Cerebral Vascular Responses to Physiological Stimulation of Sympathetic Pathways in Cats

PAUL M. GROSS, DONALD D. HEISTAD, M. RANDALL STRAIT, MELVIN L. MARCUS, AND MICHAEL J. BRODY

SUMMARY This study was designed to determine whether physiological activation of sympathetic pathways affects cerebral blood flow (CBF) and the integrity of the blood-brain barrier during hypertension. Increased sympathetic activity was induced in anesthetized cats by sinoaortic deafferentation (SAD), a procedure that results in acute, severe neurogenic hypertension. Sympathetic innervation of one cerebral hemisphere was interrupted by sectioning the superior cervical ganglion or the cervical sympathetic trunk. CBF was measured using microspheres, and disruption of the blood-brain barrier was evaluated using Evans blue dye. Following SAD, mean arterial pressure increased abruptly from 142 ± 8 (mean ± se) to 226 ± 9 mm Hg, and total CBF increased from 31 ± 2 to 99 ± 15 ml/min per 100 g. Blood flow and the extent of disruption of the blood-brain barrier were lower in the innervated hemibrain than in the denervated hemibrain. The influence of sympathetic nerves was most pronounced in cortical gray matter: blood flow was 24 ± 4% less (P < 0.06) in the cortical gray matter of the innervated hemibrain 1 minute following SAD. Additional studies were performed to determine whether physiological stimulation of sympathetic nerves constricts cerebral vessels during hemorrhagic hypotension in cats. During hypotension, blood flow was significantly lower in several regions of the innervated hemibrain compared to the denervated hemibrain, but the effect was very small. These results provide evidence for neural regulation of CBF when sympathetic tone is augmented by physiological stimuli during acute hypertension and hypotension. The data represent the first demonstration of a reduction in CBF when sympathetic nerves are activated physiologically during acute severe hypertension. Circ Res 44: 288-294, 1979

SEVERAL studies have suggested that electrical stimulation of cerebrovascular sympathetic nerves under normal conditions has minimal (Raper et al., 1972; Heistad et al., 1976) or no effect (Alm and Bill, 1973; Traystman and Rapela, 1975; Heistad et al., 1977) on cerebral blood flow (CBF) in cats and dogs. Recent experiments, however, indicate that electrical stimulation of sympathetic pathways during severe drug-induced hypertension decreases CBF and also reduces the extent of disruption of the blood-brain barrier (Reuter et al., 1978; Bill and Linder, 1976; Edvinsson et al., 1976; Boisvert et al., 1977; MacKenzie et al., 1977; Edvinsson et al., 1977). Previous studies have examined cerebrovascular responses to electrical stimulation of sympathetic nerves, but the effect of "physiological" (i.e., endogenous) increases in sympathetic tone on CBF during hypertension is not known. It is difficult to evaluate the effects of physiological increases in sympathetic activity during hypertension, because when hypertension is induced by pressor agents (Heistad et al., 1978; Bill and Linder, 1976; Edvinsson et al., 1977; Boisvert et al., 1977; MacKenzie et al., 1977; Edvinsson et al., 1977), one would expect a generalized reduction of sympathetic tone. In the present study, however, sympathetic nerves were activated by sinoaortic deafferentation, a procedure that produces marked elevation of peripheral sympathetic tone and acute extreme hypertension of neurogenic origin. The purpose of these experiments was to determine whether physiological stimulation of sympathetic pathways constricts cerebral vessels and reduces disruption of the blood-brain barrier during acute hypertension in cats.

Additional studies were undertaken to determine whether physiological stimulation of sympathetic nerves constricts cerebral vessels during hemorrhagic hypotension. Although in a previous study no effect of sympathetic denervation on CBF was found during hypotension in dogs (Mueller et al., 1977), cerebral vessels appear to be more responsive to sympathetic stimulation in cats than in dogs (Heistad et al., 1978). Therefore, in the present study, we attempted to determine whether sympathetic stimulation by hemorrhage decreases CBF in cats.

