Effects of sympathetic nerves on cerebral blood flow in awake dogs

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CEREBRAL VESSELS are innervated densely by sympathetic nerve fibers. Although cerebral blood vessels are densely innervated by sympathetic nerve fibers, the functional significance of these nerves is controversial. Because previous studies have been primarily performed in anesthetized animals, it is possible that failure to observe prominent neural control of the cerebral circulation was secondary to anesthetic-induced depression of the sympathetic nervous system. Therefore, we studied sympathetic control of the cerebral circulation in 11 awake chronically instrumented dogs. Total and regional cerebral blood flow was measured with 15-μm microspheres at control blood pressure and during three levels of progressive hemorrhagic hypotension. Sympathetic nerves had only a small effect (11% decrease; P < 0.05) on flow to the cerebrum during moderate hypotension (mean arterial pressure 49 ± 2 mmHg). Also, during severe hypotension, there was a bilateral redistribution of brain blood flow that tended to preserve flow to the medulla. Although these studies suggest that sympathetic nerves have a definite constrictor effect on cerebral vessels, the data support the concept that the functional importance of sympathetic nerves to cerebral vessels is limited.

MARCUS, MELVIN L., AND DONALD D. HEISTAD. Effects of sympathetic nerves on cerebral blood flow in awake dogs. Am. J. Physiol. 236(4): H549-H553, 1979 or Am. J. Physiol.: Heart Circ. Physiol. 5(4): H549-H553, 1979.—Although cerebral blood vessels are densely innervated by sympathetic nerve fibers, the functional significance of the nerves is controversial. Because previous studies have been primarily performed in anesthetized animals, it is possible that failure to observe prominent neural control of the cerebral circulation was secondary to anesthetic-induced depression of the sympathetic nervous system. Therefore, we studied sympathetic control of the cerebral circulation in 11 awake chronically instrumented dogs. Total and regional cerebral blood flow was measured with 15-μm microspheres at control blood pressure and during three levels of progressive hemorrhagic hypotension. Sympathetic nerves had only a small effect (11% decrease; P < 0.05) on flow to the cerebrum during moderate hypotension (mean arterial pressure 49 ± 2 mmHg). Also, during severe hypotension, there was a bilateral redistribution of brain blood flow that tended to preserve flow to the medulla. Although these studies suggest that sympathetic nerves have a definite constrictor effect on cerebral vessels, the data support the concept that the functional importance of sympathetic nerves to cerebral vessels is limited.

METHODS

Surgical preparation. Eleven mongrel dogs weighing 18–25 kg were anesthetized with sodium pentobarbital (30 mg/kg iv), intubated, and ventilated with a Harvard respirator and room air. A left thoracotomy was performed under sterile conditions, and cannulas were placed in the left atrium and internal thoracic artery. The cannulas were filled with heparin (1,000 U/ml) and exteriorized with a skin button placed between the scapulae. The chest was closed, and the dogs were given 4–12 days to recover. Eighteen hours prior to the study, the dogs were again anesthetized with sodium pentobarbital (15–20 mg/kg iv). In nine of the dogs the left superior cervical ganglion was cut and the wound was closed; in the other two dogs, a small device was implanted that allowed us to acutely crush the cervical sympathetic nerves. The end of this device was exteriorized and the wound was closed.

Measurement of cerebral blood flow. Cerebral blood flow was measured with 15-μm labeled microspheres as we have previously described (12). Briefly, microspheres labeled with either 46Sc, 85Sr, 141Ce, 125I, or 95Nb were injected into the left atrium in about 10 s; the cannula was flushed with 5 ml saline at 37°C during the subsequent 20 s. The vial containing the spheres (mixed with Tween-80) was vigorously agitated in a vortex mixer for 3–5 min prior to injection. The number of microspheres in each injection varied between 1 and 3 × 10⁶. A reference arterial sample was withdrawn from the cannula in the internal thoracic artery beginning just before the microsphere injection and continuing for 2–3 min thereafter. The interval between subsequent injections was...
15-45 min. At the end of the study, the dog was anesthetized with sodium pentobarbital and killed with KCl given intravenously. The brain was removed and divided into 39 tissue samples, including left and right medulla, pons, diencephalon-midbrain, and multiple samples of the left and right cerebrum and cerebellum. Samples of cortical cerebral gray and white matter were also obtained from both sides of the brain. The weight of the individual brain samples was 0.5-4.6 g. Flows from multiple samples are reported as the weighted mean.

