Regional Cerebral Blood Flow Autoregulation in Normotensive and Spontaneously Hypertensive Rats — Effects of Sympathetic Denervation

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SUMMARY The present study was designed to investigate the effect of acute sympathetic denervation on the regional cerebral blood flow (CBF) autoregulation during acute elevation of blood pressure in spontaneously hypertensive rats (SHR) and normotensive Wistar Kyoto rats (WKY). CBF to the parietal cortex and thalamus was measured by the hydrogen clearance method and, to test autoregulation, systemic arterial blood pressure was elevated by intravenous infusion of phenylephrine. Superior cervical ganglia were removed on both sides to interrupt sympathetic innervation in the deeper structures of the brain.

Acute bilateral sympathetic denervation did not alter the resting blood pressure or CBF in either SHR or WKY. In innervated SHR, resting mean arterial pressure (MAP) was 165 ± 5 mm Hg (mean ± SEM) and the upper limit of autoregulation in the cortex was 210 ± 3 mm Hg, which was significantly lower than that in the thalamus (229 ± 3 mm Hg, p < 0.02). In bilaterally denervated SHR, the upper limits were lowered to 193 ± 4 mm Hg in the cortex (p < 0.02 vs. innervated SHR) and to 207 ± 5 mm Hg in the thalamus (p < 0.02 vs. innervated). In WKY, resting MAP was approximately 55 mm Hg lower than that in SHR. Acute denervation reduced the upper limits from 142 ± 3 mm Hg to 130 ± 4 in the cortex (p < 0.05) and from 158 ± 4 to 145 ± 4 in the thalamus (p < 0.05).

These results indicate in either SHR or WKY that vasoconstrictive responses to an increase in blood pressure are greater in the thalamic than in the cortex and that acute interruption of sympathetic nerves alters the autoregulatory function of cerebral vessels in the cortex and thalamus as well.

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Although cerebral arteries have dense adrenergic nerve fibers, the physiologic significance of innervation in cerebral blood flow (CBF) regulation has not been fully understood. In the resting condition, sympathetic nerves exert little or minimal effect on CBF and cerebral vessels. During acute elevation of systemic arterial pressure, however, it has been shown that an increase in CBF is attenuated by overlapping sympathetic nerves which originate from the basal and medial areas of the brain are innervated by the deeper structures of the brain. In the present study, we have examined the effect of acute sympathetic denervation on CBF autoregulatory function in the cerebral cortex of spontaneously hypertensive rats (SHR).

In relation to the sympathetic innervation, less attention has been paid to the regulation of CBF in the deeper structures of the brain. In the present study, we have examined the effect of acute sympathetic denervation on CBF autoregulatory function in the thalamus as well as in the cortex in SHR. Blood vessels in the basal and medial areas of the brain are innervated by overlapping sympathetic nerves which originate from the ipsilateral and contralateral superior cervical ganglia. Thus, prior to the study, bilateral ganglionectionomy was performed simultaneously, and CBF was measured during acute increases in systemic arterial pressure. We also investigated the changes in CBF of Wistar Kyoto rats (WKY) to examine whether there are any differences in the effect of sympathetic denervation on vascular resistance to intraluminal pressure between hypertensive and normotensive animals.

Materials and Methods

We used 18 male SHR and 19 male WKY at 6 months of age. All the rats were housed with 12 hours light and 12 hours darkness alternatively at 25°C. They were fed stock chow diet (Oriental Co. Tokyo, Japan) and tap water ad libitum. Thirty minutes prior to the CBF measurement, the rats were anesthetized with isoflurane (amobarbital 100 mg/kg, i.p.). After midline incision of the neck, both superior cervical ganglia were removed in 9 SHR and 12 WKY to examine the effect of acute denervation on CBF regulation. In the other rats (9 SHR and 7 WKY), the ganglia were exposed but not removed (sham operated). One femoral artery was cannulated for infusion of drug and for blood sampling for determination of arterial acid-base parameters. One femoral vein was cannulated for continuous measurement of systemic arterial pressure and blood sampling for determination of arterial acid-base parameters. One femoral vein was cannulated for infusion of drug and for blood transfusion from donor rats.

