Upper Limit of Cerebral Autoregulation During Development of Hypertension in Spontaneously Hypertensive Rats — Effect of Sympathetic Denervation

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SUMMARY The upper limit of cerebral autoregulation was studied in pre- or early established hypertension in spontaneously hypertensive rats (SHR). Cerebral blood flow (CBF) was measured with the hydrogen clearance method, and wall/lumen ratio of cerebral arteries was morphometrically measured with the freeze-substitution technique. To test autoregulation, phenylephrine was intravenously infused to cause stepwise increments of blood pressure. Unilateral superior cervical ganglionectomy was carried out to examine the effects of sympathetic denervation on CBF autoregulation and thickness of vascular wall.

Resting blood pressure at 4 weeks, 3 months and 6 months of age were 89 ± 3 mm Hg (mean ± SEM), 140 ± 6 and 165 ± 6, respectively. Baseline CBF was slightly diminished with age; 50.6 ± 9.2 ml/100 g/min at 4 weeks, 49.8 ± 8.1 at 3 months and 44.1 ± 5.6 at 6 months. The upper limit of autoregulation was markedly raised with age; 118 ± 5 mm Hg at 4 weeks, 180 ± 7 at 3 months and 208 ± 10 at 6 months. Acute sympathetic denervation lowered the upper limits to 105 ± 2, 162 ± 4 and 185 ± 7 mm Hg, respectively. On the other hand, in chronic denervation which was made at 4 weeks of age, the upper limit of autoregulation in the denervated hemisphere was slightly lower than that in innervated hemisphere at 2 months (165 ± 5 and 178 ± 6 mm Hg), and at 5 months (202 ± 8 and 215 ± 8 mm Hg) after ganglionectomy.

Wall to lumen ratio was increased with the elevation of basal blood pressure; 0.131 ± 0.008 at 4 weeks, 0.170 ± 0.005 at 3 months and 0.223 ± 0.007 at 6 months. Chronic denervation reduced such increased ratio to 0.128 ± 0.004 at 2 months and 0.196 ± 0.007 at 5 months after ganglionectomy.

We conclude that during development of hypertension, the upward shift of CBF autoregulation is closely related to an elevation of basal blood pressure, which is also correlated to the thickness of vessel wall. By means of tonic and trophic effects, sympathetic innervation appears to play an important role in regulation of CBF in response to acute elevations of systemic blood pressure.

IN CHRONIC HYPERTENSION, the upper as well as lower limits of cerebral autoregulation are raised as tested by acute elevations of basal blood pressure. Although the mechanisms are not fully understood, results obtained from observations in both humans and experimental animals indicate that hypertensive vascular alterations are primarily responsible for these shifts of autoregulation. Therefore long-term antihypertensive treatment lowers the limits of autoregulation towards those seen in normotensives, which supports the concept that structural adaptations of cerebral vessels to high blood pressure are reversible.

The present study was designed to investigate the changes of autoregulatory capacity in early, middle and late phases of sustained hypertension in spontaneously hypertensive rats (SHR). Recent findings of Nordborg and Johansson have shown that the muscularis media/radius ratio of cerebral arteries in early or prehypertensive 15 days old SHR is greater than that in age-matched normotensive rats. Mulvany et al reported that the medial thickness is not increased in mesentric arteries of 4 or 6 weeks old SHR, despite the fact that these vessels have a reduced lumen. Therefore long-term antihypertensive treatment lowers the limits of autoregulation towards those seen in normotensives, which supports the concept that structural adaptations of cerebral vessels to high blood pressure are reversible.

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hours darkness), and fed stock chow diet (Oriental Co, Japan) and tap water ad libitum. The animals, prepared for chronic denervation, were anesthetized with amobarbital, 100 mg/kg i.p. at one month of age. After a cervical midline incision, unilateral superior cervical ganglionectomy was performed. On the opposite side, superior cervical ganglion was exposed but not dissected (sham-operated). After operation, all the animals developed eye-lid ptosis and enophthalmos on the side ipsilateral to ganglionectomy, but none on the contralateral side. They were returned to their cages and fed for another 2 or 5 months under the same conditions as described. A group of animals was prepared for acute denervation of the superior cervical ganglion which was removed in a similar manner but on the day of experiment at 3 or 6 months of age.

A modified glyoxylic acid technique was used to demonstrate histochemically the presence or absence of vascular innervation at 2 or 5 months after unilateral ganglionectomy. Coronal sections of the brain, 18 μ in thickness, were cut on the cryostat, and specific yellow-green fluorescent varicosities were observed under fluorescent microscope.

