The Role of the Peripheral Sympathetic Nervous System in Cerebral Blood Flow Autoregulation

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Abstract: The effect of chronic, unilateral superior cervical ganglionectomy on cerebral blood flow and blood flow autoregulation to changes in perfusion pressure was examined in seven pencyclidine anesthetized monkeys. Ten to 14 days prior to the experiments Doppler ultrasonic flow transducers were placed on both carotid arteries after ligation of the external carotid branches and removal of one superior cervical ganglion. Autoregulation was tested by exsanguination and metaraminol infusion with the monkeys inspire air, 9% and 12% carbon dioxide in air. Immediately following experimentation the cerebral vessels were examined for the presence of noradrenergic fibers. The results of the study demonstrate that: (1) superior cervical ganglionectomy produces a significant reduction in the noradrenergic innervation of ipsilateral extraparenchymal arteries; (2) the peripheral sympathetic nervous system contributes to overall cerebral vascular resistance primarily by affecting resistance in extraparenchymal arteries; and (3) as a result, it determines the contribution of the extraparenchymal arteries to overall cerebral blood flow autoregulation.

Additional Key Words cerebral vascular innervation superior cervical ganglion arterial carbon dioxide Doppler flow transducers

Introduction
Autoregulation of blood flow has occasionally been broadly defined as the ability of an organ to regulate its blood supply in accordance with its needs. Usually, as here, it is used in the more restricted sense to refer to the capacity of an organ to maintain constant blood flow despite changes in perfusion pressure. This phenomenon of autoregulation has been recognized as a property of the cerebral vascular bed since the early work of Fog and Forbes, and has been confirmed by others in several species, including man.

The role of the sympathetic nervous system in cerebral blood flow autoregulation (CBFA) is unclear. Both fluorescence histochemical and electron microscopy studies have revealed rich sympathetic innervation on the extraparenchymal cerebral arteries. Physiological observations in man as well as laboratory animals have produced conflicting results about the role of these nerves in CBFA. Fortunately, in no instance has the degree of sympathetic innervation been verified histologically and compared with the state of CBFA either in patients with acquired autonomic insufficiency or in sympathectomized animals.

In this study employing chronically sympathectomized monkeys, CBFA was simultaneously tested in denervated and innervated cerebral vascular beds in the same animal by means of chronically implanted flow probes. The degree of sympathetic innervation was histologically verified. Our results support the view of Harper and his associates that the sympathetic nervous system contributes to overall cerebral vascular resistance primarily by affecting the resistance in the extraparenchymal arteries. As such, it affects the functional distribution of blood flow among the major feeding vessels of the brain and the level of perfusion pressure and blood flow over which autoregulation can be demonstrated in these vessels.

Methods
ANIMAL PREPARATION
Seven adult rhesus monkeys (Macaca mulatta) weighing 3 to 5 kg were anesthetized with halothane or nitrous oxide after thiopental sodium induction, intubated and prepared for sterile surgery. An incision was made from the angle of the mandible caudally, exposing the left common carotid artery and its branches. The branches of the left external carotid artery were identified and doubly ligated. The left superior cervical ganglion was dissected free and removed.

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along with all apparent sympathetic nerve fibers along the carotid artery. A Doppler ultrasonic flow transducer was placed around the left common carotid artery. The wires from the flowmeter were buried subcutaneously on the dorsal surface of the neck and the skin incision closed. The right common carotid artery was similarly approached. The right superior cervical ganglion was left intact and a Doppler flow transducer implanted on the right common carotid artery after ligation of all branches of the right external carotid artery. Again, the lead wires from the probe were buried subcutaneously on the dorsal surface of the neck and the skin incision closed.

Following a 9-day to 15-day recovery period, the animals were anesthetized with phencyclidine hydrochloride (0.5 mg per kilogram body weight) and intubated with a cuffed endotracheal tube. The lead wires from both Doppler flow probes were exteriorized and connected to appropriate electronics for processing.26 A femoral artery and vein were exposed and cannulated. The signals from both flow probes, arterial blood pressure, end-tidal CO₂, and rectal temperature were continuously recorded on magnetic tape. Rectal temperature was maintained between 37° to 39° C with a heating pad.

