Regional Cerebral Blood Flow During Hypotension in Normotensive and Stroke-Prone Spontaneously Hypertensive Rats: Effect of Sympathetic Denervation

Seizo Sadoshima, M.D.,* and Donald D. Heistad, M.D.

SUMMARY This study was performed to determine whether, in hypertensive and normotensive rats, chronic sympathetic denervation impairs cerebral vasodilator responses during hypotension, and to determine whether there are regional differences in the autoregulatory response of brain vessels during hypotension. The superior cervical ganglion was removed on one side in stroke-prone spontaneously hypertensive rats (SHRSP) and normotensive (WKY) rats. Cerebral blood flow (CBF) was measured with microspheres when the rats were 5–6 months old. Chronic sympathetic denervation had little or no effect on cerebral vasodilator responses during acute hypotension in SHRSP and WKY. We suggest that the increase in incidence of ischemic infarction that we have observed previously after chronic sympathetic denervation in SHRSP probably is not the result of ischemia during episodes of hypotension. We also observed major regional differences in the response of cerebral vessels during acute hypotension in SHRSP: blood flow to brainstem was preserved better than flow to cerebrum and cerebellum. Thus the “lower limit” of the autoregulatory plateau differs in various regions of the brain in SHRSP.

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SYMPATHETIC DENERVATION in young stroke-prone spontaneously hypertensive rats (SHRSP) impairs development of cerebral vascular hypertrophy and increases the incidence of cerebral hemorrhage and ischemic infarction. Chronic sympathetic denervation also impairs the cerebral vasoconstrictor response to increases in arterial pressure. It seems likely that impairment of the cerebral vasoconstrictor response during increases in pressure may be an important mechanism by which sympathetic denervation results in cerebral hemorrhage in SHRSP.

Hemodynamic mechanisms by which sympathetic denervation leads to ischemic infarction in SHRSP are not clear. One possibility is that sympathetic denervation impairs autoregulatory cerebral vasodilatation during hypotension and thereby leads to ischemia. In the rabbit ear artery, vessels are less distensible after chronic sympathetic denervation. In SHRSP, cerebral vasodilator responses to seizures are not increased after sympathetic denervation despite a reduction in wall/lumen ratio. This finding suggests that sympathetic denervation may decrease the compliance of cerebral vessels and impair dilator responses. Thus, the first goal of this study was to determine whether chronic sympathetic denervation impairs cerebral vasodilator responses during hypotension, and thus might be a mechanism by which sympathetic denervation leads to ischemic infarction in SHRSP.

The pressure-flow relationship of cerebral vessels is shifted to the right in chronic hypertension. Thus, the “lower limit” of autoregulation is increased in chronic hypertension, so that reduction in pressure to levels that do not decrease cerebral blood flow (CBF) in normotensive patients results in decreases in CBF in hypertensives. Because total CBF was measured in previous studies, it is not known whether there are regional differences in the pressure-flow relationship. In previous studies we have demonstrated regional differences in the response to decreases in cerebral perfusion pressure in normotensive dogs and rabbits. We found that hypotension redistributes CBF, so that blood flow to brainstem is maintained better than flow to cerebrum or cerebellum. Thus, the second goal of this study was to determine whether there are regional differences in the autoregulatory response to hypotension in SHRSP.

Methods

We studied nine SHRSP and eight WKY that were fed American rat chow and 1% saline drinking water. When the rats were one month old, they were anesthetized with pentobarbital (3 mg/100 g, i.p.). The superior or cervical ganglion was removed on one side. On the other side the ganglion was exposed but not removed.

The rats were studied at five to six months of age. The SHRSP weighed 251 ± 13 gm and the WKY weighed 302 ± 6 gm. The rats were anesthetized with pentobarbital (4 mg/100 gm, i.p.), intubated and ventilated with room air and supplemental oxygen. Heparin (500 U/kg, i.v.) and gallamine triethiodide (2 mg/kg, i.v.) were administered for anticoagulation and skeletal muscle paralysis, respectively. Body temperature was controlled with a heating pad.

