{-1}$.h$^{-1}$) decreased immediately just after the contamination and returned to control levels after 18 h of exposure to deltamethrin. During this period, an abundant mucus secretion was observed on the gills. Respiratory frequency (f$_R$ - breaths.min$^{-1}$) remained nearly constant for the whole experimental period. The reduced VO$_2$, however, was accompanied by significant increases in V$_T$ (mLH$_2$O.kg$^{-1}$.breath$^{-1}$) which was reached by concomitant and significant increases in V$_G$ (mLH$_2$O.kg$^{-1}$.min$^{-1}$). The f$_H$(bpm) decreased significantly just after the contamination and the fish remained bradycardic for next 12 h, after which f$_H$ returned to the control frequencies.

Conclusions:

The data lead to the conclusions that just after the deltamethrin contamination the VO$_2$ was impaired and fish presented typical symptoms of hypoxemia (hyperventilation reached by higher increases in V$_T$ while the f$_R$ remained constant and bradycardia). Deltamethrin is a lipophilic compound, which is adsorbed by the membranes of gill epithelium, impairing the O$_2$ diffusion from the water ventilatory flow to the blood. Another additional barrier to the O$_2$ diffusion is the mucus layer over the gill observed in the present study.

Keywords: DELTAMETHRIN, BUTOX® CE25, CARDIORESPIRATORY, Oreochromis niloticus, NILE TILAPIA

Financial Support: CAPES

AMBLYOMIN-X INHIBITS ANGIOGENESIS IN VIVO BY IMPAIRING VEGF-INDUCED ENDOTHELIAL CELLS FUNCTIONS

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Objectives:

Amblyomin-X is a recombinant protein inhibitor of serinoprotease that inhibits the blood coagulation Factor Xa and in vivo tumors development. Here it was investigated the role of Amblyomin-X on new vessels formation induced by VEGF in vivo and the Amblyomin-X actions on endothelial cells in vitro.

Methods and Results:

The effects of Amblyomin-X on angiogenesis were in vivo investigated using dorsal chambers implanted in male Swiss mice after anesthesia (ketamine/xylazine) and in chorioallantoic membrane (CAM) of Galus galus embryos. Microcirculatory endothelial cell lineage (t-End lineage) was used to evaluate cell viability and cycle, proliferation, and expression of adhesion molecules by flow cytometry; cell adhesion on Matrigel® by colorimetric assay; tube formation by optical microscopy and gene expression of adhesion molecules by RT-PCR. Previous and simultaneous topical administration of Amblyomin-X (100ng/10µL) reduced the formation of new vessels in dorsal subcutaneous microcirculation (47% and 54%, respectively) in relation to VEGF (10ng/10µL). In addition, simultaneous topical administration of Amblyomin-X (100ng/10µL) and VEGF (0,25ng/10µL) reduced the formation of new vessels in CAM (42%). The Amblyomin-X treatment, which did not presented cytotoxicity, simultaneously incubated with VEGF, reduced the endothelial cell proliferation at 48 and 72h (10ng/mL: 40% and 109%; 100ng/mL: 53% and 108% and 1000ng/mL: 121% and 115%, respectively) and delayed the cell cycle, maintaining in phase G1/G0. The treatment with Amblyomin-X reduced significantly the endothelial cell adhesion (49%) and tube formation (85%) on Matrigel®; and the adhesion molecules expression PECAM-1 (26%), VCAM-1 (30%) and ICAM-1 (45%) induced by VEGF, independently of alterations in their mRNA content, but did not alter integrin beta 3 expression.

Conclusions:

Based on our findings, data obtained show that in presence of the growth factor VEGF, Amblyomin-X impaired new vessels formation in vivo, which may be dependent, at least in part, on decrease in endothelial cell proliferation and interference on adherence and tube formation process, which are mediated by expression of adhesion molecules. Future investigations will be carried out to clarify the intracellular mechanisms involved in Amblyomin-X actions on the angiogenesis process.

Keywords: Angiogênese, VEGF, Amblyomin-X, Célula endotelial

Financial Support: FAPESP (08/57850-8; 08/56072-1)
Objectives:

The evaluation of adenosine deaminase activity (ADA) is of great importance, since its substrate has an important role in the modulation of neurotransmission, neuroprotection and cell survival or death. This enzyme is found on cell surface, mainly in lymphocytes, and it is susceptible to cytotoxic drugs such as dexamethasone, or its degenerate insertion site. Thus, it is relevant to assess ADA enzymatic activity in lymphocytes in a model of experimental immunosuppression using dexamethasone.

Methods and Results:

A total of 20 male Wistar rats were used, divided into two groups (n=10). The control group (C) received a subcutaneous dorsal injection of saline (NaCl 0.9%), and the immunosuppressed rats (I) received a single dorsal subcutaneous injection of dexamethasone 25mg/kg. After three days, the animals were anesthetized with isoflurane and the blood was collected in tubes containing EDTA. The lymphocytes were separated by a Ficoll-Hypaque gradient density. The ADA activity was determined by a colorimetric method, in which 50μl of sample reacted with adenosine (21 mmol/L), pH 6.5. The samples were incubated by 60 minutes at 37°C. The results were analyzed by the t-test and expressed as U/L. ADA activity was showed to be decreased in the immunosuppressed rats (C: 2.480 ± 0.3764, SEM; I: 1.494 ± 0.2432, SEM).

Conclusions:

We conclude that the ADA activity was modified in the immunosuppressive state, possibly to compensate the changes that occur in this state and to avoid the adverse effects of therapy.

Keywords: Adenosine deaminase, Dexamethasone, Immunosuppressive state, Lymphocytes

Financial Support: UFSM

QuebraPagina

Resumo:31-107

EFFECT OF AQUEOUS EXTRACT FROM MAHOGANY LEAVES (SWIETENIA MACROPHYLLA) IN AN IN VITRO MODEL OF PARKINSON'S DISEASE (PD)

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Objectives:

Preliminary studies showed high content of antioxidants in the aqueous extract from the leaves of S. macrophylla. In this study, we aim to investigate the potential cytoprotective effects of this extract on an in vitro model of Parkinson disease induced by rotenone, a mitochondrial inhibitor and generator of oxidative stress in animal models, on mesencephalic primary cultures from neonatal (PND2-PND5) Wistar rats.

Methods and Results:

Primary mixed mesencephalic cultures were obtained from Wistar rat neonates (PND2-PND5) according to the method described previously (J Neurosci 22: 782, 2002). The cultures were treated on the 8th day of culture. The cultures were divided into four groups: control (CONT), treated with 40nM of rotenone (ROT), 20μg/ml of aqueous extract of S. macrophylla (MOG), and 40nM of rotenone plus 20μg/ml of aqueous extract of S. macrophylla (R + M) for seven days (n ≥ 4). At the end of treatment, cell
viability was analyzed by MTT and LDH assays. Additionally, we performed western blot to analyze the expression and cleavage of α-spectrin, in order to evaluate the occurrence of apoptosis and/or necrosis. We performed statistical analysis with one way ANOVA followed by Student’s t test. Cell loss, as assayed by MTT, resulted in values as follow: 33.22% ± 8.34%, 36.37% ± 8.72% and 41.08% ± 6.80% for groups ROT, MOG and R + M, respectively, compared to CONT. LDH test for the same conditions resulted in cell death of 11.50% ± 3.97%, 14.03% ± 1.86%, 21.54% ± 2.56% and 28.99% ± 2.76% for CONT, ROT, MOG and R + M, respectively. Western blot revealed levels of 72%, 122% and 117% for the fragment of calpain (145/150 kDa) protein, an indicator of necrosis, in groups ROT, MOG and R + M, respectively, compared to CONT (100%). For the fragment of caspase (120 kDa) protein, an indicator of apoptosis, the analysis showed levels of 166%, 280% and 293% in groups ROT, MOG and R + M, respectively, compared to the CONT (100%).

Conclusions:

As expected, rotenone promoted significant cell loss, as evidenced by MTT and LDH assays. Also, our results with the expression of calpain (145/150 kDa) and caspase (120 kDa) protein strongly suggests that rotenone induces apoptotic cell death more than necrotic. Since our preliminary results with the use of mahogany extract resulted in an overall toxic effect, additional experiments are going to be performed in order to evaluate toxic limits for the use of this antioxidant mixture.

Keywords: Parkinson's disease, Rotenone, Swietenia macrophylla

Financial Support: FAPESPA - FAPESP - CNPq - CAPES

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ANALYSIS OF FECAL ANDROGEN METABOLITES IN PUBERTAL RATS EXPOSED TO DI-(2-ETHYLHEXYL) PHTHALATE (DEHP).

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Objectives:

Phthalates are industrial chemicals that add flexibility to polyvinyl chloride plastics. In spite of their low acute toxicity, several phthalates, such as DEHP, are able to inhibit androgen production in rats and other species, and disrupt pre- and post-natal androgen dependent development. The aim of this study was to characterize the androgen insufficiency induced by a high DEHP dose in pubertal rats by measuring the concentration fecal androgen metabolites at different time points during treatment.

