The Relevance of In Vitro Smooth Muscle Experiments to Cerebral Vasospasm

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SUMMARY An overview of the possible factors that might contribute to the development of cerebral vasospasm is presented, with particular emphasis on the possibility that spasm arises from a malfunction of the regulatory or contractile processes in smooth muscle cells. This possibility is emphasized because the evidence for cellular damage and the delayed occurrence of vasospasm are suggestive of pathological alteration. Data regarding the development of spasm in vivo has been reviewed and, to the extent possible, correlated with in vitro studies of cerebrovascular smooth muscle contractility. Short-term in vitro studies of normal cerebral arteries may be of little relevance to the prolonged and severe cerebral vasoconstriction that occurs only after a delay of several days from the initial insult.

The Delays Neurological Deficits that frequently develop in patients with ruptured intracranial aneurysms are widely attributed to severe cerebral vasospasm. Despite considerable effort, however, effective therapy for such patients remains unavailable. In part, this failure is because of the need to dilate only those cerebral vessels that are constricted, a fact that has been emphasized by others.

In an attempt to study some of the factors possibly contributing to the etiology of spasm, many investigators have turned to the use of segments or strips of major cerebral arteries in vitro. Such an approach provides a system amenable to close experimental control. On the other hand, in vitro experimental simplicity may not well represent the in vivo situation.

This review considers the use of in vitro cerebroarterial tension measurements in the study of cerebral vasospasm and discusses the contribution of such studies to potential treatment.

Possible Factors Involved in the Etiology of Cerebral Vasospasm

Factors directly involved in the production of cerebral vasospasm are, at this time, a matter of speculation. At the heart of the problem is the following question: Is spasm simply a vasoconstrictive response of normal arteries to a particular set of constrictive stimuli, or is it an expression of abnormal physiology or anatomy (possibly, though not necessarily, arising in conjunction with the surrounding environment)? The concept that cerebral vasospasm occurs as a result of structural pathology within the smooth muscle cells has been suggested.

It is crucial to distinguish between these two fundamental possibilities. To date, most in vitro experiments have been carried out using normal arteries taken from untreated animals. If however, spasm occurs as a result of slowly developing pathological changes in the vessel wall, such model systems may be at best questionable and at worst misleading.

1. Evidence for Morphological Changes in Arteries in Spasm

Studies of human cerebral vessels that have been in spasm indicate that between several days and several weeks after subarachnoid hemorrhage (SAH), some smooth muscle cell necrosis occurs, leading to a reduction in the number of layers of smooth muscle cells in the media. During this period, cells tentatively identified as macrophages can be seen in the medial layer. Swelling of the intima becomes apparent during this time and after several weeks develops into a subendothelial fibrosis that narrows the lumen.

By electron microscopic examination of arteries in monkeys and dogs with a single experimentally-induced SAH, the majority of smooth muscle cells remained normal in appearance during the study periods. Some cells, however, became necrotic, with certain changes being observable as soon as 2 hours after hemorrhage and others apparently persisting as long as 6 to 8 months after hemorrhage. These changes included an increase in the number of vacuoles and dense bodies. Degenerating mitochondria were noted in one study, but not in another. Anucleated cells and smooth muscle cell debris were apparent several days after the initial event and persisted for 6 to 8 months.

More recent studies have demonstrated that a “two-hemorrhage” canine model of cerebral vasospasm more closely resembles the human pathology in time-course of development, severity of vasoconstriction, and unresponsiveness to drug therapy. Our laboratory has documented a variety of morphological changes in vessels shown to be in “irreversible” spasm by angiography. Most notable was the presence of blood components in the adventitia of vessels most...
severely constricted in situ. Extensive smooth muscle cell necrosis, endothelial cell vacuolization, disruption of the internal elastic lamina, and intimal changes were noted in both studies. 13, 60

The extent of intimal changes in different animal models is inconsistent. Several groups have reported the development of endothelial cell damage and loss in response to constriction of cerebral arteries after induced SAH. 1, 96

