Evidence for the Direct Effect of Adrenergic Drugs on the Cerebral Vascular Bed of the Unanesthetized Goat

BY SALVADOR LLUCH, M.D., CHARLES REIMANN, M.D., AND GERALD GLICK, M.D.

Abstract: Despite considerable research, the question of whether adrenergic drugs exert direct effects on the cerebral circulation has remained unresolved. With the development of a method for monitoring continuously the entire blood flow to one hemisphere in the unanesthetized goat, we have been able to study this problem directly. The effects of epinephrine, norepinephrine, and isoproterenol administered by close intra-arterial injection were investigated in 15 goats in which an electromagnetic flowmeter had been implanted previously on the internal maxillary artery, which, in this animal, provides the sole blood supply to a hemisphere. Both epinephrine and norepinephrine (0.1 to 5.0 μg) produced dose-dependent reductions in cerebral blood flow, a decrease of 55 ± 3% (SEM) occurring with the highest dose. Alpha receptor blockade of the ipsilateral hemisphere with phenoxybenzamine totally or partially abolished this cerebral vasoconstriction. Isoproterenol (0.01 to 1.0 μg) produced dose-dependent increases in cerebral blood flow, an increment of 75 ± 6% occurring with the highest dose. Beta blockade with propranolol totally or partially abolished the cerebral vasodilation induced by isoproterenol. Thus, epinephrine, norepinephrine, and isoproterenol exert powerful direct effects on the cerebral circulation of the unanesthetized goat, and these effects appear to be mediated by alpha and beta receptors.

Additional Key Words cerebral blood flow epinephrine cerebral vascular resistance norepinephrine alpha adrenergic receptors isoproterenol beta adrenergic receptors 5-hydroxytryptamine popaverine phenoxybenzamine propranolol electromagnetic flowmeter

The cerebral circulation, encased as it is within the bony cranium and generally furnished by a dual blood supply from the carotid and vertebral arterial systems, has been slow to reveal the regulatory mechanisms that control it. Thus, previous reports concerning the effects of vasoactive substances on cerebral blood flow have been inconclusive and conflicting.1-6 Recent reviews point out the difficulties of differentiating between the direct effects of drugs on cerebral vessels and the secondary effects resulting from changes in heart rate, systemic arterial pressure, and cardiac output.7-10 Definitive delineation of the pharmacological mechanisms that control cerebral blood flow has not been possible because of the lack of an experimental model that permits the effects of various interventions to be assessed on a beat-to-beat basis in an unanesthetized animal in which the effects of extracerebral blood flow have been eliminated. We have recently developed in our laboratory an experimental preparation, utilizing the goat, that obviates these difficulties.11 In this animal, each internal maxillary artery, a branch of the external carotid artery, provides the total blood flow to each cerebral hemisphere via the rete mirabile; vertebral arteries do not contribute to the cerebral blood supply.

It was the objective of the present study to evaluate, in the unanesthetized goat, the effects on the cerebral circulation of the adrenergic drugs, epinephrine, norepinephrine, and isoproterenol, before and after selective, localized blockade of the alpha and beta adrenergic receptors.

Methods

Fifteen female goats ranging in weight from 23 to 35 kg were used in these experiments. The operative procedure has been described previously.11 Briefly, the extracerebral vessels from one of the internal maxillary arteries...
were ligated or thrombosis was induced by the intra-arterial injection of 1,000 N.I.H. units of thrombin (Thrombin, topical, Parke, Davis and Co., Detroit, Michigan) dissolved in 1 to 2 ml of saline. An electromagnetic flow transducer that had been calibrated previously in vivo (Biotronex, Model BL-610, Silver Spring, Maryland) was placed on the internal maxillary artery to measure blood flow to the ipsilateral cerebral hemisphere; a balloon occluder was placed on the external carotid artery to obtain zero flow baselines. A fine polyethylene catheter placed in the temporal artery permitted the injection of drugs directly into the internal maxillary artery. 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. Aortic pressure was obtained from a catheter introduced through the femoral artery which was brought to the back of the animal through a subcutaneous tunnel and secured to the skin with silk sutures for future pressure measurements. The experiments on the unanesthetized animals started three to five days after the operative procedure, at which time the goats were fully recovered and in a steady cardiorespiratory state.