Methods and Results:

30 Wistar male rats, aged 22 or 23 days, were divided into two groups (n = 15/group), which were treated for 30 days by oral gavage with corn oil (vehicle control) or 750 mg DEHP/kg/day. Fifteen pellets of feces were randomly collected from cage bedding on days 1, 5, 10, 15, 20, 25 and 30 of treatment. Bedding was cleaned 24 hours before the collection of fecal samples. From days 1 to 19 of treatment, animals were kept at groups of three rats per cage, so that fecal material from days 1, 5, 10 and 15 represents a “pool” of three different rats. From day 19 onwards animals were kept isolated. Twenty four hours after the final administration, animals were sacrificed for collection of reproductive organs and tissues. Androgen metabolites were extracted from fecal samples with ethanol, resuspended in phosphate buffer and quantified by enzyme immunoassay. In control group, there was a sharp increase in fecal androgen metabolites throughout treatment, compatible with the rise in gonadal androgen production normally seen during puberty. Such increase, however, was impaired by DEHP treatment. As expected, concentration of fecal androgen metabolites on day 1, which reflects the 24 hour period before initial dosing, was not significantly different between control and DEHP groups - median (Q1, Q3): 76.5 (58.3, 87.8) ng/g feces (n=5) and 69.4 (50.0, 80.0) ng/g feces (n=5), respectively (p= 0.31, Mann Whitney test). However, at the end of treatment (day 30) controls exhibited significantly higher values than DEHP treated rats – median (Q1,Q3): 138.4 (112.1, 189.6) ng/g feces (n=15) and 59.2 (51.1, 62.5) ng/g feces (n=14),
respectively (p < 0.0001, Mann Whitney test). In accordance with these results, DEHP treated rats also displayed significant reductions in mean absolute and relative weights of reproductive organs and tissues when compared to controls: testis (53% reduction for both absolute and relative weights), epididymis (24% and 21% for absolute and relative weights, respectively), ventral prostate (23% and 21%), seminal vesicle (42% and 40%), glans penis (16% and 15%) and levator ani plus bulbocavernous muscle (32% and 30%).

Conclusions:

The present results indicate that measurement of androgen metabolites in fecal samples can be used as a noninvasive indicator of the androgen insufficiency induced by DEHP in pubertal rats. Quantitative analysis of fecal steroid hormone metabolites may be a good alternative for monitoring hormonal status in pharmacological and toxicological studies.

Keywords: phthalates, fecal androgens, reproductive toxicology, rats, endocrine disruptors

Financial Support: CNPq and UFPR

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Resumo:31-109

EFFECT OF BISPHENOL A IN THE ZOEA I LARVAE OF NEOHELICE GRANULATA (DANA, 1851) AND ARTEMIA SP NAUPLII

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Objectives:
Bisphenol A (BPA) is the main constituent of polycarbonate, epoxy resins, dental sealants, etc. The widespread use of BPA and its presence in the aquatic environment causes concern about the effects upon exposed populations. The aim of this study was to investigate the effect of BPA on the phototaxis activity of the crab Neohelice granulata Zoea I stage and Artemia sp nauplii stage.

Methods and Results:
The exposure to different concentrations of BPA (40μg/L, 400μg/L, 1.600μg/L and 3.200μg/L) on the phototaxis activity was evaluated for 24h and 48h in Zoea I (horizontal system and velocity - mm/s), and after 96 hours in nauplii (vertical system and displacement – centimeters after 10 min in dark). A vertical system was used because of the geotropism behavior presented by Artemia sp. BPA exposition was carried out at 22°C, and 12-h light/dark photoperiod. Results are presented as mean±standard error (n=10 to each treatment and time exposure). Larvae exposed for 24h showed no differences in velocity. After 48 hours, the BPA exposure affected the displacement of Zoea I. Velocity (mm/s) was higher (p

Conclusions:
Exposure to BPA (≥1600μg/L) impaired significantly the phototaxis reflex in Zoea I and nauplii. The present results show that the phototaxis response in Neohelice granulata Zoea I and Artemia sp nauplii is a sensitive indicator of the presence of BPA polymers.

Keywords: Bisphenol A, Neohelice granulata, Zoea, Artemia, Nauplii

Financial Support: CAPES, INCT-TA
NEUROPROTECTIVE EFFECTS OF ATORVASTATIN AND MK-801 AGAINST THE TOXICITY INDUCED BY 6-HYDROXYDOPAMINE IN VITRO

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Objectives:

Statins are a class of 3-hydroxy-3-methylglutaryl coenzyme A ((HMG-CoA) reductase inhibitors, which are widely prescribed to reduce cholesterol levels in hyperlipidemic patients (Bucher et al., 1999). More recently, the neuroprotective effects of statins such as Atorvastatin (ATOR) have been described (Piermatiri et al., 2009; 2010). Indeed, other studies have also implicated an excitotoxic component to the damage induced by dopaminergic neurotoxins since the N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 protects against the loss of dopaminergic neurons caused by MPTP (Turski et al., 1991). In the present study we investigated changes in cellular viability in slices from hippocampus, striatum and cerebral cortex after co-incubation of ATOR, MK-801 and 6-OHDA in vitro.

Methods and Results:

Rats were killed by decapitation and the hippocampus, striatum and cortex were rapidly removed and placed in ice-cold Krebs–Ringer bicarbonate (KRB) buffer. Slices (0.4 mm thick) were rapidly prepared using a McIlwain Tissue Chopper, separated in KRB at 40 C and allowed to recover for 30 min in KRB at 37oC (Oliveira et al., 2002). After pre-incubation time (30 min), the slices were co-incubated with 6-OHDA (100 uM) and MK-801 and/or ATOR for 1 h in KRB. After this period, the medium was withdrawn and replaced by a nutritive incubation (3h) medium composed of 50% of KRB, 50% of Dulbecco’s modified Eagle’s medium (DMEM, Gibco), 20 mM of HEPES and 100 ug/ ml of gentamicine, at 37 0C in a CO2 atmosphere (Molz et al., 2008).

Cell viability was determined through the ability of cells to reduce MTT (3-4,5- dimethylthiazol-2-yl-diphenyltetrazolium bromide, Sigma) (Mosmann, 1983) resulting in a colored compound from which optical density was measured in an ELISA reader (550 nm). The groups were: 1. Control; 2. 6-OHDA (100uM); 3. ATOR (10 uM); 4. MK-801 (50uM); 5. ATOR + 6-OHDA; 6. MK-801 + 6-OHDA; 7. ATOR + MK-801 + 6-OHDA. Our results demonstrated that 6-OHDA incubation reduced the cellular viability in the hippocampus (50%), striatum (61%) and cortex (63%). The incubation with ATOR or MK-801 per se did not alter the cellular viability. However, increased cell viability was observed after incubations with ATOR + 6-OHDA in the hippocampus (65%), striatum (85%) and cortex (80%). Similarly, incubations with MK-801 + 6-OHDA increased cell viability in the hippocampus (79%), striatum and cortex (81%). Of high importance, a synergistic effect on increasing cell viability was observed after co-incubation of ATOR + MK-801 + 6-OHDA in the hippocampus (97%), striatum (102%) and cortex (93%) versus 6-OHDA group.

Conclusions:

These results showed that 6-OHDA is neurotoxic in an in vitro evaluation in cerebral slices. Moreover, the neuroprotective effects of atorvastatin and MK-801 seem to converge to similar molecular mechanisms which are under investigation.

Keywords: Atorvastatin, Statins, 6-OHDA, MK-801, Parkinson's Disease

Financial Support: CAPES, CNPq, INCT-EN, FAPESC, UFSC.
ANEUPLOIDY AND CELL CYCLE ARREST INDUCED BY ZIDOVUDINE IN EMBRYONIC STEM CELLS

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Objectives:

Acquired Immune Deficiency Syndrome (AIDS) affects 33 million of people around the world. Guidelines recommend the use of Zidovudine (AZT) by pregnant women in order to reduce vertical transmission of virus. Although the efficacy of AZT treatment is well known, little is known about its side effects on embryo development. We used ES cells as a model to investigate the effects of AZT treatment on embryogenesis.

Methods and Results:

First, ES colonies were exposed to AZT during four days and cell cycle profile was evaluated. Untreated group presented 27.27% (± 5.43) of cells in G2/M phase and in AZT 50 and 100 μM the percentage of G2/M accumulated cells raised to 47.63% (± 1.98) and 45.96% (± 4.18), respectively. G2/M checkpoint blocks the progression to mitosis when DNA is damaged, to ensure that chromosome collection is correct. To identify if accumulation of cells in G2/M could be related to altered chromosome number, aneuploidy rate was evaluated after AZT treatment. Untreated colonies presented 39.6% (± 8.4) of aneuploidy, while after treatment with AZT 100 μM the proportion of aneuploid cells raised to 67.8% (± 3.4) with prevalence of hypoploidy. The event was accompanied by micronuclei formation once AZT 100 μM treated ES cells presented a 2 fold increase compared to untreated ones.

