Cerebral Autoregulation in Young Spontaneously Hypertensive Rats
Effect of Sympathetic Denervation

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SUMMARY Autoregulation of cerebral blood flow was studied with the hydrogen clearance method during development of hypertension in young spontaneously hypertensive rats. To examine the influence of sympathetic nerves on autoregulatory range, the unilateral superior cervical ganglion was removed 2 hours or 2 or 5 weeks before the study. Wall-to-lumen ratio of cerebral arteries was determined with freeze substitution technique. Basal blood pressures were 87 ± 1 mm Hg (mean ± SEM) at 4 weeks of age, 105 ± 2 at 6 weeks, and 126 ± 3 at 9 weeks, although resting cerebral blood flow was unchanged. Initially, cerebral blood flow remained relatively constant when the blood pressure was raised by intravenous infusion of phenylephrine. The upper limits of cerebral blood flow autoregulation in these groups were 110 ± 4 mm Hg, 126 ± 7, and 159 ± 6 respectively. Acute ganglionectomy significantly lowered the upper limits (p < 0.05), but chronic denervation did not affect the autoregulatory range. The wall-to-lumen ratios of cerebral arteries were 0.136 ± 0.007 at 4 weeks and 0.130 ± 0.005 at 9 weeks. These differences were not significant, nor did sympathetic denervation alter the ratio. These results indicate that (1) the upward shift of the autoregulation is closely related to a rise in the basal blood pressure, (2) acute interruption of sympathetic nerves modulates the autoregulatory range, and (3) adaptation of cerebral blood flow autoregulation to early developmental hypertension may be attributed to factors other than vascular smooth muscle hypertrophy. (Hypertension 7: 392-397, 1985)

KEY WORDS • autoregulation • cerebral blood flow • spontaneously hypertensive rat • sympathetic nerve

CEREBRAL autoregulation is the adaptive reaction of the vessels to maintain cerebral blood flow (CBF) at relatively constant rates despite variations in perfusion pressure. Although its mechanism is still under dispute, autoregulation is seen as vasoconstriction in response to increased intraluminal pressure and vasodilatation in response to pressure reduction.1 Strandgaard et al.,2 who studied CBF in severely hypertensive and in normotensive subjects, found that both the upper and lower limits of autoregulation in chronic hypertensive subjects were shifted to higher levels of blood pressure.

The effects of chronic hypertension on CBF have also been observed in experimental animals.3-5 Our previous study6 in normotensive rats and spontaneously hypertensive rats (SHR) demonstrated a close relationship between basal blood pressure level and autoregulatory capacity of cerebral vessels. In recent years, long-term antihypertensive treatment has been found to shift CBF autoregulation back toward the normal range.6 This finding suggests that the structural changes in resistance vessels caused by hypertension may be reversible and may be the primary contributors to the alteration of autoregulatory response.6-7

Research has been focused on the autoregulatory function of the brain in the later phase of established hypertension rather than in the early phase of developmental hypertension.8 In this study, we investigated CBF autoregulation in young SHR and examined whether vascular hypertrophy is essentially responsible for the shift in autoregulatory range, if present, during the early developmental stage of hypertension. As higher sympathetic nerve activity has been reported...
to be involved in the development of hypertension in younger SHR (although the effect of innervation on cerebral arteries is still controversial), we also studied the effect of sympathetic nerves on CBF in these rats. Prior to CBF study, a superior cervical ganglionectomy was performed to examine whether acute or chronic interruption of sympathetic nerves modulates CBF autoregulation.

