Magnetic resonance imaging quantification of regional cerebral blood flow and cerebrovascular reactivity to carbon dioxide in normotensive and hypertensive rats

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ABSTRACT

Hypertension afflicts 25% of the general population and over 50% of the elderly. In the present work, arterial spin labeling MRI was used to non-invasively quantify regional cerebral blood flow (CBF), cerebrovascular resistance and CO_2 reactivity in spontaneously hypertensive rats (SHR) and in normotensive Wistar Kyoto rats (WKY), at two different ages (3 months and 10 months) and under the effects of two anesthetics, α-chloralose and 2% isoflurane (1.5 MAC). Repeated CBF measurements were highly consistent, differing by less than 10% and 18% within and across animals, respectively. Under α-chloralose, whole brain CBF at normocapnia did not differ between groups (young WKY: 61 ± 3 ml/100 g/min; adult WKY: 62 ± 4 ml/100 g/min; young SHR: 70 ± 9 ml/100 g/min; adult SHR: 69 ± 8 ml/100 g/min), indicating normal cerebral autoregulation in SHR. At hypercapnia, CBF values increased significantly, and a linear relationship between CBF and $PaCO_2$ levels was observed. In contrast, 2% isoflurane impaired cerebral autoregulation. Whole brain CBF in SHR was significantly higher than in WKY rats at normocapnia (young SHR: 139 ± 25 ml/100 g/min; adult SHR: 104 ± 23 ml/100 g/min; young WKY: 55 ± 9 ml/100 g/min; adult WKY: 71 ± 19 ml/100 g/min). CBF values increased significantly with increasing CO_2; however, there was a clear saturation of CBF at $PaCO_2$ levels greater than 70 mm Hg in both young and adult rats, regardless of absolute CBF values, suggesting that isoflurane interferes with the vasodilatory mechanisms of CO_2. This behavior was observed for both cortical and subcortical structures. Under either anesthetic, CO_2 reactivity values in adult SHR were decreased, confirming that hypertension, when combined with age, increases cerebrovascular resistance and reduces cerebrovascular compliance.

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Introduction

Hypertension is a disease that afflicts 25% of the general population and more than half of the elderly population (Pedelty and Gorelick, 2008). It is the most important modifiable risk factor for cerebrovascular diseases and the second most important risk factor, after age, for hemorrhagic and ischemic stroke (Veglio et al., 2009). It is a leading cause of cognitive decline and dementia and an important risk factor for Alzheimer's disease. Hypertension modifies the intricate mechanisms of cerebral blood flow (CBF) regulation, including functional hyperemia, cerebrovascular autoregulation, and endothelial regulation (Iadecola and Davisson, 2008). In patients with symptomatic atherosclerotic disease, hypertension has been shown to be associated with elevated CBF (van Laar et al., 2008). For all of the above, continued research on the effects of hypertension on cerebrovascular function is a crucial step in the design of preventive therapies aimed at minimizing the risk of development of cerebrovascular disease.

The spontaneously hypertensive rat (SHR) is an important experimental model in the study of stroke and other cerebrovascular diseases (Amenta et al., 2003). The presence of chronic hypertension in this rat strain results in vascular remodeling, specifically in form of thickening of the arterial wall (hypertrophy), narrowing of the lumen inner diameter (eutrophy) and vascular stiffening (Amenta et al., 2003). This remodeling causes alterations in the limits of autoregulation (Fujishima et al., 1984; Harper and Bohlen, 1984) and leads to lower cerebral rate of glucose utilization (Katsuta, 1997; Wei et al., 1992), impaired cerebrovascular reactivity to carbon dioxide (CO_2) (Nakajima et al., 2007; Tamaki et al., 1995) and higher susceptibility to stroke (Barone et al., 1992; Duverger and MacKenzie, 1988; Hom et al., 2007) in SHR compared to the normotensive Wistar-Kyoto

(WKY) rat. However, the chronic effects of hypertension on resting CBF in this animal model are less clear. Contradictory findings of similar (Tamaki et al., 1995; Wei et al., 1992), smaller (Grabowski et al., 1993; Katsuta, 1997), or larger (Heinert et al., 1998) basal CBF values in SHR versus WKY rats have been reported. A possible reason for the significant discrepancy of findings in animal models is methodological. The various studies were conducted under diverse experimental conditions, including the use of animals of different ages, sedated under different anesthetics. Both age (Dai et al., 2008; Martin et al., 1991) and anesthesia (Hoffman et al., 1991; Scheller et al., 1986; Strebel et al., 1995) are known to have a strong influence on CBF. In addition, previous studies of the effect of hypertension on the functional status of the cerebral vasculature in SHR have utilized invasive techniques to measure CBF, including the hydrogen clearance method (Fujishima et al., 1984; Heinert et al., 1998), quantitative autoradiography (Grabowski et al., 1993; Ito et al., 2002; Katsuta, 1997; Wei et al., 1992), or laser Doppler flowmetry (Barone et al., 1992; Dupuis et al., 2005). These techniques are impractical for repeated measurements, making it difficult to carryout longitudinal studies.