Others noted a loss of endothelial cell tight junctions, which often had adhering platelets. 32, 51, 67 Still other investigators have not observed alterations in the endothelial cells of vessels in spasm. 46 The latter observation argues against the necessity of endothelial cell-platelet interaction being involved in all instances of spasm. It has been reported that endothelial cell integrity may be required to mediate the vasodilatory response of arterial smooth muscle to acetylcholine 77 and other vasodilators. 56

The reasons for the above-mentioned differences may be several, including severity of the induced spasm and species differences. Current thinking suggests that PGI2 synthesis is initiated in endothelial cells in response to a vasoconstrictive stimulus. 56, 69, 70 Recent evidence suggests that similar responses also occur in the smooth muscle cells themselves. 90 The reactive oxygen free radicals, which are produced as a result of prostaglandin synthesis during the conversion of PGG2 to PGH2, may overwhelm the normal cellular defenses against free radicals thereby incurring cellular damage. 35, 56 Enzymes involved in the destruction of superoxide and its metabolite peroxide are superoxide dismutase, glutathione peroxidase, and catalase. Considerable species variation in the activity of these enzymes, particularly glutathione peroxidase and catalase, has been reported in the erythrocyte. 64 Should this variability apply as well to endothelial or smooth muscle cells, a species variation in predisposition to endothelial cell damage or smooth muscle reaction during severe vasoconstriction might be expected.

2. Possible Alterations in Vascular Smooth Muscle Contraction and Its Control

Contraction of smooth muscle, like other types of muscle, develops as a result of thick (myosin) and thin (actin) filaments being driven to slide past each other in a motion coupled to the cell body, hydrolyzing adenosine triphosphate (ATP) for chemical energy. How this activity is initiated and controlled, however, differs for the different types of muscle cell. In the case of smooth muscle, the mechanism of activation that has received the most attention recently is the phosphorylation of a regulatory subunit of myosin, the 20,000-dalton myosin light chain. 14, 83

In this mechanism, the binding of the myosin head to actin (which generates contractile activity) can occur only when the inhibition afforded by the presence of a myosin light chain is removed. Under normal conditions, this is achieved by phosphorylation of the light chain from ATP catalysed by the enzyme myosin light chain kinase. MLCK is active only in conjunction with calcium ions and calmodulin, a ubiquitous calcium-binding regulatory protein. 10 Calcium binding to calmodulin takes place over a calcium concentration range of 10^-7 to 10^-5 M, and it is control of this factor that normally initiates contraction or relaxation. 87 This model is shown schematically in figure 1.

That ATP is required for phosphorylation of the myosin light chain before contraction can take place is the likely reason why rigor does not normally occur in smooth muscle as a result of ATP depletion. In smooth muscle, a lack of ATP would inhibit the rate of phosphorylation, allowing dephosphorylation of the light chain via phosphatase activity to predominate; hence, relaxation would ensue.

One can hypothesize several ways in which pathological alterations in the scheme of figure 1 might lead to intractable vasoconstriction.

Proteolytic digestion of the myosin regulatory light chain with papaain has been shown to prevent its inhibitory action, thereby permitting the binding of myosin to actin and consequent development of smooth muscle force or shortening. 72 Following such proteolytic attack, the need for normal calcium dependent activation is removed. Another way in which calcium control
might be lost is through proteolysis of myosin light chain kinase. After limited proteolysis using chymotrypsin, myosin light chain kinase no longer requires calcium and calmodulin to effect phosphorylation of the myosin light chain. Each of these conditions, other things remaining unaffected, would lead to progressive contraction as Ca\(^{2+}\)-regulated control of the contractile process was lost. Furthermore, in the former case, it is conceivable that a rigor contraction may develop if ATP production in the smooth muscle were impaired, since ATP is not required for actin-myosin association but is required for their dissociation. Normally, the continued need for myosin regulatory light chains to be phosphorylated to permit the actin-myosin interaction that leads to contraction negates the possibility of rigor developing.

Not only does the activity of myosin light chain kinase normally depend on calcium binding by calmodulin, but the kinase is also further regulated by a cAMP-dependent protein kinase, which can be stimulated by the activation of β-adrenergic receptors in the cellular membrane. Phosphorylation of myosin light chain kinase, which is promoted by cAMP, reduces the affinity of the kinase for Ca\(^{2+}\)/calmodulin, thus effectively reducing its ability to phosphorylate the myosin light chain and thereby reducing smooth muscle contraction. A failure of this endogenous inhibitory system could also lead to enhanced contraction.