Methods

Sympathetic Denervation

Forty-three male SHR were housed at 25°C and exposed to light 12 hours each day. They were fed stock chow diet (Oriental Co., Tokyo, Japan) and tap water ad libitum. At 4 weeks of age, the rats were anesthetized with amobarbital (Isomytal, 100 mg/kg intraperitoneal [i.p.]). Both superior cervical ganglions were exposed, but only the unilateral ganglion was removed. When recovered from anesthesia, all the animals had ptosis and enophthalmos on the side ipsilateral to gangliectomy. The neck incision was closed, and the animals to be used in chronic denervation studies were returned to their cages and fed for another 2 or 5 weeks. In the animals to be used in acute denervation studies, the superior cervical ganglion was removed in a similar manner at 4, 6, or 9 weeks of age; CBF determinations were made 2 hours after denervation.

Measurement of Cerebral Blood Flow

As cerebral autoregulation has been reported to be well preserved under barbiturate anesthesia, the animals in the present study were anesthetized with amobarbital (Isomytal 100 mg/kg i.p.). One femoral artery was cannulated for recording arterial pressure and sampling blood for acid-base balance, and a femoral vein was cannulated for blood and drug infusion. The hydrogen clearance technique was chosen for cortical CBF determination. This method enables direct and repeated measurement, and the values obtained with this method are compatible with those measured by 133Xe clearance technique, a radioautographic method, and a microsphere technique. Details for this method have been described previously. Briefly, the animal's head was fixed in a head holder, and two small bur holes were made on the skull 2 mm lateral to the bregma on each side. Two Teflon-coated platinum electrodes (200 μm in diameter, with a 1-mm portion at its tip uncoated and plated with a platinum black) were placed stereotaxically in the bilateral parietal cortex (1 mm in depth from the surface of the brain). 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, and the body temperature was kept close to 37°C with a heat lamp. The CBF was calculated from the clearance curve by using the formula of Aukland et al.

As Isomytal is an intermediate-acting barbiturate, stable anesthetic condition was ensured when basal blood pressure was maintained at almost the same level for over 30 minutes. At least three baseline measurements of CBF were obtained at intervals of about 15 minutes. The systemic arterial blood pressure was raised approximately 10 mm Hg in steps by intravenous infusion of phenylephrine with a Harvard infusion pump (Harvard Apparatus, Millis, MA) and maintained at each level for at least 5 minutes during CBF measurement. Arterial blood gas values and pH were determined at resting state and three times during elevation of blood pressure. Blood from strain-matched donor rats was infused into the femoral vein during blood sampling.

After the last CBF measurement, all the animals were killed with saturated KCl injected into the femoral vein and the brains were examined macroscopically. The CBF data were excluded from the present results if the electrode was improperly placed or if tissue was grossly damaged by insertion of the electrode.

Morphometry of the Vessel Wall

Eleven male SHR were used for measurement of a wall-to-lumen ratio of cerebral arteries by a freeze substitution technique. Ganglionectomy was performed in the same manner as previously described. To study acute and chronic denervation, five and six 4-week-old SHR underwent ganglionectomy. Morphometrical study was performed when the rats were 9 weeks old. With the rats under Isomytal anesthesia, a femoral artery was cannulated for blood sampling for gas analysis and arterial pressure recording. The animal's head was fixed in a head holder, and a plastic funnel was fitted into a skin incision over the skull bone for subsequent freezing of the brain in situ. After more than 30 minutes were allowed for a steady state, isopentane cooled by liquid nitrogen was poured into the funnel. The brain was then carefully chiseled out and fixed with 2% osmic acid in acetone at −70°C for 4 weeks. After dehydration in graded alcohol, brain tissues were embedded in Epon 812 and were cut 1 μm thick. The specimens were stained with toluidine blue, and the thickness of muscular layer and the diameter of cerebral arteries were estimated by eye piece micrometer.

The difference in CBF and wall-to-lumen ratios between denervated and sham-operated hemisphere was analyzed using paired t test in each age group of rats. The resting CBF in each group was compared by analysis of variance.