The purpose of the present study was to investigate the effects of hypertension and aging on the regional cerebrovascular status of the SHR and WKY rats. CBF was quantified in the whole brain and in the cortex, caudate putamen, and thalamus, with continuous arterial spin labeling (CASL), using a separate labeling coil. In addition to CBF, cerebrovascular resistance (CVR) and cerebrovascular reactivity to CO_2 were assessed during graded hypercapnia under the effects of two commonly used anesthetic agents, α-chloralose and isoflurane.

Materials and methods

Animal preparation

All experiments were approved by the Animal Care and Use Committee of the National Institute of Neurological Disorders and Stroke, National Institutes of Health. Male spontaneously-hypertensive rats (SHR) and normotensive rats (Wistar Kyoto − WKY) were acquired from a commercial vendor (Harlan Laboratories, Somerville, NJ) and aged to a minimum of 12 weeks. Measurements of whole brain CBF were performed in 32 rats (8 WKY − 235 ± 21 g, 8 SHR − 285 ± 33 g, 3–4 months old; 8 WKY − 418 ± 23 g, 8 SHR − 402 ± 46 g, 10 months old) in association with a hypercapnic (CO_2) challenge.

On the day of the experiments, the rats were anesthetized with isoflurane (5% induction, 2% maintenance), orally intubated, mechanically ventilated in oxygen-enriched air and equipped with indwelling femoral arterial and venous lines. Following surgery, animals were secured to an MRI-compatible stereotaxic head frame by means of ear-pieces and a bite bar. Rectal temperature was monitored and maintained at 37 ± 1 °C using circulating warm water. Heart rate (HR), respiration rate (RR), end-tidal CO_2 (EtCO$_2$) and arterial oxygen saturation (spO$_2$) were continuously monitored by means of a pulse oximeter (Surgivet, Waukesha, WI, USA).

The animals were further divided into two different anesthetic regimens. Four rats of each strain and age were kept anesthetized under 2% isoflurane anesthesia (1.5 MAC − minimum alveolar concentration) delivered in the inhaled gas mixture. This concentration was found necessary to maintain the 10-month-old SHR rats properly anesthetized in the MRI scanner in the presence of the loud MRI sounds. Therefore, to avoid confounds introduced by the use of different levels of anesthesia across the different groups, this concentration was uniformly applied to both WKY and SHR rats of all ages. For the remaining rats, isoflurane was discontinued and anesthesia was switched to α-chloralose (80 mg/kg initial bolus, 27 mg/kg/h continuous infusion) injected intravenously. Hypercapnia was achieved by adding 1.5%, 3%, 4.5%, 6% and 10% CO_2 to the inhaled gas mixture. A 5-minute adjustment was allowed between switching

the gas and starting the imaging acquisition. Arterial blood gasses were sampled at the end of each CO_2 level through the PE-50 catheter inserted into the right femoral artery.

MRI methods

MRI experiments were conducted in a horizontal 7 T/30 cm AVIII MRI system (Bruker-Biospin, Inc., Billerica, MA, USA), equipped with gradients capable of 450 mT/m amplitude (Resonance Research Inc., Billerica, MA, USA). RF excitation was accomplished via a home-built, transmit-only birdcage volume RF coil, 12 cm internal diameter. For signal reception, a home-built single surface coil, equipped with dedicated RF preamplifiers, was used. For ASL, a small home-built figure-8 labeling coil (Silva et al., 1995) was positioned under the neck of the animal, approximately 2 cm away from the magnet's isocenter, and connected to the second RF transmit channel of the spectrometer. All coils were equipped with active decoupling circuits to minimize coil-to-coil interferences during the labeling and imaging stages of the experiment, and to minimize off-resonance saturation of water in the acquisition region (Zhang et al., 1995).

Coronal ASL perfusion-weighted images were acquired using a spin-echo echo-planar-imaging (EPI) sequence with the following parameters: TR = 10000 ms, TE = 28 ms, FOV = 32 × 32 mm², matrix size = 64 × 64, slice thickness = 2 mm, number of slices = 5. These parameters yielded an in-plane spatial resolution of 500 μm. A post-labeling delay of 994 ms was employed to prevent ASL signals in the feeding arteries from causing overestimation of CBF (Alsop and Detre, 1996). A labeling RF pulse of 8183 ms was applied in the presence of a longitudinal gradient $G_z = 1$ G/cm at the appropriate labeling frequency offset. The RF power used for ASL was optimized in a subset of animals to produce the highest labeling efficiency. The optimal power required to achieve the maximum labeling efficiency of 75 ± 2% in the brain was 2 W. The 5 contiguous slices were acquired according to a sequential anterior–posterior order to avoid interferences from the acquisition in the magnetization of labeled blood. Sixteen averages (NA = 16) were acquired resulting in an acquisition time of 5 min 20 s.

