Experimental catecholamine-induced chronic cerebral vasospasm

Myonecrosis in vessel wall

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The authors report a new experimental model for the study of delayed intracranial arterial spasm in monkeys. The injection of norepinephrine into the prepontine cistern produces an intense immediate vasospasm that disappears in minutes and is followed by a second stage of vasospasm that persists for 8 to 10 days. Electronmicroscopic examinations of the basilar artery removed during the second stage of this spasm reveal myonecrosis of the media, with fragmentation of myofibrils, dissolution of the sarcolemma, and interstitial edema.

KEY WORDS - intracranial vasospasm - norepinephrine - electronmicroscopy - myonecrosis

ALTHOUGH radiographically demonstrated cerebral arterial spasm after subarachnoid hemorrhage (SAH) is a well-recognized clinical entity, its etiology and pathophysiology are unknown. Clinical observation has established that vasospasm may be delayed in onset but that, once present, does not respond to any known pharmacological therapy and usually lasts for many days. Experimental vasospasm has been produced by numerous investigators using either arterial injury, subarachnoid blood injection, or both. The resultant vasospasm is biphasic, with the first phase beginning immediately and lasting for minutes and the second phase beginning after many hours and lasting for days. The delayed or persistent vasospasm appears to be the process that is associated with morbidity in patients following SAH.

The observation that the subarachnoid blood injection that produces experimental vasospasm causes the immediate disappearance of the catecholamines located in the nerve endings of cerebral arteries and the report that pretreatment of animals with alpha adrenergic blocking agents will prevent the acute vascular contraction secondary to direct blood application to the basilar artery have implicated the sympathetic nervous system in the etiology of cerebral vasospasm. Nevertheless, most investigators have concluded that, although the immediate vasoconstriction seen after blood injection may be secondary to neural discharge, the delayed or persistent spasm is most likely due to a chemical liberated from the clotted blood itself. Extensive investigations have failed to identify this chemical, however, and the mechanism of persistent cerebral vasospasm
Myonecrosis with norepinephrine-induced vasospasm

remains obscure.

A number of complex events, such as the electrocardiographic (ECG) changes and cardiac arrhythmias that occur at the time of subarachnoid hemorrhage, are secondary to the liberation of catecholamines from peripheral sympathetic nerves and nerve endings at the time of SAH, and have been shown to induce severe injury to myocardial cells. Our hypothesis, based upon the reactions seen in the heart to massive catecholamine release, is that sympathetic discharge at the time of SAH may damage smooth muscle cells of the cerebral arterial wall, and that this intrinsic damage, or the reaction to it, produces the second or delayed phase of vasospasm. To test this hypothesis, we have administered norepinephrine directly into the subarachnoid space without inducing either vascular injury or SAH and compared the effects radiologically and structurally to those induced by subarachnoid blood injection.

Methods

All experiments have been performed on monkeys under Surital anesthesia. Under sterile technique a C-2 laminectomy is performed, and under magnification the dura and arachnoid are opened. A Silastic catheter is inserted immediately beneath the C-2 root and directed cephalad ventral to the spinal cord so that its distal end rests in the preponine cistern. The proximal end of the catheter is brought out between the paraspinous muscles, occluded, and left beneath the skin for subsequent injections.

Vertebral angiography is then performed by the transfemoral approach, the catheter being positioned in the left subclavian artery at the origin of the left vertebral artery.

After satisfactory control angiograms have been obtained, the test material is injected into the preponine cistern through the Silastic catheter. Experimental animals receive a standard dose of 2 mg of

Fig. 1. Subtraction vertebral arteriograms. Left: Pretreatment control film. Right: Film taken 2 days after intracisternal norepinephrine injection showing a 50% reduction in the caliber of the intracranial vessels. The angiogram was performed with a rapid cassette changer at two films per second.
norepinephrine (an alpha-adrenergic stimulator) either as supplied in its commercial base or dissolved in the animal's own spinal fluid. One animal received 5 cc of its own arterial blood intracisternally in order to reproduce the standard vasospasm model. Control animals received either the injection base alone without norepinephrine, or isoproterenol, a β-adrenergic stimulator.

Immediately after injection of the test material, vertebral arteriography was repeated; it was also done at 1 hour and at 2, 4, 8, and 10 days following injection. All arteriograms were reviewed independently by both investigators and graded for the presence or absence of arterial spasm.