Results

Autoregulation

The resting blood pressure, arterial blood gas values, CBF, and cerebral vascular resistance (CVR; calculated by mean arterial pressure/CBF) for each group of rats are presented in Table 1. Blood pressure was significantly raised with advance of age from 87 ± 1 mm Hg (mean ± sem) at 4 weeks to 126 ± 3 at 9 weeks of age (p < 0.01). There were no differences of blood gas or CBF values among the groups, although
TABLE 1. Average Values for Mean Arterial Pressure, Arterial Acid-Base Parameters, Cerebral Blood Flow, and Cerebral Vascular Resistance at Resting State

<table>
<thead>
<tr>
<th>Value</th>
<th>Age of rats (wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 (n = 6)</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>87 ± 1</td>
</tr>
<tr>
<td>pH</td>
<td>7.34 ± 0.03</td>
</tr>
<tr>
<td>PaCO₂ (mm Hg)</td>
<td>35.5 ± 1.8</td>
</tr>
<tr>
<td>PaO₂ (mm Hg)</td>
<td>78.5 ± 3.3</td>
</tr>
<tr>
<td>CBF (ml/100 g/min)</td>
<td>50.6 ± 9.2</td>
</tr>
<tr>
<td>CVR (mm Hg·100 g·ml⁻¹·min⁻¹)</td>
<td>1.71 ± 0.18</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

MAP = mean arterial pressure; CBF = cerebral blood flow; CVR = cerebral vascular resistance; PaCO₂ = arterial CO₂ tension; PaO₂ = arterial O₂ tension.

* p < 0.05, 9 weeks versus 6 weeks.
† p < 0.01, 9 weeks versus 4 weeks.
‡ p < 0.01, 9 weeks versus 6 weeks.

CVR was significantly increased in 9-week-old rats (p < 0.01) compared with the CVR in 6-week-old rats.

Figure 1 shows the pressure-flow relationship in each group. In spite of the stepwise increase in blood pressure, CBF initially was relatively constant. When CBF was increased approximately 15 to 20% above baseline levels, the upper limits of autoregulation began to increase steeply with each rise in blood pressure. These upper limits of autoregulation were 110 ± 4 mm Hg at 4 weeks of age, 126 ± 7 at 6 weeks, and 159 ± 6 at 9 weeks respectively. The resting blood pressures were increased by 18 mm Hg from 4 to 6 weeks of age and by 21 mm Hg from 6 to 9 weeks, while the upper limit increased 33 mm Hg from 6 to 9 weeks, and 16 mm Hg from 4 to 6 weeks. Figure 2 depicts the relationship between the blood pressure and CVR in each group of rats. The CVR initially was slightly increased, and the blood pressure levels beyond which CVR started to decrease were 112 ± 4 mm Hg at 4 weeks, 129 ± 5 at 6 weeks, and 150 ± 6 at 9 weeks. Thus, the upper limits of autoregulation, based on CVR changes, were essentially the same as those obtained by pressure-flow patterns.

Sympathetic Denervation

The mean values for CBF in innervated and denervated hemispheres are shown in Table 2. Sympathetic denervation did not alter CBF at resting state. The upper limit of autoregulation in the acutely denervated hemisphere shifted to a significantly lower blood pressure level in rats of each age (p < 0.05). Figure 3 shows the autoregulation curves in these animals. During a rise in arterial pressure, a greater increase in the CBF was observed in the denervated hemisphere than in the innervated hemisphere. Such a tendency was more prominent in 4-week-old SHR than in 6- or 9-week-old rats. As shown in Figure 4, there was no difference of autoregulatory pattern between the denervated and innervated hemisphere in animals with chronic denervation.
TABLE 2. Effect of Sympathetic Denervation on Cerebral Blood Flow and Upper Limit of Autoregulation

