Cerebral Blood Flow and Vascular Reactivity after Removal of the Superior Cervical Sympathetic Ganglion in the Goat

ENRIQUE ALBORCH, BERNARDINO GÓMEZ, GODOFREDO DIEGUEZ, JESÚS MARÍN, AND SALVADOR LLUCH

SUMMARY We studied the effects of removal of the superior cervical sympathetic ganglion on cerebral blood flow and vascular reactivity to adrenergic agonists and antagonists in 11 unanesthetized goats. Cerebral blood flow was measured by an electromagnetic flow transducer previously implanted on the internal maxillary artery. Ganglionectomy produced an increase of 66 ± 8.26% (SEM) in cerebral blood flow; this increment decreased gradually, and 15-25 days later values for cerebral blood flow were similar to those obtained before ganglionectomy. The administration of norepinephrine (0.03-9 μg) and tyramine (50-500 μg) into the internal maxillary artery in normal goats produced dose-dependent reductions in cerebral blood flow. At 6-8 days after ganglionectomy the reduction of cerebral blood flow produced by norepinephrine was markedly increased, whereas the effects of tyramine were diminished. Before ganglionectomy the administration of phentolamine (1 mg) into the internal maxillary artery produced a 31% increase in cerebral blood flow, whereas the injections of propranolol (1 mg) into the same site reduced cerebral blood flow by 14%. After removal of the superior cervical ganglion the effects of the same doses of the adrenergic blocking drugs were considerably lessened. These results support the view that the perivascular sympathetic nerve endings play an active role in the overall regulation of cerebrovascular resistance in the goat and indicate that both α- and β-receptors display a tonic adrenergic activity in the cerebral blood vessels.

THE VASOMOTOR effects of the sympathetic nerve fibers present in the cerebral blood vessels have been explored directly in vivo by means of stimulation of or injury to the sympathetic system, and indirectly by means of the administration of drugs with known catecholamine-releasing properties. We recently have shown in the unanesthetized goat that both electrical stimulation of the cervical sympathetic nerves and injections of tyramine into the cerebral circulation produce a considerable reduction in cerebral blood flow which may be partially or totally abolished by pretreatment with reserpine or the α-blocker, phentolamine; furthermore, the administration of phentolamine directly into the arterial blood supply to the brain produces a decrease in cerebrovascular resistance which may be eliminated by previous treatment with reserpine. These experiments demonstrate the catecholamine-mediated effects of sympathetic stimulation and suggest the presence of an active adrenergic tone in the cerebral blood vessels.

The present studies were designed to examine further the adrenergic activity of the cerebral vascular bed by means of a double approach: (1) evaluation of the effects of removal of the superior cervical sympathetic ganglion on resting cerebral blood flow and its time course, and (2) analysis of the participation of α- and β-adrenergic components in the regulation of cerebral vascular tone by using small doses of phentolamine and propranolol directly injected into the arterial blood supply of the brain. In addition, the availability of the neurotransmitter present in the cerebral blood vessels was tested by the administration of tyramine, whereas the response of the vascular effector system was investigated by the administration of norepinephrine.

All experiments were carried out in the goat because its unique arterial supply to the brain permits the measurement of cerebral blood flow and other hemodynamic variables in the unanesthetized animal.

Methods

Eleven female goats weighing between 30 and 42 kg were used in this study. In this species each internal maxillary artery, a branch of the external carotid artery, provides the total blood flow to each hemisphere via the rete mirabile (Fig. 1); the vertebral arteries do not contribute to brain blood flow and the extracranial internal carotid artery is absent. The rete mirabile is a netlike arrangement of small arteries interposed between the internal carotid artery and the distal remnant of the internal carotid artery. The circle of Willis in the goat is similar to that of man except that the blood flows in a caudal direction in the basilar artery, which has only insignificant communications with the vertebral artery. Analysis of the distribution of radioactively labeled microspheres in the cerebral circulation of the goat after the surgical procedure described by Reimann et al. indicates that nearly all of the blood carried by the internal maxillary artery passes directly to cerebral tissue. Extracerebral blood flow is minimal and measures less than 5% of total flow.
The various measurements were made with the goat unrestrained in a large cage, except for a Lucite stock fitted loosely around the neck that limited forward and backward motion.

