Published in final edited form as:

Neuroscience. 2014 June 6; 269: 223–231. doi:10.1016/j.neuroscience.2014.03.031.

Impaired CBF Regulation and High CBF Threshold Contribute to the Increased Sensitivity of Spontaneously Hypertensive Rats to Cerebral Ischemia

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Abstract

The correlation between temporal changes of regional cerebral blood flow (rCBF) and the severity of transient ischemic stroke in spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY) was investigated using T₂-, diffusion- and perfusion-weighted magnetic resonance imaging at six different time points: before and during 1 hour of unilateral middle cerebral artery occlusion (MCAO), 1 hour after reperfusion, and 1 day, 4 days and 7 days after MCAO. Regional CBF values were measured in both hemispheres, and the perfusion deficient lesion (PDL) was defined as the area of the brain with a 57% or more reduction in basal CBF. Within the PDL, regions were further refined as ischemic core (rCBF = 0–6 mL/100 g/min), ischemic penumbra (rCBF = 6–15 mL/100 g/min) and benign oligemia (rCBF > 15 mL/100 g/min). SHR and WKY had identical initial volume of the PDLs (WKY: 32.52±4.08% vs. SHR: 33.95±3.68%; *P*>0.05) and the maximum rCBF measured within those lesions (WKY: 38.20±3.57 mL/100 g/min vs. SHR: 38.46±6.22 mL/100 g/min; *P*>0.05) during MCAO. However, in SHR virtually all of the PDL progressed to become the final ischemic lesion (33.02±5.41%, *P*>0.05), while the final ischemic lesion volume of WKY (12.62±9.19%) was significantly smaller than their original PDL (*P*<0.01) and similar to the ischemic core (13.13±2.96%, *P*>0.05). The region with the lowest range of rCBF was positively correlated with the final ischemic lesion volume (*r*=0.716, *P*<0.01). Both during ischemia and after reperfusion, rCBF in either ipsilesional and contralesional brain hemispheres of SHR could not be restored to pre-ischemic levels, and remained lower than in WKY until up to 4 days after MCAO. The data suggests that impaired CBF regulation and

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relatively high CBF threshold for ischemia are strong contributors to the increased susceptibility of SHR to ischemic stroke.

Keywords

arterial spin labeling; cerebral blood flow; ischemic stroke; magnetic resonance imaging; spontaneously hypertensive rats

Introduction

Hypertension is a major risk factor for ischemic stroke as 80% of patients suffering from acute ischemic stroke are hypertensive (Leonardi-Bee et al.). Among various animal models of chronic arterial hypertension, the spontaneously hypertensive rat (SHR) shares many features with human essential hypertension (Folkow, 1982, Amenta et al., 2003). Therefore, SHR is relevant to stroke research and has been widely used to evaluate the effect of hypertension on ischemic stroke (Takaba et al., 2004, Yao and Nabika, 2012). SHR were developed by systematic inbreeding from Wistar-Kyoto rats (WKY), which are commonly used as the normotensive control strain (Okamoto and Aoki, 1963).

It is well known that the susceptibility to cerebral ischemia is greater in SHR than that in WKY (Barone et al., 1991, Dogan et al., 1998, Letourneur et al., 2011). This increased vulnerability of SHR is frequently explained by a deficit in collateral circulation compared to normotensive rats (Coyle, 1987, Grabowski et al., 1993). Because chronic arterial hypertension induces both structural and functional vascular alterations (Iadecola and Davisson, 2008), hypertension has been considered as the main factor contributing to increased sensitivity of SHR. However, several studies suggested that hypertension-independent genetic factors also contribute to the increased infarct volume in hypertensive rats, because infarct volume was not different among SHR-related strains irrespective of the presence of hypertension (Gratton et al., 1998, Takaba et al., 2004, Lecrux et al., 2007, Sakurai-Yamashita et al., 2010). In addition, intrinsic vulnerability to stroke was observed in neurons and glial cells of SHR and stroke-prone SHR (SHR-SP) (Lecrux et al., 2007, Sakurai-Yamashita et al., 2010). Therefore, the decreased blood supply arising from hypertension-induced vascular changes may not be the sole contributor of increased susceptibility to ischemic stroke in SHR.