Data analysis

Quantitative CBF maps were calculated using MATLAB R2009a (The MathWorks, Natick, MA, USA). Control and labeled images were separately averaged and then subtracted. CBF values (ml/100 g/min) were calculated for each CO_2 level using the following equation (Zhang et al., 1995):

$$CBF = \frac{\lambda}{T_1} \frac{S_C - S_L}{S_L + (2\alpha - 1)S_C}, \tag{1}$$

where λ is the brain/blood partition coefficient, T_1 is the longitudinal relaxation time of the brain water magnetization in the absence of perfusion and cross-relaxation, S_C is the equilibrium signal intensity, S_L is the brain water signal intensity from the labeled image, and α is the labeling efficiency. The labeling efficiency was determined experimentally and the values of S_C and S_L were measured for all subjects; λ = 0.9 ml/g and $T_1 = 1.75$ s were used in all calculations.

Cerebrovascular reactivity to CO_2 (%/mm Hg) was calculated using the following equation:

$$CO_2 \, reactivity = 100 \frac{\Delta CBF / CBF_0}{\Delta PaCO_2}, \tag{2}$$

where CBF_0 is the resting cerebral blood flow measured before adding CO_2 to the inhaled gas mixture, and CBF and $PaCO_2$ are, respectively, the cerebral blood flow and the arterial pressure CO_2 for each level of CO_2 added to the gas mixture.

Cerebrovascular resistance (mm Hg/ml/100 g/min) was calculated using (Eq. (3)):

$$CVR = \frac{MABP}{CBF},\qquad (3)$$

where MABP is the mean arterial blood pressure.

Four regions of interest (ROIs) were analyzed: whole brain, cortex, caudate putamen and thalamus (Fig. 1a). For each rat, CBF, CO_2 reactivity and CVR values of the four ROIs were calculated for each level of CO_2 (six levels, from 0 to 10%). Then, the values were divided in two groups (normocapnia, for $PaCO_2 \leq 45$ mm Hg; and hypercapnia, for $PaCO_2 > 60$ mm Hg), and averaged across individuals.

Statistics

Physiologic parameters, CBF, CO_2 reactivity, and CVR were compared by analysis of variance (ANOVA) and post-hoc Bonferroni's test, or corresponding non-parametric test, using SYSTAT 12 2007 (Systat Software, Inc., Chicago, IL, USA). All values and error bars are reported as mean ± standard deviation. Statistical significance was set at $P < 0.05$.

Results

To assess the effect of hypertension and age on the cerebrovascular reactivity and resistance, graded CO_2 was added to the breathing gas mixture of the rats. Initially, once the animals were in the magnet, the respiration rate and the amount of oxygen in the breathing gas mixture were adjusted for each animal to bring arterial blood gasses to normal physiological conditions. Thereafter the respiration parameters were fixed and, throughout the remainder of the experiments, heart rate, rectal temperature, oxygen saturation and end-tidal CO_2 were monitored continuously. Arterial blood gasses were sampled periodically after the CBF measurements at each CO_2 level. Physiologic measurements under basal ($PaCO_2 \leq 45$ mm Hg) and hypercapnic ($PaCO_2 > 60$ mm Hg) conditions are listed in Table 1. Mean arterial blood pressure (MABP) was higher in SHR than in WKY ($P < 0.05$), and for all groups of rats the MABP values under α-chloralose were higher than under isoflurane ($P < 0.05$). During hypercapnia, $PaCO_2$ increased and pH decreased compared to normocapnia ($P < 0.05$).

Representative coronal EPI images from a SHR under isoflurane are shown in Fig. 1a. Five adjacent 2 mm thick slices are shown from posterior to anterior. Overlaid on the EPI images are three representative ROIs, which include the cortex, caudate putamen and thalamus.

Corresponding whole-brain CBF maps obtained under normocapnia are shown in Fig. 1b. To ensure maximum SNR, we acquired MRI data under fully relaxed conditions both for the control and the labeling phases of the CASL experiment. There was equal sensitivity to perfusion of both cerebral hemispheres, with good gray versus white matter contrast, and CBF distributed heterogeneously across the brain.

To evaluate the reproducibility of CBF measurements within the same animal, each rat was measured twice under normocapnia. The average percentage differences were similar between rat strains and anesthesias, with whole brain averages of 9% and 13% for rats under α-chloralose and isoflurane, respectively. Consistency of CBF measurements across animals in the same group was also similar between rat strains and anesthesias. It was 16% for rats under α-chloralose and 20% for rats under isoflurane, which are comparable with previous studies (Danker and Duong, 2007; Duong et al., 2000).