After a left thoracotomy, a flanged PE 10 cannula was placed in the left atrium for injection of microspheres. Polyethylene catheters were inserted into both brachial arteries for withdrawal of reference blood samples. A femoral artery was cannulated for continuous measurement of arterial pressure. Arterial blood...
gases and pH were measured before each injection of microspheres.

Microspheres (15 μm in diameter) were injected into the left atrium to measure blood flow. Injections of microspheres labelled with 85Sr, 46Sc, 113Sn, or 141Ce allowed us to make three separate measurements of CBF in each rat. The vials containing microspheres were shaken vigorously for several minutes on a Vortex mixer prior to injection. We injected approximately 104 microspheres into the left atrium in 10–15 seconds and flushed the injection line with 0.1 ml of warmed saline. Beginning 10 seconds before injection of microspheres and continuing for 60 seconds afterwards, reference arterial blood samples were withdrawn with a Harvard pump at 0.206 ml/min.

After injection of microspheres under control conditions, we withdrew blood from the femoral arterial cannula to decrease arterial pressure by approximately 1 mmHg/sec. When arterial pressure was approximately 70% of the initial value, pressure was maintained constant for about 10 seconds before a second batch of microspheres was injected. Arterial pressure was maintained constant for 4–6 minutes, and then reduced to approximately 50% of the initial value. After about 10 seconds, microspheres were injected for a third measurement of CBF.

At the end of each experiment, the rat was killed with intravenous KCl. The brain was cut into eight samples: right and left brainstem, cerebellum, anterior cerebrum (frontal and parietal cortex) and posterior cerebrum. We obtained and examined separately tissue samples from the anterior cerebrum because sympathetic innervation appears to be particularly dense in this region. The brain samples weighed 0.1 to 0.5 gm. The tissues were placed in plastic tubes and counted in a gamma counter. Cerebral blood flow was calculated from the equation: CBF = C \(_B\) \times RBF + C \(_R\), where CBF = cerebral blood flow in ml/min per 100 gm, C \(_B\) = counts per gm of brain, RBF = reference blood flow in ml/min, and C \(_R\) = total counts in the reference arterial blood samples.

We have estimated the number of spheres in each tissue sample. Based on the assumption that cardiac index = 250 ml/min × kg in rats, we estimate that the brain receives 2,000 spheres under control conditions. We estimate that the number of spheres per region (left and right anterior and posterior cerebrum, cerebellum, and brainstem) is 100–750. We estimate that the upper 95% confidence limit, based on 100 to 750 spheres/region, is 10–30%. The number of counts for each sample was approximately 400–6,000, so that the maximum statistical error based on counts/ samples = \(\frac{\sqrt{400}}{400}\) to \(\frac{\sqrt{6000}}{6000}\) = 1–5%.

Statistical analysis was performed with the t-test for paired data, to compare blood flow to the innervated and denervated hemispheres. We used analysis of variance and Tukey’s test to compare blood flow to brainstem, cerebrum, and cerebellum during hypotension.

**Results**

**Effects of Hypotension in SHRSP**

Autoregulatory dilatation of cerebral vessels during hypotension was impaired in SHRSP (table 1). Reduction of mean arterial pressure to 140 mmHg produced only a small decrease in cerebral vascular resistance with a significant reduction in CBF. Further reduction of arterial pressure to 109 mmHg tended to increase cerebral vascular resistance, presumably from passive collapse of the vessels, with a further reduction in CBF. Blood flow was not significantly different in the innervated and denervated sides in any area of the brain. Thus, sympathetic denervation did not alter the response to hypotension in SHRSP.

