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, phentolamine 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. Mulyavan 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. We speculated, therefore, that during development of hypertension the reactivity of cerebral arteries to changes in blood pressure might be different from those seen in chronic hypertension, and it may be influenced by factors other than structural adaptation, i.e. pressure independent aberration of arteries. Cerebral arteries have a dense sympathetic nerve innervation although the sympathet- ic effects on CBF have been the subject of intense controversy. Increased sympathetic activity and hyperinnervation are evident in young SHR, suggesting that if an exaggerated sympathetic discharge is interrupted, vascular constrictor response to a rise in blood pressure might be altered. In the present study, we examined whether the upper limit of autoregulation is altered with a rise of resting blood pressure during states of development of hypertension, and whether acute or chronic sympathetic denervation influences CBF autoregulation. Concurrently, we investigated whether autoregulatory changes in hypertension are accompanied by morphological alterations of the cerebral vascular wall.

Materials and Methods

Cervical Ganglionectomy

Fifty-three SHR, aged 4 weeks, 3 months and 6 months, were used for the present study. The animals were housed in air-conditioned quarters (25° C) with light control (12 hours of light alternating with 12
hours darkness), and fed stock chow diet (Oriental Co.,
Japan) and tap water ad libitum. The animals, prepared
for chronic denervation, were anesthetized with amo-
obarbital, 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 dissect-
ed (sham-operated). After operation, all the animals
developed eye-lid ptosis and enophthalmos on the side
ipsilateral to ganglionectomy, but none on the contra-
lateral 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 \( \mu \) in
thickness, were cut on the cryostat, and specific yel-
low-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 measure-
ment 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 electrode, 200 \( \mu \) in diameter, with a 1 mm
portion at its top uncoated and plated with platinum
black, were stereotactically 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 elec-
trode 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\( ^\circ \) 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 intra-
venous 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 previous-
ly 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 anesthesitized with amobarbi-
tal (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, fro-
zened brain was chiselled out carefully and fixed with 2% OsO2 in acetone at \(-70^\circ\) C for 4 weeks. After dehydra-
tion in graded alcohol, the fronto-parietal cortex was
cut into 10–25 blocks and embedded in Epon 812. For
each sample 1 \( \mu \) 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 \( \times 1000 \) 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
TABLE 1  Average Values for Mean Arterial Pressure (MAP), Arterial Acid-base Parameters, Cerebral Blood Flow (CBF), Vascular Resistance (CVR) and Upper Limit of Autoregulation

<table>
<thead>
<tr>
<th>Age of rats</th>
<th>4 weeks</th>
<th>3 months</th>
<th>6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>9</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>89 ± 3</td>
<td>140 ± 6</td>
<td>165 ± 6</td>
</tr>
<tr>
<td>PaCO2 (mm Hg)</td>
<td>35.5 ± 1.8</td>
<td>35.8 ± 1.3</td>
<td>34.0 ± 0.7</td>
</tr>
<tr>
<td>PaO2 (mm Hg)</td>
<td>88.5 ± 2.9</td>
<td>92.5 ± 1.9</td>
<td>95.4 ± 1.8</td>
</tr>
<tr>
<td>pH</td>
<td>7.34 ± 0.03</td>
<td>7.37 ± 0.01</td>
<td>7.40 ± 0.01</td>
</tr>
<tr>
<td>CBF at rest (ml/100g/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Innervated hemisphere</td>
<td>50.6 ± 9.2</td>
<td>49.8 ± 8.1</td>
<td>44.1 ± 5.6</td>
</tr>
<tr>
<td>Denervated hemisphere</td>
<td>52.1 ± 7.1</td>
<td>48.4 ± 7.1</td>
<td>47.0 ± 7.8</td>
</tr>
<tr>
<td>CVR at rest (mm Hg/ml/100g/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Innervated hemisphere</td>
<td>1.69 ± 0.15</td>
<td>2.79 ± 0.47</td>
<td>3.80 ± 0.17</td>
</tr>
<tr>
<td>Denervated hemisphere</td>
<td>1.64 ± 0.05</td>
<td>2.83 ± 0.33</td>
<td>3.74 ± 0.10</td>
</tr>
<tr>
<td>Upper limit of CBF autoregulation (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Innervated hemisphere</td>
<td>118 ± 5</td>
<td>180 ± 7</td>
<td>208 ± 10</td>
</tr>
<tr>
<td>Denervated hemisphere</td>
<td>105 ± 2*</td>
<td>162 ± 4*</td>
<td>185 ± 7*</td>
</tr>
</tbody>
</table>

*p < 0.05 vs. Innervated hemisphere.

fluorescent varicosities than did the small cortical arteries. On the denervated side, however, the plexi of fluorescent varicosities were present on only 14.3% of the pial and cortical arteries (13 among 91 arteries) at 3 months of age and 8.9% (5 among 56 arteries) at 6 months. In addition, the fluorescence was, when present, weaker and more sparse than that observed in the innervated hemisphere. These results indicate that unilateral cervical ganglionectomy produces sustained and significant reductions of fluorescent varicosities in the ipsilateral pial and cortical arteries.

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 in 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 sympathetomy on CBF autoregulation in 3 and 6 months old SHR, in which unilateral cervical ganglionectomy 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. Johansson 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 thicker.
stiffer 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, and Heistad et al. 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, 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 and attenuates cerebral vascular hypertrophy in stroke-prone SHR. 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. 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, 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.

References
12. Mulvany MJ, Hansen PK, Aalkjaer C: Direct evidence that the greater contractility of resistance vessels in spontaneously hypertensive rats is associated with a narrowed lumen, a thickened media, and an increased number of smooth muscle cell layer. Circ Res 43: 854–864, 1978
Upper limit of cerebral autoregulation during development of hypertension in spontaneously hypertensive rats--effect of sympathetic denervation.
S Sadoshima, F Yoshida, S Ibayashi, O Shiokawa and M Fujishima

Stroke. 1985;16:477-481
doi: 10.1161/01.STR.16.3.477

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