After weighing, the brain specimens and reference blood samples were placed in plastic tubes and counted for 5 min in a 3-in, well-type gamma counter. The energy windows used were: 20-50 keV (125I); 125-175 keV (141Ce); 650-800 keV (60Nb), and 800-1500 keV (68Sc). Isotope separation was performed according to standard techniques (18).

Tissue weights and output from the gamma counter were punched on a paper tape that was subsequently processed with a PDP-11 computer. Cerebral blood flow was calculated as:

\[ \text{CBF} = \frac{C_b \times 100 \times \text{RBF}}{C_r} \]

where \( C_b \) is counts per gram of brain tissue, RBF is reference blood flow (the rate of withdrawal of blood samples from the reference artery), and \( C_r \) is total counts in the reference arterial blood. Cerebrovascular resistance was calculated by dividing mean arterial pressure by cerebral blood flow.

**Environmental chamber.** The dimensions of the environmental chamber were 1.2 \times 0.7 \times 1.1 m. A 4000 BTU air conditioner with a recirculating fan was used to maintain constant temperature and to ensure adequate mixing of the gases within the chamber. One side of the chamber was Plexiglas and contained two circular access ports (12 cm diam) so that the dog could be observed and was accessible to the investigator during the study. The \( \text{CO}_2 \) and \( \text{O}_2 \) content in the chamber could be varied by forcing \( \text{CO}_2 \) and \( \text{O}_2 \) gas into the chamber, and in turn, blood gases could be kept relatively constant even though the dogs varied their respiratory minute volume. The chamber, which was relatively air tight, had a pressure-relief valve that prevented air pressure within the box from rising more than 1-2 mmHg. The \( \text{O}_2 \) and \( \text{CO}_2 \) content in the chamber was monitored continuously with a Beckman \( \text{O}_2 \) analyzer OM-11 and a Beckman medical gas analyzer LB-1, respectively. Arterial blood gases were monitored frequently with an Instrumentation Laboratory ultramicro-gas analyzer and just prior to and after each injection of microspheres.

**Protocol.** On the day of the study the dog was placed in the chamber. A harness kept the dog in a standing position and prevented excessive movement. In eight of the dogs, 7-15 mg iv morphine were given to calm the animals. In the dogs with the implanted denervating device, it was activated 30 min prior to the control measurement of cerebral blood flow. Ptoxia, enophthalmos, and pupillary constriction on the side of the sympathectomy were present in all the dogs in the study and appeared abruptly after we activated the denervating device in the two dogs so studied. The control measurement of cerebral blood flow was not made until the dog had been in the chamber for more than 1 h. Arterial pressure was measured with a P23 Statham pressure transducer leveled at the midchest position and attached to an oscillographic recorder. After injecting microspheres under control conditions, the dogs were progressively bled by withdrawing blood via the arterial cannula. We attempted to obtain cerebral blood flow measurements at three levels of mean arterial pressure during hemorrhage: 60-75 mmHg, 45-60 mmHg, and less than 45 mmHg. Because of technical difficulties in adjusting the level of hemorrhage, chamber, and blood gas concentrations nearly simultaneously, it was not possible to obtain measurements at all three levels of hypotension in all the dogs. In addition, only five of the dogs tolerated extreme hypotension (arterial pressure <45 mmHg).

In general, the blood pressure and blood gases were stabilized for a 5- to 15-min interval at each level of hemorrhage and then the microspheres were injected.