Previous studies showed that the reduction in cerebral blood flow (CBF) is not significantly different between SHR and normotensive rats (WKY and Sprague-Dawley rats) after middle cerebral artery occlusion (MCAO) using a laser Doppler flowmetry (LDF) technique (Dogan et al., 1998, Lecrux et al., 2007). However, LDF only allows measurements of relative changes in regional CBF (rCBF) in a very limited volume of the cortex. In addition, due to the need to perform a craniotomy to allow access of the LDF probe to the surface of the brain, LDF makes longitudinal studies of rCBF difficult to implement. To circumvent this limitation while allowing non-invasive assessment of stroke outcome, magnetic resonance imaging (MRI) can be utilized. Nevertheless, while there have been a few MRI studies aimed at understanding the underlying causes of the increased susceptibility of SHR to ischemic stroke (McCabe et al., Henning et al., Lee et al., 2011, Letourneur et al.), most of

these studies were performed in the hyper-acute or acute phase of stroke and thus the temporal changes of whole brain CBF after MCAO have not been systematically evaluated in SHR.

In the present work, to further investigate the contributing factors of the increased susceptibility of SHR to brain ischemia, we used T2-, diffusion- and perfusion-weighted MRI to examine the correlation between temporal changes of whole-brain rCBF and the severity of ischemic stroke in SHR and WKY at six different time points: before and during 1 hour of middle cerebral artery occlusion (MCAO), 1 hour after reperfusion, and 1 day, 4 days and 7 days after MCAO.

Experimental Procedures

Animal Preparation

All procedures were approved by the Animal Care and Use Committee of the National Institute of Neurological Disorders and Stroke and the National Institute on Deafness and Other Communication Disorders, in accordance with guidelines established by the National Institutes of Health, and have been described in detail in a previous publication (Kang et al., 2012). Briefly, male, adult WKY and SHR (13–17 weeks old, 250–350 g body weight) were acquired from a commercial vendor (Harlan Laboratories, Frederick, MD) in age-matched pairs, and housed with a 12-hour light-dark cycle under constant temperature conditions. The number of animals ($n = 12$, 6 per strain) was estimated to be the minimum necessary to evaluate differences between two strains.

Animals were allowed to acclimate to the facility for a minimum of 1 week prior to being used in the present study. On the day of the surgery, each animal was brought to the laboratory after being fasted overnight and then anesthetized with isoflurane (5% induction, 2.5% maintenance of surgical plane), delivered via a facemask in a 2:2:1 mixture of medical air, nitrogen, and oxygen. Rectal temperature was monitored and kept at 37 – 38°C using a thermostatically controlled heating pad (Gaymar Industries Inc., Orchard Park, NY). The tail artery was catheterized to allow for periodic sampling of arterial gases (ABL80 Flex, Radiometer America Inc., Westlake, OH) and continuous monitoring of mean arterial blood pressure (MABP) and heart rate (Biopac Systems Inc., Goleta, CA).

Focal Cerebral Ischemia

Transient occlusion of the right middle cerebral artery (MCAO) was achieved by advancing a 4-0 silicone-coated nylon suture (Doccol Corporation, Redlands, CA) into the right internal carotid artery until resistance was met (Longa et al., 1989). Reperfusion was achieved by withdrawing the filament after 1-hour occlusion. All catheters were removed, the incisional wound was sutured closed and the rats were allowed to recover consciousness prior to being brought back to their housing facility.

Neurological Evaluation

Neurological deficits were assessed at 1 day, 4 days and 7 days after MCAO, as described previously (Barone et al., 2001, Chu et al., 2008). An experienced observer who was blinded

to group assignment performed scoring of postural reflexes, circling and proprioceptive placement of contralateral forelimb and hindlimb, according to the scale shown in Table 1 (Kang et al., 2012).