Conclusions:

These data suggest that AZT exerts genotoxic effects even at early stages of development. Furthermore, we consider that ES cells can be used as a tool for understanding genotoxic effects of nucleoside analogues in order to improve therapy and drug design.

Keywords: ANEUPLOIDY, AZT, CELL CYCLE ARREST, EMBRYONIC STEM CELLS, MICRONUCLEI

Financial Support: CNPq, Ministério da Saúde, FAPERJ

ASSESSMENT OF THE ACUTE TOXICITY OF THE AQUEOUS FRACTION OF LEAVES OF ALBIZIA INOPINATA (HARMS) G. P. LEWIS (LEGUMINOSAE) IN RATS

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Objectives:
Assess of the acute toxicity of the aqueous fraction of leaves of Albizia inopinata (Harms) G.P.Lewis (AF-AI) in rats.

Methods and Results:

The acute oral toxicity study was performed in rats Wistar, a single dose of 2000 mg/kg of AF-AI was orally administered to male and female groups (n=6). Animals receiving the vehicle (distilled water) served as control. After treatment, the behavior parameters were observed, during 30, 60, 90, 120, 180 and 240 minutes in the first day and once daily in the following 14 days. At the end of the period, the number of survivors was recorded for the determination of the LD50. The weight, organs, food and water consumption were evaluated in both sexes (ALMEIDA et al., Rev. Bras. Farmacog., v.80, 72-76, 1999). The blood was collected by puncturing the brachial plexus. Tubes with anticoagulant ethylenediamine tetracetic acid (EDTA) were used for determination of hematological parameters. Employing standard techniques were performed biochemical measurements of serum. The results are expressed as the mean + S.D. Statistical significance was assessed by t tests. The level of significance was p

Conclusions:

Throughout the experiment was demonstrated that the aqueous fraction of Albizia inopinata has low toxicity. However it is necessary further studies to better assess of toxicity for the plant in continuous use.

Keywords: Toxicidade Aguda, Albizia inopinata, Toxicologia, Plantas Medicinais

Financial Support: CNPq-CCS-UFPB

GENOTOXIC DAMAGES CAUSED BY MERCURY POISONING IN THE CENTRAL NERVOUS SYSTEM CELLS

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Objectives:

Mercury is an important source of environmental contamination and human intoxication, where his main focus is the central nervous system, causing various disorders such as ataxia, paresthesia, delay in development among others. Recently it was suggested that poisoning by very low doses of methylmercury would induce genotoxicity in humans. Our objective was to investigate the impact of mercury exposure directly on cells of the nervous system by conducting tests for the detection of cytogenetic alterations.

Methods and Results:

Human neuroblastoma cells (B103) were cultured and intoxicated with methylmercury chloride (0, 0.1μM, 1μM and 5μM) for 24 hours. For detection of chromosomal aberrations, demecolcine (10 μg/ml) was added to cultures, 2 h before completing 72 h of incubation. After that the cells were fixed and stained with 5% Giemsa. We analysed metaphases for detection of chromosomal abnormalities (gaps, breaks and other deformations) and the proportion of metaphases (mitotic index, M/N). The statistical analysis was performed using a one–way analysis of variance (ANOVA), followed by a Tukey post hoc test (p

Conclusions:

Positive relationship between mercury concentration and MI CNS cells are consistent with data of other authors. In addition, it has described a negative relationship between mercury concentration and MI cells of the peripheral tissue. So, we observed that mercury cause changes in the IM with different patterns in the CNS and peripheral tissue.
GUARANA, CAFFEINE AND TAURINE: REDOX PROFILE OF THREE MAJOR COMPONENTS OF ENERGY DRINKS IN NEURONAL-LIKE CELLS (SH-SY5Y)

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2 Department of Psychiatry and Behavioral Neurosciences, McMaster University

Objectives:

Energy drinks are commercially available highly-caffeinated beverages that have recently become very popular among adults and teenagers. Besides caffeine, energy drinks may contain different aminoacids (e.g., taurine), vitamins (e.g., B vitamins) and a number of herbal compounds, which in some cases, may provide extra caffeine doses to the drink (e.g., guarana). In spite of the available number of research works exploring any behavioral and/or cognitive effects these beverages may exert, very little is known about their actual biochemical and physiological effects. The aim of the present study is to evaluate the effects of three major components of energy drinks in a well-described in vitro model for neurotoxicological research: SH-SY5Y cells.

Methods and Results:

SH-SY5Y cells were treated for 2-4h with 5 concentrations of guarana (Gua), caffeine (Caf) or taurine (Tau), ranging from 3.125 to 50.0, 0.125 to 2.0 and 1.0 to 16.0 mg/ml, respectively. After each treatment, SOD, CAT, GPx and GST activities were evaluated, as well as thiobarbituric acid-reactive substances (TBARS) and sulfidril content (SH). Preliminary results indicate that Gua prevents lipid peroxidation and diminishes GPx and GST activity. Cell morphology was observed using inverted phase-contrast optic microscopy and scanning electronic microscopy. Tau has no apparent effect on cell morphology, while increasing doses of Gua was able to promote neurite regression and plasma membrane blebbing (zeiosis), a common feature of the apoptotic program. High doses of Caf (2 mg/ml) also induced neurite regression. Cell integrity was evaluated by propidium iodide staining followed by flow citometry. Maximum cell integrity was reached at concentrations of 12.5 mg/ml of Gua, 0.5 mg/ml of Caf, and 4.0 mg/ml of Tau; approximately the same concentrations one could find in popular energy drinks like Red Bull® and Monster®. Such concentrations were tested in co-treatment experiments and our preliminlar results showed that for instance, Gua + Tau co-treatment (at 12.5 and 4 mg/ml respectively) was able to induce neurite swelling. Looking for a putative molecular mechanism triggering these observed effects, we determined the antioxidant capacity of all drugs by using TRAP (Total Reactive Antioxidant Potential) method. Gua was shown to be a very powerful antioxidant, in a dose-dependent manner, while Caf and Tau antioxidant capacity was only detected at high doses.

Conclusions:

Gua is a powerful antioxidant and might imbalance the cell redox signaling, inducing apoptosis and relevant morphological alterations, like neurite regression and membrane blebbing. Combinations of these drugs can potentiate their effects at the cellular level.

Keywords: Caffeine, Energy drinks, Guarana, SH-SY5Y, Taurine

Financial Support: CNPq, FAPERGS, PRONEX
EXHALED BREATH CONDENSATE: DIFFERENCES IN PH BETWEEN NON-SMOKING STREET -TRAFFIC OPERATORS AND CONTROLS EXPOSED TO AIR POLLUTION OF SAO PAULO CITY

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Objectives:

Exhaled breath condensate (EBC) and nasal lavage (NL) have been used to assess biomarkers of inflammation. The aim of this study was to determine pH and cytokines in EBC and in NL from non-smoking male street traffic-operators and office-workers in Sao Paulo city.

Methods and Results:

EBC samples were obtained from 74 street traffic-operators (traffic-operators, 27 - 56 years) and 14 controls, healthy office workers (controls, 21 - 42 years). EBC samples were collected with a modified condenser system, and pH was measured after deaeration with ultrapure argon gas (350 ml/min for 15 min) in a room with ambient temperature of 23°C and relative humidity of 65%. Differences between groups were assessed by Mann-Whitney test. Traffic-operators were older than controls (42 ± 6 and 30 ± 5 years, p < 0.001). Clinical data were similar between traffic-operators and controls: systolic blood pressure (119 ± 13 and 118 ± 12 mmHg, respectively), diastolic blood pressure (82 ± 9 and 78 ± 9 mmHg, respectively), heart rate (69 ± 9 and 70 ± 10 bpm, respectively), pulse oximetry (97 ± 1 and 98 ± 1%, respectively), body temperature (36.3 ± 0.2 and 36.3 ± 0.1°C, respectively), and respiratory rate (15 ± 3 and 14 ± 2 rpm, respectively). In contrast, EBC pH values of traffic-operators were significantly lower than controls (7.80 ± 0.47 and 8.12 ± 0.14, respectively, p = 0.001). In addition, exhaled breath carbon monoxide values were greater in traffic-operators compared with controls (4.5 ± 1.5 and 2.4 ± 0.9, respectively, p < 0.001).

Conclusions:

This study indicates that street traffic-operators may be under increased risk of airway inflammation in São Paulo City compared with office workers.

Keywords: air pollution, exhaled breath condensate, inflammation

Financial Support: FAPESP 07/51605-9

REPEAT-DOSE TOXICITY OF COPAIFERA MULTIJUGA STEM OIL ON HEMATOLOGIC PARAMETERS OF WISTAR RATS.