Addition of CO_2 significantly increased CBF in all rat groups under either anesthetic (Fig. 2). Under α-chloralose, a linear relationship between CBF and $PaCO_2$ levels was observed for all analyzed brain regions (Fig. 2a shows the whole brain CBF x $PaCO_2$ curves for all groups). At normocapnia, there was no difference in CBF values between brain regions or between rat groups under α-chloralose (Fig. 3a), which indicates normal cerebral autoregulation in SHR. Average whole brain CBF values were 61 ± 3 ml/100 g/min for young WKY, 62 ± 4 ml/100 g/min for adult WKY; 70 ± 9 ml/100 g/min for young SHR, and 69 ± 8 ml/100 g/min for adult SHR. At hypercapnia, CBF values for all brain regions significantly increased when compared to normocapnia, but still did not differ between rat groups (whole brain CBF — young WKY: 108 ± 21 ml/100 g/min; adult WKY: 98 ± 16 ml/100 g/min; young SHR: 131 ± 39 ml/100 g/min; adult SHR: 114 ± 25 ml/100 g/min).

Under isoflurane, there was a clear saturation of CBF at $PaCO_2$ levels greater than 70 mm Hg in both young and adult rats (Fig. 2b), regardless of absolute CBF values, which suggests isoflurane interferes with the vasodilatory mechanisms of CO_2. The same behavior was observed for the cortex, caudate putamen and thalamus. Furthermore, under isoflurane, CBF in both young and adult SHR were significantly higher than the respective groups under α-chloralose (Fig. 2b), demonstrating that isoflurane impairs cerebral autoregulation. There was no difference in CBF in WKY rats between the two anesthetics, which suggests that the effects of isoflurane on autoregulation are specific to the hypertensive animals. Young SHR presented significantly higher CBF than WKY rats in all brain regions, both at normocapnia (Fig. 3b) (whole brain CBF — young SHR: 139 ± 25 ml/100 g/min; young WKY: 55 ± 9 ml/100 g/min; adult WKY: 71 ± 19 ml/100 g/min, $P < 0.05$), as well as at hypercapnia (whole brain CBF — young SHR: 194 ± 22 ml/100 g/min; young WKY:
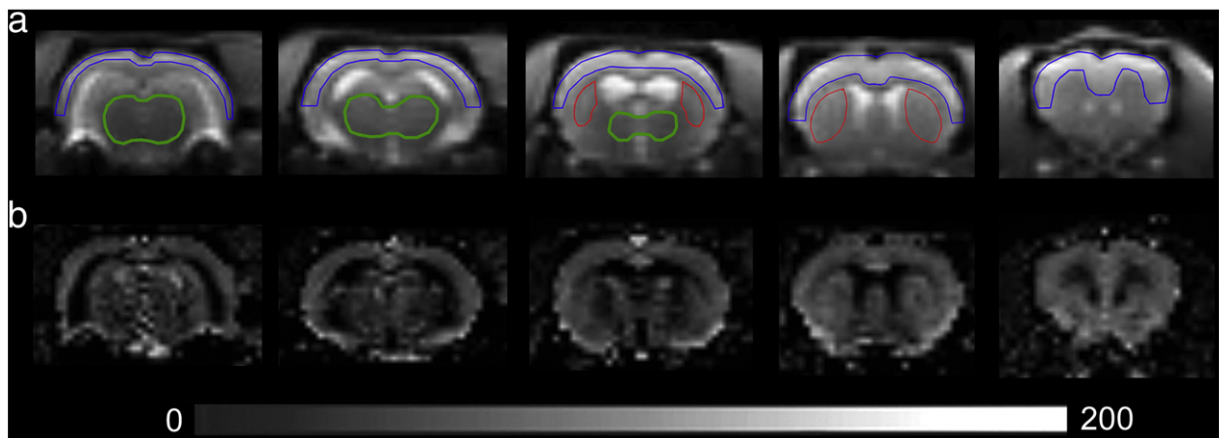

Fig. 1. (a) Coronal EPI images of a spontaneously hypertensive rat under isoflurane. Five adjacent 2 mm thick slices are shown from posterior (left) to anterior (right). Overlaid on the EPI images are three regions of interest: cortex (blue), caudate putamen (red), and thalamus (green). (b) Corresponding cerebral blood flow (CBF) images acquired under normocapnia show equal sensitivity to perfusion on both cerebral hemispheres, good gray versus white matter contrast, and heterogeneous distribution of CBF across the brain. Grayscale bar expresses the CBF values in ml/100 g/min.

Table 1
Physiologic parameters at basal conditions (PaCO$_2 \leq 45$ mm Hg) and at hypercapnia (PaCO$_2 > 60$ mm Hg) under two different anesthetics for all groups of rats.