MRI

MRI was performed on a 7T/30 cm USR Avance III spectrometer (Bruker Biospin Corp., Ettlingen, Germany), equipped with a 15 cm gradient set of 450 mT/m strength (Resonance Research Inc., Billerica, MA, USA). For signal excitation, a home-built, transmit-only birdcage volume RF coil, 12 cm internal diameter, was used. For signal reception, a home-built single surface coil, equipped with a dedicated RF preamplifier, was used. For arterial spin labeling (ASL), a small home-built 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 three RF coils were equipped with active decoupling circuits to prevent coil-to-coil interactions.

MR data was acquired at six serial time points: before and during MCAO, 1 hour after reperfusion, and 1 day, 4 days and 7 days after MCAO. The animals were placed on an MR compatible cradle equipped with an MRI compatible stereotaxic frame. Anesthesia was maintained at 2% and animal physiology was continuously monitored by end tidal CO₂, heart rate, and SPO₂ using a capnograph and pulse oximeter (Surgivet, Waukesha, WI, USA). Three MRI sequences were used: a T2-weighted (T2W) sequence for anatomical imaging and delineation of ischemic lesion volumes 24 hours and later following MCAO; a continuous ASL (CASL) sequence (Silva et al., 1995, Leoni et al., 2011) for measurements of rCBF at all time points; and a diffusion weighted imaging (DWI) for measurements of regional apparent diffusion coefficients (ADC) during MCAO. All three MRI sequences were run to cover the entire brain in 15 × 1-mm thick consecutive coronal slices with an in-plane field-of-view (FOV) of 25.6 × 25.6 mm². Other MRI parameters were as follows. For T2W, a rapidly-acquired relaxation enhancement (RARE) sequence with TR/TE = 12000/72 ms and matrix = 128 × 128 was used. For DWI, a single-shot spin-echo echo-planar imaging (SE-EPI) sequence with TR/TE = 6000/30 ms, matrix = 64 × 64, and *b*-values = 0, 1600 s/mm² acquired in 3 directions (x, y, z) was used, from which ADC trace maps were estimated using a computer software (DPTools, Denis Ducreux, Paris, France). And for rCBF measurements, a multi-slice SE- EPI sequence was used with TR/TE = 10000/30 ms, matrix = 64 × 64, and a labeling RF pulse of 8183 ms applied in the presence of a longitudinal gradient *G_z* = 1 G/cm at the appropriate labeling frequency offset. Intravascular contamination was attenuated by using a post-labeling delay of 994 ms (Alsop and Detre, 1996). Quantitative rCBF maps were obtained by subtraction of the ASL images from control images using MATLAB (The MathWorks, Natick, MA). Following the imaging studies (7 days post-stroke), all rats were euthanized with an overdose of sodium pentobarbital (Nembutal Sodium Solution, Akorn, Inc., Lake Forest, IL, 100 mg/kg, IV).

Image Analysis

Image analysis was also performed by the same expert, who was blinded to group assignment, as previously described (Kang et al., 2012), using MIPAV (Biomedical Imaging Research Services Section, National Institutes of Health, Bethesda, MD). During MCAO,

the ischemic lesion was determined from the ADC maps using the criterion of 23% reduction in mean contralesional ADC (McCabe et al., 2009). Following MCAO, ischemic lesion volumes were determined from the T2W images by manual tracing of the hyperintense lesions. Brain edema and the edema-corrected lesion volumes were calculated and expressed as percentage of the hemispheric volume (Gerriets et al., 2004). The perfusion deficient lesion (PDL) was determined from the rCBF map obtained during MCAO, by using a 57% reduction relative to the contralesional side (Meng et al., 2004). Regions of interest (ROI) outlining these areas were transferred to the corresponding rCBF maps obtained at different time points (before MCAO, 1 hour after reperfusion, and 1 day, 4 days and 7 days after MCAO). rCBF values were measured within and outside the PDL and in the corresponding regions of the contralesional hemisphere. Within the PDL, the volume of the ROI with rCBF decreased to three ranges (0–6 mL/100 g/min (ischemic core), 6–15 mL/100 g/min (ischemic penumbra), and 15 mL/100 g/min to 57% reduction relative to the contralesional side (benign oligemia) was expressed as percentage of the hemispheric volume as described previously (Drummond et al., 1989).

