Noradrenergic Mediation of Experimental Cerebrovascular Spasm

BY R. A. R. FRASER, M.D., B. M. STEIN, M.D., R. E. BARRETT, M.D., AND J. L. POOL, M.D.

Abstract: Noradrenergic Mediation of Experimental Cerebrovascular Spasm

Catecholamine fluorescent techniques demonstrate an abundant noradrenergic periarterial nerve plexus in the adventitia of the major intracranial vessels. After repeated spasm of these vessels, a marked reduction to complete absence of catecholamine fluorescence is noted. Seemingly, an exhaustion of noradrenaline stores from the nerve terminals has taken place. This has suggested the possibility of noradrenergic mediation of cerebrovascular spasm. Pharmacological depletion of the noradrenaline stores of this plexus produces a dilated vessel, but blood-induced vasospasm is not prevented. Alpha adrenergic blockade at the receptor prevents the induction of vasospasm and dilates both normal and spastic vessels above normal caliber.

These data suggest that cerebral vasospasm is produced by substances acting at the alpha adrenergic receptor of the vessel wall, and that blood contains a vasoconstrictor substance capable of acting at the receptor site.

ADDITIONAL KEY WORDS
subarachnoid hemorrhage
adrenergic blockade
periarterial nerves
neurocirculatory control

Studies in our laboratory using cats, dogs, monkeys and humans, utilizing the catecholamine fluorescent technique of Falck,1 have revealed the presence of an abundant noradrenergic fiber plexus in all the major intracranial vessels. This confirms the previous finding of Neilsen and Owman2 in the cat. Surgical and pharmacological manipulation of this noradrenergic fiber system suggests that it plays an important role in the mediation of experimentally induced vasospasm. In addition, tonic vasoconstriction of the major intracranial vessels by this noradrenergic fiber system is demonstrated.

Despite the careful description of intracranial arterial nerve supply 70 years ago3 and its subsequent reconfirmation4-7 and description of both sympathetic and parasympathetic sources of these fibers,8-10 it is generally agreed that while neural mediation of vasomotor mechanism in cerebral vessels is demonstrable in the laboratory, the significance of these nerve fibers in the regulation of cerebral blood flow is probably minimal and, at best, obscure.11-14

Early animal studies have revealed that a minor reduction in the caliber of ipsilateral pial vessels occurred after the stimulation of the superior cervical ganglion15 and only slight dilatation of the cerebral vessels after cervical sympathectomy.16 These effects have been confirmed by some workers in man,17,18 demonstrating an increase in cerebral blood flow with surgical or pharmacological ablation of these sympathetic fibers. Recently, using Xenon188 in baboons, James et al.10 demonstrated what they considered important vaso-dilator and vasoconstrictor effects after vagus and sympathetic stimulation respectively.

It is the purpose of this report to describe...
EXPERIMENTAL CEREBROVASCULAR SPASM

the effect of surgical sympathetic denervation and of various catecholamine releasing and blocking agents on experimentally induced spasm of the vertebral and basilar arteries. The resulting catecholamine morphology is described.

Method

Twenty-eight Rhesus monkeys have undergone exposure of the basilar and vertebral arteries after the method described by Echlin. Under barbiturate anesthesia a submandibular transclival exposure of the brain stem is carried out, using the dental drill, operating microscope and microsurgical techniques. A cannula is inserted via the femoral artery into the aortic arch for continuous recording of blood pressure via a transducer strain gauge. Blood aliquots are also obtained by this route for frequent sampling of pH, $P_{O_2}$, and $P_{CO_2}$, as well as samples for application to the basilar and vertebral arteries in order to induce vasospasm. The animal is maintained at 38°C throughout the experiment. Continuous recordings are made of blood pressure, EKG, respiration and temperature of each animal.

After removal of the clivus from the foramen magnum to the intercavernous sinus, with small drills and bone rongeurs, the dura is carefully opened and tented with small stay sutures. The ventral surface of the pons and medulla is thus exposed, with the basilar and vertebral arteries lying in the subarachnoid space. After ensuring that meticulous hemostasis of the previously dissected tissues is attained, the arachnoid is carefully removed from its attachments to the underlying vessels.