Methods

Twenty-four mongrel cats (1.5–6 kg) of both sexes were studied. In the cats undergoing baroreceptor denervation, anesthesia was induced with halothane...
thanes, and chloralose (60 mg/kg, iv) was administered as needed during the experiment. In 10 of 12 cats that were studied during hypotension, anesthesia was induced with sodium methohexital (30 mg/kg, ip), and chloralose (50 mg/kg, iv) and urethane (500 mg/kg, iv) were given as needed during the experiment. In two other cats, halothane and chloralose (60 mg/kg, iv) were used for anesthesia. The cats were ventilated artificially with room air and supplemental oxygen via tracheal intubation. Polyethylene catheters were inserted into brachial and femoral arteries to measure arterial blood pressure and to withdraw reference arterial blood samples during microsphere injections. Heparin (500 U/kg, iv) was administered for anticoagulation, and skeletal muscle paralysis was induced with decamethonium bromide (0.3 mg/kg, iv) or gallamine triethiodide (4 mg/kg, iv) before the protocol was started. Arterial blood gases and pH were measured frequently, and ventilation was adjusted to maintain normal values for blood gases.

Measurement of Cerebral Blood Flow

Our procedure for measurement of CBF has been described in detail (Heistad et al., 1977, 1978; Mueller et al., 1977; Marcus et al., 1976). Between 0.5 and 3 million 15-μm microspheres were injected slowly into the left atrium over a 20-second period for each determination of CBF. Warm saline was used to flush microspheres from the catheter. Reference arterial blood samples were withdrawn at the rate of 1.03 ml/min from brachial and femoral arteries beginning before the microspheres were injected and continuing for approximately 90 seconds thereafter. Microspheres were injected four times in each experiment, and each bolus was labeled with a different isotope.

At the end of each study, the brain was removed and sectioned by region and structure. The samples were weighed, placed in plastic tubes, and counted with the blood samples for 2–5 minutes in a gamma counter. Energy window settings and separation of the different isotopes were established according to standard techniques (Marcus et al., 1976; Rudolph and Heyman, 1967).

The output from the gamma counter was processed by computer. Blood flow for individual segments or regions was computed by the formula: 

\[ F = (w_1 f_1 + w_2 f_2 + \cdots + w_n f_n) \times \frac{1}{(C_b \times 100 \times RBF) + (w_s \times C_c)} \]

where \( f_s \) is the blood flow to a brain segment, \( C_b \) is counts obtained from the brain segment, \( RBF \) is reference blood flow (rate of blood withdrawal from reference arteries), \( w_s \) is weight in g of the brain segment, and \( C_c \) is average counts from the two reference blood samples. The difference in counts between the two reference arterial blood samples was less than 10% in all measurements of blood flow.

Total and hemibrain blood flow were derived from the equation:

\[ F = (w_1 f_1 + w_2 f_2 + \cdots + w_n f_n) + (w_1 + w_2 + \cdots + w_n), \]

where \( F \) is total or hemibrain blood flow, \( w = \) weight of a segment, and \( f = \) flow to a segment. Thus, total and hemibrain blood flow were calculated from the flows to individual segments.

Statistical analysis was performed by comparing blood flow in the denervated and intact cerebral hemispheres by the nonparametric Wilcoxon sign-rank test (Steel and Torrie, 1960).

Evaluation of the Blood-Brain Barrier

In the cats undergoing sinoaortic deafferentation, we evaluated the effect of physiological stimulation of sympathetic nerves in protecting the blood-brain barrier during acute hypertension. Albumin-bound Evans blue dye, used as a marker of blood-brain barrier permeability, does not cross the barrier under normal conditions but extravasates into the brain during severe hypertension and stains cerebral structures (Haggendahl and Johansson, 1972; Hansson et al., 1975; MacKenzie et al., 1976). In the present studies, Evans blue dye (2.5%, 3 ml/kg, iv) was injected during the control period. After the study, the brain was examined for extent of staining. Staining was graded subjectively on the basis of a system consisting of the following ranks: none, minimal, moderate, and extensive. Comparisons were made between the innervated and denervated cerebral hemispheres.