Statistical analysis

Data are presented as mean $\pm$ standard deviation. Statistical analysis was performed using the SPSS package (version 12.0, SPSS Inc., Chicago, IL). Unpaired t-tests were used for comparison of the areas of the three ranges of rCBF determined above. Mann-Whitney U-test and Wilcoxon signed rank tests were used to evaluate non-parametric data. Two-way repeated measures analysis of variance (ANOVA) with the Bonferroni post-hoc test was used to compare sequential changes of physiological parameters, lesion volumes, brain edema, and rCBF between the two strains. Pearson's correlation was used to investigate the relationship between the final ischemic lesion volume estimated 7 days after MCAO and rCBF of the PDL and the PDL regions within three determined ranges of rCBF. Statistical significance was set at $P < 0.05$.

Results

Physiological parameters

All 12 rats survived the surgical procedure and participated in all measurements performed before and during MCAO, after reperfusion, and at 1 day, 4 days and 7 days after stroke. In all rats, physiological parameters were maintained within normal ranges before, during, and after MCAO (Table 2). There were no significant differences in physiological parameters between the two strains, except for MABP, which was significantly higher in SHR than in WKY at each measurement time ($P < 0.05$).

Evolution of ischemic lesion volumes and brain edema

Ischemic lesion volumes as determined from ADC maps and T₂-weighted MRI were significantly larger in SHR than in WKY at each measurement time (Fig. 1; $P < 0.01$). Already during the MCAO, the mean lesion volume in SHR was 9.9% larger than in WKY, and the difference between the two strains grew to 17.8% on Day 1, 18.3% on Day 4 and 20.4% on Day 7. In both strains, maximal lesion volumes were observed on Day 4 (WKY:

15.67±10.99%, SHR: 33.97±4.16%), but the evolution of lesion volumes was not significantly different across strains (during MCAO vs. Day 7: $P>0.05$).

During MCAO, the formation of brain edema was not apparent in either strain, but it was clearly present on Days 1 and 4 after reperfusion. The amount of edema was significantly higher in SHR than in WKY on Day 4 (Fig. 2; $P<0.01$). On Day 7, the presence of edema was strongly subdued in both strains and not different from the extent measured during MCAO ($P>0.05$). Across individuals, the variation of both lesion volume and edema was smaller in SHR than that in WKY at each measurement time.

Figure 3 shows representative ADC maps, T2-weighted images and CBF maps demonstrating the temporal evolution of ischemic lesions, along with the regional perfusion status of brain tissue. In both strains, lesions were characterized by both ADC deficits and T2 hyperintensities, and they comprised both cortical and subcortical areas. The extent of the ischemic lesions was larger in SHR than in WKY, especially in the dorsolateral cortex. As well, SHR presented more severe compression of the ventricles and shift of the midline caused by edema than WKY.

Evolution of neurological deficits

In both strains, neurological deficits were observed after reperfusion. The neurological scores were highest on Day 1 (WKY: 3.7±3.1, SHR: 7±0) and gradually improved with time (Day 1 vs. Day 7: $P<0.05$). Neurological scores were significantly higher in SHR than in WKY at each measurement time (Day 1 and 4: $P<0.05$, Day 7: $P<0.01$; Fig. 4). Across individuals, the variation in neurological scores was smaller in SHR than in WKY.

Evolution of rCBF

For both SHR and WKY strains, rCBF in the ipsilesional hemisphere was evaluated within the PDL (Fig. 5A-1) and in the region outside this lesion (Fig. 5A-2), as well as in homologous regions of the contralesional hemisphere (Fig. 5B-1 and 5B-2, respectively). Before ischemia, baseline rCBF in SHR was significantly higher than in WKY in these four ROIs (WKY vs. SHR: $P<0.05$).