Dose-response curves were obtained to epinephrine HC1, norepinephrine bitartrate, and isoproterenol HC1. Phenoxybenzamine HC1 was used to block alpha adrenergic receptors and propranolol HCl was used to block beta adrenergic receptors. The effects of epinephrine and norepinephrine were studied in six goats, isoproterenol in five other goats, and all three drugs in the remaining four animals. Drugs were injected through the temporal catheter in a volume of 0.5 ml or less and washed in with an additional 1 ml of physiological saline. Control injections of 1 ml of saline were administered and had no detectable effect on the recorded measurements.

We produced selective adrenergic blockade of the cerebral circulation in 12 goats. Five were subjected to alpha blockade, four to beta blockade, and the other three to alternate beta and alpha blockade. In the latter three goats a period of at least 24 hours was allowed between beta and alpha blockade. In order to prevent spurious effects such as hypotension and tachycardia that ensue when large doses of phenoxybenzamine are administered intravenously,12 we attempted to block selectively the vessels of the brain. Such blockade was achieved by slow infusion of phenoxybenzamine into the internal maxillary artery (200 to 400 μg over a period of 20 to 30 minutes). Because of the small doses of phenoxybenzamine used, no detectable changes in heart rate or aortic pressure occurred. Significant blocking effects appeared one-half to one hour after the end of the infusion of phenoxybenzamine. Alpha blockade of the cerebral vessels was successfully accomplished in eight experiments. Similarly, selective beta blockade of the brain vessels was obtained in seven goats by the injection of relatively small amounts of propranolol (250 μg) into the internal maxillary artery.

The ability of the cerebral vessels to react to other drugs after adrenergic blockade was tested in three goats by the injection of 5-hydroxytryptamine and papaverine HCl into the internal maxillary artery before and after blockade.

Arterial blood was analyzed before and during the effects of the injections of drugs for pH, Pco2, and Po2 by standard electrometric methods (Instrumentation Laboratories, Model 123, Watertown, Massachusetts).
Effects of Epinephrine, Norepinephrine and Isoproterenol on Cerebral Blood Flow and Cerebral Vascular Resistance in the Unanesthetized Goat

<table>
<thead>
<tr>
<th></th>
<th>Control (N = 15)</th>
<th>Epinephrine (N=10)</th>
<th>Norepinephrine (N=10)</th>
<th>Isoproterenol (N=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral blood flow (ml/min/100 gm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p value (versus control)</td>
<td>124 ± 7.3</td>
<td>105 ± 4.7</td>
<td>87 ± 4.0</td>
<td>58 ± 3.4</td>
</tr>
<tr>
<td>p value (versus next lower dose)</td>
<td>&lt;0.02</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cerebral vascular resistance</td>
<td>0.83 ± 0.01</td>
<td>0.99 ± 0.01</td>
<td>1.19 ± 0.01</td>
<td>1.79 ± 0.02</td>
</tr>
<tr>
<td>p value (versus control)</td>
<td>&lt;0.005</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>p value (versus next lower dose)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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</tr>
</tbody>
</table>

Values listed are means ± standard error. Dosages in micrograms. N = number of experiments. NS = not significant.

Heart rate was measured from the aortic pressure pulse using a rate meter. Blood flow, aortic pressure, and heart rate were recorded on an Electronics for Medicine recorder. Cerebral vascular resistance was calculated as the mean aortic pressure in mm Hg divided by cerebral blood flow in ml/min/100 gm.

**Results**

Representative dose-response curves to epinephrine, norepinephrine, and isoproterenol are shown in figures 1 through 3, and the data from all the experiments are tabulated in table 1 and are illustrated graphically in figure 4. The values listed are means ± standard error.