To measure CBF repeatedly in the cortex and thalamus in each rat, the hydrogen clearance technique was applied. The details of this method have been described elsewhere. Briefly, after the rat's head was fixed in a head holder, two small burr holes were made on the skull 2 mm lateral and 2 mm posterior to the bregma on each side. A fine platinum electrode (200 µm in diameter) was placed stereotaxically in the cortex on one side and thalamus on the other side; 2 mm in depth from the surface on the brain for the parietal cortex and 7 mm in depth for the thalamus. The reference Ag-AgCl electrode was inserted under the skin.
Hydrogen gas, a 10% mixture with room air, was administered to the spontaneously breathing rat for 2 minutes, and the body temperature was kept close to 37°C with a heat lamp. At least 3 baseline CBF measurements were made at intervals of about 10 minutes. Then, systemic arterial pressure was increased approximately 10 mm Hg in steps by infusion of phenylephrine through the femoral vein with a Harvard infusion pump, and maintained at each level for at least 5 minutes during CBF measurement. The CBF at each level of blood pressure was calculated from the clearance curve by using the formula at Auckland et al.\(^{13}\) When blood pressure reached a certain level, CBF started to increase with further elevations of blood pressure. That blood pressure level was defined as the upper limit of autoregulation.

Blood gases and pH were determined at rest and 2 or 3 times during elevation of blood pressure. Blood from strain-matched donor rats was infused into the femoral vein to protect against reduction of blood pressure during blood sampling. After the last CBF measurement, the rats were killed by a bolus injection of saturated KCl into the femoral vein, and the brain was examined carefully. Rats in whom macroscopical hemorrhage, brain damage, or improper placement of the electrode was found were excluded from this study.

Differences in the upper limit of autoregulation between the denervated and innervated (sham operated) rats, and in the upper limit between the cortex and thalamus in each group were analysed using unpaired t-tests. The autoregulatory range and the reduction in the upper limit following denervation in SHR were compared with those in WKY by analysis of variance. Analysis of variance was also used to compare the resting CBF and blood pressure in each group.

**Results**

The resting mean arterial pressure (MAP), arterial blood gas values, CBF and cerebral vascular resistance (CVR; calculated by MAP/regional CBF) for each group of rats are presented in Table 1. Resting MAP and CBF in the denervated rats were substantially the same as those in the innervated ones. Blood gases and pH were not different among the animals, nor did change during acute elevation of systemic arterial pressure.

**CBF Autoregulation in SHR**

Resting CBF in the denervated rats were 56 ± 7 ml/100 g/min in the parietal cortex and 58 ± 4 in the thalamus, and in the innervated rats 53 ± 9 and 53 ± 6, respectively (Table 1). In spite of the stepwise increase in blood pressure, the blood flow to the cortex and thalamus was maintained relatively constant (only 10–15% increase over baseline) until blood pressure was raised beyond a certain level, when CBF increased passively indicating the upper limits of autoregulation (figures 1 and 2). In the innervated rats, these upper limits were 210 ± 3 mm Hg (128% of the resting MAP) in the parietal cortex and 229 ± 3 (136%) in the thalamus, its difference being of a statistical significance (p < 0.02) (table 1). In rats with bilateral denervation, the upper limits were lowered to 193 ± 4 mm Hg in the cortex (p < 0.02 vs. innervated cortex) and to 207 ± 5 in the thalamus (p < 0.02 vs. innervated thalamus); the average reduction being 17 mm Hg and 22, respectively. The difference in the upper limits of blood pressure between the denervated cortex and the denervated thalamus was also significant (p < 0.05).

**CBF Autoregulation in WKY**

MAP at rest was about 55 mm Hg lower than that in SHR. CBF to the cortex and thalamus was around 55 ml/100 g/min, being not different from those in hypertensive rats. Calculated CVR was, therefore, approximately 63% of that in SHR. When MAP was raised 31% above the resting levels in the cortex, and 39% in the thalamus, CBF started to increase passively. In the innervated rats, these upper limits of autoregulation were 142 ± 3 mm Hg in the cortex and 158 ± 4 in the thalamus (p < 0.02 vs. cortex). Acute denervation significantly lowered the upper limits to 130 ± 4 mm Hg in the cortex (p < 0.05 vs. innervated cortex) and 145 ± 4 in the thalamus (p < 0.05 vs. innervated thalamus). The upper limits between the cortex and thalamus following denervation were also significantly different (p < 0.05).

The autoregulatory ranges in the cortex, namely MAP ranges from the resting blood pressure levels to the upper limits, were 45 mm Hg for SHR and 33 for WKY (p < 0.05) and those in the thalamus were 64 and 49, respectively (p < 0.05). Acute denervation in SHR narrowed the ranges more than in WKY in the cortex (17 mm Hg vs. 12, p < 0.05) and also in the thalamus (22 mm Hg vs. 13, p < 0.05).