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Objectives:

Copaifera multijuga Hayne (Leguminosae) stem oil has been used as anti-inflammatory, and gastroprotective in folk medicine. Despite its wide use, there are no studies evaluating its possible toxic effects. Thus, we evaluated the repeated dose toxicity of C. multijuga stem oil on hematologic parameters of rats.

Methods and Results:

Four groups of female Wistar rats (n= 10/group) received orally water (control group, C) or C. multijuga stem oil at the doses of 200 (T1), 500 (T2) and 2500 mg/kg (T3) for 8 consecutive weeks. Animals were observed for signs of abnormalities and body weight gain during whole treatment period. At the end of 4th and 8th week of treatment, the animals were anesthetized with sodium pentobarbital (0.135 g/kg, i.p.) and blood samples were obtained for determinations of hematological parameters. No toxic effects or deaths, neither changed body weight gain were observed during whole period of treatment. Four weeks of treatment with C. multijuga did produce any alteration in hematological parameters: erythrocytes count (C = 7.9±0.1, T1 = 7.8±0.2, T2 = 7.5±0.2 and T3 = 8.2±0.2 106/L); hemoglobin (C = 14.9±0.4, T1 = 15.3±0.2, T2 = 14.5±0.2 and T3 = 15.1±0.2 g/dL); hematocrit (C = 42.3±0.3, T1 = 43.4±0.5, T2 = 41.0±0.7 and T3 = 43.5±0.8 %); platelets count (C = 780.5±48.4, T1 = 943.2±75.9, T2 = 802.4±99.7 and T3 = 896±41.4 103/L); white blood cell (C = 9.1±0.4, T1 = 9.4±1.0 and T3 = 8.5±0.9 103/L); neutrophils (C = 10.4±1.1, T1 = 10.0±1.9, T2 = 11.5±1.6 and T3 = 11.1±2.5 %); eosinophils (C = 0.8±0.4, T1 = 0.9±1.9, T2 = 1.5±1.6 and T3 = 0.4±0.2 %); basophils (C = 0.3±0.1, T1 = 0.7±0.4, T2 = 0.8±0.2 and T3 = 0.3±0.1 %); lymphocytes (C = 86.0±1.7, T1 = 85.6±2.7, T2 = 84.8±2.0 and T3 = 84.7±3.6 %); and monocytes (C = 2.5±0.3, T1 = 2.5±0.3, T2 = 2.3±0.4 and T3 = 3.2±0.8 %).

After 8 weeks of treatment, it was observed an increase (*p

Conclusions:

Our data showed that oral treatment up to 8 weeks with C. multijuga stem oil produced no toxic effects on the body weight gain and hematological profile of female Wistar rats, suggesting its safety use.

Keywords: Leguminosae, Copaifera multijuga, REPEAT-DOSE TOXICITY

Financial Support: FINEP

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Resumo:31-117

REPEAT-DOSE TOXICITY STUDIES OF THE HYDROALCOHOLIC EXTRACT OF OPERCULINA ALATA TUBER.

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2 Department of Pharmaceutical Sciences, UFPE
3 Department of Physical Education and Sport Sciences, UFPE

Objectives:
The hydroalcoholic extract of *Operculina alata* tuber has been used in the traditional medicine for treating diseases of digestive tract (functional constipation). Despite its wide use, there are few studies evaluating the toxic effects due to chronic intake. Herein, we assessed the effects of the chronic administration of *Operculina alata* on hematological and biochemical profiles of male Wistar rats.

Methods and Results:

Male Wistar rats were divided in 4 groups (n=10/group), which received orally water (control group, C) or *Operculina alata* at the doses of 25 (T1), 125 (T2) and 625 mg/kg (T3) for 12 consecutive weeks. During whole treatment period, animals were observed for signs of toxicity and body weight gain. At the end of 12th week of treatment, the animals were anesthetized and blood samples were obtained for determinations of hematological and biochemical profiles by automatic hematological analyzer and dimension automation with Boehringer Ingelheim™ biochemical kits, respectively. No acute toxicity signs or deaths were recorded during whole period of treatment with *Operculina alata*. After 12 weeks of treatment, it was observed statistical differences in relation to control group in neutrophils (C=19.4 ± 1.9, T1=29.9 ± 2.8*, T2=24.6 ± 2.4, T3=19.4 ± 1.3 %, *pOperculina alata.

Conclusions:

Altogether, the results showed that the intake of *Operculina alata* for until 12 weeks was not toxic in male Wistar rats, suggesting its safety use.

Keywords: HYDROALCOHOLIC EXTRACT, MALE WISTAR RATS, *Operculina alata* TUBER, REPEAT-DOSE, TOXICITY STUDIES

Financial Support: SOBRAL Laboratory

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Resumo:31-118

**ACUTE AND SUBCHRONIC CADMIUM EXPOSURE EFFECTS ON ZEBRAFISH (DANIO RERIO) BEHAVIOR**

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Objectives:

Cadmium is a highly toxic heavy metal released into the environment by human activity. Organisms tend to bioaccumulate when exposed to this metal and it may reach humans through food chain and inhalation of tobacco smoke, mainly. Once in the cells, it is known that cadmium induces formation of reactive oxygen species (ROS), damaging many proteins and interfering in many intracellular signaling pathways, including the Wnt signaling pathway that is prominently active in the central nervous system. The aim of the present study is to characterize the acute and subchronic effects of cadmium exposure on zebrafish behavior and its underlying mechanisms.

Methods and Results:

Adult zebrafish (>6 months-old) of both sexes were exposed to three different doses of cadmium carbonate (10 ug/l, 100 ug/l and 1000 ug/l) for 24, 96 and 168h. Subsequently, animals were placed in cadmium-free tanks and, after 24h of each treatment, were filmed individually for 5 minutes. The records were evaluated in each treatment using ANY-maze software for the following parameters: traveled distance (m), mean speed (m/s) and mobile periods (s). Data were analyzed by ANOVA followed by Tukey’s test and a p < 0.05 was considered to be significant. Acute exposure increased the traveled distance, mean speed and mobile periods in treated groups when compared to controls but no effect was observed in subchronic treatments.
Conclusions:
The increased general locomotor activity observed in acutely treated animals resembles neurobehavioral effects previously described in humans, including hyperactivity in children (J. Abnorm. Child Psychol. 13;185, 1985) and neurobehavioral defects in attention, psychomotor speed and memory in adults (Biochem. Biophys. Res. Commun. 328;326, 2005). These results are being further characterized regarding neuronal cell death and ROS and the Wnt pathway activity.

Keywords: Behavior, Cadmium, Central Nervous System, Heavy Metal, Zebrafish

Financial Support: CNPq 567483/2008-8, 305060/2009-0 and CAPES (PROSUP)

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Resumo:31-119

HOW CHRONIC AND PERSISTENT EXPOSURE TO ROUNDU® MODULATE SOME PARAMETERS OF CARBOHYDRATE ABSORPTION AND METABOLISM DURING DEVELOPMENT IN RATS? AN ONTOGENETIC INVESTIGATION

Depto de Bioquímica/ CCB, UFSC

Objectives:
The most important herbicide used worldwide is Roundup®, a commercial preparation of glyphosate. It has been demonstrated that exposition of the commercial preparation could induce cell death in several human cell types. The aim of this study was to investigate the effect of chronic exposure of glyphosate-Roundup® in glycemia, intestinal disaccharidase activities, hepatic glycogen content, and hepatic glucose-6P-desidrogenase (G6PD) activity throughout development.

Methods and Results:
Dams were treated orally with water or 1% glyphosate-Roundup® (0.38% glyphosate) from pregnancy until 15, 30 and 60 day-old. The duodenum, the liver and the blood were collected. The disaccharidase activities were determined by incubating intestinal homogenate with specific disaccharide. The serum glucose levels and G6PD activities were determined by using commercial “kits”. The hepatic glycogen levels were measured by a colorimetric method. The G6PD activity was inhibited in the liver from animals in all developmental stages studied. Results also showed that chronic treatment with Roundup® leads to hypoglycemia and reduced duodenal lactase activity only in 15-day old animals, without alter these parameters in serum/duodenum of 30 and 60 day-old animals. However, maltase activity was affected in duodenum of 30 and 60 day old rats and unaltered in 15 day-old animals. In addition, the hepatic glycogen levels were determined in animals from different developmental stages. Our data demonstrated diminished glycogen content in liver from 15 day-old rats opposite to the increased levels observed in 30 day-old rat liver. On the other hand, in 60 day-old rats, the glycogen levels were unaltered.

Conclusions:
These intriguing results suggest that the suckling period is the most affected by glyphosate exposure, showing hypoglycemia, inhibited lactase, decreased levels of glycogen and lower G6PD activity. Probably there is some type of hepatic adaptation to the chronic and persistent exposure to the pesticide. We might postulate that the presence of the pesticide in the milk might be more toxic than its presence in drinking water. New experiments are needed to better understand the mechanisms underlying such effects.