There were regional differences in the cerebral vascular response to hypotension (table 1 and fig. 1). Hypotension produced larger reductions in blood flow

**TABLE 1 Effects of Hypotension in SHRSP**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Hypotension 1</th>
<th>Hypotension 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>195 ± 6</td>
<td>140 ± 6</td>
<td>109 ± 6</td>
</tr>
<tr>
<td>Cerebral vascular resistance (mmHg × ml/min × 100 gm)</td>
<td>2.82 ± 0.21</td>
<td>2.60 ± 0.25</td>
<td>2.79 ± 0.33</td>
</tr>
<tr>
<td>Arterial blood gases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PaCO2 (mmHg)</td>
<td>36 ± 2.6</td>
<td>37 ± 2.9</td>
<td>35 ± 2.2</td>
</tr>
<tr>
<td>pH</td>
<td>7.30 ± 0.04</td>
<td>7.31 ± 0.04</td>
<td>7.34 ± 0.03</td>
</tr>
<tr>
<td>PaO2</td>
<td>169 ± 16</td>
<td>164 ± 20</td>
<td>161 ± 18</td>
</tr>
<tr>
<td>Brain blood flow (ml/min per 100 gm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cerebrum</td>
<td>D = 76 ± 8</td>
<td>I = 73 ± 6</td>
<td>D = 58 ± 7</td>
</tr>
<tr>
<td>anterior cerebrum</td>
<td>65 ± 5</td>
<td>64 ± 5</td>
<td>55 ± 6</td>
</tr>
<tr>
<td>brainstem</td>
<td>65 ± 9</td>
<td>66 ± 10</td>
<td>68 ± 10</td>
</tr>
<tr>
<td>cerebellum</td>
<td>75 ± 14</td>
<td>73 ± 12</td>
<td>64 ± 12</td>
</tr>
</tbody>
</table>

Values are mean ± SE obtained in nine SHRSP.
D = blood flow to denervated side
I = blood flow to innervated side
*no values were significantly different on the denervated and innervated sides
During hypotension in WKY, blood flow tended to be better preserved to brainstem than to cerebrum or brainstem (fig. 1), but there were no statistically significant differences ($p = 0.07$).

**Discussion**

There are two major new findings in this study. *First*, chronic sympathetic denervation in SHRSP has little or no effect on cerebral vasodilator responses during acute hypotension. Thus, the increase in incidence of ischemic infarction after chronic sympathetic denervation in SHRSP probably is not the result of ischemia during episodes of hypotension. *Second*, there are important regional differences in the autoregulatory response of cerebral vessels in SHRSP during acute hypotension: blood flow to brainstem is preserved better than flow to cerebrum and cerebellum. Thus the “lower limit” of autoregulation differs in various regions of the brain in SHRSP.

In this section we will focus on 1) effects of sympathetic nerves on cerebral vessels and 2) regulation of CBF in chronic hypertension.

**Effects of Sympathetic Nerves on Cerebral Vessels**

Electrical stimulation of sympathetic pathways appears to have little effect on CBF during normotension. Recently we have obtained direct evidence that sympathetic stimulation increases resistance of large arteries, but resistance of distal vessels either tends to decrease or decreases significantly; thus, total cerebral vascular resistance remains unchanged during sympathetic stimulation. In contrast, numerous studies indicate that, during acute increases in arterial pressure, sympathetic stimulation constricts cerebral vessels, attenuates increases in CBF, and protects the blood-brain barrier against disruption.