Within the PDL (Fig. 5A-1), rCBF values measured during MCAO were significantly reduced to 30.6±9.0% (WKY) and 18.3±2.5% (SHR) of their respective pre-ischemic values (baseline rCBF vs. during MCAO: $P<0.01$). Because of the larger rCBF reduction in SHR than in WKY, during ischemia the absolute rCBF value in SHR was lower than in WKY (WKY: 24.4±4.05 mL/100 g/min vs. SHR: 17.56±3.4 mL/100 g/min; $P<0.05$). One hour after reperfusion, WKY exhibited a complete recovery of rCBF to pre-ischemic values (baseline vs. reperfusion: $P>0.05$), while in SHR full recovery of baseline rCBF values did not occur until Day 4 following stroke (baseline vs. reperfusion: $P<0.05$, baseline vs. Day 1: $P<0.01$). Therefore, rCBF values within the ischemic lesion remained higher in WKY rats than in SHR during reperfusion ($P<0.05$) and Day 1 ($P<0.01$), but this difference disappeared on Day 4 and Day 7 ($P>0.05$).

Outside the PDL (Fig. 5A-2), there were no rCBF changes in WKY for all measurement times (baseline vs. during MCAO, reperfusion, Day 1, 4, and 7: $P>0.05$). On the other hand,

SHR presented a significant rCBF reduction from its pre-ischemic value during MCAO (baseline vs. during MCAO: $P<0.05$) that did not recover until Day 4 (baseline vs. Day 1: $P<0.05$), indicating a failure of the collateral circulation from anterior cerebral artery (ACA)-MCA anastomoses to sustain rCBF outside the ischemic region. This failure was only present in SHR and not in WKY, and it was large enough that rCBF in WKY was significantly higher than that in SHR 1 hour after reperfusion ($P<0.05$) and on Day 1 ($P<0.01$). The difference in collateral rCBF between SHR and WKY was only resolved after Day 4 ($P>0.05$).

When rCBF was measured in corresponding regions of the contralesional hemisphere (Fig. 5B), WKY rats showed no significant changes from pre-ischemic values (baseline vs. during MCAO, reperfusion, Day 1, 4, and 7: $P>0.05$). On the other hand, contralesional rCBF in SHR was significantly decreased from its pre-ischemic value during MCAO (baseline vs. during MCAO: $P<0.05$), and remained decreased until Day 1 (baseline vs. Day 1: $P<0.05$), so that absolute rCBF values in SHR were lower than in WKY after reperfusion and on Day 1 ($P<0.05$), and not different on Day 4 and Day 7 ($P>0.05$).

The correlation between rCBF and ischemic lesion volume

The volume of the PDLs (WKY: $32.52\pm4.08\%$ vs. SHR: $33.95\pm3.68\%$; $P>0.05$) and the maximum rCBF measured within those lesions (WKY: 38.20 ± 3.57 mL/100 g/min vs. SHR: 38.46 ± 6.22 mL/100 g/min; $P>0.05$) during MCAO were not different between the two strains (Fig. 6), indicating that the territory of the MCA was the main determinant of the initial lesion volume. However, within this initial PDL, the region with the lowest rCBF range (0–6 mL/100 g/min) was significantly larger in SHR than in WKY (WKY: $13.28\pm2.28\%$ vs. SHR: $17.8\pm2.15\%$; $P<0.0001$), while the region with the highest rCBF range (15 mL/100 g/min to 57% reduction relative to the contralesional side) was significantly smaller in SHR than in WKY (WKY: $13.41\pm2.70\%$ vs. SHR: $10.04\pm1.77\%$; $P<0.01$). There were no differences between two strains in the region with the middle rCBF range (6–15 mL/100 g/min) ($P>0.05$).