Stimulation of Sympathetic Pathways by Baroreceptor Deafferentation

In 12 cats, posterior structures in the neck were exposed by evasion of the esophagus and trachea. Bilateral isolation of the carotid sinus, aortic depressor, and vagus nerves was accomplished with a dissecting microscope. The aortic depressor nerve was identified in each animal at the base of the superior laryngeal nerve where it leaves the vagal trunk. The vagus, aortic depressor, and carotid sinus nerves were identified and cut separately because preliminary experiments suggested that the increase in arterial pressure after sinoaortic deafferentation was greater if the nerves were cut in this sequence. One cerebral hemisphere was denervated by sectioning the superior cervical ganglion or the cervical sympathetic trunk caudal to the ganglion. Sympathetic nerves to the contralateral hemisphere remained intact.

Microspheres were injected during the control period and at 1, 10, and 30 minutes after cutting the carotid sinus, aortic depressor, and vagus nerves. In eight of the 12 cats, sinoaortic deafferentation produced severe arterial hypertension that lasted less than 30 minutes. In four of the 12 cats, arterial blood pressure, vascular resistance of several organs, and total CBF did not increase significantly during 30 minutes after sinoaortic deafferentation, even though responses to bilateral carotid occlusion were abolished. There were no significant differences in blood flow between the innervated and denervated hemispheres. These results suggest that any differences in blood flow between innervated
and denervated hemispheres in the other eight cats were dependent on hypertension and/or sympathetic activation, and that these differences were not the result of other experimental procedures. Because sinoaortic deafferentation did not produce hypertension in these four cats, the data were excluded from the study.

Stimulation of Sympathetic Pathways by Hemorrhagic Hypotension

Responses to hemorrhagic hypotension were examined in 12 cats. To interrupt sympathetic innervation to one side of the brain, one superior cervical ganglion was removed before the control period. The cats were bled rapidly until arterial pressure was about 60% and 45% of control pressure, and were allowed to equilibrate for at least 5 minutes before measurements were obtained. Microspheres were injected during the control period, at each of the two levels of hypotension (at a time when the tachycardia was maximal), and after blood pressure was restored to normal by reinfusion of the blood, small volumes of dextran (average molecular weight = 73 × 10^6), and small volumes of sodium bicarbonate.

Results

Effect of Unilateral Sympathetic Denervation after Sinoaortic Deafferentation

Physiological activation of sympathetic pathways by sinoaortic deafferentation resulted in an abrupt increase in arterial blood pressure (Table 1). Calculated vascular resistance in several organs indicated intense sympathetic stimulation following sinoaortic deafferentation. Control blood flows for liver, kidney, and small bowel were 48 ± 1, 290 ± 58, and 38 ± 9 ml/min per 100 g, respectively. One minute after sinoaortic deafferentation, vascular resistance in these tissues had increased by 78 ± 8%.

TABLE 1 Cerebral Blood Flow during Neurogenic Hypertension after Unilateral Sympathetic Denervation