A photograph is then taken through a reticule eyepiece on the operating microscope to record vessel size and establish a baseline diameter (fig. 1).

Arterial reactivity is established by the application of blood to the adventitial surface of the vessels. Two to three cubic centimeters of fresh arterial blood is gently irrigated from a syringe, or applied in a blood-soaked pledget to the vessels for one to two minutes. This constantly results in a 30% to 50% reduction in external arterial diameter. A saline-soaked cotton pledget will not change the vessel diameter when applied in the same way. Vascular spasm, so induced, will persist for 30 to 50 minutes unless pharmacological agents are used to remove the constriction. Photographs of changes in vessel size, and its return to normal diameter, are taken through the microscope. In some experiments, various pharmacological agents: (1) adrenergic blocking agents acting at either presynaptic or receptor sites, (2) noradrenaline-releasing agents, and (3) sympathomimetic agents were topically applied to the vessel. Three cubic centimeter aliquots of each drug used were irrigated from a syringe into the subarachnoid space surrounding the basilar and vertebral arteries. Before its application, each agent was dissolved in isotonic saline, buffered to pH 7.35 (TRIS), and maintained at 37.5°C in a constant temperature water bath. The pH of all concentrations of each solution used was recorded prior to its use. Each drug was allowed to bathe the vessels for three minutes, then was removed by suction and irrigation with lactated Ringer's solution (37°C). The brain stem and vessels remained submerged in cerebrospinal fluid, supplemented by normothermic lactated Ringer's solution except when photographs were taken. While the initial concentration of each of the pharmacological agents applied was known, because of dilution by cerebrospinal fluid surrounding the brain stem, the actual concentration of the agent in contact with the vessels was considerably reduced.

Upon completion of the experiment, the basilar and vertebral arteries are removed, placed on a glass slide and prepared according to the
When blood is applied to the adventitia of a normal (previously untreated) vessel in the manner described, marked vasospasm is consistently produced. In addition to the 30% to 50% reduction in overall diameter, the visible intraluminal blood column, an index of the lumen diameter, is noted to be reduced to an even greater degree (fig. 2) (table 1).

Noradrenalin was irrigated into the subarachnoid space in varying concentrations (1 × 10^{-8} M - 1 × 10^{-5} M) for three minutes in four animals. Vasoconstriction was immediate, with thickening of the vessel wall. A dose response phenomenon was noted with maximal constriction occurring with highest concentration of the drug. Serotonin (5-hydroxytryptamine) in similar concentration produces virtually identical results. Neither the degree of spasm nor its duration was equal to that produced by blood.

Tyramine, a monamine known to displace noradrenalin from the nerve terminals, was also applied to the vessel wall in (1 × 10^{-3} - 1 × 10^{-6} M) concentrations for two-minute periods. Transient vasoconstriction occurred at the site of the application, followed by a dilation to a larger than normal diameter (table 2). The subsequent application of blood resulted in immediate vasoconstriction of this previously dilated artery. The character of this spasm and its duration was identical to that following the application of blood to a normal vessel.

Bretylium, when applied to the vessel in a (1 × 10^{-3} M) concentration, resulted in transient dilatation of the basilar and vertebral arteries lasting from ten to twenty minutes. The vessels subsequently returned to normal size. If blood is placed in the subarachnoid space around the vessels during the period of dilation, immediate constriction occurs. The character of the spasm and its duration did not appear to differ from that resulting when blood was applied to a previously untreated vessel.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Initial diameter</th>
<th>Minimum diameter</th>
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<tbody>
<tr>
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<td>6</td>
</tr>
<tr>
<td>Rhesus 412</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>Serotonin</td>
<td>11</td>
<td>8</td>
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<td></td>
</tr>
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<tr>
<td>Noradrenalin</td>
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<td></td>
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<tr>
<td>Rhesus 21270</td>
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</table>

\[ \text{FIGURE 2} \]

Basilar and vertebral arteries. Spasm of basilar artery after blood application. Note greater diminution in blood column than in overall diameter in spastic area (×10).
When a known antagonist to the effects of serotonin on smooth muscle is applied to the adventitia of a normal basilar artery, an immediate dilatation of the vessel results. 2-Brom LSD in (1 × 10^{-8}) concentration consistently produces these results. In this instance the dilatation is to a far larger than normal diameter and cannot be reversed by the subsequent application of blood, serotonin or noradrenalin.