There was no correlation between rCBF measured within the PDL during MCAO and the final ischemic lesion volume measured 7 days after MCAO ($r=-0.443$, $P>0.05$). However the region with the lowest range of rCBF was positively correlated with the final ischemic lesion volume ($r=0.716$, $P<0.01$). Interestingly, the final ischemic lesion volume of WKY was not different from the volume of the region with the lowest rCBF range during MCAO (volume of the regions with the lowest rCBF: $13.13\pm2.96\%$ vs. final ischemic lesion volume: $12.62\pm9.19\%$; $P>0.05$). By contrast, the final ischemic lesion volume of SHR was significantly larger than the region with the lowest range of rCBF (volume of region with the lowest rCBF: $17.5\pm2.13\%$ vs. final ischemic lesion volume: $33.02\pm5.41\%$; $P<0.0001$).

Discussion

The present study shows that impaired collateral blood flow, higher CBF threshold and prolonged impairment in cerebrovascular autoregulation in SHR after ischemic stroke are major contributing factors for the larger ischemic lesion volumes observed in this strain. While most of the perfusion deficient lesion progressed to become the final ischemic lesion

in SHR, in normotensive WKY rats the final ischemic lesion volume was significantly smaller than the perfusion deficient lesion and similar to the region with the lowest rCBF range. Both during ischemia and after reperfusion, rCBF in either ipsilesional and contralesional brain hemispheres of SHR could not be restored to pre-ischemic levels, and remained lower than in WKY until up to 4 days after MCAO.

Collateral circulation, defined as the supplementary vascular network that maintains rCBF when the main blood vessels fail, is a major defense mechanism of the brain against stroke (Liebeskind, 2003). Collateral circulation is formed from anastomoses between all types of blood vessels, providing an alternative route for blood to reach brain tissue when the principal supply or drainage are compromised. Collateral circulation greatly contributes to the outcome of ischemia, because it prevents tissue infarction if flow is maintained above the critical level. The luminal width of collateral vessels is a major determinant of blood flow into the territory of the occluded artery by collateral circulation (Coyle and Heistad, 1991). Because SHR have smaller internal diameter of collateral vessels than normotensive rats, reduced collateral circulation through the narrowed and stiff vessels of hypertensive rats has been considered as a major contributing factor to increased susceptibility of cerebral ischemia (Coyle, 1987, Grabowski et al., 1993). The present data corroborates the previous findings.

Using the criterion of a 57% reduction in rCBF measured during MCAO relative to the contralesional side (Meng et al., 2004), we observed that SHR had identical PDL volumes to WKY rats during the occlusion. However, already at that time point the ADC maps predicted a significantly larger lesion volume in SHR (smaller diffusion-perfusion mismatch), indicating that both within and outside the perfusion deficient lesion collateral flow was not able to compensate for the perfusion deficit. Two previous studies explained the increased susceptibility of cerebral ischemia in SHR-related strains using the diffusion-perfusion mismatch method, because hypertensive strains had a significantly smaller ischemic penumbra than WKY on MR images (McCabe et al., 2009, Letourneur et al., 2011). However, they couldn't pinpoint the exact cause of the difference in mismatch between normotensive and hypertensive strains. The smaller mismatch volume of SHR than WKY during MCAO was reproduced in the present study. Much of the perfusion-diffusion mismatch in SHR occurred in the dorsolateral cortex, in the border between the territories of the MCA and the ACA. Previously we showed that the ACA territory has large variability in both WKY and SHR rats, and that WKY rats show more variations of the ACA territory than SHR (Leoni et al., 2012). This limited flexibility of the ACA territory in SHR may explain its failure to compensate for perfusion deficits in the MCA territory during stroke.