<table>
<thead>
<tr>
<th>After baroreceptor deafferentation</th>
<th>Control</th>
<th>1 Min</th>
<th>10 Min</th>
<th>30 Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>142 ± 8</td>
<td>226 ± 9</td>
<td>162 ± 10</td>
<td>128 ± 15</td>
</tr>
<tr>
<td>Arterial blood gases and pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PO2 (mm Hg)</td>
<td>34 ± 1.1</td>
<td>36 ± 1.9</td>
<td>34 ± 1.2</td>
<td>30 ± 2.0</td>
</tr>
<tr>
<td>pH</td>
<td>7.28 ± 0.04</td>
<td>7.33 ± 0.02</td>
<td>7.31 ± 0.02</td>
<td>7.27 ± 0.02</td>
</tr>
<tr>
<td>P02 (mm Hg)</td>
<td>211 ± 27</td>
<td>170 ± 30</td>
<td>204 ± 25</td>
<td>190 ± 24</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Regional cerebral blood flow (ml/min per 100 g)</th>
<th>Control</th>
<th>1 Min</th>
<th>10 Min</th>
<th>30 Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemibrain</td>
<td>30 ± 3</td>
<td>31 ± 2</td>
<td>31 ± 4</td>
<td>31 ± 4</td>
</tr>
<tr>
<td>Brainstem</td>
<td>31 ± 4</td>
<td>31 ± 3</td>
<td>32 ± 3</td>
<td>32 ± 3</td>
</tr>
<tr>
<td>Thalamus-midbrain</td>
<td>7 ± 3</td>
<td>7 ± 3</td>
<td>7 ± 3</td>
<td>7 ± 3</td>
</tr>
<tr>
<td>Pons</td>
<td>33 ± 3</td>
<td>33 ± 3</td>
<td>33 ± 3</td>
<td>33 ± 3</td>
</tr>
<tr>
<td>Medulla</td>
<td>28 ± 3</td>
<td>28 ± 3</td>
<td>28 ± 3</td>
<td>28 ± 3</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>34 ± 3</td>
<td>34 ± 3</td>
<td>34 ± 3</td>
<td>34 ± 3</td>
</tr>
<tr>
<td>Cerebrum</td>
<td>28 ± 3</td>
<td>28 ± 3</td>
<td>28 ± 3</td>
<td>28 ± 3</td>
</tr>
<tr>
<td>Cerebral gray matter</td>
<td>38 ± 3</td>
<td>38 ± 3</td>
<td>38 ± 3</td>
<td>38 ± 3</td>
</tr>
<tr>
<td>Cortex</td>
<td>43 ± 3</td>
<td>43 ± 3</td>
<td>43 ± 3</td>
<td>43 ± 3</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>19 ± 3</td>
<td>19 ± 3</td>
<td>19 ± 3</td>
<td>19 ± 3</td>
</tr>
<tr>
<td>Cerebral white matter</td>
<td>4 ± 3</td>
<td>4 ± 3</td>
<td>4 ± 3</td>
<td>4 ± 3</td>
</tr>
<tr>
<td>Temporalis muscle flow (ml/min per 100 g)</td>
<td>±0.3 ± 0.3</td>
<td>±0.3 ± 0.3</td>
<td>±0.3 ± 0.3</td>
<td>±0.3 ± 0.3</td>
</tr>
</tbody>
</table>

Values are mean ± SE for eight cats.

* Significantly different from corresponding denervated side (P < 0.05).
blood-brain barrier in the innervated cerebrum dur-
ing severe hypertension. In five of eight cats, ex-
vavasation of dye was less extensive in
in the innervated hemisphere (Table 1). Ten minutes after sinoaortic deafferentation, arterial pressure and total CBF were increased above control, but were decreased from the peak values at 1 minute after sinoaortic deafferentation. Blood flow to cortical gray matter was significantly lower in the innervated side of the brain. The maximum difference in blood flow to the cerebrum occurred 10 minutes after sinoaortic deafferentation. Blood flow to the innervated cerebrum was 16 ± 6% lower than in the denervated cerebrum. Thirty minutes after sinoaortic deafferentation, CBF remained slightly elevated above control, and blood flow to cortical gray matter was lower in the hemisphere with intact sympathetic nerves. An unexplained observation was that blood flow to the cerebellum was significantly higher on the innervated side after nerve section. There were no signif-
cic differences between innervated and denerv-
ated subcortical white matter during the experi-
ment.

Sympathetic nerves appeared to protect the
blood-brain barrier in the innervated cerebrum dur-
ing severe hypertension. In five of eight cats, extrava-
sation of Evans blue dye was less extensive in the innervated hemisphere: staining was graded moderate or extensive in the denervated hemibrain and was minimal or moderate in the innervated hemibrain. In three cats, staining was similar on the two sides: extravasation of dye was minimal bi-
larly in one cat, moderate in one cat, and severe in one cat. In the cat that developed the highest.
arterial pressure after sinoaortic deafferentation (mean arterial pressure = 263 mm Hg), visible hemorrhage occurred in the occipital lobe of the denervated hemisphere.