As described above, phosphorylation of the myosin regulatory light chain is thought to permit the binding of P-myosin heads to actin to form active crossbridges. Further studies suggest, however, that phosphatase action on P-myosin can take place while actin and P-myosin are still bound together, forming an actomyosin complex that dissociates only very slowly and is somewhat analogous to molluscan "catch" muscles. In this pathway, the rate of dissociation of crossbridges is much reduced, so that contraction can be maintained at a substantially lower ATP utilization rate.

Disturbances in the contractile mechanism, such as might result from changes in intracellular ionic concentrations, presumably affect the contraction or relaxation process. In particular, a reduction in the rate of dissociation of the actomyosin complex prolongs contraction under conditions in which little energy is being consumed. In the extreme situation, contraction could be maintained with virtually no ATP utilization. Cerebrovascular smooth muscle can maintain contraction without external calcium and at a much restricted rate of energy utilization. For example, rabbit basilar arteries, when stretched to twice their initial length, rapidly developed localized rings of constriction that persisted for as long as the vessels were maintained in vitro, i.e., for 72 hours. Such constrictions were associated with rupture of the elastic lamina, localized disruption of the media where the elastic lamina was absent, and disturbance of the smooth muscle cells at these sites. Furthermore, the constrictions produced in this manner were not reversed by cyanide or by calcium deple-

3. Membrane Alterations and Calcium

Another point of control, and possibly the most susceptible to malfunction, is regulation of the intracellular calcium concentration. The cytoplasmic calcium concentration in relaxed smooth muscle cells is approximately 10\(^{-7}\) M, while that of plasma and cerebrospinal fluid is approximately 10\(^{-3}\) M, giving rise to a concentration ratio of approximately 10\(^{4}\)-fold across the sarcolemma. In addition to the concentration gradient, there is also a transmembrane potential of about −50 to −70 mV, which acts in the same direction as the calcium concentration gradient. Damage to the membrane of the smooth muscle cell might increase its permeability to calcium which, in turn, would activate smooth muscle contraction. Evidence from electron probe analysis supports this contention and indicates that the calcium concentration in smooth muscle cells can increase in arteries that are atherosclerotic or have sustained damage in other ways.

In our laboratory, we have found that canine basilar arteries shown angiographically to be in spasm in situ following the double-SAH canine model are depolarized relative to control basilar arteries. In seven such vessels studied in vitro, membrane potential measured by microelectrode puncture averaged −42 mV under the same in vitro conditions in which normal vessels averaged −53 mV. This persistent depolarization of vessels subjected to subarachnoid blood may render the vessels more reactive to vasoactive materials.

Other observations also indicate that the contractile response is modified by alterations in the membrane. For instance, prior stretching of vascular smooth muscle preparations in vitro increases the sensitivity of the tissue to various agonists. This might arise as a result of slight conformational changes in the receptors which increase their affinities for the agonists. Changes in the number of cell surface receptors and their coupling to membrane-bound enzymes are also influenced by hormonal and other chemical factors, and it is conceivable that mechanical influences can exert a similar effect. Very low levels of cholesterol, 10\(^{-10}\) M to 10\(^{-12}\) M, were reported to produce small sustained contractions of canine coronary arteries in vitro and, furthermore, to enhance the effects of potassium and calcium. One would expect cholesterol to be liberated extracellularly as a result of myonecrosis. Its inclusion in cell membranes is known to modulate membrane fluidity, which, in turn, would be expected to affect membrane function.

One mechanism by which a reduction in membrane fluidity, such as cholesterol produces, can be translated into a contraction of smooth muscle is suggested by studies showing that membrane alterations associated with a change in membrane fluidity also change the membrane Ca\(^{2+}\)-ATPase activity. Ca\(^{2+}\)-ATPase, a protein that transports calcium across cellular membranes at the expense of ATP hydrolysis, is dependent

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for its activity on a surrounding annulus of about 90 lipids. An increase in the activity of this protein in the membrane of the smooth muscle cell decreases sarcoplasmic calcium concentrations. Conversely, a decrease in its activity, such as has been observed after a reduction in membrane fluidity with exogenous phospholipids, would cause an increase in sarcoplasmic calcium concentration and hence an activation of smooth muscle contraction.