Keywords: pesticides, disaccharidase, glycogen, glycemia, glucose-6-phosphate dehydrogenase
EFFECTS OF INORGANIC SELENIUM ADMINISTRATION IN METHYLMERCUry-INDUCED BEHAVIORAL EFFECTS DURING DEVELOPMENT IN RATS.

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Objectives:

Aim: To evaluate potential protective actions of sodium selenite (selenium) on behavioral (motor and memory) deficits after exposure to methylmercury (MeHg) in two (early and late) different postnatal neurodevelopmental periods of the Wistar rats.

Methods and Results:

Methods and Results: Wistar (P1 and P21) rats received orally vehicle, selenium (5ppm), MeHg (10ppm) or selenium (5ppm) plus MeHg (10ppm) during 20 and 10 days, respectively (n = 6 per group). After treatment, the subjects were submitted to the open field test and Morris water maze in order to evaluate motor and memory/learning deficits, respectively, following the described experimental procedures. Analysis and statistics were made by using the software Any Maze tracking system, Stöelting, analysis of variance (ANOVA) and descriptive statistics. As expected, our preliminary data showed significant reduction (% ± standard deviation) in the locomotor activity when younger rats (P1) were exposed to MeHg (26 ±16%). Additionally, we also observed similar reduction in rats who received selenium + MeHg (37 ±20%), when compared to control group. Subjects treated with selenium alone showed no difference in the motor activity when compared to control rats. Same groups analyzed in the water maze showed learning/memory deficits in the groups treated with MeHg or MeHg + selenium. Reductions in the latency time were only observed in the control and selenium-treated animals (61 ±16% and 36 ±20%, respectively). As for the older rats (P21), subjects treated with MeHg showed significant increase in the locomotor activity (35 ±32%) when compared to control rats. Concomitant treatment with MeHg and selenium abolished this effect. When submitted to the water maze, we also observed task-specific deficits in the MeHg and MeHg + selenium groups. Also, we only observed reduction in the latency time in the control rats and those exposed to selenium (57 ±30% and 64% ±22%, respectively).

Conclusions:

Conclusions: Our results showed that MeHg exposure under our experimental paradigm resulted in hypokinetic or hyperkinetic effects, depending on the age of the subjects tested. Selenium administration was only able to interfere with these deficits in older animals. Also, we showed that selenium administration was not able to interfere with the memory/learning deficits induced by MeHg exposure. Possible mechanisms associated protective actions of selenium in older stage of neural development remains to be elucidated.

Keywords: behavioral, Methylmercury, Selenium

Financial Support: Capes, UFPA
EFFECTS OF CADMIUM, N-ACETYLCYSTEINE OR THEIR COMBINATION ON CD CONCENTRATION IN SPLEEN AND THYMUS OF MALE RATS.

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2 Departamento de Química, UFSM. RS, UFSM/RS

Objectives:
Cadmium (Cd) is known to be one of the most toxic environmental and industrial pollutants. In fact, Cd is a potent immunotoxicant which damages humoral and cell mediated immunity. Cd exposure causes disruption of immune system which affects other vital systems. However, in spite of many studies, the mechanism of its toxicity has not yet been well elucidated and in contrast to other metals, there is no effective therapy for its poisoning. Therefore, considering that Cd impairment is associated with immunotoxicity and that N-acetylcysteine (NAC) has important antioxidant actions, the aim of this study was to investigate the effects of this compound on Cd concentration in spleen and thymus (important immune organs) of Cd-exposed rats.

Methods and Results:
In the present study the rats received CdCl2•H2O (2 mg/kg) and NAC (150 mg/kg) by gavage every other day for 30 days. The animals were randomly divided into four groups: control/saline, NAC, Cd and Cd/NAC. The last group received NAC 30min after Cd. The solutions were freshly prepared in saline and were administered (1 mL/kg) between 9 and 11 a.m. (Chem. Biol. Interact. 186:53, 2010). After the treatment, the animals were anesthetized and submitted to euthanasia. The spleen and thymus were gently removed and weighted in glass vessels and 3–8 mL of HNO3 was added for digestion. Digestion was performed using a block (Velp Scientifica, Milano, Italy) heated at 130 °C during 3 h. After this time, 2 mL of H2O2 was added and the samples were heated for 1 h. Digested samples were then transferred to polypropylene flasks for Cd determination. Cd determination was performed by inductively coupled plasma mass spectrometry (ICP-MS). An ICP-MS equipment (PerkinElmer Sciex,model ELAN DRC II, Thornhill, Canada), equipped with a concentric nebulizer (Meinhard Associates, Golden, USA), a cyclonic spray chamber (Glass Expansion, Inc.,West Melbourne, Australia) and a quartz torch with an injector tube of 2 mm i.d. was used. (Chem. Biol. Interact. 186:53, 2010). The Cd concentration was expressed as µg Cd/g tissue. The statistical analysis was performed using two-way ANOVA–Duncan's Test (p

Conclusions:
Taken together, the results of the current study demonstrate that NAC administration was able to modulate Cd concentration in both spleen and thymus. Considering that both organs are important immune components, we can suggest that NAC is a promising drug which should be investigated in order to improve therapeutic alternatives against Cd intoxication.

Keywords: Cadmium, NAC, rats

Financial Support: CAPES, CNPq, FAPERGS.
Objectives:

The action of drugs in humans and/or in animals can be divided into three phases: pharmaceutical, pharmacokinetic and pharmacodynamic. The pharmacokinetic phase includes the processes of absorption, distribution, metabolism and excretion (ADME). Many drugs entering clinical trials still fail in late stage development due to inadequate pharmacokinetic properties. Since metabolic stability and/or drug metabolism are a pivotal contributor to drug efficacy, assays using liver microsomes of different species are an indispensable component in any drug screening process, aiming to minimize the failures observed in transposition of pre-clinical phase to clinical phase. In this scenario, we describe the hepatic microsomal metabolism in vitro of LASSBio-448, a sulfonamide derivative, designed as a new phosphodiesterase-4 (PDE4) inhibitor, recently described as a new antiasthmatic drug candidate.

Methods and Results:

The antiasthmatic prototype LASSBio-448 was selected for studies of hepatic microsomal metabolism in vitro using the liver of rats to establish its kinetics towards metabolic enzymes present in the microsomal fraction (FM) (i.e. CYP450, monoflavin oxygenases (FMO) and carboxylesterases). The assays were performed in the presence and absence of cofactors, since the catalytic activity of CYP450 and FMO depends on these cofactors. LASSBio-448 was metabolized in the presence of cofactors, with a t1/2 of approximately 16 minutes, suggesting a metabolism by CYP450 or FMOs. To investigate which enzyme system would be acting in metabolism of LASSBio-448, assays were conducted in the presence and absence of non-selective CYP450 inhibitor (ketoconazole (30uM)) and FMOs inhibitor (methimazole (100uM)). The results indicated the involvement of CYP450 in the metabolism of LASSBio-448. Next, experiments were performed in the presence of selective inhibitors of the major CYP450 isoforms, namely ketoconazole (10uM; CYP3A4/5), sulfafenazole (50uM; CYP2C9), quinidine (5uM; CYP2D6), furafyline (20uM; CYP1A2) and ticlopidine (40uM; CYP2C19). The results obtained indicate that the hepatic metabolism of LASSBio-448 is dependent of the isoenzymes CYP2D6, CYP1A2, CYP2C19. The two principal metabolites were characterized using MS-MS spectrometry.

Conclusions:

We described the in vitro hepatic microsomal metabolism of a new inhibitor of PDE-4, LASSBio-448, previously identified as antiasthmatic drug candidate. The results indicate a very rapid metabolism (t1/2 of 16 minutes), dependent of the participation of CYP450 isoenzymes. The two principal metabolites were characterized and now are being synthesized aiming to verify their eventual antiasthmatic activity.

Keywords: microsomal metabolism, Antiasthmatic, LASSBio-448

Financial Support: FAPERJ, INCT-INOFAR, CNPq, CAPES.

Resumo:32-012

NONLINEAR ANALYSIS OF THE ON AND OFF PATHWAYS IN THE HUMAN ELECTRORETINOGRAM

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Objectives:
Instead of amplitudes, latencies and power spectra traditionally measured in full field electroretinogram (ERG), we seek to identify and quantify the excitatory (ON) and inhibitory (OFF) mechanisms of the human retina, using the Fractal and Chaos Theory.