**Statistical analysis.** All data are presented as means ± SE. Data were analyzed using a paired t test or analysis of variance. Intergroup differences were tested with Duncan's test (20). In analyzing the effects of hypotension on the distribution of cerebral blood flow, flow to a region of the brain during hypotension was expressed as a percent of the control flow to that region.

**RESULTS**

**Effects of hemorrhage on mean arterial pressure and blood gases.** Mean arterial pressure did not fall to the 60-75 mmHg range until blood loss was 38 ± 8 ml/kg. This suggests that intense activation of the sympathetic nerves prevented blood pressure from falling in response to less severe hemorrhage (21). Despite the stimulation of ventilation associated with hemorrhagic hypotension (2), blood gases were maintained relatively constant by altering the gas content of the environmental chamber (see Table 1). A mild metabolic acidosis developed in the dogs subjected to severe hypotension.

**Effects of hemorrhagic hypotension on total cerebral blood flow.** Total cerebral blood flow did not fall until mean arterial pressure had decreased to a moderate level of hypotension and, as expected, hypotension was associated with a decrease in cerebral vascular resistance (Table 1 and Fig. 1). Thus, autoregulation was very effective in maintaining cerebral blood flow during changes in mean arterial pressure. During severe hypotension, cerebral vascular resistance tended to increase rather than decrease, perhaps from passive collapse of the vascular channels.

**Effects of sympathetic nerves on cerebral blood flow during hemorrhagic hypotension.** Because the data from the dogs subjected to denervation 30 min and 18 h prior to study were similar, the results of the two groups were combined. Under control conditions, blood flow to the denervated side of the brain was nearly identical to that to the innervated side of the brain in all regions. Thus, the level of resting sympathetic tone to cerebral blood vessels was probably very low. As blood pressure was lowered, the only significant difference noted between the innervated and denervated sides of the brain occurred in the cerebrum during moderate hemorrhagic hypotension. Under these conditions the flow to the innervated cerebrum was about 11% less (P < 0.05) than the flow to the denervated cerebrum (see Table 1 and Fig. 1).
TABLE I. Effects of hemorrhage

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Mild Hypotension</th>
<th>Moderate Hypotension</th>
<th>Severe Hypotension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>110 ± 4.8</td>
<td>69 ± 1</td>
<td>49 ± 2</td>
<td>37 ± 3</td>
</tr>
<tr>
<td>Total brain blood flow, ml/ (min-100 g)</td>
<td>75.9 ± 10.4</td>
<td>72.5 ± 8.0</td>
<td>57.5 ± 5.7</td>
<td>33.0 ± 7.5</td>
</tr>
<tr>
<td>Total brain vascular resistance, (X arterial pressure/[ml/ (min-100 g)])</td>
<td>1.77 ± 0.25</td>
<td>1.01 ± 0.12</td>
<td>0.88 ± 0.07*</td>
<td>1.12 ± 0.6</td>
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Systemic blood gases

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<tbody>
<tr>
<td>PO₂, mmHg</td>
<td>100.6 ± 3.8</td>
<td>102.7 ± 5.1</td>
<td>100.8 ± 7.3</td>
<td>100.6 ± 7.7</td>
</tr>
<tr>
<td>PCO₂, mmHg</td>
<td>34.4 ± 0.7</td>
<td>35.2 ± 0.5</td>
<td>33.6 ± 1.0</td>
<td>36.6 ± 3.4</td>
</tr>
<tr>
<td>pH</td>
<td>7.38 ± 0.02</td>
<td>7.31 ± 0.04</td>
<td>7.31 ± 0.03</td>
<td>7.25 ± 0.04*</td>
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Regional cerebral blood flow, ml/(min-100 g)