Effect of Unilateral Sympathetic Denervation during Graded Hypotension

Total CBF did not change significantly when arterial pressure was reduced by hemorrhage (Table 2). Total cerebral vascular resistance de-
creased in both the innervated and denervated hemispheres during progressive hypotension, which indicates that autoregulation was intact in both hemispheres. During hypotension, however, CBF was lower in several regions of the innervated hemi-
sphe than in the denervated hemisphere (Table 2).

During the control measurement, blood flow to temporalis muscle was significantly higher on the denervated side, but flow was not significantly dif-
ferent at the two levels of hypotension or during the recovery period (Table 2).

Discussion

This study indicates that physiological activation
of sympathetic pathways produces modest vasocon-
striction in some regions of the cat brain. Activation of sympathetic pathways during neurogenic hyper-
tension produced by sinoaortic deafferentation elic-
ited moderate constriction in the innervated hemi-
brain, particularly in cerebral cortex. In contrast to the sizeable response to electrical stimulation during hyper-
tension in the cat (Heistad et al., 1978; Bill and Linder, 1976; Boisvert et al., 1977), physiologi-
ical stimulation of cerebrovascular sympathetic nerves during hypertension produced a much less pronounced effect on the cerebral vasculature. El-
vation of sympathetic tone by hemorrhage reduced CBF slightly in several regions of the innervated hemibrain.

In this study we compared blood flow in a hemi-
isphere in which sympathetic neural pathways to cerebral vessels were interrupted and in a hemi-
sphere in which sympathetic nerve traffic presum-
ably was increased. It is important to discuss two
aspects of the study: the effectiveness of sympa-
thetic denervation, and the magnitude of sympa-
thetic stimulation. Concerning effectiveness of de-
nervation, histochemical studies indicate that vas-
cular sympathetic innervation in the cerebrum arises primarily from the ipsilateral superior cervi-
cal ganglion (Nielson and Owman, 1967), so that interruption of this pathway should provide effec-
tive sympathetic denervation. Furthermore, in the
present studies, sympathetic denervation produced vasodilation in temporalis muscle (Tables 1 and 2), which provides evidence for interruption of sym-
pathetic innervation. Electrical stimulation of these sympathetic pathways produced cerebrovascular constriction in another series of experiments (Heis-
tad et al., 1978).

Concerning the magnitude of increases in sym-
pathetic neural activity in these experiments, activ-
ity in renal and splanchnic sympathetic nerves in-
creased approximately 2-fold after sinoaortic deaff-
erentation (unpublished observations); this indi-
cates that sympathetic neural pathways were acti-
vated intensely. The increase in vascular resistance in temporalis muscle (which, like the cerebrum, receives its innervation from the superior cervical
ganglion) was similar in magnitude to the increases in resistance that were observed in other organs. It seems likely, therefore, that increases in sympathetic nerve traffic were similar in the superior cervical ganglion and in the renal and splanchnic nerves. However, we have not been able to demonstrate that activation of sympathetic nerves to cerebral vessels was similar in magnitude to the increase in sympathetic activity to temporalis muscle.

In this study we found that the maximum reduction in CBF during physiological stimulation of sympathetic nerves was 16 ± 6%, and, in another study (Heistad et al., 1978), we found that electrical stimulation of sympathetic nerves reduced CBF by 29 ± 7%. The finding that electrical stimulation of sympathetic nerves produced greater constriction of cerebral vessels than did physiological stimulation may be related to the intensity of the stimuli. It is likely that the intensity of stimulation of sympathetic pathways after sinoaortic denervation was less than during electrical stimulation at 20 Hz (Heistad et al., 1978). An alternative or additional explanation may be that, after sinoaortic deafferentation, dilator as well as constrictor nerve fibers were activated. In cats, cerebral vessels have a large number of nonadrenergic vesicles that presumably represent dilator nerve terminals (Neilson et al., 1967; Duckles et al., 1977). If vasodilator neurons are activated after sinoaortic deafferentation, an antagonistic effect of cholinergic (Edvinsson et al., 1977) or other vasodilator influences could attenuate the constrictor response to sympathetic stimulation.