Methods and Results:

We recorded ERGs in healthy human retinas (4 individuals, Ethical Committee Approval number CEP-HU/USP: 642/06), using rapid on/ramp off and rapid off/ramp on saw tooth wave stimuli, to isolate, respectively, the retinal responses of the ON and OFF pathways [Kremers, Vision Res. 33, 1997 (1993)], presented at 4Hz in a Ganzfeld stimulator (Q450 SC Roland–Consult, Germany). The signals were analyzed using typical methods for characterizing nonlinear systems: Takens time delay reconstruction [Lect. Notes in Math. 898, 366 (1981)], Lyapunov exponents [Phys. Rev. E 65, 036702/1-10 (2002)], Kaplan-York dimension [Peitge & Walther, ‘Functional Differential Equations and Approximation of Fixed Points,’ Springer, New York (1987)] and Haussdorff fractal dimension [Physica D 124, 210-224 (1998)]. Our results for the ON pathway, applying the Takens reconstruction over the signal, treated using a 32.60 Hz digital RC low-pass filter, indicate the Haussdorff fractal dimension $D_{zero}$ value 1.86(0.16), i.e. we may reconstruct the attractor associated with the ERG in a two-dimensional phase space. The Lyapunov exponents in two-dimension for ON pathway are $L_1=8.1(1.5)/sec$ and $L_2=-10.2(4.7)/sec$. The $L_1$ and $L_2$ results are consistent with $D_{zero}$ ($p=0.7$), since the Kaplan-York dimension $DKY$, estimated from $L_1$ and $L_2$, value 1.79(0.39). Concerning the OFF pathway, we compute $D_{zero}=1.72(0.05)$, $L_1=5.5(1.6)/sec$, $L_2=-8.9(4.1)/sec$, and $DKY=1.56(0.15)$. Again, $DKY$ and $D_{zero}$ are the same for the OFF channel with $p=0.5$. Finally, $D_{zero}$ of the ON pathway are bigger than OFF with $p=0.008$, indicating that the electrophysiological mechanisms of the ON and OFF visual processing are not the same.

Conclusions:
The nonlinear methods are viable for the ERG signal analysis, since the ON and OFF electrical responses are low dimensional (small fractal dimension), i.e. these pathways could be represented by few variables. Moreover, we have one positive and one negative Lyapunov exponent associated respectively with the expansion and contraction of the phase space in chaotic systems. We conjecture that they are related with the excitatory and inhibitory process of the ganglion cells by the bipolar cells. Since the positive exponent is finite, we have sensitive dependence to initial condition (chaos). As expected, retina has an operational memory and a feedback mechanism. We will extend our analysis to additional retinas.

Keywords: Electroretinogram, Chaos, Fractal, ON/OFF Pathways


Resumo:32-013

THE EFFECTS OF BRIMONIDINE ON RETINAL SPREADING DEPRESSION

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3 Faculdade de Medicina de Petrópolis, FMP

Objectives:

Spread depression (SD) was originally described by Leão (1994) in the rabbit cerebral cortex and first identified on retina by Gouras (1958). The retinal SD is a propagating wave in an excitable medium in neuronal tissue of the retina. Its velocity is about 3 mm/min and it is accompanied by a variety of changes in the tissue, including electrical and optical events. Our purpose was to investigate the influence of brimonidine tartarate ($\alpha_2$ agonist) on the propagation of retinal SD waves as it has been postulated to have protective effects on neuronal tissue.
Methods and Results:

We performed 30 experiments on fragments of retinal preparations of White Leghorn chicks from 3 to 8 days after hatching. Immediately after decapitation the eyeballs were removed and sectioned along the equator. Fragments of retina were transferred to a chamber and infused with Ringer solution (Rs) driven by a peristaltic pump in order to maintain the Rs flowing at a rate from 0.8 to 0.85 ml/min. The temperature in the chamber was set at 30 °C by means of a thermostatic bath. Rs composition in mM was: NaCl 100, KCl 6, CaCl 2, MgSO4 1, NaHPO4 1, NaHCO3 30 and glucose 20. The presence or absence of SD was detected by recording its concomitant slow voltage variations through two pore electrodes connected to a Grass polygraph. All experiments started with a control procedure. Firstly, the retina infused with Rs was mechanically stimulated by a sharpened tungsten wire, triggering a SD. Next, the infusion was changed to Rs containing 0.1% brimonidine tartarate (0.1% Rs/Bt) and the retina was mechanically stimulated twice with an interval of 15 min. Afterwards, the infusion was returned to Rs and 15 min later the retina was mechanically stimulated. Our data demonstrated that brimonidine tartarate reduces the amplitude of the negative potential shift in 28.83% (from 21.37mV ± 2.24 to 16.33 ± 2.82mV; p

Conclusions:

Brimonidine tartrate reduces the amplitude and duration of the negative potential shift on SD. A possible explanation is that when the brimonidine tartrate binds to an α2 adrenergic receptor, this receptor activates a G protein that inactivates the enzyme adenylate ciclase. This reduces the intracellular cAMP levels and the protein Kinase A (PKA) resulting in an inactivation of the Ca2+ channels and in the closure of the K+ channels at the same time. This pathway would be also responsible for brimonidine tartrate neuroprotector effect.

Keywords: Spreadin Depression, Brimonidine, Retinal

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Resumo:32-014

RESTING POTENTIAL OF THE RABBIT'S LENS

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Objectives:

The resting potential of the lens is measured by the difference of ions distribution between the surrounding medium and the lens. It is believed that the lens behave as a big cell and the resting potential is equally distributed throughout its entire surface. Our purpose was to measure the resting potential of the lens in rabbits (Oryctolagus cuniculus).

Methods and Results:

We performed 40 experiments (eighty eyes were measured but only one eye was counted) on rabbit’s lens. Immediately after decapitation the eyes were removed and the posterior pole and vitreous were excised. The anterior part of the eye was placed in a modified Ringer solution (RS) with the cornea downwards. The temperature in the chamber was set at 30°C by means of a thermostatic bath. The RS composition in mM was: NaCl 100, KCl 6, CaCl 2, MgSO4 1, NaHPO4 1, NaHCO3 30 and glucose 20. The membrane potential was measured using microelectrode consisting of a thin recording electrode encased in a very fine-tipped glass pipette that was inserted inside the lens through the posterior pole. The microelectrode was connected via a voltmeter to a reference electrode that was immersed in the solution outside the lens. The voltmeter measures the voltage drop across the circuit caused by the membrane potential (mV). The signal was amplified and displayed on a Grass polygraph and chart recorder.
The external electrode is outside the lens capsule. When the microelectrode crosses the lens capsule, whichever be its position, it sends a signal that is recorded as a negative potential in relation to the potential measured by the reference electrode. The difference between the two electrodes gives the resting potential. The resting potential measured was 44.05 mV (± 4.14), ranging from 33 mV to 53 mV. Brindley (1956) found a resting potential of the rabbit’s lens of 66 mV and 74 mV in frog lens. The difference might be due because the ionic content present in their experiments was different from the modified RS used in our experiments.

Conclusions:

In this study, the resting potential of the rabbit’s lens was equal to 44.05 mV (± 4.14) ranging from 33 mV to 53 mV. The precise nature of the lens resting potential is a rather confusing issue. We believe that our data should encourage further studies related to the electrical properties of the lens membrane, not only in rabbits but particularly in monkeys (and possibly in human eyes). It may eventually help to elucidate permeability changes during cataracts formation.

Keywords: Lens, Resting Potential, Rabbit

Resumo:32-015

DIRECTION TUNING OF THE EXTRA CLASSICAL RECEPTIVE FIELD IN VISUAL WULST NEURONS

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Objectives:

The response of neurons in the primary visual cortex (V1) to stimuli presented within their classical receptive field (CRF) can be modulated by stimuli presented in surrounding regions that do not evoke response on their own. This contextual modulation depends on the relative direction of motion between the center and surround stimuli. Colinear stimuli usually induce suppression, while orthogonal surrounding field enhance the response up to facilitatory levels. Here, we investigate the prevalence of such effects in visual wulst neurons of an owl species that is atypically active during the day, namely the burrowing owl. The visual wulst constitutes a very interesting comparative model to the study of V1 as these two structures show remarkable hodological and physiological similarities despite their presumably independent evolutionary origin.

Methods and Results:

Our results are based on 172 well-isolated neurons extracellularly recorded from eight awake burrowing owls, Athene cunicularia. Manual mapping was initially used to determine the location of the CRF center. All subsequent measurements were made through the dominant eye, with sine wave gratings centered on the CRF and optimized for direction of motion, spatial and temporal frequencies. The average receptive field size recorded in foveal/parafoveal areas was 1.9º of visual angle, while the average surround size effect was 7.8º. Colinear surround suppression was widely distributed with a population mean of 37.1%. Lack of suppression was found in only 8% of our cell sample. No correlation was found between CRF size and suppression strength. The Difference of Gaussians model (DoG), proposed by several investigators working in V1, fitted well our dataset, as 75% presented an R-squared value higher than 0.8. No cell response could be evoked by presenting grating annuli of inner diameter larger than the cell receptive field. As grating annuli progressively encroached the CRF, response increased but never reached that obtained with a grating patch centered on the CRF and of corresponding size. Introducing a direction-of-drift contrast between the CRF and its surround modulated the degree of suppression. Half of the cell population showing surround direction selectivity (~ 55% of the dataset) showed significantly different responses between colinear and orthogonal surrounds. A direction-of-drift contrast of 90º elicited a robust increase in activity for 75% of these cells. No unit stimulated by an orthogonal surround responded more than when stimulated within the CRF.
Conclusions:

Our results demonstrate that surround suppression is an expressive phenomenon in the owl visual wulst. Interesting considerations for future work would be to determine the extent to which feedforward versus feedback mechanisms, as well as intrinsic connectivity contribute to this phenomenon.