Vascular smooth muscle contraction has been linked to active sodium transport across the sarcolemma, a process associated with the functioning of the membrane-embedded protein (Na, K)-ATPase (sodium and potassium ion-stimulated adenosine triphosphate). Conditions that lead to a reduction in the activity of (Na, K)-ATPase can induce vascular smooth muscle contraction, such as a reduction in external potassium. If the potassium is then replaced, a relaxation occurs which can be prevented by the presence of the (Na, K)-ATPase inhibitor. Presumably, the fact that ouabain stimulates vascular smooth muscle to contract reflects such an action.

Several mechanisms have been proposed to explain these effects: (a) neuronal depolarization leading to release of vasoconstrictive neurotransmitters, (b) partial depolarization of the smooth muscle cells leading to calcium influx through voltage-dependent channels, and (c) interruption of sodium ion effusion in the smooth muscle cell leading to a rise in internal sodium ion concentration and hence a reduction in the transmembrane sodium ion electrochemical gradient. This sodium ion gradient is believed to be linked to calcium ion extrusion. Consequently, its reduction will increase the intracellular calcium concentration.

In the case of cerebrovascular smooth muscle, canine vessels showed a dose-dependent response to ouabain that exceeded 30% of the contraction elicited by 30mM potassium. This response was only slightly attenuated by pretreatment of the animals with reserpine or treatment of the vessels with phenolamine, indicating that the neuronal mechanism of the response in these vessels is of limited importance. A direct action on the smooth muscle (Na, K)-ATPase is therefore suggested, leading to both a partial depolarization and a reduced sodium gradient across the membrane. As with the Ca-ATPase, changes in membrane fluidity can be expected to modulate the activity of (Na, K)-ATPase and hence to alter both internal calcium concentrations and membrane polarization.

The above discussion suggests the possibility that cerebral vasospasm arises as a consequence of pathological alterations of the smooth muscle cell membranes which lead to an activating calcium ion influx. Because this influx may not be limited to the normal routes of calcium entry, i.e., calcium channels, we have recently used our "two-hemorrhage" canine model to evaluate the effectiveness of calmodulin blockade in preventing the development of cerebral vasospasm. The strategy in these experiments was to inhibit the intracellular calcium receptor (calmodulin, cf. fig.1) and thereby inhibit vasoconstriction irrespective of possible damage to the smooth muscle membranes. In 4 untreated animals subjected to SAH, basilar artery diameter was reduced to 55 (± 13)% of control diameter. Following the same protocol, but with an added prophylactic regimen of the calmodulin antagonist trifluoperazine, basilar artery diameter after experimental SAH averaged 89 (±9)% of control diameter.

4. Neuronal Factors

A. In Vitro Considerations

The presence of a well established plexus of adrenergic fibers within the adventitial layer of the pial vasculature has been documented using the sensitive technique of histochemical fluorescence (see for instance). Denervation and histochemical experiments have shown that these nerves originate in the superior cervical sympathetic ganglia and terminate as a series of varicosities approaching within 80 to 100 nm of the smooth muscle cells. The size and density of the varicosities vary considerably depending on the particular cerebral vessel. In rats, rabbits, and humans, the adrenergic fibers of the basilar artery are thin, narrow, and contain an abundance of non-varicose preterminal axons. On the other hand, the anterior cerebral arteries and the carotid, middle, and posterior cerebral arteries are richly endowed with large varicose terminals. The presence of cholinergic nerves has also been demonstrated in the walls of cerebral blood vessels, based on the presence of acetylcholinesterase and electron-microscopic evidence. These neurons closely follow the adrenergic fibers and both fiber types are concentrated at the bifurcations of the vessels. The origin of the cholinergic fibers is less well understood than that of the adrenergic neurons. Those terminating on the carotid, anterior, and middle cerebral arteries arrive via the petrosal and oculomotor nerves from the geniculate ganglion. Those of the vertebral, basilar and posterior cerebral arteries originate in the glosopharyngeal and, possibly, vagus nerves. Other neuronal systems have been reported in addition to these two types of neurons. Two systems can be visualized by immunohistochemical techniques because their fibers contain vasoactive intestinal polypeptide (VIP) in one case and substance P in another. Mayberg et al. have localized perivascular sensory afferents with cell bodies in the trigeminal ganglia of cats using retrograde transport of horseradish peroxidase.