At the termination of each experiment, the monkey was sacrificed by transcardiac perfusion with glutaraldehyde-paraformaldehyde fixative. The intra-arterial pressure was monitored continually during the perfusion to insure that it did not go above the animal's normal systolic pressure. The brain was immediately removed and the basilar artery excised, postfixed in phosphate buffered osmium tetroxide, imbedded in epon and sectioned for electronmicroscopy. Comparable samples of artery were imbedded in paraffin for light microscopy.

**Results**

All animals that received subarachnoid norepinephrine injections showed biphasic vasospasm although the degree of spasm varied from animal to animal (Fig. 1). The first phase of spasm began immediately and

![Fig. 2. Electron micrograph showing the intima and media of the basilar artery from an animal sacrificed 3 days after subarachnoid norepinephrine injection. Vertebral angiography performed immediately prior to fixation revealed the basilar artery to be in severe chronic spasm. The endothelium (E) and the internal elastic lamina (I) appear normal. The smooth muscle cells of the media (M) show fragmentation of myofilaments (F) and degeneration of an entire smooth muscle cell (X). × 5000.](image-url)
Myonecrosis with norepinephrine-induced vasospasm lasted for 10 to 15 minutes. The second phase began between 4 and 24 hours post injection and lasted 8 to 10 days. The monkeys showed no abnormal clinical effects during this interval.

Electronmicrography of the basilar arteries of all animals sacrificed during the chronic phase of angiographically demonstrated vasospasm showed significant changes in the smooth muscle cells of the media consisting of fragmentation of myofilaments and disruption of the sarcolemma in the animals sacrificed at 3 or 4 days (Figs. 2 and 3). The animals sacrificed after 5 to 7 days showed lipid inclusions within abnormal smooth muscle cells that had lost their usual myofilamentous structure (Fig. 4). The abnormal cells were scattered randomly throughout the vessel wall; markedly distorted cells frequently lay adjacent to normal ones. Abnormal cells were more frequently found close to the internal elastic lamina than to the adventitia. In both intact and fragmented muscle fibers, the mitochondria appeared normal.

The one monkey that had a subarachnoid blood injection showed a similar angiographic picture of biphasic spasm. Samples of basilar arterial wall from this animal also showed myonecrosis with lipid inclusions in the smooth muscle cells.

No inflammatory cells were seen in any of the specimens.

Discussion

These experiments demonstrate that the subarachnoid administration of norepinephrine is capable of producing severe cerebral vasospasm with a radiographic appearance and time course similar to that produced by the injection of blood.
Others have shown that pretreatment with alpha-adrenergic blocking agents will prevent the immediate vasoconstriction in response to blood and have suggested that sympathetic discharge is responsible for the acute phase of vasospasm. Our work suggests that catecholamines have a role in the production of the chronic phase as well.

Although the presence of catecholamines in cerebral vessels is well known, the prolonged time course of the late phase of clinical vasospasm has been difficult to understand in terms of neurotransmitter release. At the same time, no chemical agent capable of producing the late stage of vasospasm has been identified. Our data suggest that persis-

Fig. 4. Electron micrograph from a section of the media of the basilar artery removed from an animal 7 days after intracisternal norepinephrine injection. Angiography immediately prior to fixation revealed chronic vasospasm. Note the abnormal muscle fiber with loss of myofilaments and electron dense inclusion, lying adjacent to a normal muscle fiber that shows well-preserved myofilaments, mitochondria, and nucleus. The dark inclusions are presumed to be lipid collections from cellular degeneration. × 40,000.
tent vasospasm is due neither to continuous release of neurotransmitter nor to a principle in the blood, but rather to events within the blood vessel wall induced by the hemorrhage. It is possible that a substance originating within the smooth muscle cells of the arterial wall is released into the extracellular space as a result of catecholamine-induced myofibrillar fragmentation and destruction of the sarcolemma and that this substance causes neighboring normal smooth muscle cells to contract. The contraction would then persist as long as the intrinsic irritation persisted and could not be expected to respond to sympathetic blocking agents.

If this experimental model has clinical significance, one must be pessimistic about any pharmacological therapy designed to treat vasospasm; the arterial injury we have described is produced at the time of the initial hemorrhage, and treatment with blocking agents may not be of much benefit. Nevertheless, future investigations might uncover a method to reverse or arrest the process once it has begun. Additional studies are needed to determine if this model is applicable to human vasospasm and, if so, to further characterize the cause of the myonecrosis and its relation to the persistent vasoconstriction.

References

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