EXPERIMENTAL PROCEDURE

CBFA was evaluated by changing the mean arterial blood pressure (MABP). MABP was elevated by the intravenous infusion of metaraminol (306 to 764 μg per minute) and lowered by withdrawal of blood (40 to 80 ml) from the venous cannula into a heparinized glass syringe. The order of procedure, hemorrhage or metaraminol first, was randomly varied without appreciable effect on the results.

The relationship between MABP and cerebral blood flow was first established with the animals spontaneously breathing room air. The endotracheal tube was then connected to a low resistance demand valve to administer 9% CO₂ in air. The relationship between MABP and cerebral blood flow was re-examined under the influence of each gas mixture. Ten to fifteen minutes were allowed at each new level of inspired carbon dioxide to insure a steady state. This was additionally insured by awaiting stability in the continuously recorded cerebral blood flow and end-tidal CO₂ tension. Measurements of arterial pH, Pco₂, and P0₂ accompanied all tests of CBFA.

Data from a single animal are shown in figure 1. At all levels of MABP in excess of 60 mm Hg and at all levels of inspired carbon dioxide tension, blood flow on the denervated side was greater than on the innervated side. CBFA was clearly present in both innervated and denervated vascular beds at MABP greater than 60 mm Hg during normoxic, normocarboxic conditions. Inhalation of 9% carbon dioxide in air increased CBF, although autoregulation was still maintained, particularly at higher MABP levels. Breathing 12% CO₂ abolished autoregulation in the pressure range tested in both innervated and denervated sides.

The results from all seven animals are presented in figure 2. Arterial blood gases at the various levels of inspired carbon dioxide are given in table 1. Blood flow on the denervated side was significantly greater (P < 0.001) than the innervated side at all levels of inspired carbon dioxide tension for MABP in excess of 80 mm Hg. Significant differences in blood flow between the two sides disappeared when MABP fell below 80 mm Hg because of the convergence of the curves at lower perfusion pressure levels.

Cerebral blood flow autoregulation was present in both the innervated and denervated vascular beds when the animals inspired room air (fig. 2A). Inspiration of 9% carbon dioxide in air shifted the mean autoregulation curves to a higher flow level (fig. 2B). Autoregulation was essentially lost on the denervated side but still preserved at a MABP in excess of 140 mm Hg on the innervated side. CBFA was abolished on the innervated as well as the denervated side when the animals inspired 12% carbon dioxide in air for MABP up to 160 mm Hg (fig. 2C). After the acute withdrawal of blood (~40 to 80 ml) to reduce MABP, the blood was re-infused as...
Relationship between mean carotid blood flow and mean arterial blood pressure in a single animal breathing room air (A), 9% CO₂ in air (B), and 12% CO₂ in air (C). Denervated side is denoted by the broken line and the innervated side by the continuous line.

Figure 1

Rapidly as possible (~30 seconds). During the reduction in blood pressure, CBF on the control and denervated side declined in a nearly parallel fashion, converging slightly as zero MABP was approached. During the restoration of pressure, however, CBF on the denervated side increased more rapidly for the same incremental change in blood pressure than did the innervated side (fig. 3). When control pressure was reestablished, the denervated side returned toward control CBF more slowly than the innervated side. Figure 3 shows this phenomenon for a single animal. This was a consistent finding in all monkeys breathing room air.

**TABLE 1**

<table>
<thead>
<tr>
<th>Arterial Blood Gases (± SD)</th>
<th>Room air</th>
<th>9% CO₂</th>
<th>12% CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.45 ± 0.04</td>
<td>7.35 ± 0.07</td>
<td>7.26 ± 0.09</td>
</tr>
<tr>
<td>P_{A,CO₂} (torr)</td>
<td>38 ± 5</td>
<td>52 ± 5</td>
<td>67 ± 8</td>
</tr>
<tr>
<td>P_{A,CO₂} (torr)</td>
<td>74 ± 7</td>
<td>104 ± 26</td>
<td>111 ± 11</td>
</tr>
</tbody>
</table>

HISTOLOGICAL RESULTS

Unilateral superior cervical ganglionectomy produced a nearly total loss of innervation on the ipsilateral side in three monkeys, both in the number of fibers and in the concentration of fluorescent bodies contained in the fibers. In the four remaining monkeys a notable decrease of fibers and fluorescent bodies was observed. The remaining fibers were very thin and contained few varicosities. The ipsilateral middle cerebral and vertebral arteries were the most susceptible to the denervation. A typical example of the degree of denervation compared to control is shown in figure 4.