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<tbody>
<tr>
<td>Cerebrum</td>
<td>78 ± 11.0</td>
<td>75 ± 10.4</td>
<td>74 ± 8.8</td>
<td>76 ± 10.2</td>
<td>60 ± 5.3</td>
<td>54 ± 6.2†</td>
</tr>
<tr>
<td>Cerebral gray</td>
<td>105 ± 18.1</td>
<td>94 ± 1619</td>
<td>96 ± 1617</td>
<td>94 ± 1816</td>
<td>64 ± 4.9</td>
<td>66 ± 4.7</td>
</tr>
<tr>
<td>Cerebral white</td>
<td>29 ± 4.4</td>
<td>29 ± 5.6</td>
<td>29 ± 3.3</td>
<td>30 ± 5.2</td>
<td>18 ± 1.7</td>
<td>19 ± 2.6</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>75 ± 9.4</td>
<td>81 ± 10.0</td>
<td>77 ± 5.3</td>
<td>78 ± 3.9</td>
<td>66 ± 2.8</td>
<td>65 ± 3.9</td>
</tr>
<tr>
<td>Medulla</td>
<td>55 ± 6.7</td>
<td>50 ± 5.6</td>
<td>47 ± 3.5</td>
<td>46 ± 3.7</td>
<td>43 ± 3.2</td>
<td>44 ± 5.3</td>
</tr>
<tr>
<td>Pons-midbrain-thalamus</td>
<td>73 ± 9.0</td>
<td>73 ± 9.9</td>
<td>59 ± 3.6</td>
<td>62 ± 7.9</td>
<td>52 ± 4.5</td>
<td>56 ± 3.4</td>
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</table>

No. of dogs 11 6 5 5

Values are means ± SE. D, denervated; I, innervated. * P < 0.05 vs. control. † LT vs. RT (P < 0.05).

FIG. 1. Effects of hemorrhagic hypotension on total brain flow and on percent difference in flow between denervated and innervated cerebrum. During moderate hemorrhagic hypotension, cerebral blood flow to denervated cerebrum was significantly greater than flow to innervated cerebrum. * P < 0.05.

EFFECTS OF SYMPATHETIC NERVES ON CEREBRAL BLOOD FLOW

FIG. 2. Hemorrhagic Hypotension

Mean Arterial Pressure mmHg

Control 110±5  Mild 69±1  Moderate 49±2  Severe 37±3

Total Brain Blood Flow ml/min x 100 g

% Difference Denervated-Innervated Cerebrum

The most important observation in this study is that intense sympathetic stimulation induced by a physiological stress (hemorrhagic hypotension) had only a very small effect over a narrow range of hypotensive pressures on cerebral blood flow in awake dogs. Thus, these data support the concept that the functional importance of sympathetic nerves to cerebral blood vessels is limited (5). In addition, these studies suggest that in awake dogs, cerebral autoregulation extends to regional areas within the brain. When severe hypotension occurs, there is redistribution of brain blood flow that preserves flow to the medulla.

DISCUSSION

The three aspects of this study require further discussion: design of the experiments, factors affecting the response of cerebral vessels to sympathetic stimulation, and the effects of hemorrhagic hypotension on the distribution of cerebral blood flow.

Experimental design. In designing these experiments, we attempted to maximize the possibility of detecting significant effects of sympathetic stimulation on cerebral blood vessels. The method we have used to measure cerebral blood flow—labeled microspheres—permits us to completely separate intracranial from extracranial...
flow. This is of great importance because intense sympathetic stimulation will decrease flow to the extracranial vascular bed (7). In addition, the microsphere method has another major advantage: because regional blood flow can be measured, flow to the control and denervated hemispheres can be assessed simultaneously, which presumably improves the sensitivity of the studies in detecting small changes. To decrease variability, we maintained blood gases relatively constant during our studies. Also, the sympathetic nerves were intensely stimulated and sympathetic tone was not depressed by anesthesia. Nonetheless, even under these conditions, sympathetic stimulation had only a limited effect on the regulation of cerebral blood flow.