Sympathetic denervation has minimal effect on CBF under normal conditions. Acute denervation or 1–3 weeks of denervation has been

**Table 2. Effects of Hypotension in WKY**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Hypotension 1</th>
<th>Hypotension 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>105 ± 6</td>
<td>75 ± 5</td>
<td>48 ± 4</td>
</tr>
<tr>
<td>Cerebral vascular resistance (mmHg x ml/min x 100 gm)</td>
<td>1.76 ± 0.27</td>
<td>1.23 ± 0.22</td>
<td>1.27 ± 0.17</td>
</tr>
<tr>
<td>Arterial blood gases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PaCO₂ (mmHg)</td>
<td>36 ± 1.2</td>
<td>37 ± 0.6</td>
<td>36 ± 1.9</td>
</tr>
<tr>
<td>pH</td>
<td>7.34 ± 0.02</td>
<td>7.33 ± 0.02</td>
<td>7.36 ± 0.03</td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
<td>95 ± 1.9</td>
<td>117 ± 17</td>
<td>100 ± 17</td>
</tr>
<tr>
<td>Brain blood flow (ml/min per 100 gm)</td>
<td>D</td>
<td>I</td>
<td>D</td>
</tr>
<tr>
<td>cerebrum</td>
<td>67 ± 10</td>
<td>66 ± 12</td>
<td>65 ± 8</td>
</tr>
<tr>
<td>anterior cerebrum</td>
<td>64 ± 10</td>
<td>62 ± 11</td>
<td>63 ± 10</td>
</tr>
<tr>
<td>brainstem</td>
<td>50 ± 8</td>
<td>50 ± 9</td>
<td>46 ± 6</td>
</tr>
<tr>
<td>cerebellum</td>
<td>63 ± 7</td>
<td>61 ± 7</td>
<td>49 ± 4</td>
</tr>
</tbody>
</table>

Values are mean ± SE obtained in eight WKY
D = blood flow to denervated side
I = flow to innervated side
*no values were significantly different on the denervated and innervated sides
observed to have small effects22, 23 or no effect10, 24 on CBF during hypotension. Thus, the role of sympathetic tone in cerebral vascular responses to hypotension appears to be small.

Sympathetic denervation at one month11, 2 but not at three months3 of age inhibits development of cerebral vascular hypertrophy in SHRSP. Thus, sympathetic nerves appear to exert a "trophic" effect on cerebral vessels, during their rapid period of growth, which promotes the development of vascular hypertrophy. This trophic effect of sympathetic nerves apparently contributes to augmented vasoconstrictor responses of cerebral vessels during acute increases in arterial pressure.3 The present study indicates that, although chronic sympathetic denervation alters cerebral vasoconstrictor responses to acute increases in arterial pressure, cerebral vasodilator responses to acute decreases in pressure are unchanged.

We would like to speculate about possible mechanisms by which sympathetic denervation leads to ischemic infarction in SHRSP. First, it is unlikely that thrombi or emboli account for this effect. We are not aware of evidence that sympathetic denervation predisposes to thrombosis and, in our experience, thrombi are common but small in ischemic infarction of SHRSP.3 Second, because the "lower limits" of the autoregulatory plateau are shifted to a higher pressure in chronic hypertension, SHRSP presumably have increased susceptibility to ischemic infarction during episodes of hypotension. Data in the present study, however, indicate that sympathetic denervation does not impair the cerebral autoregulatory responses during hypotension. We suggest, therefore, that ischemic infarction after sympathetic denervation in SHRSP probably is not produced by episodes of hypotension. Third, we suggest that ischemic infarction after sympathetic denervation may be related to impaired cerebral vasoconstrictor responses to acute increases in arterial pressure. Cerebral autoregulatory responses to increases in pressure are impaired by chronic sympathetic denervation in SHRSP.3 It is possible that acute increases in pressure produce passive dilatation in chronically denervated cerebral vessels, disruption of the blood-brain barrier, and that disruption of the barrier leads to ischemia and infarction. This hypothesis about the pathogenesis of ischemic infarction has been proposed previously by other investigators and, we should point out, is not yet supported by direct evidence.