Dose-response curves were obtained for tyramine (tyramine hydrochloride, Sigma) and norepinephrine (l-norepinephrine hydrochloride, Sigma). Phentolamine (Regtine, Ciba) and propranolol (Sumial, ICI Farma) were used to block the α- and β-receptors, respectively. Tyramine (50-500 µg) and norepinephrine (0.03-3 µg) were injected through the temporal catheter in a volume of 0.25 ml and washed in with an additional 0.5 ml of physiologic saline. Maximal responses to tyramine and norepinephrine were not studied because preliminary experiments had shown that large doses of these drugs produced changes in systemic arterial blood pressure and heart rate that masked the direct effects on the cerebral vessels. Similar observations have been reported for relatively large doses of other adrenergic drugs in the same animal preparations.20

α-Adrenergic blockade of the cerebral circulation was produced in eight goats by slow infusion of phentolamine into the internal maxillary artery (1 mg in 1 ml of saline over a period of 10-15 minutes). Propranolol (1 mg) was used in a similar manner in the same goats. These doses of phentolamine and propranolol produce selective blockade of the adrenergic receptors of the cerebral vessels without altering systemic hemodynamics.8,20

In all the goats removal of the left (seven goats) or right (four goats) superior cervical sympathetic ganglion was performed under intravenous sodium thiopental anesthesia 5-11 days after the first operative procedure. In these goats the effects of norepinephrine, tyramine, phentolamine, and propranolol were evaluated at various times postoperatively.

The superior cervical sympathetic ganglion was exposed but not removed (sham operation) in five additional goats and the time course of cerebral blood flow was compared with that obtained from ganglionectomized goats.

After termination of the experiments the goats were killed by an overdose of intravenous pentobarbital and the whole brain was removed from the skull and weighed to permit accurate calculation of cerebral blood flow per 100 g of brain tissue. Brain weight ranged from 89 to 112 g (97 ± 1.78, mean ± SEM).

Results

After ganglionectomy two distinct phases in the evolution of cerebral blood flow were observed: there was a prompt, pronounced increase in ipsilateral cerebral blood flow which lasted 1-2 days, and a slower decline in blood flow which tended to attain the control values 15-20 days after ganglionectomy. The maximum percentage increase in cerebral blood flow achieved in the different goats ranged from 25 to 106% (66% on the average). Ganglionectomy did not produce any changes in arterial blood pressure or heart rate. Figure 2 is a recording of left hemispheric blood flow and systemic arterial blood pressure before left ganglionectomy and at several times postoperatively and Table 1 illustrates the results from all the experiments. Figure 3 represents the time course of resting cerebral blood flow after ganglionectomy; for comparison,
FIGURE 2 Representative tracings of left hemispheric cerebral blood flow (CBF) and systemic arterial blood pressure (AP) before removal of the left superior cervical sympathetic ganglion (control) and at several times postoperatively. Zero flow is obtained by occluding the external carotid artery as illustrated in the middle of each panel.

the time course of cerebral blood flow from five sham-operated goats have been included.

Tyramine and norepinephrine were injected into the internal maxillary artery in seven goats before and after removal of the superior cervical ganglion. The vasoconstriction produced by tyramine before ganglionectomy was significantly diminished 6-8 days after the operation. Concomitantly, the decrease in cerebral blood flow induced by injections of norepinephrine before ganglionectomy was markedly intensified 6-8 days postoperatively. Figure 4 illustrates the dose-response curves to tyramine and norepinephrine before and after removal of the superior cervical ganglion.

To estimate the α- and β-adrenergic participation in control of resting cerebral blood flow, phentolamine and propranolol were injected directly into the internal maxillary artery before and after ganglionectomy. Figure 5 shows the changes in left hemispheric blood flow which occur with the administration of phentolamine and propranolol before and after removal of the ipsilateral superior cervical ganglion in one goat, and Table 2 summarizes the results from eight goats. Before ganglionectomy the administration of phentolamine produced a 31% increase in cerebral blood flow, whereas the injections of propranolol into the same site reduced cerebral blood flow by 14%. After removal of the superior cervical ganglion, the effects of the same doses of the adrenergic blocking drugs were markedly diminished: phentolamine increased cerebral blood flow by 2% and propranolol decreased cerebral blood flow by 4%.

Discussion

A number of investigations in vivo and in vitro have shown that tyramine and sympathetic nerve stimulation produce an increase in tension or in resistance to flow in cerebral blood vessels which is partially prevented by reserpine or α-adrenergic blockade.4, 5, 21-22 The results suggest that both tyramine and nerve stimulation release endogenous norepinephrine from the perivascular sympathetic nerve endings; this, in turn, activates the α-adrenergic receptors present in the cerebral blood vessels.20, 23-25 This approach provides information concerning the nature of the vasoconstrictive effects of such procedures but neglects the tonic activity of the nerve terminals and the degree of involvement of the α- and β-adrenergic recep-
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Development of supersensitivity of the \( \alpha \)-adrenergic receptors to circulating catecholamines after denervation. 26