The quantitative CBF measurements performed in the present study demonstrated that SHR showed a larger drop in CBF during ischemia than normotensive rats, and lacked the capacity to restore CBF to pre-ischemic values following reperfusion, providing further evidence for the compromised collateral flow to respond to ischemia in the hypertensive strain. While in WKY rats the decreased blood flow due to ischemia was spatially confined to within the perfusion deficient region and it was immediately restored to its pre-ischemic value upon reperfusion, in SHR there was a global decrease in CBF that extended to the contralesional hemisphere. Chronic hypertension in SHR leads to vascular remodeling,

including hypertrophy of the arterial wall, eutrophy of the arterial lumen and stiffening of the blood vessels (Amenta et al., 2003). Such remodeling strongly impairs autoregulation (Fujishima et al., 1984, Harper et al., 1984), and we demonstrated here that this impairment was not resolved until 4 days after reperfusion. The present data suggests that the impaired CBF autoregulation in both ipsilesional and contralesional hemispheres contribute not only to the decrease collateral circulation in SHR, but also lead to a larger ischemic lesion volume and worse neurological deficits compared to the normotensive rats.

In the present study, the region with the lowest CBF range was significantly smaller in WKY than in SHR and not different from the final ischemic lesion volume, which was significantly smaller than the final ischemic lesion volume in SHR. These findings suggest that the CBF threshold for ischemia is higher in SHR than in WKY, and explain the exacerbated ischemic lesion on the ADC map and smaller mismatch volumes in SHR. In a previous study, the CBF threshold for SHR was around 50 mL/100 g/min when ischemia persisted for more than 3 hours (Jacewicz et al., 1992). In the present study, the maximum rCBF measured in SHR within the PDL (38.46 ± 6.22 mL/100 g/min) was lower than 50 mL/100 g/min and the PDL volume was not different from the final ischemic lesion volume. On the other hand, in WKY, the PDL volume was significantly larger than the final ischemic lesion volume, even though the maximum rCBF (38.20 ± 3.57 mL/100 g/min) and the PDL volume in WKY were not different from those in SHR. Based on these present findings, we postulate that the CBF threshold for ischemia in WKY and SHR following a 1-hour MCAO is about 6 and 38 mL/100 g/min, respectively.

Even though a failure of collateral circulation has been suggested as the primary cause for the SHR's increased susceptibility to stroke, it is important to notice that the development of structural vascular alterations appear to involve genetic components that are phenotypically expressed regardless of the presence of hypertension (Barone et al., 1992, Mies et al., 1999). For example, genetic abnormalities in the stroke-prone SHR strain are associated with diffuse vascular processes resulting in global decompensation of CBF that could be detected before the manifestation of brain ischemia (Mies et al., 1999). In addition, several studies have suggested that exacerbated ischemic lesion of SHR-related strains is independent of arterial hypertension and that their increased susceptibility to ischemia is merely due to the intrinsic characteristics of those strains (Honda et al., 1990, Yang and Raizada, 1998, Yamagata et al., 2000, Veerasingham et al., 2005). In the present study, SHR had smaller collateral circulation and higher CBF threshold than WKY. The relative contribution of each deficit to the increased susceptibility of SHR to ischemia needs to be further investigated in larger sized cohorts of animals.

Acknowledgments

The authors would like to thank Ms. Xianfeng (Lisa) Zhang for her excellent technical skills in support of this work. This research was supported by the Intramural Research Program of the NIH, NINDS.

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- We investigated the severity of transient ischemic stroke in SHR and WKY rats
- During ischemia, perfusion deficient lesions did not differ between WKY and SHR
- In WKY, the final ischemic lesion was smaller than the perfusion deficient lesion
- In SHR, most of the perfusion deficient lesion became the final ischemic lesion
- Harmed CBF control, higher floor contribute to larger SHR susceptibility to stroke

Fig. 1.

Temporal evolution of ischemic lesion volumes as estimated from ADC maps during MCAO and T₂-weighted MRI for Days 1–7 in WKY and SHR. Lesion volumes, expressed as a percentage of the hemispheric volume, were significantly larger for SHR compared to WKY, indicating a higher susceptibility of SHR to brain ischemia. **: $P < 0.01$ (two-way repeated measures ANOVA with Bonferroni *post-hoc* test).

Fig. 2.