Measurement of CBF

After amobarbital anesthesia (100 mg/kg i.p.), one femoral artery was cannulated for continuous measurement of arterial pressure and for sampling blood to measure arterial pH, PCO2, and PO2, with an IL meter. A femoral vein was cannulated for infusion of drug or blood. The animal’s head was fixed in a head holder, and two small burr holes were made on the skull 2 mm lateral to the bregma on each side. Two teflon-coated platinum electrodes, 200 μ in diameter, with a 1 mm portion at its top uncoated and plated with platinum black, were stereotaxically placed in the right and left parietal cortices. The depth of the electrode tip was 1 mm from the surface of the brain. The reference electrode was Ag-AgCl electrode put under the skin of the neck. The animals breathed spontaneously room air and their body temperature was kept close to 37°C with a heat lamp.

At least 3 base-line CBF measurements were made at intervals of approximately 10 min, and then, the systemic arterial pressure was raised in a step-wise manner approximately 10 mm Hg per step by an intravenous infusion of phenylephrine utilizing a Harvard infusion pump, and maintained at each level for at least 5 min during CBF measurement. Arterial gases and pH were determined in the resting state and 3 times during elevation of blood pressure. Blood, obtained previously from strain-matched donor rats, was infused into a femoral vein during blood sampling. After completing CBF measurement, the animals were sacrificed with saturated KCl into femoral vein, and the brain was examined macroscopically. When either an improper placement of the electrode or gross tissue damage caused by inserting the electrode was found, that series of CBF data were excluded from analysis.

Morphometric Study

One cervical ganglion was removed at 4 weeks of age for the chronic experiments. Either two hours (acute study), or 2 or 5 months (chronic study) after denervation, the rats were anesthetized with amobarbital (100 mg/kg) prior to the following procedure. A plastic funnel was fitted on the skull after incision of the scalp. Then, liquid nitrogen cooled isopentane was poured into the funnel on the calvarium to rapidly freeze the brain. The details of this freeze substitution method have been described elsewhere. Briefly, frozen brain was chiselled out carefully and fixed with 2% OsO2 in acetone at −70°C for 4 weeks. After dehydration in graded alcohol, the fronto-parietal cortex was cut into 10–25 blocks and embedded in Epon 812. For each sample 1 μ section was made and stained with toluidin blue. The thickness of muscular layer and the diameter of cerebral arteries were measured with an eye-piece micrometer at ×1000 magnification.

Statistics

Paired t-tests were used for statistical analysis of differences in CBF and cerebrovascular morphometry between the denervated and sham-operated intact hemispheres. The differences of resting CBF in each group of rats were evaluated by analysis variance.

Results

Vascular Innervation

Specific yellow-green fluorescent varicosities on the intact hemisphere were observed in 51.6% of pial and cortical arteries (32 among 62 arteries) at 3 months of age and 41.7% (30 among 72 arteries) at 6 months (fig. 1). Large pial arteries had more abundant plexi of
CEREBRAL AUTOREGULATION IN YOUNG SHR/Sadoshima et al

### Table 1

<table>
<thead>
<tr>
<th>Age of rats</th>
<th>No. of animals</th>
<th>MAP (mm Hg)</th>
<th>PaCO2 (mm Hg)</th>
<th>PaO2 (mm Hg)</th>
<th>pH</th>
<th>CBF at rest (ml/100g/min)</th>
<th>CVR at rest (mm Hg/ml/100g/min)</th>
<th>Upper limit of CBF autoregulation (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 weeks</td>
<td>9</td>
<td>89 ± 3</td>
<td>35.5 ± 1.8</td>
<td>88.5 ± 2.9</td>
<td>7.34 ± 0.03</td>
<td>50.6 ± 9.2</td>
<td>1.69 ± 0.15</td>
<td>118 ± 5</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>140 ± 6</td>
<td>35.8 ± 1.3</td>
<td>92.5 ± 1.9</td>
<td>7.37 ± 0.01</td>
<td>49.8 ± 8.1</td>
<td>2.79 ± 0.47</td>
<td>180 ± 7</td>
</tr>
<tr>
<td>6 months</td>
<td>7</td>
<td>165 ± 6</td>
<td>34.0 ± 0.7</td>
<td>95.4 ± 1.8</td>
<td>7.40 ± 0.01</td>
<td>44.1 ± 5.6</td>
<td>3.80 ± 0.17</td>
<td>208 ± 10</td>
</tr>
</tbody>
</table>