Phenoxybenzamine (1 × 10^{-8} to 1 × 10^{-5}), when applied to the adventitia of the basilar and vertebral arteries, results in immediate dilatation to a larger than normal diameter. The response occurs in both vasospastic and normal vessels. This effect is irreversible—an unreactive dilated vessel results. The subsequent application of blood, serotonin or norepinephrine is without effect; the caliber of the vessel remains unchanged (fig. 3).

After bilateral surgical removal of the superior cervical ganglion, the source of the intracranial noradrenergic fibers, similar experiments were performed. A normal ability to constrict after the application of blood, noradrenalin and serotonin was noted. Neither the character of the spasm nor its duration seemed affected by prior sympathetic denervation, and was apparently identical to that seen in those animals with intact sympathetic supply. Not only were the basilar and vertebral arteries of normal diameter in these animals when first exposed, they were also capable of further dilatation after the application of dibenzyline. Again, the resulting dilatation was irreversible.

An untreated vessel, or one placed in spasm by the application of blood, noradrenalin or serotonin for one or two occasions, demonstrates an abundant adventitial noradrenergic fiber plexus when studied by the fluorescent technique of Falck\(^1\) (fig. 4). After repeated induction of spasm, an exhaustion phenomenon appears, usually after several hours of repeated testing and induction of vasospasm. Such vessels, when removed and studied with the fluorescent technique, reveal a marked

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**FIGURE 3**

Basilar artery dilated from spastic state by dibenzyline (1 × 10^{-8}M) application. Vertebral arteries not treated with dibenzyline remain in a constricted state. (Fine oblique black line—silk suture markers [×10]).

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**TABLE 2**

<table>
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<tr>
<th>Agent</th>
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<tr>
<td>Rhesus 3870</td>
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<tr>
<td>(1 × 10^{-8} molar)</td>
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</tbody>
</table>
depletion to complete absence of the normally rich catecholamine containing nerve fiber plexus. Vessels treated with a catecholamine releasing agent, such as tyramine, reveal an almost complete absence of these catecholamine containing fibers (fig. 5). Similar experiments carried out in the cat and dog have revealed virtually identical results with similar catecholamine morphology.

Vessels treated with an alpha adrenergic blocking agent (dibenzyline) reveal a normally abundant catecholamine nerve fiber plexus when examined with this technique.

In two animals treated with prior removal of the superior cervical ganglion, fluorescent studies revealed a complete absence of the catecholamine fluorescence.

**Discussion**

Since the recent demonstration of an abundant noradrenergic periarterial nerve fiber plexus in catst and in dogs, primates and humans in our laboratories in all the major intracranial vessels, it is suggested that these nerves may exert an important effect on cerebral vascular tone. Nielsen and Owman report a relatively deficient adrenergic innervation of the posterior circulation in the cat. Our studies in the monkey, however, indicate that the basilar and vertebral arteries enjoy an abundant adrenergic nerve supply, by inspection equal to that seen on the large vessels of the anterior circulation. These nerves become markedly reduced in number in the smaller vessels, with only one or two fibers being seen in vessels smaller than 15 to 20 μm.

In view of the rich adrenergic innervation of all the major intracranial vessels, it might be expected that pharmacological manipulation of their sympathetic fibers would produce greater effects than on pial vessels with their much more scanty innervation. It is reported that vasoconstriction of the pial vessels caused by...
hyperventilation may be released by the administration (route not specified) of beta adrenergic blocking agents (INPEA). These same authors suggest that cervical sympathectomy will prevent the pronounced vasoconstriction of the pial vessels that occurs following hyperventilation. Rosenblum, utilizing barium chloride, induced vasoconstriction of pial vessels in mice, and found that beta adrenergic blockade could prevent spasm so induced. Alpha adrenergic blockade (phentolamine), on the other hand, did not consistently dilate these vessels. Neither of the investigators cited above reported on the effect of these substances on the larger intracranial vessels.

The present report is, to the best of our knowledge, the first to describe the effects of alpha adrenergic blockade applied via the subarachnoid route to the major intracranial vessels.