<table>
<thead>
<tr>
<th>Value</th>
<th>Age of rats (wk)</th>
<th>Acute denervation</th>
<th>Chronic denervation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4 (n = 6)</td>
<td>6 (n = 9)</td>
</tr>
<tr>
<td>MAP at rest (mm Hg)</td>
<td>87 ± 1</td>
<td>104 ± 3</td>
<td>127 ± 4†</td>
</tr>
<tr>
<td>CBF at rest (ml/100 g/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Innervated hemisphere</td>
<td>50.6 ± 9.2</td>
<td>48.3 ± 4.4</td>
<td>46.0 ± 3.7</td>
</tr>
<tr>
<td>Denervated hemisphere</td>
<td>52.1 ± 7.1</td>
<td>48.7 ± 3.1</td>
<td>47.0 ± 2.1</td>
</tr>
<tr>
<td>Upper limit of autoregulation (mm Hg)</td>
<td>110 ± 4‡</td>
<td>127 ± 4‡</td>
<td>168 ± 7‡</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

MAP = mean arterial pressure; CBF = cerebral blood flow.

* p < 0.05, 9 weeks versus 6 weeks.
† p < 0.01, 9 weeks versus 4 weeks.
‡ p < 0.05, innervated versus denervated hemisphere.

Morphometry

Wall-to-lumen ratios were measured in 118 pial and cortical arteries of 4-week-old SHR and in 190 arteries of 9-week-old SHR. Mean arterial blood pressure was 93 ± 6 mm Hg in the former group and 131 ± 14 in the latter (p < 0.01). Average values for wall-to-lumen ratio in 4-week-old rats were 0.139 ± 0.008 in the denervated hemisphere and 0.136 ± 0.007 in the innervated hemisphere (p < 0.05). The ratio in 9-week-old rats did not differ between the denervated hemisphere (0.123 ± 0.005) and the innervated hemisphere (0.130 ± 0.005; Figure 5). The average ratio at 9 weeks was similar to that for younger SHR. Thus, chronic denervation for a period of 5 weeks had a minimal effect on the structural development of the vascular wall with advancing age.

Discussion

The major findings of the present study are that (1) CBF at resting condition actually was unchanged among young SHR of 4, 6, and 9 weeks of age, al-

FIGURE 3. Effect of acute denervation of cervical sympathetic nerve on CBF autoregulation. The upper limit of autoregulation (arrows) was lower in the denervated hemisphere (D) compared with innervated hemisphere (I). Values are means ± SEM. Numbers in parentheses show number of rats. MAP = mean arterial pressure; *p < 0.05, CBF in the denervated versus innervated hemisphere.

FIGURE 4. Effect of chronic denervation of cervical sympathetic nerves on CBF autoregulation. There were no differences in the upper limits of autoregulation (arrows) between innervated (I) and denervated (D) hemispheres. Values are means ± SEM. Numbers in parentheses show number of rats. MAP = mean arterial pressure.

FIGURE 5. Wall-to-lumen ratio in 4-week-old and 9-week-old SHR. Chronic denervation exerted little effect on the ratio. Values are means ± SEM. I = innervated hemisphere; D = denervated hemisphere.
though arterial pressure was significantly increased with advance of age; (2) the upper limit of the autoregulatory range was closely related to an increase in basal blood pressure level; and (3) acute superior cervical ganglionection altered the autoregulatory responses of cerebral vessels, whereas chronic denervation had no influence on CBF. To raise systemic arterial pressure, we infused phenylephrine. As previous reports have indicated that the α-adrenergic receptor in cerebral vessels is enormously less sensitive (about 1000 times less) to agonist than is usual for adrenergic receptors, we assumed that the effect of phenylephrine on the responsiveness of cerebral arteries to blood pressure might be minimal. Surprisingly, the wall-to-lumen ratio of cerebral arteries in 4-week-old SHR did not differ from that in 9-week-old rats. In the prehypertensive or early hypertensive state, it is unlikely that a change in medial thickness would lead to a change in vascular resistance and consequently to a shift in the autoregulation range of a higher level. On the contrary, we suggest that sympathetic nerve activity may participate in autoregulatory vasoconstrictor responsiveness in the growing SHR.

