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

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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[1–3] have been explored directly in vivo by means of stimulation[4–9] of or injury[10–15] to the sympathetic system, and indirectly by means of the administration of drugs with known catecholamine-releasing properties.[5] 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.[5] 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[16–19] permits the measurement of cerebral blood flow and other hemodynamic variables in the unanesthetized animal.[5, 20]

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.[16–18] The rete mirabile is a netlike arrangement of small arteries interposed between the internal maxillary 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.[16, 18] Analysis of the distribution of radioactively labeled microspheres in the cerebral circulation of the goat after the surgical procedure described by Reimann et al.[18] indicates that nearly all of the blood carried by the internal maxillary artery passes directly to cerebral tissue.[19] Extracerebral blood flow is minimal and measures less than 5% of total flow.

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Supported in part by the Comisión Asesora de Investigación Científica y Técnica and by the Instituto Nacional de Previsión.

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Received November 9, 1976; accepted for publication February 17, 1977.

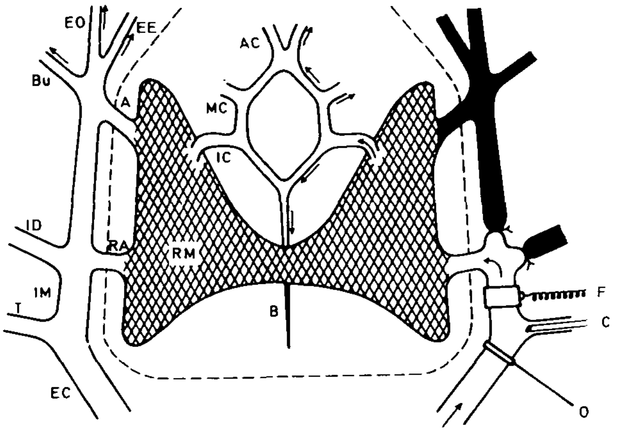

FIGURE 1 *Schematic representation of the intracerebral and extracerebral branches of the internal maxillary artery of the goat. Arrows indicate the direction of blood flow; cross-hatched area = rete mirabile (RM); filled area = extracerebral vessels deprived of blood; dashed line = skull; F = electromagnetic blood flow transducer; C = temporal artery catheter; O = snare type occluder; EE = external ethmoidal artery; EO = external opthalmic artery; Bu = buccinator artery; A = arteria anastomotica; ID = inferior dental artery; RA = ramus anastomoticus; IM = internal maxillary artery; T = temporal artery; EC = external carotid artery; AC = anterior cerebral artery; IC = internal carotid artery; MC = middle cerebral artery; B = basilar artery.*

The operative procedure to measure blood flow has been described in detail before.[18] Briefly, the extracerebral vessels from one of the internal maxillary arteries were ligated and thrombosed with 1,000 NIH units of thrombin (thrombin, topical, Parke, Davis) dissolved in 0.5 ml of saline. This maneuver produces an almost immediate obliteration of the ethmoidal, ophthalmic, and buccinator arteries and thus eliminates blood flow to the eye and other facial structures. This is confirmed on recovery from surgery by the presence of ipsilateral blindness. An electromagnetic flow transducer (Biotronex) was placed on the internal maxillary artery to measure blood flow to the ipsilateral cerebral hemisphere (Fig. 1). A polyethylene catheter was inserted in the temporal artery and permanently fixed to permit injection or infusion of drugs directly into the internal maxillary artery in the awake goat; the same catheter was used to measure arterial blood pressure with a Statham transducer. A snare-type occluder was placed on the external carotid, close to the temporal artery, to obtain zero-flow baselines. The external connecting leads from the flow transducer and occluder and the temporal artery catheter were led out subcutaneously and secured to the goat's horn.

Heart rate was measured from the arterial pressure pulse with a rate meter. Flow measurements were made with a Biotronex electromagnetic flowmeter (model BL-610). Cerebral blood flow, arterial blood pressure, and heart rate were recorded on a Beckman recorder.