In all the experiments both epinephrine and norepinephrine produced dose-dependent decreases in cerebral blood flow and increases in cerebral vascular resistance that were statistically significant (table 1, figs. 1 and 2). Contrariwise, isoproterenol produced dose-dependent increases in blood flow and decreases in cerebral vascular resistance (table 1, fig. 3). With all three drugs, no detectable systemic effects were observed with the two lowest dosages used. However, systemic effects were noted after the injection of the highest dose of each drug. These systemic effects occurred after the direct effects of the drugs on the cerebral vascular bed had become manifest, and although the systemic action partially counterbalanced the direct action, cerebral blood flow never returned to control levels. That is, the reduction in cerebral blood flow that had been induced by epinephrine or norepinephrine did not return to control levels despite the rise in perfusion pressure produced by the 5 μg dose of these drugs (figs. 1 and 2). The augmentation in cerebral blood flow induced by isoproterenol remained despite the
EFFECTS OF ADRENERGIC DRUGS ON THE CEREBRAL CIRCULATION

TABLE 1 (Cont'd)

<table>
<thead>
<tr>
<th>Dose (µg)</th>
<th>Norepinephrine (N = 10)</th>
<th>Isoproterenol (N = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>108 ± 3.7</td>
<td>193 ± 10.1</td>
</tr>
<tr>
<td></td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>1.0</td>
<td>92 ± 6.1</td>
<td>220 ± 8.5</td>
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<tr>
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<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>5.0</td>
<td>62 ± 4.4</td>
<td>0.67 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>0.53 ± 0.01</td>
<td>0.47 ± 0.01</td>
</tr>
<tr>
<td>0.01</td>
<td>1.03 ± 0.01</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>0.67 ± 0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1.0</td>
<td>155 ± 8.2</td>
<td>0.47 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>5.0</td>
<td>1.67 ± 0.02</td>
<td>0.60 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Reduction in cerebral perfusion pressure that resulted from the 1 µg dose of this drug. For these larger doses, the variables were measured before the appearance of the cardiac and peripheral vascular effects.

Alpha receptor blockade markedly diminished the responsiveness of the cerebral circulation to epinephrine and norepinephrine, and beta blockade greatly decreased the responsiveness to isoproterenol (fig. 4). Thus, whereas before phenoxybenzamine the highest doses of epinephrine and norepinephrine produced decreases in cerebral blood flow averaging 53 ± 3%, after alpha blockade the reduction in cerebral blood flow averaged only 15 ± 4%. In no

EFFECTS OF ISOPROTERENOL ON CBF

FIGURE 3

Effects of increasing doses of isoproterenol (Isop) injected into the left internal maxillary artery on left cerebral blood flow (LCBF, pulsatile and mean), aortic pressure (AoP) and heart rate (HR). With the two smallest doses, 0.01 and 0.1 µg, the increase in cerebral blood flow was not associated with an increase in heart rate or a decrease in aortic pressure. With the largest dose, 1.0 µg, the increase in cerebral blood flow was followed in 20 seconds by an increase in heart rate and a slight drop in aortic pressure resulting from the cardiac and peripheral vascular effects of the drug after passage through the brain. Concomitant with the transient drop in aortic pressure, a decrease in LCBF occurred. In spite of this decrease in perfusion pressure, however, cerebral blood flow remained above the control values.
experiment was the extent of alpha blockade great enough to produce reversal of the effects of epinephrine and norepinephrine. That is, we did not unmask the beta stimulating properties of these drugs. The presence of beta receptors in the cerebral vascular bed was evidenced, however, by the increase in cerebral blood flow produced by isoproterenol, which averaged 75 ± 6% for the highest dose, and the marked reduction in this response after the administration of propranolol, the largest increase produced now being only 12 ± 1% (fig. 4).

The ability of the cerebral vascular bed to constrict and dilate after selective adrenergic blockade was demonstrated by the fact that injections of 5 μg of 5-hydroxytryptamine into the internal maxillary artery of three goats produced a 40 ± 4% reduction in cerebral blood flow both before and after alpha blockade. Similarly, an increment in cerebral blood flow of 30 ± 4% was observed with 0.5 mg of papaverine before and after propranolol. Thus, the cerebral vessels continued to respond to drugs that do not depend upon activation of adrenergic receptors.