Factors affecting the autoregulatory capacity of CBF have been studied extensively in humans as well as in experimental animals with sustained hypertension. In these hypertensive subjects, both the upper and lower limits of autoregulation are shifted to higher levels and are well correlated with a rise in resting blood pressure and CVR. As long-term antihypertensive treatment can restore the autoregulation toward normal range, the vascular resistance appears to be primarily increased by hypertension-related structural alterations, such as medial hypertrophy and connective tissue proliferation, that are reversible in part by lowering the blood pressure.

In contrast, in 4-week-old to 9-week-old rats in an early hypertensive state, the enhanced medial thickness of cerebral arteries, represented as wall-to-lumen ratio, was not evident, although their basal blood pressure was elevated with age. Therefore, the age-related increase in the calculated CVR in young SHR cannot be explained by a structural adaptation to hypertension. Nordborg and Johansson found that the media to radius ratio in even 15-day-old SHR was greater than that in age-matched normotensive rats. They suggested that a non-pressure-dependent aberration of vascular walls caused, for example, by the trophic influence of the sympathetic nervous system may occur in young SHR. Isolated mesenteric vessels from 4-week-old or 6-week-old SHR have a smaller relaxed lumen, but unlike vessels from adult SHR, the medial thickness is not increased. According to Bohlen and Lobach, there are no differences in the lumen and wall thickness of small resistance vessels between SHR and normotensive rats after the vessels are totally relaxed by both denervation and application of nitroprusside. These findings and those of the present study indicate that an increase in CVR in young hypertensive rats is functional in origin, that is, in the case of genetically increased vasoconstriction or abnormally high responsiveness to extrinsic factors such as sympathetic activity or circulating norepinephrine.

The characteristic pressure-flow relationship in the brain has been proposed to be partially modified by neurogenic factors, although the effect of sympathetic nerves on CBF is not evident at steady state. Young SHR, such as those used in the present study, have increased dopamine β-hydroxylase activity in blood vessels and high norepinephrine concentration in plasma, which result in increased sympathetic activity. Such sympathetic involvement is more important for the initiation of developmental hypertension than for its maintenance. Therefore, we speculated that interruption of sympathetic nerves during development of hypertension would alter the reactivity of cerebral vessels and affect CBF autoregulation in young SHR. Although resting CBF remained unchanged after acute superior cervical ganglionection, a stepwise rise in blood pressure beyond a certain level caused a greater increase in CBF to the denervated hemisphere than to the innervated one. The greater increase indicates that the upper limit of CBF autoregulation is lowered. This finding suggests that sympathetic vasoconstriction plays an important role in altering vascular resistance during an acute rise in systemic arterial pressure.

In contrast to acute denervation, chronic sympathetic interruption had a minimal effect on CBF. Two possible mechanisms might reduce the influence of chronic denervation on CBF autoregulation. First, it could be a denervation hypersensitivity that reaches a maximum about 2 weeks after ganglionection and lasts for several weeks. Second, it could be the trophic effect of sympathetic nerves. Two studies have found that 1 month after unilateral superior cervical ganglionectomy in the rabbit, the number of smooth muscle cells decreased and elasticity was reduced in the ipsilateral ear artery. Consequently, it became less distensible than did the artery contralateral to denervation. If this is the case in cerebral arteries, acute hypertension-induced vasodilatation is attenuated and CBF is kept constant.

In summary, the present study indicated that during the developmental stage of hypertension, the upper limit of CBF autoregulation is shifted to a higher level as resting blood pressure rises with age. Such an upward shift is modulated by functional but not structural adaptation of cerebral vessels to hypertension. Cervical sympathetic nerves seem to be one of the important factors affecting CBF autoregulatory capacity.

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Cerebral autoregulation in young spontaneously hypertensive rats. Effect of sympathetic denervation.
S Sadoshima, M Fujishima, F Yoshida, S Ibayashi, O Shiokawa and T Omae

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