*<p < 0.05 vs. Innervated hemisphere.*

**CBF Autoregulation**

Mean values for resting blood pressure, blood gases, CBF and cerebral vascular resistance (CVR) are summarized in table 1. The blood pressure was elevated significantly from 89 ± 3 mm Hg at 4 weeks of age to 165 ± 6 mm Hg at 6 months. Baseline CBF averaged 50.6 ± 9.2 ml/100 g/min at 4 weeks of age, 49.8 ± 8.1 at 3 months and then became reduced to 44.1 ± 5.6 at 6 months of age, although trends between the groups were not significant. CVR, calculated from mean arterial pressure/CBF, increased significantly with age, 1.69 ± 0.15 mm Hg/ml/100 g/min at 4 weeks, to 2.79 ± 0.47 at 3 months and 3.80 ± 0.17 at 6 months. Acute or chronic sympathetic denervation had no effects on baseline CBF or CVR.

Pressure-flow relationships in animals with acute denervation are shown in figure 2. The blood flow was maintained relatively constant (only 15-20% increase over baseline) until blood pressure was raised beyond a certain level when CBF increased passively indicating the upper limit of autoregulation. In innervated hemispheres, the upper limits of autoregulation were 118 ± 5 mm Hg at 4 weeks of age, 180 ± 7 at 3 months and 208 ± 10 at 6 months, respectively, as shown in table 1. In the denervated hemisphere autoregulation limits were shifted to lower levels such as to 105 ± 2 mm Hg at 4 weeks, 162 ± 4 at 3 months and 185 ± 7 at 6 months of age. These values were significantly lower than those seen in the innervated hemisphere (<p < 0.05). An increase in CBF in response to an acute rise in blood pressure, so-called “break-through” phenomenon, was much greater in the denervated hemisphere than in the normally innervated hemisphere, and was also steeper in younger rats than in older ones.

Chronic interruption of sympathetic nerves also influenced CBF during the rise in the blood pressure (fig. 3). At two months after ganglionectomy, the upper limit of autoregulation was 165 ± 5 mm Hg in denervated hemisphere, being lower than 178 ± 6 in intact hemisphere. Similarly, at 5 months, the upper limits were 202 ± 8 and 215 ± 8 mm Hg, respectively. Differences in the upper limits of CBF autoregulation between denervated and innervated hemispheres were smaller after chronic denervation than after acute sympathectomy.

**Figure 2.** Effect of acute sympathectomy on cerebral blood flow (CBF) autoregulation in SHR of different ages. The upper limits of autoregulation are lowered in the innervated hemisphere. CBF rises more steeply in response to an acute rise in the blood pressure in rats aged 4 weeks than those aged 3 or 6 months. D = denervated; I = innervated hemisphere. *<p < 0.05 vs. innervated hemisphere. Numbers in parentheses show number of rats.
Effects of chronic sympathectomy on CBF autoregulation in 3 and 6 months old SHR, in which unilateral cervical ganglionection was made at 4 weeks of age. A greater increase in CBF is observed in denervated hemisphere during an acute rise in arterial pressure. D = denervated; I = innervated hemisphere. *p < 0.05 vs. innervated hemisphere. Numbers in parentheses show number of rats.

Wall to Lumen Ratios

Wall to lumen ratios were determined in 96 pial and cortical arteries of chronically denervated hemispheres and 84 arteries in innervated hemispheres in 5 SHR aged 4 weeks, in 140 arteries of denervated and 124 of innervated hemispheres in 6 SHR aged 3 months and in 96 arteries of denervated and 87 of innervated hemispheres in 5 SHR aged 6 months (fig. 4). The ratio in younger animals was 0.131 ± 0.008 in the denervated hemisphere, being not significantly smaller than 0.139 ± 0.007 in the innervated hemisphere. In 3 month-old rats, the ratios were 0.128 ± 0.004 in the denervated hemisphere and 0.170 ± 0.005 in the innervated, respectively, the former being 33% smaller than the latter (p < 0.02). At 6 months of age, the ratios were increased to 0.196 ± 0.007 in the denervated and to 0.223 ± 0.007 in innervated hemisphere, respectively. These differences became smaller (12%) compared with those in animals aged 3 months, but it was still statistically significant (p < 0.05).

Discussion

The major findings in the present study were as follows; the upper limits of CBF autoregulation were shifted to higher levels of blood pressure during development of hypertension. Either acute or chronic sympathetic denervation led to alterations of the autoregulatory range in response to acutely raised blood pressure. Sympathetic activity seems to have greater influences on CBF regulation in younger animals with developing hypertension than in older animals. Morphometric observations revealed that wall to lumen ratio of cerebral arteries was augmented with rise in basal blood pressure even during development of hypertension, and this was diminished by chronic sympathetic denervation.