The results indicate that sympathetic activation by sinoaortic deafferentation produced constriction in gray matter but not white matter in the cerebrum. We are not aware of differences in density of innervation, or in responsiveness of cerebral vessels, between gray and white matter which would account for this finding. It is possible that the responses to sympathetic stimulation in gray matter, but not white matter, are related to the extent of disruption of the blood-brain barrier in these re-
gions. We speculate that disruption of the blood-brain barrier may allow transport of a substance, such as histamine (Bevan et al., 1975), to the vascular adventitia, and this substance augments sympathetic neural responses. Our observation that disruption of the barrier was greatest in gray matter, and minimal in white matter, is consistent with this hypothesis.

Electrical stimulation of sympathetic pathways during hypertension in cats not only elicited cerebrovascular constriction in some regions but also reduced disruption of the blood-brain barrier (Heistad et al., 1978; Bill and Linder, 1976; Edvinsson et al., 1977). In the present study, neurogenic hypertension produced extravasation of Evans blue dye throughout the brains of these cats, which indicates disruption of the blood-brain barrier. Extravasation of the dye was less in the innervated hemisphere in five of eight cats, and the same in three cats. These results indicate that physiological activation of sympathetic nerves reduces disruption of the blood-brain barrier. It is possible that sympathetic stimulation constricts large cerebral arteries during hypertension, reduces pressure in the distal smaller vessels, and thereby decreases disruption of the blood-brain barrier. We have not been able to demonstrate constriction of large cerebral arteries in normotensive dogs (Heistad et al., 1977), but it is possible that this response occurs in cats during acute hypertension. In comparison to results obtained with electrical stimulation (Heistad et al., 1978; Bill and Linder, 1976; Edvinsson et al., 1977), physiological stimulation of sympathetic pathways was less effective in maintaining the integrity of the blood-brain barrier during hypertension.

Activation of sympathetic pathways by hemorrhagic hypertension produced mild vasoconstriction in several regions of the innervated hemisphere. It is likely that activation of sympathetic neural activity was of smaller magnitude during hemorrhagic hypertension than after sinoaortic deafferentation. Increases in vascular resistance in muscle were smaller during hypertension and were probably produced by circulating vasoconstrictor hormones as well as by increased neural activity.

A small effect of sympathetic nerves on CBF also has been observed during hypertension in baboons (Fitch et al., 1975). In a previous study, we observed that, in the dog, activation of sympathetic nerves during severe hypertension had minimal effect on CBF (Mueller et al., 1977). The difference in response to hemorrhage in anesthetized cats and dogs provides another example of species difference in cerebrovascular responses to neural stimuli (Heistad et al., 1978).

We observed previously that CBF is redistributed during severe hypertension in anesthetized dogs (Mueller et al., 1977). The decrease in blood flow to brainstem was less than in other cerebral structures when blood pressure was lowered by hemorrhage, and the decrease in flow was less in cerebral gray matter than in white matter. This effect was not mediated by sympathetic nerves, since the response was seen in both the denervated and innervated hemispheres. Redistribution of CBF was seen only when the lower limits of autoregulation were exceeded and total CBF decreased. In the present studies in cats, total CBF did not decrease during hypotension (Table 2), and there was no significant redistribution of CBF, perhaps because the lower limits of autoregulation were not exceeded.

In summary, these experiments provide the first evidence that physiological activation of sympathetic nerves constricts vessels in some regions of the brain and protects the blood-brain barrier during acute hypertension. However, the response of cerebral vessels to physiological stimulation of sympathetic pathways is small compared to the marked constriction in other vascular beds.

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