Keywords: contextual modulation, visual wulst, owl

Financial Support: Fapemig, FINEP, CNPq, Capes.

Objective: To investigate refractive services outcomes in low-income school children, using visual acuity (VA) and need/usage of glasses as outcome measurements.

Methods and Results:

The study was approved by the UNIFESP Committee on Ethics in Research protocol No.0810/08, São Paulo. A population-based study design was conducted from June to November 2005 using cluster sampling which was based on a geographically-defined school census. School children aged 11 to 14 years from 9 of 76 public schools (grades 5-8) in 3 districts in the East Zone of São Paulo City were randomly selected. Children were assessed for visual acuity (VA) measurement and ocular examination. The definition of visual impairment (VI) was presenting/uncorrected VA = 20/40 or worse in either eye. Children were queried about previous usage of glasses. Clinical eye exam included presenting visual acuity for distance (PVA), uncorrected visual acuity for those wearing glasses (UCVA), VA measurement by using LogMar chart, biomicroscopy, and on those with PVA/UCVA of 20/40 or worse in either eye, cycloplegic auto-refraction, subjective refraction and fundus examination were performed. From the total of 2825 children enumerated, 2441 (86.4%) were examined and 25 children of them with VI causes other than RE were excluded from the analysis. The need/benefit of glasses were analysed for those children (2416) that were wearing or not wearing glasses. Among those 136 students who used glasses, 66 (2.73%) children needed and were wearing appropriate correction, 10 (0.01%) children needed and were wearing inappropriate correction and 60 (2.48%) children were wearing glasses but did not need them. Among those 128 with visual impairment, 66 (51.56%) children were wearing appropriate correction, 10 (7.81%) were wearing inappropriate correction, 1 (0.79%) was wearing glasses without any need of them and 51 (39.84%) were not wearing glasses but need optical correction.

Conclusions:

Unmet need for glasses was evident by the substantial number of children with visual impairment due to uncorrected refractive error without appropriate refractive correction. Contrarily, there was a group of children who were wearing glasses without visual impairment. These outcomes demonstrate that better quality of refractive services in this population is desirable, reinforcing the need for specific eye care programs to improve access and affordability for glasses in school children in Brazil.

Keywords: REFRACTIVE ERROR, SCHOOL CHILDREN, VISUAL IMPAIRMENT

Resumo:32-016

“REFRACTIVE SERVICES OUTCOMES IN LOW-INCOME SCHOOL CHILDREN IN SÃO PAULO CITY”


DEPTO OFTALMOLOGIA, UNIFESP

Objectives:

To investigate refractive services outcomes in low-income school children, using visual acuity (VA) and need/usage of glasses as outcome measurements.

Methods and Results:

The study was approved by the UNIFESP Committee on Ethics in Research protocol No.0810/08, São Paulo. A population-based study design was conducted from June to November 2005 using cluster sampling which was based on a geographically-defined school census. School children aged 11 to 14 years from 9 of 76 public schools (grades 5-8) in 3 districts in the East Zone of São Paulo City were randomly selected. Children were assessed for visual acuity (VA) measurement and ocular examination. The definition of visual impairment (VI) was presenting/uncorrected VA = 20/40 or worse in either eye. Children were queried about previous usage of glasses. Clinical eye exam included presenting visual acuity for distance (PVA), uncorrected visual acuity for those wearing glasses (UCVA), VA measurement by using LogMar chart, biomicroscopy, and on those with PVA/UCVA of 20/40 or worse in either eye, cycloplegic auto-refraction, subjective refraction and fundus examination were performed. From the total of 2825 children enumerated, 2441 (86.4%) were examined and 25 children of them with VI causes other than RE were excluded from the analysis. The need/benefit of glasses were analysed for those children (2416) that were wearing or not wearing glasses. Among those 136 students who used glasses, 66 (2.73%) children needed and were wearing appropriate correction, 10 (0.01%) children needed and were wearing inappropriate correction and 60 (2.48%) children were wearing glasses but did not need them. Among those 128 with visual impairment, 66 (51.56%) children were wearing appropriate correction, 10 (7.81%) were wearing inappropriate correction, 1 (0.79%) was wearing glasses without any need of them and 51 (39.84%) were not wearing glasses but need optical correction.

Conclusions:

Unmet need for glasses was evident by the substantial number of children with visual impairment due to uncorrected refractive error without appropriate refractive correction. Contrarily, there was a group of children who were wearing glasses without visual impairment. These outcomes demonstrate that better quality of refractive services in this population is desirable, reinforcing the need for specific eye care programs to improve access and affordability for glasses in school children in Brazil.

Keywords: REFRACTIVE ERROR, SCHOOL CHILDREN, VISUAL IMPAIRMENT
Objectives:
Amblyopia is a form of cerebral visual impairment in the absence of an organic cause. It is a consequence of retinal image degradation associated with abnormal visual experience during development of the visual system in childhood. It can occur when the difference in refractive power (anisometropia) and/or misalignment of the visual axes (strabismus) in both eyes interfere with the critical period of development, causing abnormal processing of vision in the affected eye due to interocular competition and leading to reduction in visual acuity. The aim of this study is to investigate possible abnormalities in the pattern reverse VEP (PRVEP) in amblyopic children.

Methods and Results:
This study was approved by the Ethics Committee of the Federal University of São Paulo (0503/08). A group of 38 children with amblyopia (19 girls), aged 5-14 years (mean 8.6±2.3 years), including 10 anisometropic, 21 strabismic and 7 with anisometropia and strabismus was studied. A group of 19 healthy children (13 girls) aged 5-15 years (mean 8.2±2.6 years) was used as control. PRVEP recording was obtained with checkerboard stimuli subtending visual angles of 1°, 30' and 15'. Latency in milliseconds (ms) for components N75, P100 and N135, and amplitude between the peaks of N75 and P100 in microvolts (μV) were calculated. For stimulus 1°, the latency of P100 was comparable between amblyopic eyes (mean 102.5±10.1ms), control eyes (mean 98.2±5.3ms) and fellow eye of the amblyopic group (average 99.3±7.0ms). There were statistically prolonged P100 latencies (p < 0.001) in amblyopic eyes for both stimuli of 15' (115.8±19.7ms) and 30' (125.6±23.7ms) compared to controls (respectively, 101.4±5.5ms and 103.2±6.8ms). Amplitudes were significantly reduced (p < 0.001) in amblyopic eyes for stimuli of 15' (13.7±8.1μV) compared with control eyes (19.0±3.0μV).

Conclusions:
There was delayed conduction and reduced amplitude of PRVEP responses in amblyopic eyes of children with strabismus and/or anisometropia. These findings are consistent with functional deficits in the maculo-occipital pathway in amblyopic eyes. PRVEP can be an ancillary tool in monitoring patching therapy in this condition.

Keywords: Electrophysiology/methods, Visual acuity, Amblyopia
Objectives:

In adult mammals, the regeneration of the optic nerve is very limited and to date there are no efficient therapies to generate neuroprotection and axon outgrowth after injuries. Using the optic nerve crush of rats as a model for CNS injury, we investigated the effect of intravitreal transplantation of syngeneic bone-marrow mononuclear cells (BMMCs) on retinal ganglion cell (RGC) survival and on the regeneration of optic axons.

Methods and Results:

We used Lister-hooded adult rats (3 to 5 months old) and the optic nerve was crushed using frozen tweezers. The transplantation was performed shortly after optic nerve crush and control animals received intravitreal saline injections. Analysis of mRNA levels was performed using qRT-PCR. We demonstrated an increase of 1.6 fold in retinal-ganglion cell (RGC) survival and an increase of 3.7 fold in the axon outgrowth 14 days after injury, besides a reduced Müller glia activation. We also showed an increase of 5.2 fold in the axon outgrowth 28 days after lesion, but the BMMCs effect on RGC survival was not sustained. Analysis by qRT-PCR revealed an increase in levels of fibroblast growth factor 2 (FGF-2) mRNA in treated animals 1 and 14 days after injury. There was also an increase in the brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CTNF), vascular endothelial growth factor (VEGF) and oncomodulin mRNA levels in the saline-injected animals 3 days after injury. To investigate whether the regenerated axons could reach visual targets, we have retrograde labeled the RGCs by injecting a lipophilic tracer in the superior colliculus. We have also analyzed the expression of NGFI-A in the superficial layers of the superior colliculus to assess glutamate release by the RGCs axons. We found evidences that a higher number of RGCs were able to reach the superior colliculus after the treatment. We have also shown that NGFI-A expression was higher in the treated animals 60 days after injury.