Effects of hypertension on cerebral arterial wall structure and CBF regulation have been extensively studied. Russell demonstrated an elevated wall to lumen ratio of small cerebral arteries in hypertensive humans at post mortem examination. Joehansson and Nordborg reported an increase in wall to lumen ratio of cerebral arteries in 6.5 month-old SHR. Recent morphometric studies by Ichijima, Mulvany et al. and Hart et al. provided evidence that vascular hypertrophy is an accompaniment of chronic hypertension in animals. This structural alteration, such as medial hypertrophy and increased fibrous connective tissue, appears to be physiologically important for limiting CBF at control conditions and for protection against forced dilatation of cerebral vessels during sudden rises in blood pressure, resulting in shift of autoregulatory range to higher levels.

Previous observations, however, have focused on cerebral vascular responsiveness to blood pressure in chronic and sustained hypertension. In the present study, relatively younger animals were used with SHR in which hypertension was just developing at 4 weeks of age. An interesting finding was that even during development of hypertension, wall to lumen ratios were increased and correlated well with the rise in resting blood pressure. An increase in the thickness of vascular walls with advancing age or hypertension leads to encroachment of the arterial lumen and increased vascular resistance, which is a primary factor in maintaining resting CBF at similar levels even in differently aged animals. Increased volumes of vascular smooth muscle cells has been reported to cause enhanced vascular responses to changes of intramural pressure or effects of vasoactive substances such as the catecholamines. In addition, a concomitant increase in mass of the extracellular collagen was observed as an accompaniment of age- and hypertension-related adaptations, which may cause vessel walls to become...
stiffer\textsuperscript{15} and prevents excessive vasodilatation in acute hypertension, as discussed above.

The functional importance of neurogenic regulation of the cerebral circulation has been a matter of controversial debate for many years. However, in recent years, several observations suggest the importance of sympathetic vasoconstriction in regulating CBF during acute changes in systemic blood pressure. Bill and Linder,\textsuperscript{16} and Heistad et al.\textsuperscript{17} found that electric stimulation of sympathetic nerves attenuates increases in CBF during acute elevations of systemic arterial pressure. Besides, excessive sympathetic innervation is evident in the mesenteric arteries of prehypertensive young SHR,\textsuperscript{18} suggesting that a high sympathetic tone contributes to elevated vascular resistance. In addition to such tonic effects, the trophic influence of sympathetic nerves on vascular smooth muscle cells has attracted attention recently. Chronic interruption of vascular innervation is shown to inhibit normal growth of the ear artery of the rabbit\textsuperscript{19} and attenuates cerebral vascular hypertrophy in stroke-prone SHR.\textsuperscript{20} In the present study, superior cervical ganglion was dissected acutely or chronically to examine whether sympathetic activity plays a role in regulating CBF, and if so, whether sympathetic tone or trophic influences are more important. Baseline CBF was unchanged after either acute or chronic denervation, confirming that sympathetic tone has little effect on resting CBF. During abrupt rises in systemic arterial pressure, however, both the tonic and trophic effects of sympathetic nerves play an important role for CBF regulation. Such sympathetic influences were greater in younger SHR than in older animals. These findings suggest that cerebrovascular resistance in early hypertensive SHR is more dependent on sympathetic tone activity than occurs in sustained hypertension.

Compared with innervated vessels in this study, the wall to lumen ratios of the chronically denervated vessels were not increased even 2 months after ganglionectomy, indicating that chronic denervation is able to reduce the age- and hypertension-related increase in the ratios for a certain period of time. In other words, medial thickness observed during early development of hypertension might be directly related to sympathetic trophic effects.\textsuperscript{6} Denervated vessels have a smaller wall to lumen ratio and are more easily subjected to forced dilatation than intact vessels, and thus lower the upper limit of CBF autoregulation. Sympathetic overactivity in the hemisphere contralateral to ganglionectomy may be another factor to cause different CBF responses to blood pressure changes between innervated and chronically denervated vessels,\textsuperscript{21} although we are unable to clarify this question from results of the present experimental design.

In summary, the present study indicates that autoregulatory range of CBF is substantially influenced by the level of basal blood pressure and the resistance of cerebral vessel walls, the latter may be modulated in part by two different results of sympathetic innervation; one acute tonic effect and another chronic effect associated with medial hypertrophy and augmented responses.

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