The experiments on the unanesthetized goat started 2-3 days after the operative procedure, at which time the goats had fully recovered and were in a stable cardiorespiratory state. The various measurements were made with the goat unrestrained in a large cage, except for a Lucite stock fitting 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 (Regitine, 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.[5, 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,

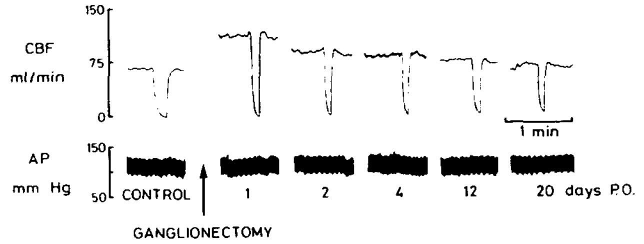

FIGURE 2 *Representative tracings of left hemispheric cerebral blood flow (CBF) and systemic arterial blood pressure (AP) before removal of the left superior cervical sympathetic ganglion (control) and at several times postoperatively. Zero flow is obtained by occluding the external carotid artery as illustrated in the middle of each panel.*

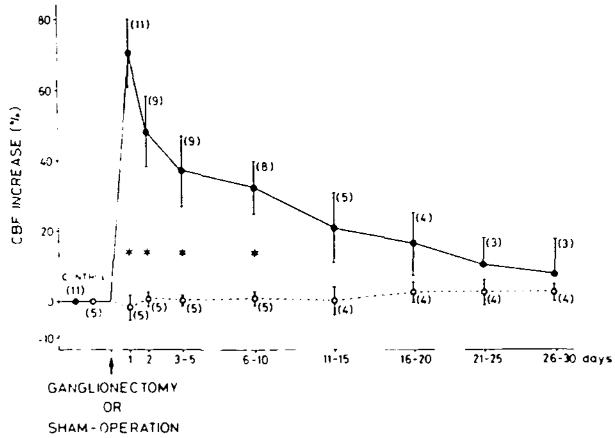

FIGURE 3 *Sequential changes in cerebral blood flow (CBF) in ganglionectomized (solid line) and sham-operated (broken line) goats. Values for CBF are expressed as percent change from control and depict mean ± SEM. The numbers in parentheses denote number of goats. The asterisks represent statistically significant differences (Student's t-test; $P < 0.05$) between the ganglionectomized and sham-operated goats at a given time.*

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%.

TABLE 1 *Cerebral Blood Flow and Mean Arterial Blood Pressure before and 1 Day after Removal of the Superior Cervical Sympathetic Ganglion*

Goat no.	Cerebral blood flow (ml/min per 100 g)		Mean arterial pressure (mm Hg)	
	Before	After	Before	After
44	150	250	100	95
46	100	140	105	105
50	132	266	95	105
57	90	186	125	110
58	144	244	90	90
65	98	146	95	110
68	146	294	80	100
69	132	196	105	100
72	118	174	110	105
75	140	176	90	90
80	126	224	80	90
Mean	125	208	97	100
SE	6.30	15.17	4.00	2.33

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-

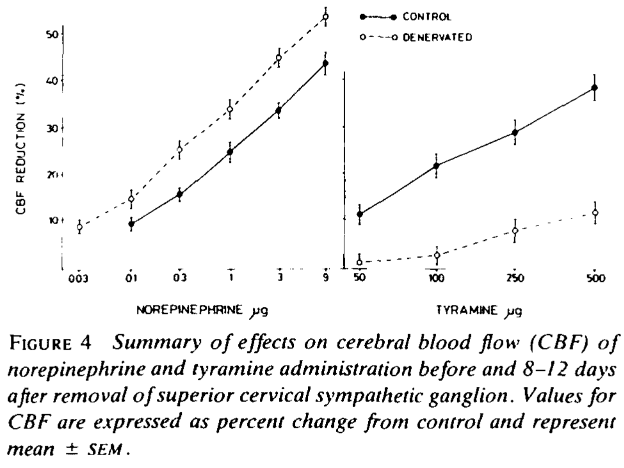

FIGURE 4 *Summary of effects on cerebral blood flow (CBF) of norepinephrine and tyramine administration before and 8–12 days after removal of superior cervical sympathetic ganglion. Values for CBF are expressed as percent change from control and represent mean ± SEM.*