Discussion

VALIDATION OF METHOD EMPLOYED TO DETERMINE CBF

The measurement of cerebral blood flow in non-human primates, especially the rhesus monkey, employing a flow transducer on the common carotid artery with the branches of the external carotid artery ligated, has been employed by numerous investigators. This approach to the continuous
arteriovenous differences for oxygen across brain by sampling blood from a peripheral artery and from the posterior end of the sagittal sinus through a chronically implanted device designed for this purpose. We used the reciprocal of the arteriovenous oxygen content difference as an index of CBF, the justification of which has been discussed by others, and induced changes in CBF by having the animals inspire different concentrations of carbon dioxide in air (range: 6% to 12%). The correlation between the two methods (fig. 5) of measuring CBF was a close one (R = 0.94, P < 0.001). Additionally, during the development of our model, experiments were carried out on chronically prepared monkeys in which one Doppler flow transducer had been placed on the middle cerebral artery with a second either on the internal carotid or on the common carotid, or sometimes all three. From the internal carotid or from the common carotid with external branches chronically ligated, changes in blood flow induced by carbon dioxide inhalation, hypoxia, or changes in arterial blood pressure correlated closely, both in degree and direction with simultaneous measurements made from the middle cerebral artery probe. If the external carotid branches were left unoccluded, measurements from the common carotid artery did not, as expected, correlate closely with those from the Doppler flow probe on the middle cerebral artery. Because of its ease of implantation and its durability, the common carotid artery probe with the external carotid branches ligated was chosen over either a middle cerebral or internal carotid artery probe.

VASCULAR INNERVATION

In all animals in this study unilateral removal of the superior cervical ganglion caused a marked ipsilateral decrease in the number of fluorescent nerve fibers and the quantity of fluorescent granules contained in the remaining fibers on the major arteries at the base of the brain. The most severely affected vessels were the middle cerebral artery, its branches, and the vertebral artery. These findings are similar to those reported by Nielsen and Owman in the cat. Superior cervical ganglionectomy in the cat resulted in a reduction in the number of fluorescent fibers on the major arteries of the base of the brain. The results of our survey of the sympathetic innervation of the major cerebral arteries have been published elsewhere.

CAROTID BLOOD FLOW

Carotid blood flow was significantly greater on the denervated than the innervated side, implying a difference in resistance distal to the two flow transducers. Two possible explanations for this difference exist. It could be the result of a significant fixed difference in the size of the arteries within or distal to the flowmeters, either as the result of a natural asymmetry or acquired scarring. We excluded this ex-
An example of the vascular innervation of an innervated (A) and denervated (B) middle cerebral artery demonstrated by fluorescent histochemistry (100 X).
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![Graph](image)

Relationship between changes in the brain arteriovenous oxygen content difference and the output in centimeters per second of a chronically implanted Doppler ultrasonic flowmeter on the common carotid artery of a rhesus monkey with all branches of the external carotid ligated. The equation for the regression line is $1/A-V\times100 = 1.005\times MCV\times\%A + 20\ (%A = 0.94,\ P < 0.001)$.

The difference in flow between the denervated and innervated sides of the cerebral vasculature we have observed in this study may not fully reflect the difference in resistance between sides, in the same animal. This strongly suggests that the observed difference in flow was the result of a significant functional difference in the extraparenchymal resistance in the cerebral vessels distal to the flow probe. These data are in agreement with data from other laboratories suggesting that resistance in extraparenchymal cerebral vessels is significantly influenced by the peripheral sympathetic nervous system.

Support for this thesis comes, first, from our observation of an asymmetrical hyperemic response following acute hypovolemic hypotension (fig. 3). During acute, severe hypovolemic hypotension, a transient hyperemic response when perfusion pressure is restored. Extraparenchymal vessels, because of their relative remoteness from tissue metabolic activity, are not likely to participate significantly in this acute hyperemic response. The effect of differences in extraparenchymal resistance on CBF would be accentuated under these circumstances. As perfusion pressure is restored, we conclude that CBF would be higher on the denervated side. This is exactly what was observed. We believe this observation is analogous to that of Harper, that cervical sympathetic stimulation produces a greater decline in
blood flow during acute hypercapnia than normocapnia.