Despite the evidence for the existence of such nerves, however, their functional effect on the cerebral vasculature is not well understood and, indeed, is currently a matter of debate. Some investigators have observed diminution in cerebral blood flow after sympathetic stimulation, whereas others have not. Heistad and Marcus concluded that, although some constriction of blood vessels can occur during sympathetic stimulation, the magnitude of this effect is normally too small to produce significant changes in cerebral blood flow. A number of in vitro studies involving transmural stimulation of sympathetic nerves in ves-
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sels have shown that contraction occurs in rabbits, dogs, sheep, and monkeys. In contrast, transmural neuronal stimulation of cerebral vessels from cats consistently produced dilatation, as did stimulation of vessels from dogs and sheep after sympathetic denervation or treatment with guanethidine. Surprisingly, α-adrenergic antagonists were often ineffective in blocking the contractile effects of transmural stimulation. Phentolamine (10^-6 M) potentiated the contractile response in the canine vessels, although it blocked the effect in sheep cerebral arteries. One possible explanation for this finding is that phentolamine at 10^-6 M is not effective in blocking the post-junctional receptors at the high norepinephrine concentrations elicited by the electrical stimulation of the canine vessels, whereas it is effective in blocking the prejunctional uptake, which potentiates the contractile effect. These observations also demonstrate the significant behavioral differences that cerebral arterial preparations from different species can exhibit.

B. After Subarachnoid Hemorrhage

Although there is disagreement concerning the role of neurons in controlling cerebral blood flow under normal circumstances, the possibility exists that in certain disease states this factor assumes increased importance. In the case of SAH, the loss of catecholamine-induced fluorescence of the adrenergic fibers innervating the cerebral vessels has been well documented. This change may not revert to normal for 4 to 8 weeks. Massive release of catecholamines from adrenergic fibers after SAH has been implicated as the cause of myonecrosis, which can be prevented by prior catecholamine depletion. Prevention of myonecrosis, however, does not necessarily prevent vasoconstriction, nor does myonecrosis per se lead to vasoconstriction. Abnormalities in cholinergic neurons have also been reported as a result of SAH, in particular, the loss of acetylcholinesterase. Several studies have shown that cerebral arteries that have been exposed to blood develop an altered sensitivity to vasoactive stimuli. In one study, delayed spasm was induced in cats by the injection of blood into the cisterna magna. Three days later, topical applications of incubated blood and norepinephrine induced significantly greater contractions in these cats than in untreated animals. Application of norepinephrine alone at a concentration of 10 ng/ml was reported to reduce the caliber of these cerebral vessels to 50.3 ± 8.2%, while concentrations of less than 50 ng/ml norepinephrine caused no significant vasoconstriction in control rats. Increased sensitivity to serotonin and decreased responsivity to pCO2 have also been reported in the cerebral arteries of baboons 5 to 7 days after SAH induced by puncture of the middle cerebral artery.

In other experiments, cerebral arteries of both cats and rabbits that had been exposed to subarachnoid blood in vivo and then examined in vitro 3 days later showed an increased contractile sensitivity to both norepinephrine and serotonin. The increase in the sensitivity of the arteries to these amines correlated with the decrease in norepinephrine-induced fluorescence with a reduced uptake of norepinephrine by the vessels, and with dopamine-β-hydroxylase activity. A similar increase in sensitivity to both norepinephrine and serotonin was obtained by chemical denervation of cerebral arteries by the use of 6-hydroxy-dopamine. In contrast to these studies, Morooka observed an increase in the norepinephrine content of feline cerebral vessels 3 days after subarachnoid blood treatment. In this particular study, the norepinephrine content of the arteries was found to decrease only transiently after treatment with blood and to reach a maximum after 3 days. The observation correlated with the dopamine-β-hydroxylase activity.