**Discussion**

There are three major findings in this study. First, the upper limits of CBF autoregulation were shifted to higher levels of blood pressure in SHR compared with WKY, and the autoregulatory range from the resting MAP to the upper limits were greater in SHR than WKY. Secondly, the thalamus showed a wider range than did the cortex, suggesting that the autoregulatory function in the deeper structures is better operated than that in the superficial area of the brain. Thirdly, acute bilateral ganglionectomy significantly lowered the upper limits in the cortex and the thalamus as well, indicating the responsiveness of the cortical and thalamic vessels to an acute rise in arterial pressure is, at least in part, modulated by sympathetic tone. Although resting MAP was higher in SHR than WKY, baseline CBF did not differ between the two groups, resulting in an increased CVR in SHR.

CBF autoregulation is defined as maintenance of relatively constant blood flow in face of moderate changes in perfusion pressure and the autoregulatory activity is closely related to the changes in CVR.\(^{11}\) In response to an increase in intraluminal pressure, cerebral arteries constrict, while they dilate when blood pressure is decreased.\(^{14,15}\) Thus CVR seems to be pri-
TABLE 1  Average Values for Mean Arterial Pressure (MAP), Arterial Acid-base Parameters, Cerebral Blood Flow (CBF), Cerebral Vascular Resistance (CVR) and Upper Limits of Autoregulation in the Cortex and Thalamus

<table>
<thead>
<tr>
<th></th>
<th>SHR Denervated (9)</th>
<th>SHR Innervated (9)</th>
<th>WKY Denervated (12)</th>
<th>WKY Innervated (7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP mm Hg</td>
<td>166 ± 4</td>
<td>165 ± 5</td>
<td>112 ± 6</td>
<td>109 ± 5</td>
</tr>
<tr>
<td>PCO₂ mm Hg</td>
<td>35.6 ± 1.5</td>
<td>34.8 ± 1.9</td>
<td>36.7 ± 1.3</td>
<td>36.4 ± 1.6</td>
</tr>
<tr>
<td>PO₂ mm Hg</td>
<td>103 ± 3</td>
<td>97 ± 3</td>
<td>92 ± 2</td>
<td>94 ± 3</td>
</tr>
<tr>
<td>pH</td>
<td>7.40 ± 0.02</td>
<td>7.39 ± 0.01</td>
<td>7.39 ± 0.02</td>
<td>7.37 ± 0.03</td>
</tr>
<tr>
<td>CBF at rest</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cortex</td>
<td>56 ± 7</td>
<td>53 ± 9</td>
<td>59 ± 8</td>
<td>54 ± 6</td>
</tr>
<tr>
<td>thalamus</td>
<td>58 ± 4</td>
<td>53 ± 6</td>
<td>56 ± 2</td>
<td>55 ± 7</td>
</tr>
<tr>
<td>CVR at rest</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cortex</td>
<td>3.06 ± 0.21</td>
<td>3.09 ± 0.18</td>
<td>1.90 ± 0.18</td>
<td>2.11 ± 0.20</td>
</tr>
<tr>
<td>thalamus</td>
<td>3.01 ± 0.20</td>
<td>3.07 ± 0.16</td>
<td>2.00 ± 0.08</td>
<td>1.98 ± 0.16</td>
</tr>
<tr>
<td>Upper limit of</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>autoregulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cortex</td>
<td>193 ± 4§</td>
<td>210 ± 3*‡</td>
<td>130 ± 4 §</td>
<td>142 ± 3*‡</td>
</tr>
<tr>
<td>thalamus</td>
<td>207 ± 5</td>
<td>229 ± 3*</td>
<td>145 ± 4</td>
<td>158 ± 4*</td>
</tr>
</tbody>
</table>

Numbers in parentheses show number of rats. Values are presented as mean ± SEM.

*p < 0.02, †p < 0.05 vs. upper limit of autoregulation in denervated rats.

It has been shown that cerebral arteries undergo hypertrophy in chronic hypertension. Our present study in SHR demonstrated that the wall-to-lumen ratios of cerebral arteries are increased, and the upper limits of CBF autoregulation are concomitantly shifted to higher levels of blood pressure during development of hypertension. Thus, the increase in CVR and the upward shift of upper limits in SHR, as shown in the present study, seem to primarily depend on the structural adaptation of the vessels to the high blood pressure.