Conclusions:

These results demonstrate that BMMCs transplantation can promote neuroprotection and neuroregeneration 14 days after injury but the effects on RGC survival were not sustained after 28 days. Our results also suggest that the BMMC effects may be related to FGF-2 release or macrophage/monocyte activation.

Keywords: Optic nerve regeneration, retinal ganglion cells, bone marrow cells, neuroprotection

Financial Support: CNPq, CAPES, FAPERJ, Fundação Universitária José Bonifácio, DECIT, PROTECEL, INB

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Resumo:32-019

POTENCIAL VISUAL EVOCADO POR REVERSAO DE PADROES EM PACIENTES COM NEURITE OPTICA UNILATERAL

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Objectives:
A Neurite Óptica (NO) é uma neuropatia inflamatória desmielinizante, que pode estar associada ou não a doenças sistêmicas, sendo a Esclerose Múltipla (EM) a mais comum. O objetivo deste estudo é avaliar a integridade funcional do nervo óptico em pacientes com diagnóstico prévio de NO unilateral utilizando os PVERP e comparar com resultados da acuidade visual (AV).

**Methods and Results:**

Participaram deste estudo 23 pacientes de 13 a 64 anos (M = 34±15 anos) atendidos entre 2000 a 2010. Destes, 5 (22%) eram do sexo masculino e 18 (78%) do sexo feminino. O PVERP foi realizado em condição monocular de estimulação taxa de reversão = 2Hz; estímulos em xadrez com 15 e 60; contraste de 100%, num ambiente em penumbra. O PVERP foi realizado de acordo com o protocolo clínico da Sociedade Internacional de Eletrofisiologia Visual Clínica. A latência P100 (ms), a amplitude N75-P100 (mV) e a dispersão temporal (calculada pela diferença da latência entre o N135 e N75) foram determinados para ambos os tamanhos de estímulos; além da assimetria interocular tanto para amplitude (≥ 30%) e a latência (≥ 3.5ms). A AV foi medida para longe e com a melhor correção óptica utilizando a tabela ETDRS retro-iluminada. Resultados: A AV foi normal em ambos os olhos em 10 (43,4%) pacientes. No olho de melhor visão a AV variou de -0,1 a 0,3 logMAR (20/16 a 20/40), com média=0,0±0,09 logMAR; mediana=0,0 logMAR. No olho de pior visão, a AV variou de 0,0 a 1,5 (20/20 a 20/640) logMAR, com média=0,3±0,8 logMAR; mediana=0,8 logMAR. Um paciente apresentou percepção luminosa no olho de pior visão. Dos pacientes que apresentaram AV normal em ambos os olhos, um não apresentou latência prolongada e/ou amplitude reduzida no PVERP. A latência de P100 foi estatisticamente (p<0.05).

**Conclusions:**

22 pacientes apresentaram alterações nos PVEs e 1 paciente apresentou PVE normal. Os PVEsRP demonstraram atraso na velocidade de condução dos estímulos e redução na amplitude das respostas compatíveis com déficits funcionais na via mático-occipital em olhos afetados por NO. Estes resultados confirmam que o PVE é um instrumento sensível para revelar anormalidades subclínicas nas vias visuais em pacientes com NO.

**Keywords:** Acuidade visual, Eletrofisiologia Visual, Neurite óptica, Potencial visual evocado

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**Resumo:**

DECREASED FUNCTIONAL MAGNETIC RESONANCE IMAGING RESPONSE TO VISUAL STIMULI AND PERICALCARINE CORTEX VOLUME IN PATIENTS WITH GLAUCOMA

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**Objectives:**

To evaluate functional magnetic resonance imaging (fMRI) response to visual stimuli and pericalcarine gray matter (GM) volume in patients with glaucoma and controls, correlating to psychophysical tests and structural ocular findings.

**Methods and Results:**

Patients and controls performed complete ocular examination, perimetry (SAP and FDT Matrix) and Stratus OCT. fMRI was performed with a 3 Tesla MRI, with 4 reversing checkerboard stimuli: expanding ring (eccentricity), rotating wedge (polar angle), magnno and parvo, presented bilaterally in 3 cycles of 60 seconds each. Visual cortex response was obtained by changes in blood flow oxygenation (BOLD effect). BOLD response was analyzed in 3 ways: “fMRI measure” (maximum peak of the BOLD
response with a delay of 3 seconds); “BOLD90%” (largest range of cumulative distribution); and “occipital response” (average of the most significant values in the occipital pole ROI). SAP and FDT results were divided into quadrants, using the average of the total deviation for each quadrant, excluding the central point. Superior and inferior average RNFL thickness were also compared to fMRI response. MRI image processing and segmentation were performed using a surface-based morphometry analysis and FreeSurfer software. To compare both groups regarding anatomical and functional examinations, generalized estimating equation (GEE) models were performed to account for the dependence between eyes and cortical segment of the same patient. 20 individuals performed the exams, 14 with glaucoma and 6 controls. Mean age was 59.3 ± 14.8 years for control group and 61.7 ± 10.5 years for glaucoma group. Regarding polar angle stimulus, there was a statistically significant association with fMRI measure for SAP (p=)

Conclusions:

Glaucoma patients have a decreased functional cortical response to visual stimuli associated with the magnitude of the visual field loss seen in SAP and FDT and RNFL thickness by OCT. A significant association between fMRI response to magnostimulus and FDT Matrix in glaucoma patients was also observed. Pericalcarine cortex volume was reduced in glaucoma patients.

Keywords: Glaucoma, functional MRI, Visual cortex

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**QuebraPagina**

**Resumo:**

**RETINAL NERVE FIBER LAYER EVALUATION IN DEMYELINATING DISEASES WITH SPECTRAL-DOMAIN OPTICAL COHERENCE TOMOGRAPHY AND SCANNING LASER POLARIMETRY**


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Objectives:

To demonstrate whether retinal nerve fiber layer (RNFL) thickness measured by spectral-domain optical coherence tomography (SD-OCT) and scanning laser polarimetry (SLP) is a potential biomarker for demyelinating diseases (multiple sclerosis [MS] and neuromyelitis optica [NMO]).

Methods and Results:

**Methods:** In this cross-sectional study, patients with MS and NMO diagnosed according to clinical and neuroimaging criteria as well as healthy individuals were recruited. Eyes with a recent clinical diagnosis of optic neuritis (less than six months), glaucoma, optic neuropathy (other than MS and NMO-related optic neuritis), or other relevant retinal and/or optic nerve disease were excluded. The eyes had the parapapillary RNFL thickness measured by SD-OCT, using the Spectralis (software version 4.0, Heidelberg Engineering, Dossenheim, Germany), and by SLP, using the GDxVCC (software version 5.3.3, Carl Zeiss Meditec Inc., Dublin, CA). **Results:** Fifty-four eyes of 27 patients with MS, 9 eyes of 5 patients with NMO, and 45 eyes of 23 healthy individuals were included. RNFL measurements were compared between groups in Table 1 (http://files.abstractsonline.com/CTRL/a0/3/be4/e29/5ea/42a/ebb/2b3/a54/2ad/776/97/g5019_1.jpg). The percentage of abnormal results is shown in Table 2 (http://files.abstractsonline.com/CTRL/a0/3/be4/e29/5ea/42a/ebb/2b3/a54/2ad/776/97/g5019_4.jpg).

Conclusions:

The RNFL is affected in MS and NMO. SD-OCT retinal imaging may represent a high-resolution, objective, noninvasive, and
A COMPUTER SIMULATION FOR CUSTOMIZED CONTACT LENS ABLATION TO CORRECT HIGH-ORDER
ABERRATIONS

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Objectives:

To develop a computer simulation for ablation in customized soft contact lenses in order to correct high-order aberrations.

Methods and Results:

We use real data from two patients diagnosed with two different high-order aberrations, which were measured with the Alcon LADARWAVEÂ® wavefront aberrometer. Using Zernike polynomials and taking into account the optical path difference, we determined the thickness of the contact lenses that compensate these aberrations as well as the numbers of pulses required to ablate these lenses. A Gaussian profile with a 0.75 mm beam width â€“ minimum width of LADARVISIONÂ® system â€“ and a 0.3 μm ablation depth were considered as parameters in the simulation. Optical quality was expressed through both the Point Spread Functions (PSFs) and Modulation Transfer Functions (MTFs). The correction maps generated from theoretical ablation were calculated. Both simulated lenses are shown, as well as the PSFs and MTFs.

Conclusions:

Based on these simulations, we demonstrate the feasibility to construct an actual controlled laser system for manufacture customized soft contact lenses.

Keywords: Algoritmos, Computer Simulation, Contact Lenses

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