Group	CO$_2$ Level	HR	RR	pH	PaCO$_2$ (mm Hg)	PaO$_2$ (mm Hg)	SPO$_2$	MABP (mm Hg)
α-chloralose								
Young WKY	*Normocapnia*	350 ± 1	58 ± 1	7.4 ± 0.1	42 ± 3	111 ± 7	88 ± 5	137 ± 11
	Hypercapnia	350 ± 2	58 ± 1	7.2 ± 0.1^c	83 ± 19^c	120 ± 9	84 ± 11	117 ± 15
Adult WKY	*Normocapnia*	344 ± 6	59 ± 1	7.5 ± 0.1	38 ± 5	134 ± 24	92 ± 1	150 ± 6
	Hypercapnia	350 ± 1	59 ± 1	7.3 ± 0.1^c	81 ± 16^c	130 ± 20	93 ± 4	118 ± 9^c
Young SHR	*Normocapnia*	347 ± 4	59 ± 1	7.4 ± 0.1	38 ± 5	141 ± 14	95 ± 2	176 ± 11^b
	Hypercapnia	349 ± 2	58 ± 1	7.2 ± 0.1^c	81 ± 17^c	135 ± 11	92 ± 1	$143 \pm 12^{b,c}$
Adult SHR	*Normocapnia*	350 ± 1	56 ± 1	7.4 ± 0.1	35 ± 15	136 ± 9	95 ± 1	193 ± 17^b
	Hypercapnia	333 ± 8	57 ± 1	7.2 ± 0.1^c	79 ± 14^c	128 ± 12	93 ± 2	$159 \pm 15^{b,c}$
Isoflurane								
Young WKY	*Normocapnia*	271 ± 14^d	54 ± 5	7.4 ± 0.1	37 ± 5	152 ± 16	94 ± 4	68 ± 8^d
	Hypercapnia	296 ± 14^d	48 ± 8^d	7.2 ± 0.1^c	79 ± 17^c	159 ± 12^d	90 ± 5	64 ± 7^d
Adult WKY	*Normocapnia*	261 ± 22^d	56 ± 1	7.4 ± 0.1	37 ± 4	146 ± 15	94 ± 4	93 ± 18^d
	Hypercapnia	297 ± 19^d	57 ± 3	7.2 ± 0.1^c	78 ± 16^c	137 ± 9	94 ± 3	83 ± 8^d
Young SHR	*Normocapnia*	329 ± 23^b	54 ± 13	7.4 ± 0.1	36 ± 3	156 ± 14	94 ± 1	$116 \pm 25^{b,d}$
	Hypercapnia	329 ± 28^b	49 ± 13	7.2 ± 0.1^c	72 ± 15^c	$134 \pm 19^{b,c}$	90 ± 4	$114 \pm 12^{b,d}$
Adult SHR	*Normocapnia*	321 ± 22^b	53 ± 2	7.4 ± 0.1	38 ± 4	127 ± 11^a	93 ± 2	104 ± 22^d
	Hypercapnia	321 ± 30	48 ± 6^d	7.2 ± 0.1^c	73 ± 12^c	146 ± 7	94 ± 1	107 ± 11^d

Heart rate (HR), respiration rate (RR), pH, PaCO$_2$, PaO$_2$, oxygen saturation (spO$_2$), and mean arterial blood pressure (MABP).
[a] Compared to young rats of the same strain.
[b] Compared to normotensive rats of the same age.
[c] Compared to the same rat group during normocapnia.
[d] Compared to α-chloralose; P<0.05.

91 ± 12 ml/100 g/min; adult WKY: 94 ± 26 ml/100 g/min, P<0.05). On the other hand, adult SHR presented CBF values that were not different from young SHR at normocapnia (104 ± 23 ml/100 g/min, for whole brain), but significantly lower at hypercapnia (135 ± 15 ml/100 g/min, for whole brain; P<0.05), which indicates that aging further augments the effects of hypertension in compromising the cerebrovascular CO$_2$ reactivity in SHR.

Mean CVR values were calculated for both ages and strains and averaged across individuals. No significant differences were observed between ROIs for both α-chloralose (Fig. 3c) and isoflurane (Fig. 3d). Under α-chloralose, mean whole brain CVR value in adult SHR at normocapnia (2.8 ± 0.4 mm Hg/ml/100 g/min) was significantly higher than in young normotensive rats (2.2 ± 0.2 mm Hg/ml/100 g/min, P<0.05), however it was not different from other groups (adult WKY: 2.4 ± 0.2 mm Hg/ml/100 g/min; young SHR: 2.5 ± 0.2 mm Hg/ml/100 g/min). At hypercapnia, MABP decreased while CBF increased, significantly decreasing CVR when compared to normocapnia (whole brain CVR — young WKY: 1.1 ± 0.3 mm Hg/ml/100 g/min; adult WKY: 1.2 ± 0.3 mm Hg/ml/100 g/min; young SHR: 1.2 ± 0.4 mm Hg/ml/100 g/min; adult SHR: 1.5 ± 0.4 mm Hg/ml/100 g/min, P<0.05). Under isoflurane (Fig. 3d), CVR values were significantly lower when compared to the values in rats under α-chloralose (P<0.05).