Secondly, changes in PaCO₂ produced equal stepwise shifts in the CBFA axis on the innervated and denervated sides. Such changes are thought to be primarily produced by hydrogen-ion mediated dilation of intraparenchymal cerebral resistance vessels, progressively reducing their capacity to respond further to changes in perfusion pressure. Persistent asymmetries in blood flow under these circumstances must reflect independent functional asymmetries in resistance in the extraparenchymal cerebral vasculature.

Several clinical studies in patients with acquired autonomic insufficiency have reported conflicting results on the role of the sympathetic nervous system in CBFA. It is probable that the discrepant views are based, as suggested by Coronna and Plum, primarily on the nature of the autonomic lesion itself. These investigators showed, through clinical and pharmacological testing, that in the presence of a predominantly central or preganglionic lesion, CBFA remained intact. Peripheral or postganglionic autonomic lesions, however, appear to be accompanied by the loss of CBFA.

Conflicting results have come from two other animal studies employing chronic superior cervical ganglionectomy. Waltz et al. observed no difference in resting CBF or CBFA in chronically uni- or bilaterally superior cervical ganglionectomized cats. CBF and autoregulation were evaluated in both the innervated and denervated hemispheres of the same animal by the tissue clearance of 85Kr and photography of cortical surface vessels through bilateral craniectomies. Eklof and his colleagues measured CBF and CBFA by the tissue clearance of 133Xe in monkeys with chronic bilateral superior cervical ganglionectomies. Controls were a separate group of animals. CBFA was intact in the ganglionectomized animals but CBF was 29% less, a fact not commented upon by the authors.

The reason for these conflicting results in animals is not clear to us. Three factors should be weighed during their consideration. First, in each experiment including ours, superior cervical ganglionectomy was performed sufficiently in advance of the flow measurements to anticipate a significant degree of denervation. However, only in our experiment was the degree of denervation actually confirmed. Because of the variability of response to ganglionectomy from animal to animal, we believe direct confirmation of the degree of denervation is necessary for an interpretation of the results. Second, the study by Eklof et al. employed bilaterally sympathectomized animals, making necessary the use of a separate group of controls. The upward shift in the CBFA axis we observed might well be obscured in their study by anticipated variability between animals in resting CBF. Finally, and we believe most important, changes in CBF and CBFA measured by carotid flow transducers reflect the summed resistance in all vessels distal to the probe. A symmetrical fall in resistance in proximal vessels, as in the case of bilateral denervation, may, as suggested by Harper et al., be compensated for by increased resistance in the intraparenchymal resistance vessels. As a result, CBF measured either by the tissue clearance of a radioisotope or by flow transducers may remain near or at control levels. However, the autoregulatory response to rapid changes in perfusion pressure may be impaired, as suggested by the data of Yoshida et al. mentioned earlier. Such changes in CBFA are probably too transient to be detectable by tissue clearance methods of measuring CBF. An asymmetrical fall in resistance in proximal vessels, as in the case of unilateral denervation, may also be accompanied by compensatory changes in distal resistance vessels with preservation of normal tissue flow and CBFA as suggested by the work of Waltz et al. However, blood flow and CBFA simultaneously measured in both internal carotid arteries in such a preparation differ significantly, as we have shown. This, we believe, is the result of side-to-side differences in cerebrovascular resistance brought about by the decrease in sympathetic innervation after chronic superior cervical ganglionectomy. By employing bilateral carotid flow transducers with unilateral excision of the sympathetic innervation, it is possible to observe the effect of these nerves on resistance in the extraparenchymal cerebral vessels. Loss of sympathetic innervation appears to reduce the contribution of the affected vascular segment to CBFA but probably does not abolish the phenomenon of CBFA at the tissue level.

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References

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Anaesth 37:225-235, 1965


20. Mchedlishvili GI, Mitagvaria NP, Ormotsady LG: Vascular slroke, Vol. 6, May-June 1975


45. James JM, Millar RA, Purves MJ: Observations on the extrin-
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