Others, working entirely with in vitro systems, have reported that exposure of human basilar arteries to serum increases the subsequent responses of the arteries to both serotonin and norepinephrine for up to 15 minutes after the application of the serum. Platelet-derived growth hormone, an important serum protein, has been shown to potentiate the effects of serotonin on smooth muscle cells in culture by either increasing the number of serotonin receptors or increasing the efficiency of receptor coupling. Whether such acute modifications in the behavior of the arteries are related to the increase in sensitivity seen in arteries treated in vivo is open to question, however, as the time courses of the modifications in response are so different.

In contrast to the above observations, a decrease in the contractile responses of canine middle cerebral arteries has been reported to occur 24 hours and 7 days after exposure to subarachnoid blood. In this study, the vessels were examined at 2 and 24 hours and again at 7 and 42 days after SAH induced by needle puncture of the internal carotid artery. At 24 hours and 7 days, these arteries exhibited reduced contractile responses in vitro to serotonin, histamine, norepinephrine, and potassium. By 42 days, however, the responses had normalized. In no instance were the ED50 values of the arteries affected, only the magnitude of the contractions.

The enhanced sensitivity of cerebral arteries pretreated with blood, together with the reduced norepinephrine content and uptake, has been taken to imply that either a transient denervation of the arteries occurs in response to the exposure to blood, or, alternatively, a temporary inhibition of neuronal uptake takes place. Additional evidence suggesting neuronal dysfunction is that a similar supersensitivity can be induced by chemical sympathetic denervation using 6-hydroxy-dopamine. The resulting increase in sensitivity to serotonin has been taken to imply a postjunctional mechanism, as serotonin-induced contractions are known not to be mediated by α-adrenergic receptors. In these experiments, however, a direct postjunctional effect of pretreatment with blood not mediated by neuronal dysfunction cannot be excluded.

Conversely, total sympathetic denervation of the middle cerebral arteries of cats produced an increase in...
the sensitivity to norepinephrine, but no change in the response to acetylcholine; these effects were also observed when the vessels were treated with cocaine. These results indicate that only prejunctional modifications occurred and are inconsistent with the concept that denervation leads to the development of postjunctonal changes as well. Taken together, these observations suggest that treatment of cerebral arteries with blood affects both prejunctional and postjunctional mechanisms, a conclusion that recently has also been proposed by others.

A corollary of the concept that denervation increases the sensitivity of vascular smooth muscle nonspecifically is that increased innervation may cause a nonspecific decrease in sensitivity. Recent findings indicate that, in regions of arteries afflicted with severe vasospasm (induced by the application of a mixture of blood and cerebrospinal fluid incubated at 37°C for 5 days), the adrenergic neuronal distribution was rich and uniform in all parts of the adventitia. In contrast, portions of vessels with only slight spasm exhibited scanty innervation in the innermost 20-micron layer of the adventitia. This implies that parts of an artery in severe spasm may tend to be reduced in their sensitivity to vasoactive agents, whereas regions with a lesser degree of spasm might exhibit enhanced sensitivity. Such behavior is consistent with the possibility that, while vasoactive agents are not involved in the maintenance of severe spasm, they may operate on vessels that have been sensitized by blood but that are not yet fully constricted. Such variability is not inconsistent with the differences in behavior observed in arteries exposed to blood, as described previously.

It has been suggested that qualitative changes in the contractile machinery of rabbit ear arteries might occur as a result of long-term denervation.

Another aspect of prejunctional and postjunctional sensitivity that is particularly relevant to *in vitro* experimentation concerns the neuronal distribution in the blood vessel walls. As previously mentioned, the neurons are confined to the adventitial layer of the vessels. The side of the vessel to which agonists are applied can thus have a significant effect on the resultant response. This phenomenon has been demonstrated several times. Norepinephrine provides the most dramatic example of this effect. In sheep carotid arteries, a 10- to 100-fold greater sensitivity was found for norepinephrine on the intimal, as opposed to the adventitial surface, and on rabbit aortas an approximately 20-fold difference was found. The two surfaces exhibited less dramatic differences in sensitivity to other agents; histamine and serotonin exhibited approximately 5-fold and 3- to 4-fold greater sensitivity on the intimal side of rabbit aortas.