Temporal evolution of brain edema following transient MCAO in WKY and SHR. SHR had significantly larger brain edema compared to WKY at 4 days after MCAO. In both strains, edema resolved by 7 days after ischemia. **: $P < 0.01$ (two-way repeated measures ANOVA with Bonferroni *post-hoc* test).

Fig. 3. Representative ADC maps, T2-weighted images, and CBF maps demonstrating the temporal evolution of ischemic lesions and rCBF obtained from WKY and SHR after transient MCAO. SHR had larger regions of ADC deficits, as well as larger hyperintense lesions and more edema in comparison with WKY during MCAO and at 1 day, 4 days and 7 days after MCAO. In addition, while rCBF in both hemispheres was significantly decreased in SHR, higher values and rapid restoration of rCBF were observed in WKY. The scale bar expresses the CBF values in mL/100 g/min.

Fig. 4.

Temporal evolution of neurological scores evaluated following transient MCAO in WKY and SHR. Neurological deficits were significantly higher in SHR compared to WKY, indicating an increased susceptibility of SHR to brain ischemia in the acute phase of stroke. Differences between two strains were significant at the *: $P < 0.05$ and **: $P < 0.01$ levels (Mann-Whitney U-test).

Fig. 5. Temporal evolution of rCBF measured within (1) and outside (2) the perfusion deficient lesion of the ipsilesional hemisphere (A) and the corresponding regions of the contralesional hemisphere (B). The average rCBF was calculated on the 15 slices scanned throughout the brain. (C) A representative image of the CBF map shows 4 ROIs outlining those areas. *: $P < 0.05$ and **: $P < 0.01$ for differences between two strains; †: $P < 0.05$ and ††: $P < 0.01$ versus baseline rCBF of WKY; ‡: $P < 0.05$ and ‡‡: $P < 0.01$ versus baseline rCBF of SHR (two-way repeated measures ANOVA with Bonferroni *post-hoc* test).

Fig. 6.

The region within the perfusion deficient lesion with three determined ranges of rCBF during MCAO in WKY and SHR. [†] The maximum rCBF was reduced by 57% relative to the contralesional side (WKY: 38.20 ± 3.57 mL/100 g/min; SHR: 38.46 ± 6.22 mL/100 g/min). Differences between two strains were significant at the *: $P < 0.01$ and ***: $P < 0.0001$ levels (unpaired t-test).

Table 1

Scoring Scale of Neurological Deficits

Score	0	1	2
Postural Reflexes	<i>No deficit</i>	<i>Contralateral forelimb flexion when suspended by the tail</i>	<i>Reduced resistance to lateral push toward contralateral side</i>
Circling	<i>Absent</i>		<i>Present</i>
Proprioceptive Placement of Contralateral Limbs	<i>Complete and Immediate</i>	<i>Incomplete or Delayed (< 2 s)</i>	<i>Absent</i>

Table 2

Physiological parameters measured before, during, and after MCAO

	MABP (mm Hg)	pH	PaO ₂ (mm Hg)	PaCO ₂ (mm Hg)	Heart Rate (bpm)	Temperature (°C)
<i>Before MCAO</i>						
WKY	88±14	7.43±0.03	160±15	34±3	300±27	37.7±0.4
SHR	135±29**	7.43±0.04	174±11	33±2	329±21	38.2±0.5
<i>During MCAO</i>						
WKY	84±10	7.40±0.03	157±18	34±3	337±13	37.9±0.3
SHR	128±29*	7.39±0.03	165±20	36±3	334±37	38.2±0.3
<i>Reperfusion</i>						
WKY	87±6	7.39±0.03	154±17	33±4	332±16	38.0±0.6
SHR	111±22*	7.36±0.05	169±17	35±4	349±7	38.4±0.5

bpm: beats per minute; MABP: mean arterial blood pressure; MCAO: middle cerebral artery occlusion; SHR: spontaneously hypertensive rats; WKY: Wistar-Kyoto rats;

* $P < 0.05$,** $P < 0.01$ for differences between WKY and SHR (two-way repeated measures analysis of variance (ANOVA) with Bonferroni *post-hoc* test).