A new finding in this study is that the upper limits of CBF autoregulation in the thalamus were 14 to 19 mm Hg higher than those in the cortex in SHR as well as WKY. We have demonstrated previously that during hemorrhagic hypotension, blood flow to the brain stem including the thalamus was preserved well and significantly greater than that to the cerebrum or cerebellum in stroke-prone SHR and WKY. Thus our present results indicate that, similar to the lower limits, the upper limits of the autoregulatory plateau differ in various regions of the brain, and that the plateau is wider in the thalamus or in the brain stem than that in the cortex. Since the thalamus is a common site for hypertensive intracerebral hemorrhage, it might be reasonable to assume that blood vessels in this area should have more protective function against an acute elevation of blood pressure or overperfusion than other re-

![Figure 1](image1.png)  Effect of acute bilateral denervation of cervical sympathetic nerves on cerebral blood flow autoregulation in the cortex. In both SHR and WKY, the upper limits of autoregulation are lowered in the denervated rats compared with innervated ones. Arrows show the blood pressure levels of the upper limits of autoregulation. Values are presented as mean ± SEM. D: denervated, I: innervated rats, *p < 0.02, †p < 0.05, upper limits in the innervated vs. denervated rats.

![Figure 2](image2.png)  Effect of acute bilateral denervation of cervical sympathetic nerves on cerebral blood flow autoregulation in the thalamus. Upper limits of autoregulation are shifted to higher level of blood pressure in the thalamus than in the cortex in both SHR and WKY. Bilateral gangliectomy significantly lowers the upper limits. Arrows show the blood pressure levels of the upper limits of autoregulation. Values are presented as mean ± SEM. D: denervated, I: innervated rats, *p < 0.02, †p < 0.05, upper limits in the innervated vs. denervated rats.
gions of the brain. Mechanisms that account for the regional differences in the cerebral vascular responsiveness have not been clear. The differences in the density of sympathetic nerves in the regional vasculatures could be one of the factors which is mentioned later. Further studies are desired to clarify the structural or functional properties of the blood vessels in the thalamus and the cortex, including metabolic alterations in these areas, during an acute rise in systemic arterial pressure.

Although cerebral vessels are innervated by adrenergic nerve fibers from the cervical sympathetic chain, the significance of these sympathetic functions in the regulation of the regional CBF is still under dispute. 1,4,5 Very recently, Bill and Linder,4 and Heistad and his colleagues6, 5, 8-9 have found that during an acute rise in systemic arterial pressure, CBF is increased more in the denervated hemisphere than in the innervated hemisphere, and such increase in CBF is attenuated by an electrical stimulation of the cervical ganglion. Vasocostrictive response to an acutely elevated intramural pressure seems to be augmented by activation of the sympathetic tone. The blood vessels in the parietal cortex are innervated by nerve fibers which originate from the ipsilateral superior cervical ganglion, while those in the basal and medial areas of the brain are innervated by overlapping nerve fibers from the bilateral superior cervical ganglion.1, 3 Functional characteristics of this overlapping innervation on the regional CBF have not been examined before. In this study, superior cervical ganglia were removed on both sides to interrupt the sympathetic nervous system to the thalamus. The upper limits of CBF autoregulation in the denervated cortex were found to be 12 to 17 mm Hg lower than those in rats with the sham operation. The upper limits in the thalamus in both SHR and WKY with bilateral denervation were also shifted to 13 to 22 mm Hg lower levels of blood pressure compared with those in the innervated animals, indicating that the thalamic autoregulation is modulated by sympathetic innervation. It is assumed that overlapping innervation may play an important role in the effective responsiveness of the blood vessels to the changes in the blood pressure.

Basal sympathetic nerve activity has been found to increase in the conscious18 as well as anesthetized SHR.18, 19 Recently, Lee and Saito7 demonstrated that the concentration of the endogenous norepinephrine in the cerebral arteries is higher in SHR than WKY. We speculate, therefore, that in addition to the structural changes of the cerebral vessels, the enhanced neural activity leads to the increased vascular resistance in SHR and the effects of acute denervation on CBF regulation are larger in SHR compared to those in WKY. As demonstrated in the present study, the upper limits following denervation were significantly lowered in SHR (22 mm Hg in the thalamus and 17 in the cortex) than in WKY (13 mm Hg and 12, respectively), indicating that high sympathetic activity contributes to a greater neurally-derived vasoconstriction in SHR, and thus, acute interruption of sympathetic tone causes a larger reduction of the upper limits of autoregulation than in WKY.

In conclusion, the upper limits of CBF autoregulation differ in various regions of the brain. Autoregulatory function in the thalamus is better preserved against acute hypertension than that in the cortex. Sympathetic nerves play a more important role in the maintenance of CBF autoregulation for hypertensive animals than normotensive ones, or in the thalamus than in the cortex.

References
Regional cerebral blood flow autoregulation in normotensive and spontaneously hypertensive rats--effects of sympathetic denervation.
S Sadoshima, K Fujii, H Yao, K Kusuda, S Ibayashi and M Fujishima