No similar studies have been reported for cerebral vessels, but it has been shown that, in order to relax the serotonin-induced contraction of canine basilar arteries, nitroprusside must be present at a concentration of about 10⁻⁸ M when applied luminally. If, however, it is applied to the adventitial surface, a concentration 100-fold higher, or 10⁻⁷ M, must be attained to produce the same effect. In the case of papaverine, however, no difference in effect was observed between luminal and adventitial applications.

It is unclear whether these observations on cerebral arteries using nitroprusside are related to neuronal distribution, but they do emphasize the difference in response that can be obtained by using either adventitial or endothelial application. Clearly, the integrity of the endothelial lining might play an important role in the observation of any such differences in isolated arterial preparations, as well as any possible direct role in mediating the action of pharmacological agents.

We are unaware of any investigator who, in studying cerebral vasospasm using *in vitro* techniques, routinely differentiates between responses of cerebrovascular preparations derived from agonists, antagonists, or other agents. This would seem to be a methodological weakness of possibly serious consequence.

5. Spasminogens

More than two dozen known agents can elicit a contractile response in cerebral arteries. Regrettably, no conclusive evidence exists to indicate that vasoconstrictive agents are directly involved in the delayed phase of cerebral vasospasm.

One problem that arises in considering the possible role of known vasoconstrictive compounds is the diversity of mechanisms through which such substances are thought to act. Direct agonists such as serotonin, histamine, or dopamine bind to specific receptors on the membrane of the smooth muscle cell. This mechanism, however, is susceptible to variation when the concentration of receptors changes. Reduction in receptor number has been documented for other receptor agonist interactions during prolonged exposure and elevated agonist concentrations. Other agents such as thrombin or arachidonic acid appear to exert a vasoconstrictive effect because of their influence on prostaglandin or thromboxane synthesis. Still other substances such as hemoglobin act by mechanisms that have yet to be identified.

**Model Systems**

Whatever the cause or causes of clinical cerebral vasospasm, understanding will depend on the development and utilization of appropriate *in vitro* and *in vivo* model systems. At present, no single species of animal has been accepted as standard for the study of cerebral vasospasm. Rats, rabbits, cats, dogs, monkeys, and baboons have all been used. Subarachnoid hemorrhage has been simulated *in vivo* by either injection of blood or rupture of vessels, although these two techniques by no means produce the same final effect. A number of studies, purportedly of cerebral vasospasm, appear to confuse the acute contractile effects of subarachnoid blood with the phenomenon of delayed spasm. Much evidence suggests that the acute and delayed phases of constriction differ in their etiology, reducing the clinical relevance of investigations of cerebrovascular con-
strictions that occur less than 2 days after the induced subarachnoid hemorrhage.

In vitro techniques that measure the vasoactive effects of a substance on preparations of normal cerebral arteries suffer from several limitations. Some of these include (a) manipulative trauma to the vessels, which can be minimized by skilled technique; (b) denervation effects caused by severing the arterial section from the animal; these can be minimized by using the preparation while it is still fresh; (c) the lack of distinction between the luminal and adventitial surfaces of the vessel with regard to agonist application; and (d) differences that may be incurred by incubation in an artificial medium.

The effects of most vasoconstrictive agents in vitro have usually been measured only during brief periods, and almost all have been studied only on normal arteries. If such substances are to be shown to be important, their presence in appropriate concentrations during the time of spasm must be at least conceptually possible. Also, their effects must be capable of lasting for hours or days. In vitro measurements rarely provide evidence of sustained contractions lasting for more than a few hours, and usually the duration of the effect is limited to periods much shorter than this. Furthermore, relatively few studies document the occurrence of "escape" or tachyphylaxis, a phenomenon that may occur as the vessel accommodates to the presence of the agonist.

Conclusion

Any model that hopes to contribute to the understanding of clinical cerebral vasospasm must answer two questions:

1. Is spasm mainly a normal contraction of arterial smooth muscle in response to a continuously vasoconstrictive environment, or does the contraction reflect an induced abnormality of the smooth muscle wall?

2. Are the various morphological changes seen in arteries in spasm the cause or the result of spasm?

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