Because sympathetic denervation was performed 18 h prior to the study in most of the dogs, the possibility that denervation hypersensitivity influenced our results must be considered. If denervation hypersensitivity occurred, and if cerebral blood vessels constricted in response to circulating catecholamines, these effects could have obscured a differential response of the denervated and innervated cerebral hemispheres to sympathetic nerve stimulation. In the two acutely denervated dogs we studied, the increases in cerebral flow in the denervated versus the innervated sides of the brain (7% and 1%) were not exaggerated as they might have been if denervation hypersensitivity had blunted the differences in the 18-h denervated dogs we studied. Furthermore, the blood-brain barrier limits the access of circulating epinephrine and norepinephrine to cerebral vessels (4). Thus, it is unlikely that denervation hypersensitivity obscured an important effect of sympathetic nerves on cerebral blood flow in this study.

Factors affecting the response of cerebral blood vessels to sympathetic stimulation. Although under many conditions (5) sympathetic stimulation or denervation does not affect the regulation of cerebral blood flow, several important factors have recently been elucidated that unmask effects of sympathetic nerves on cerebral blood vessels. First, Bill and Linder (1) have shown that during severe hypertension, sympathetic stimulation constricts cerebral vessels and also tends to maintain the integrity of the blood-brain barrier. Second, Lacome et al. (6) have shown in awake rabbits that electrical stimulation of the superior cervical sympathetic nerves causes a change in heat clearance that is maximal during the 1st min of stimulation. Although heat clearance may not be exclusively dependent on changes in blood flow, these data suggest that transient vascular effects of sympathetic stimulation may be greater than steady-state effects. Third, in vitro studies in isolated cerebral vessels by Duckles et al. (3) and in vivo studies in our laboratory (6) have shown that the cerebral vessels of monkeys and cats are more responsive to sympathetic nerve stimulation than vessels from dogs. Recent studies in our laboratory, however, indicate that there is little species difference in responses to sympathetic stimulation during hemorrhagic hypotension because in anesthetized cats (unpublished observations), as well as in dogs (13), the effects of sympathetic stimulation on cerebral vessels were small or nonexistent. Fourth, the present study suggests that anesthesia may also mask effects of sympathetic nerve stimulation. Another potentially important factor that may affect responses of sympathetic stimulation on cerebral vessels is acidosis; in isolated veins (22), the spleen (17), and pial vessels (14) acidosis inhibits adrenergic neural transmission and responses to norepinephrine. In this study it is possible that the mild acidosis that occurred during severe hypotension reduced the effects of sympathetic stimulation on cerebral vessels.

Effects of hemorrhagic hypotension on distribution of cerebral blood flow. In a previous study (13) we noted in anesthetized dogs that autoregulation of cerebral blood flow extended to regional areas within the brain. Thus, as mean blood pressure was decreased from 120 to 70 mmHg, total cerebral blood flow remained constant and there was no redistribution of cerebral flow. The present investigation extends this concept to the awake dog. We also found that at more severe levels of hemorrhagic hypotension in anesthetized dogs, there was a redistribution of cerebral blood flow, which tends to maintain blood flow to certain areas of the brain (cortical gray matter and brain stem). This effect only occurs when cerebral vascular resistance is about 50% of control. In the present study we have extended this concept to the awake dog. During severe hemorrhagic hypotension in awake dogs, there is also a redistribution of the blood flow that maintains flow to the medulla. The redistribution occurs when cerebral vascular resistance is reduced to about half of the control value. In our studies in anesthetized dogs during severe hypotension gray matter flow was also preserved. It is possible that in the awake dogs this effect was masked by variations in cortical input that presumably are more prominent in awake dogs and are known to markedly alter gray matter blood flow (9, 10). Although the functional significance of redistribution of cerebral blood flow during hypotension is not known, preservation of flow to vital areas of the brain, which is not dependent on neural control, is presumably beneficial to the animal.
REFERENCES