Regulation of CBF in Chronic Hypertension

These experiments were performed in anesthetized rats because measurement of CBF with microspheres in unanesthetized rats requires cannulation and ligation of a carotid artery. We considered the possibility that the use of anesthesia in these experiments might contribute to differences in the pressure-flow relationship in SHRSP and WKY. Resting blood flow was similar in the two groups, however, which suggests that the level of anesthesia was similar. In addition, we have demonstrated previously normal autoregulatory responses of cerebral vessels during increases in arterial pressure in anesthetized rats.3

This study confirms numerous previous studies which indicate that the pressure-flow relationship of cerebral vessels is shifted by chronic hypertension5-9. We found normal levels of CBF when mean arterial pressure was reduced to 75 mmHg in normotensive rats. In contrast, when mean arterial pressure was reduced to 140 mmHg in SHRSP, CBF decreased. Thus, the "lower limit" of autoregulation is shifted by more than 70 mmHg in SHRSP.

A new finding in this study was that blood flow during acute hypotension was significantly greater in brainstem than in cerebrum or cerebellum of SHRSP. These regional differences in vascular responses presumably protect the brainstem during hypotension.

Mechanisms that account for regional differences in cerebral vascular responses to hypotension are not clear. We are not aware of studies that have compared structural properties of vessels in the brainstem, cerebrum, and cerebellum, or studies that have compared myogenic properties of vessels from different regions in vitro. A recent study indicates that glucose utilization increases in several areas of the brainstem, but not cerebrum or cerebellum, during acute hypotension.25 Thus, the higher levels of blood flow to brainstem than to cerebrum and cerebellum during hypotension may reflect increases in metabolism in the brainstem, rather than a more effective autoregulatory response to changes in arterial pressure.

Acknowledgments

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References

Regional CBF in Hypertensive Rats/Sadoshima & Heistad

Regional CBF in Hypertensive Rats/Sadoshima & Heistad

Experimental Cerebral Vasospasm. Part 2. Contractility of Spastic Arterial Wall

Shiro Nagasawa, M.D., Hajime Handa, M.D., Yoshito Naruo, M.D., Hitoshi Watanabe, M.D., Kouzo Moritake, M.D., and Koizaburo Hayashi, Ph.D.*

SUMMARY We studied the mechanical properties of canine basilar arteries subjected to experimental subarachnoid hemorrhage (SAH). Smooth muscle contractility was determined from pressure-diameter curves obtained after subjecting the basilar arteries to three different conditions: Krebs-Ringer solution (KRS), Krebs-Ringer solution containing serotonin (5HT), and saline solution.

Pressure-diameter curves obtained in KRS and 5HT are biphasic and have sharp flexions that yield flexion points. The pressure level at the flexion point increases as vasospasm increases. Strong constriction is retained up to that pressure above which the constriction is released abruptly. These data suggest that increasing the intraluminal pressure dilates the spastic artery nonlinearly and that induced hypertension could relieve the cerebral ischemia caused by vasospasm if blood pressure were maintained above the flexion point. The contractile response of spastic arterial wall to serotonin remains unchanged after SAH although the spastic constriction increases progressively and becomes maximal seven days after SAH. The lesser the arterial wall stiffness, the more efficiently it constricts. This means that the diminution of arterial stiffness observed after SAH might be one of the factors promoting the development of vasospasm.

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Although the phenomenon of cerebral vasospasm following the rupture of an aneurysm is well recognized and has been described in many publications, there have been few studies on the mechanical properties of arterial walls subjected to subarachnoid hemorrhage (SAH). There is a controversy as to whether the contractile response of a spastic arterial wall to vasoconstrictors increases as compared to a normal wall.1, 2 While it has been demonstrated in the intracranial and extracranial arteries that the change in connective tissue contents (collagen and elastin) produced in the processes of aging and systemic hypertension alters the contractility of walls,3, 4 there is little information on the correlation that may exist between the connective tissue contents and the contractility of arterial walls subjected to SAH.

In a previous paper, we demonstrated that the vasospasm is attributable to the constriction of vascular smooth muscle and hence is reversible, and that the passive mechanical properties of vascular walls observed under the relaxed condition of the smooth mus-
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