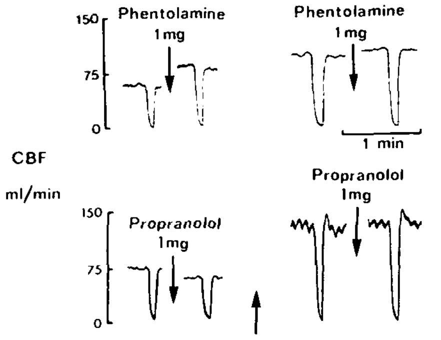

FIGURE 5 *Upper tracings: effects of phentolamine on left cerebral blood flow (CBF) in an unanesthetized goat. Phentolamine increased CBF from a control value of 65 ml/min to 85 ml/min. Removal of the superior cervical sympathetic ganglion increased control CBF to 95 ml/min. Phentolamine under these 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.*

tors in regulating resting cerebral blood flow. Data from such studies were examined in the present work. Our results show that removal of the superior cervical sympathetic ganglion produces a sizable increase in cerebral blood flow most likely due to the loss of an existing sympathetic tone. This is in accord with results of previous studies using the same experimental preparation which demonstrate that depletion of the catecholamine stores with reserpine maintains cerebral blood flow at normal values while the arterial blood pressure is clearly diminished; the findings thus indicate a decrease in cerebrovascular resistance.[5]

The marked increase in cerebral blood flow consequent to ganglionectomy was followed by a gradual return to near normal values 15-25 days after removal of the ganglion while at the same time the cerebral vasoconstrictor effects of injected norepinephrine were significantly increased. Both phenomena are probably related to the development of supersensitivity of the α-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 vasoconstriction induced by tyramine is 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,[5] and indicates the presence of an effective basal α-adrenergic activity in the cerebral blood vessels. The role of β-adrenergic activity in the maintainance of a sympathetic tone probably is less pronounced than that of α-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 β-receptor activity in regulating the cerebral blood vessels.

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

	Cerebral blood flow (ml/min per 100 g)							
	Before ganglionectomy				After ganglionectomy*			
Goat no.	C	Ph	C	Pr	C	Ph	C	Pr
46	105	152	100	86	140	140	130	130
50	132	170	125	108	260	270	250	244
57	80	120	95	85	176	186	178	176
58	140	168	138	130	234	247	230	220
69	130	180	–	–	180	186	–	–
72	128	152	125	98	134	140	160	155
75	135	182	135	126	176	185	160	150
80	120	155	130	115	190	200	180	174
Mean	121	150	121	106	186	194	184	178
SE	7.00	7.07	6.38	6.83	15.14	16.18	15.88	15.24

C = control.
* Measured 2-4 days postoperatively.

Previous experiments on anesthetized animals have shown minor changes in cerebral blood flow after sympathetic denervation of the cerebral blood vessels[14] or after bilateral cervical sympathectomy.[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,[15] cervical sympathectomy, or preganglionic conduction blockage.[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 ganglionectomy. Some of the studies related to this matter had to do with the acute effects of injury to the cervical sympathetic nerves or ganglia.[7, 9, 29] In other investigations cerebral blood flow has been studied after degeneration of the sympathetic nerve endings surrounding cerebral blood vessels.[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[30] 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 α- and β-receptors display a tonic adrenergic activity in the cerebral blood vessels.

Acknowledgments

We thank M.C. Ruiz for technical assistance and C. Muela and M.A. Traisset for help in preparing the figures.

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