The effects of anesthesia and operation may markedly affect both the baseline levels of cerebral blood flow and the responsiveness of the cerebral vasculature to drugs, as illustrated in figure 5. After recovery from operation, cerebral blood flow was greater and the vascular bed was much more sensitive to the injected vasoactive amines, indicating the importance of studying unanesthetized animals. Arterial blood samples obtained before and during the effects of injected epinephrine, norepinephrine, and isoproterenol did not show any significant difference in pH, PCO₂, and PO₂ values (table 2).
EFFECTS OF ADRENERGIC DRUGS ON THE CEREBRAL CIRCULATION

EFFECTS OF NE ON CBF IN THE GOAT

DURING ANESTHESIA AND SURGERY

![Graph showing effects of NE on CBF during anesthesia and surgery.](image)

**FIGURE 5**

Effects of anesthesia and surgery on the responsiveness of the cerebral vessels to injections of norepinephrine (NE). During these procedures (upper panel), NE 1.0 µg administered intra-arterially decreased left cerebral blood flow (LCBF, pulsatile and mean) from 50 to 41 cc/min (18% decrease from control). Two days after recovery from operation (lower panel), one-tenth the dose (0.1 µg) injected into the awake animal reduced LCBF from 75 to 50 cc/min (33% decrease from control).

Discussion

The effects of catecholamines on the cerebral circulation remain controversial because measurements of cerebral blood flow after intravenous administration of epinephrine and norepinephrine do not necessarily reflect the direct action of these drugs on the vasculature, since the induced changes in aortic pressure, heart rate, and cardiac output will themselves modify the cerebral blood flow. Investigations in anesthetized animals and in man have yielded conflicting results, with epinephrine and norepinephrine reported to increase, decreases, or have no effect on cerebral blood flow. In addition, it has been difficult to separate the effects produced on the large arteries in the neck from those on small vessels supplying the brain. The general consensus appears to be that relatively large doses of epinephrine or norepinephrine are necessary to produce significant changes in cerebral blood flow.

In the studies described here, we have utilized the unanesthetized goat as the experimental model. This preparation is advantageous because, in the goat, each cerebral hemisphere is supplied by a single artery. In addition, sources of extracerebral blood flow can be eliminated, and, by studying chronic preparations, the effects of general anesthesia and operation are obviated. With this experimental approach, we have clearly demonstrated that relatively small doses of epinephrine and norepinephrine administered directly into the arterial supply to the brain produce sizable reductions in cerebral blood flow without any obvious accompanying changes in aortic pressure or heart rate. When larger doses were used the decrease in cerebral blood...
flow occurred 10 to 15 seconds before the rise in aortic pressure. This sequence of events indicates that, contrary to some previous reports, the decrease in cerebral blood flow is due to a direct action of epinephrine and norepinephrine on the cerebral vessels rather than a consequence of changes in systemic variables. This interpretation is consonant with recent findings on the isolated cerebral circulation of the dog.4

The decrease in cerebral blood flow induced by epinephrine and norepinephrine was blocked to a significant extent after local administration of phenoxybenzamine, suggesting the presence of alpha adrenergic receptors in the brain vasculature, which is in agreement with experiments carried out on the isolated cerebral circulation of the dog. The effects of epinephrine and norepinephrine, however, were not reversed after administration of phenoxybenzamine, probably because of the relatively small dose of blocking agent used.10

Although agreement exists that intravenously administered isoproterenol increases cerebral blood flow, the mechanism responsible remains obscure. It has been suggested that the increase in blood flow results from the tachycardia and the increase in cardiac output that is produced.15,16 These conclusions, however, should be interpreted cautiously since most previous experimental preparations did not allow accurate measurement of true cerebral blood flow, and direct injections into the cerebral arteries could not be made. In the present study isoproterenol increased cerebral blood flow in doses that did not modify heart rate or aortic pressure. The increase in cerebral blood flow with the largest dose of isoproterenol preceded the observed tachycardia and the drop in aortic pressure and the presumed increase in cardiac output by 15 to 20 seconds, strongly suggesting that isoproterenol does indeed exert a direct effect on cerebral vessels.

The cerebral vasodilation produced by isoproterenol was partially abolished after propranolol, indicating the presence of beta receptors in the vessels of the brain. These results are consistent with experiments in the isolated circulation of the dog and in isolated cerebral arteries of the cat.17

In summary, with this experimental technique we have demonstrated that norepinephrine and epinephrine produce marked cerebral vasoconstriction by their direct action and isoproterenol produces vasodilation, also by a direct action. These effects can be blocked by phenoxybenzamine or propranolol and therefore are mediated by adrenergic receptors. Consequently, it does not seem appropriate to ignore the effects of vasoactive drugs on the cerebral circulation, as has been done frequently in the past.

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