Moreover, CVR values for SHR and WKY rats of both ages were not different either at normocapnia (Fig. 3d) or at hypercapnia, showing that 2% isoflurane completely masks the effects of hypertension in elevating CVR.

Mean CO$_2$ reactivity values were calculated for both ages and strains using (Eq. (2)), and averaged across individuals (Fig. 4). Under α-chloralose, no differences were observed between brain regions. However, under isoflurane, CO$_2$ reactivity values in caudate putamen were significantly lower than in cortex (P<0.05) for adult SHR. Moreover, under both anesthetics, CO$_2$ reactivity in adult SHR was significantly decreased (P<0.05), suggesting an age-dependent impairment of cerebrovascular reactivity in SHR.

Discussion

In the present work, we have successfully used ASL to investigate the combined effects of hypertension and aging on the functional status of the cerebral vasculature in a well-established rat model of chronic hypertension. CBF, CVR and CO$_2$ reactivity were quantified in SHR and their normotensive controls, the WKY rats, under two commonly used anesthetics, α-chloralose and isoflurane. We demonstrated that hypertension, when combined with age, significantly
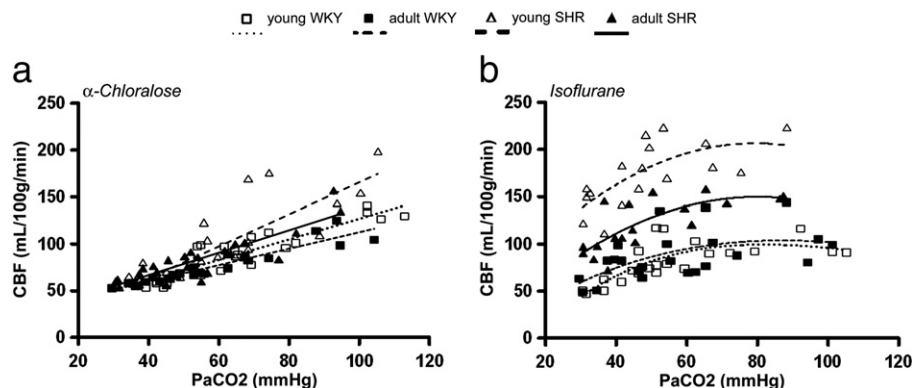

Fig. 2. Relationship between whole brain cerebral blood flow (CBF) and PaCO$_2$ measured in normotensive (Wistar Kyoto — WKY) and spontaneously hypertensive (SHR) rats under (a) α-chloralose and (b) isoflurane. While a linear increase in CBF with PaCO$_2$ levels is obtained under α-chloralose, there is a clear CBF saturation at PaCO$_2$ levels greater than 70 mm Hg under isoflurane (polynomial fit).
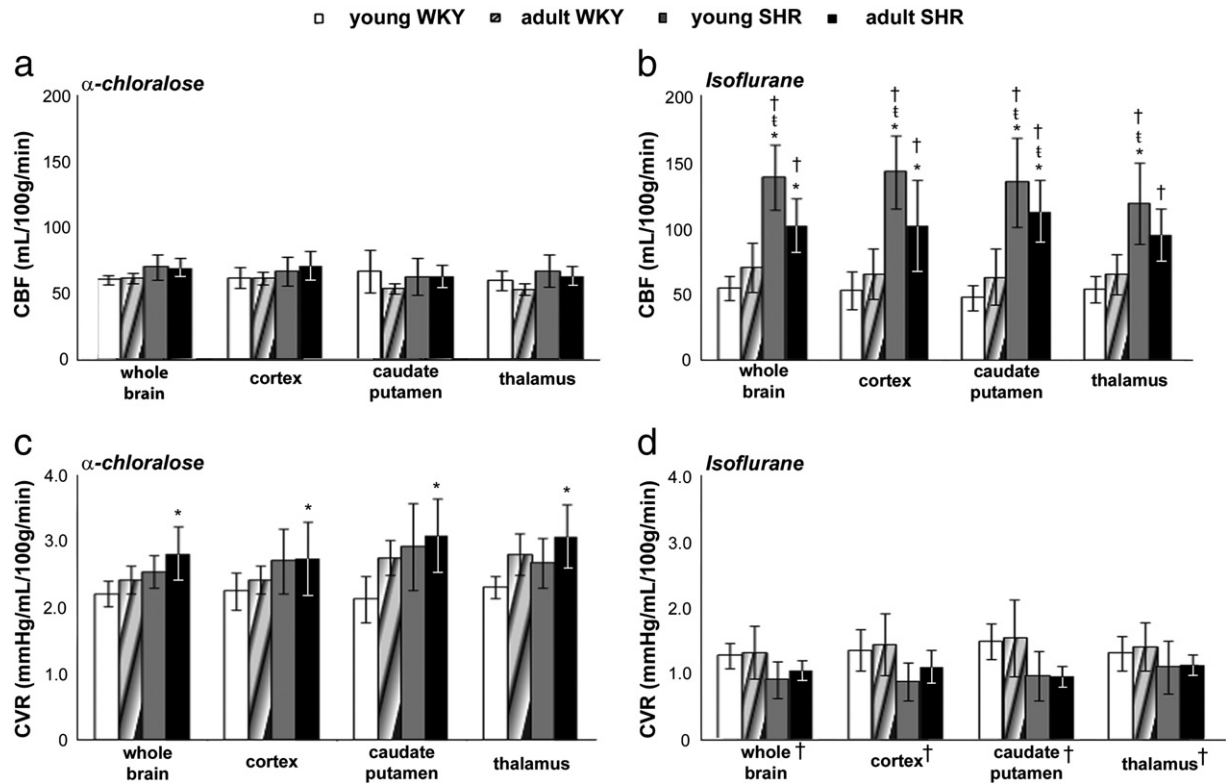
Fig. 3. Mean CBF (a–b) and CVR (c–d) for normotensive (WKY) and hypertensive (SHR) rats at normocapnia, under α-chloralose and isoflurane. ([*] compared to young WKY; [ŧ] compared to adult WKY; $P<0.05$).