Transmission at the sympathetic nerve endings was functionally impaired after ganglionectomy. This is shown by indirect activation of the perivascular nerve endings with tyramine, an agent which exerts its main effects by the release of endogenous norepinephrine from sympathetic stores. Relatively small doses of tyramine administered directly into the arterial supply of one brain hemisphere produced dose-dependent reductions in cerebral blood flow but, after ganglionectomy, these effects were practically abolished. This is in agreement with results of experiments on mice which have shown that the reduction in cerebral blood volume (used as an index of cerebral blood flow) produced by tyramine is abolished by previous removal of both superior cervical ganglia. 27 These results support the concept that the cerebral vasomotor responses induced by tyramine are mainly due to the displacement of endogenous norepinephrine from the perivascular nerve endings and indicate that after removal of the superior cervical ganglia the sympathetic nerve endings are no longer capable of releasing the neurotransmitter.

The conclusion that the cerebral vessels display, under normal conditions, a tonic adrenergic activity is also based on the effects on cerebral blood flow obtained by direct administration of adrenergic blocking drugs into the internal maxillary artery. Phentolamine produced a 30\% increase in cerebral blood flow in the normal goats but failed to change cerebral blood flow after ganglionectomy. This is consonant with observations showing the inability of phentolamine to increase cerebral blood flow after depletion of catecholamine stores with reserpine, 28 and indicates the presence of an effective basal \( \alpha \)-adrenergic activity in the cerebral blood vessels. The role of \( \beta \)-adrenergic activity in the maintenance of a sympathetic tone probably is less pronounced than that of \( \alpha \)-adrenergic activity but consistently present. The reduction in cerebral blood flow produced by propranolol was practically abolished after ganglionectomy; this suggests that normally there is a small but significant participation of \( \beta \)-receptor activity in regulating the cerebral blood vessels.

Table 2 Effects of Phentolamine (Ph) and Propranolol (Pr) on Cerebral Blood Flow in Unanesthetized Goats

<table>
<thead>
<tr>
<th>Goat no.</th>
<th>Before ganglionectomy</th>
<th>After ganglionectomy*</th>
</tr>
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<tbody>
<tr>
<td></td>
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<td>Ph</td>
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C = control.
* Measured 2-4 days postoperatively.

FIGURE 5 Upper tracings: effects of phentolamine on left cerebral blood flow (CBF) in an unanesthetized goat. Phentolamine increased CBF from a control value of 65 ml/min to 85 ml/min. Removal of the superior cervical sympathetic ganglion increased control CBF to 95 ml/min. Phentolamine under these circumstances increased CBF from 95 ml/min to 100 ml/min. Lower tracings: effects of propranolol on left CBF in another goat. Propranolol decreased CBF from a control value of 75 ml/min to 60 ml/min. Ganglionectomy increased CBF to 130 ml/min, and the effects of propranolol were abolished.
Previous experiments on anesthetized animals have shown minor changes in cerebral blood flow after sympathetic denervation of the cerebral blood vessels\textsuperscript{14} or after bilateral cervical sympathectomy.\textsuperscript{12} On the other hand, studies combining measurement of cerebral blood flow and visualization of arteriolar innervation by fluorescence histochemistry claim a significant increase in cerebral blood flow after chronic denervation,\textsuperscript{15} cervical sympathectomy, or preganglionic conduction blockade.\textsuperscript{28} The reasons for the conflicting results are not clear. Probably one of the serious obstacles to the proper evaluation of the role of the sympathetic nerves in the regulation of cerebral blood flow is the lack of understanding of the sequential changes of cerebral blood flow after ganglioneuromy. Some of the studies related to this matter had to do with the acute effects of injury to the cervical sympathetic nerves or ganglia.\textsuperscript{7, 9, 29} In other investigations cerebral blood flow has been studied after degeneration of the sympathetic nerve endings surrounding cerebral blood vessels.\textsuperscript{12-15, 29} Even in these cases, only spot measurements were possible because of the use of anesthesia. The present study attempted to provide data on acute and chronic effects of removal of the superior cervical ganglion so that they could be systematically and serially examined in awake animals. Others have also had this interest\textsuperscript{28} but have provided observations concerning changes only in local cerebral blood flow.

In conclusion, these experiments on unanesthetized goats support the concept of an active participation of the perivascular sympathetic nerve endings in the overall regulation of cerebrovascular resistance. The effects of phentolamine and propranolol on cerebral blood flow before and after removal of the superior cervical sympathetic ganglion indicate that under normal conditions both \alpha- and \beta-receptors display a tonic adrenergic activity in the cerebral blood vessels.

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**References**

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