In our experience, vasospasm induced by blood could be consistently prevented or, if present, removed by blockade at the alpha adrenergic receptor. Prior surgical removal of the source of the adrenergic plexus did not, however, in any way change the ease of induction of the spasm or its duration. This would suggest that blood produces its well-known vasoconstrictive effect by acting at the neuromuscular junction, and not by causing the periarterial nerve fibers to release a vasoconstrictor substance such as noradrenaline. Pharmacological support for this concept is provided by the effect of tyramine. This substance, while known to release noradrenaline from nerve terminal stores, did not result in more than a transient and mild degree of vasoconstriction. This is, presumably, the effect of the released noradrenaline which occupies the alpha adrenergic receptor sites. The vasodilatation that followed was presumed due to removal of the tonic vasoconstrictive effect being mediated at the noradrenergic receptor, or some other site on the vessel wall, and not via the release of noradrenaline stores in the nerve terminals. Finally, pharmacological evidence of the role of alpha adrenergic receptor sites is provided by observations of the effect of phenoxybenzamine. This substance, when applied to the adventitia, produces large dilatation—both normal (previously untreated) and spastic vessels become irreversibly dilated. Blood is no longer able to produce vasoconstriction. Presumably its site of action—the alpha adrenergic receptor—has been competitively blocked.

The noradrenergic periarterial fiber plexus would appear to function as a tonically active vasoconstrictor of the larger intracranial vessels. Substances causing depletion of the noradrenalin stores or blockade at the receptor site result in dilatation of the vessel to a larger than normal diameter—reversible in the former, irreversible in the latter instance.

In addition to the 30% to 50% reduction in external diameter of the artery, the blood column, in certain instances, virtually disappeared in the spastic segment of the vessel. It seems likely that a portion of this phenomenon is a visual artifact due to the greater mural opacity of a constricted vessel. Van Citters et al. noted that medial thickening and intimal folding associated with vasoconstriction made the intraluminal blood column less visible. Using the superior mesenteric artery, these authors showed that the lumen diameter to vessel wall thickness ratio changed markedly with alterations in vessel caliber. A relaxed vessel could have a 30:1 lumen to wall ratio. The data indicated that a reduction in external vessel diameter due to vasoconstriction was associated with an even greater decrease in the actual lumen of the vessel. In one example cited by Van Citters et al., a 1 mm superior mesenteric artery was observed to undergo a reduction in external diameter to 60% of the initial value; while the lumen diameter was reduced to 20% of the initial value. Such amounts of stenosis are of obvious importance regarding the blood-carrying capacity of the vessel.

**Summary**

Twenty-eight Rhesus monkeys (including two with prior surgical sympathectomy) underwent transclival exposure of the basilar and vertebral arteries as part of an experimental study of cerebrovascular spasm. Various pharmacological agents were applied to the adventitia of the exposed vessels prior to and after the induction of spasm. At the conclusion of the experiment the vessels were removed and studied with the catecholamine fluorescent technique.

Data derived from this study indicate that
(1) blood supplied to the adventitia of an intracranial vessel consistently results in spastic constriction; (2) serotonin and noradrenalin applied in a similar fashion produce a minor vasoconstriction of short duration; and (3) blood-induced spasm occurs only in that segment where blood is in contact with the vessel. No propagation of spasm to blood-free areas occurs.

Catecholamine fluorescent studies reveal an abundant noradrenalin containing perivascular nerve plexus in normal vessels. Repeated vascular spasm results in depletion of the catecholamines of the perivascular plexus. Pharmacological depletion of these catecholamine stores results in dilatation of the vessel to a larger than normal diameter, but subsequent blood-induced spasm is not prevented. Blockade at the alpha adrenergic receptor site dilates both normal and spastic vessels above normal caliber, and renders a vessel refractory to further spastic constriction.

From these observations we conclude that blood-induced vascular spasm is the result of a constrictor substance contained in blood that is functionally active at the alpha adrenergic receptor site. Further, cerebrovascular spasm would appear to be the result of a stimulus in excess of that provided by presynaptic sympathetic stimulation, and mediated at the smooth muscle adrenergic site.

References
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