alters cerebrovascular resistance and impairs cerebrovascular reactivity. Furthermore, our data also shows that, in contrast to α-chloralose, 2% isoflurane causes impairment of cerebral autoregulation, masks the effects of hypertension on CVR and interferes with the vasodilatory mechanisms of CO_2.

Differential physiological effects of α-chloralose versus isoflurane

Anesthesia is a necessary tool in experimental preparations to ensure compliance and to relieve pain and distress. However, anesthetic agents have a profound effect on both systemic physiology as well as on the physiological state of the brain, introducing potential confounds and making interpretability of the data difficult. While isoflurane is a commonly used anesthetic in experimental research, it decreases systemic blood pressure by significantly reducing peripheral vascular resistance (Eger, 1984). Consistently with the potent

systemic effects of isoflurane, we observed lower blood pressure in both strains and age groups, when compared to α-chloralose, a hypnotic anesthetic that preserves systemic blood pressure (Table 1). Moreover, isoflurane eliminated the blood pressure difference between SHR and WKY in the adult age group. On the other hand, under α-chloralose, SHR rats of either age group presented significantly higher blood pressure than their respective WKY controls, demonstrating that, at 3 months of age, the hypertensive state in SHR is already fully established.

Differential cerebrovascular effects of α-chloralose versus isoflurane

The divergent influence of the two anesthetics on systemic physiology translated into distinct cerebrovascular effects. Under α-chloralose, there was no difference in basal CBF values between normotensive and hypertensive rats (Fig. 3a), which indicates this anesthetic does not
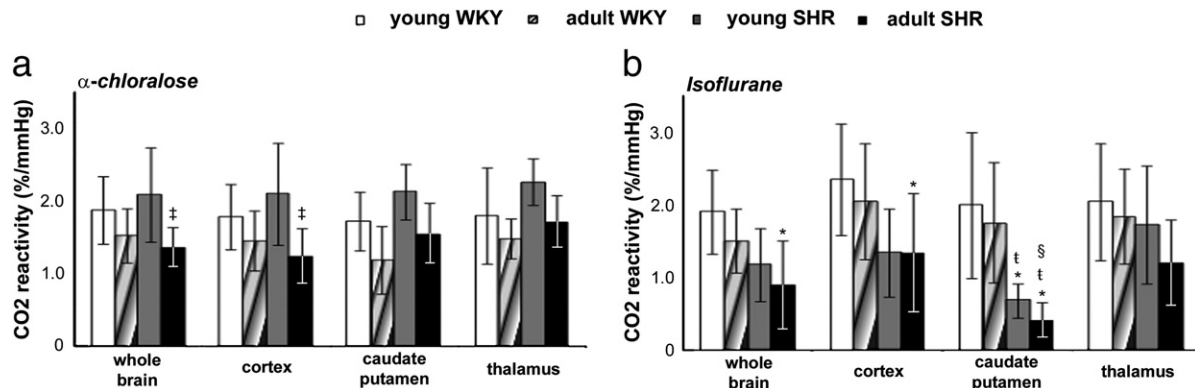

Fig. 4. Mean CO_2 reactivity for normotensive (WKY) and hypertensive (SHR) rats, under (a) α-chloralose and (b) isoflurane. ([*] compared to young WKY; [ŧ] compared to adult WKY; [†] compared to young SHR; [§] compared to cortex region of the same rat group; $P<0.05$).

impair autoregulation in SHR. This finding is in agreement with previous studies that used chloralose-urethane (Alson et al., 1985) or barbiturates (Tamaki et al., 1995). In addition, under α-chloralose, CBF increased linearly with CO_2 (Fig. 2a), and CO_2 reactivity values are in good agreement with previous LDF studies (Bakalova et al., 2001). On the other hand, under isoflurane, CBF values in young SHR were significantly higher than in WKY rats (Fig. 3b). Moreover, CBF values in SHR of either age group under isoflurane were significantly higher than the respective groups under α-chloralose (Fig. 3b). Isoflurane significantly reduced CVR in all rats and masked the effects of hypertension in elevating CVR (Fig. 3d). Taken together, these findings indicate impairment in autoregulatory mechanisms caused by isoflurane, in agreement with previous reports in rats (Hoffman et al., 1991), dogs (McPherson et al., 1989; McPherson and Traystman, 1988), monkeys (Van Aken et al., 1986) and humans (Strebel et al., 1995), and urge the use of caution when interpreting results obtained under this anesthetic.

The basal CBF values found here under isoflurane are consistent with those measured in previous studies (Danker and Duong, 2007; Heinert et al., 1998; Kusuda et al., 1996). The fact that saturation is observed in all strains and age groups regardless of the absolute value of CBF, as shown in Fig. 2b, suggests that isoflurane at 1.5 MAC interferes with the vasodilatory mechanisms of CO_2. In support of our results, Sicard et al. observed a much attenuated CBF response to 5% and 10% CO_2 challenges in rats anesthetized with 1.5 MAC isoflurane compared to awake rats (Sicard et al., 2003), and concluded that the cerebrovascular reactivity to CO_2 was markedly suppressed by isoflurane. As well, McPherson et al. observed no CBF response to hypercapnia and markedly decreased CVR and CO_2 reactivity in dogs anesthetized under isoflurane at 2 MAC (McPherson et al., 1989). In our study, CBF saturation was observed for $PaCO_2$ levels greater than 70 mm Hg, suggesting that isoflurane interferes most with nitric oxide independent vasodilatory mechanisms, which have been shown to affect the CBF response to hypercapnia at high $PaCO_2$ levels (Iadecola and Zhang, 1994). However, further studies are necessary to address this issue.

Effects of hypertension and aging on cerebrovascular resistance and reactivity

When left untreated, the chronic presence of hypertension leads to vascular remodeling in the form of hypertrophy, eutrophy, and reduced vascular compliance (Tuttle et al., 2002). In agreement with previous work (Kusuda et al., 1996), adult SHRs presented increased cerebrovascular resistance under elevated arterial blood pressure (Fig. 3c). There has been a suggestion that the increased CVR may be a mechanism to keep CBF within normal values and to protect the brain against the development of hemorrhagic stroke (Gonzalez et al., 2008). Indeed, when CVR was reduced due to the use of isoflurane anesthesia, CBF values increased substantially in SHRs (Fig. 3b).

It has been shown that normal aging leads to decreased vascular response to vasodilator stimuli (Riecker et al., 2003). Compared to their young counterparts, adult normotensive animals showed a trend towards elevated CVR and decreased CO_2 reactivity, but these were not significant. However, adult SHR presented significantly decreased CO_2 reactivity at either anesthetic agent. There is significant evidence that aging compounds the impairment in vascular relaxations induced by hypertension (Tamaki et al., 1995). As well, hypertension accelerates the vascular stiffening that naturally occurs due normal aging (Feihl et al., 2009). The data obtained in adult SHR can be fully explained by a combined effect of aging and hypertension in limiting the compliance of the cerebral vessels.

In all brain regions analyzed, regional CBF values of hypertensive rats under isoflurane were significantly higher than those of normotensive controls (Danker and Duong, 2007); however, no regional differences were observed under α-chloralose. A previous study reported greater CO_2 reactivity in the cortex than in subcortical structures in normotensive rats (Lu et al., 2009). We observed similar results in hypertensive rats under isoflurane, but not in normotensive rats under either anesthesia. These findings suggest that the CBF response to hypercapnia varies across brain regions and is dependent on rat strain and anesthesia.

Conclusions

In conclusion, we successfully used ASL to assess and characterize the compliance of the cerebral vasculature of an important animal model used in modeling stroke and other cerebrovascular diseases, the spontaneously hypertensive rat, under different anesthetics. Contrary to α-chloralose, 2% isoflurane (1.5 MAC) had profound effects on the animals' systemic blood pressure and caused impairment of the mechanisms of CBF regulation, which may introduce potential confounds on studies of cerebrovascular diseases. Chronic hypertension, when compounded by age, resulted in increased cerebrovascular resistance and reduced cerebrovascular reactivity to CO_2.

Acknowledgments

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