Pharmacologic Irreversible Narrowing in Chronic Cerebrovasospasm in Rabbits Is Associated With Functional Damage

Peter Vorkapic, MD, Rosemary D. Bevan, MD, and John A. Bevan, MD

We studied isolated basilar artery segments from a rabbit model of chronic cerebrovasospasm. Autologous blood placed around the basilar artery of rabbits killed 1, 2, 3, 4, 5, 6, 7, or 9 days later caused narrowing of the segments with a biphasic time course. The first (immediate) phase was reversed by intra-arterial papaverine; the second phase exhibited an increasing component of narrowing that was papaverine-insensitive. Based on the passive force/length curves, basilar artery segments became increasingly stiff over 9 days. By contrast, the segments' contractility decreased. Responses of the basilar artery segments were greater over the first few days, but then became less than that of saline-injected controls. Contractions in response to norepinephrine and potassium were reduced. Endothelium-based acetylcholine-induced vasodilation progressively diminished, as did the response to sympathetic nerve stimulation. There was a negative correlation between artery wall stiffness and contractility. The papaverine-insensitive component of angiographic narrowing correlated directly with loss of contractility and with artery wall stiffness. These results are consistent with the conclusion that increased artery wall stiffness is a primary determining factor in the arterial narrowing of chronic cerebrovasospasm. (Stroke 1990;21:1478-1484)

Refractoriness of chronic cerebrovasospasm to pharmacologic vasodilator therapy has been widely reported in humans and in some animal models. This feature probably reflects the primary basis of the clinical concern with cerebrovasospasm. In humans the first clinical evidence of this state is seen 3–4 days after subarachnoid hemorrhage (SAH); cerebrovasospasm reaches its maximum after approximately 7 days and reverses after several weeks. We have recently developed a model of SAH in rabbits. Papaverine-sensitive and -insensitive narrowing of the basilar artery was determined by serial angiography over 9 days after the placement of 3 ml/kg autologous blood by multiple injections around the base of the brain. The papaverine-insensitive component began 3–5 days after blood placement and subsequently progressed slowly, representing 63% of the total narrowing at day 9.

Several hypotheses have been proposed to explain the basis of cerebrovasospasm. Structural changes of the endothelial and smooth muscle cell layers of the adventitial nerves of the vessel wall have been found both in humans and in large animal models of this condition, and such damage has been proposed as being responsible. Increased active vascular muscle tone could result from vasoconstrictor substances released locally from surrounding tissues or associated with blood and blood clot. Denervation hypersensitivity of the smooth muscle cells has also been proposed to play a role. Abnormal myogenic tone of the smooth muscle cells has also been proposed to play a role. This in vitro investigation of the properties of basilar artery walls was designed to explore the hypothesis that the late pharmacologically irreversible narrowing is the result of damage to the artery wall.

Materials and Methods

The method of inducing SAH in rabbits that leads to chronic cerebrovasospasm has been described. Under general anesthesia, a Silastic tube was inserted with its tip in the prepontine cistern. In some rabbits, a total of 3 ml/kg unheparinized autologous blood from the central ear artery was injected in aliquots around the basilar artery over 4 hours.
This ensures that the basilar artery is persistently and closely encased by blood clot. Serial angiography before and after an intra-arterial bolus injection of papaverine performed daily over 9 days served to establish the total and irreversible components of cerebrovasospasm. Sham-injected control rabbits were bled through the central ear artery but received injections of only physiological saline into the base of the brain.

Approximately 12 hours after angiography, rabbits were stunned and bled. The brain was removed from the skull as rapidly as is consistent with exercising great care to preserve the arachnoid. The vertebral and carotid arteries were severed where they pierced the dura. The basilar artery was dissected from the brain and placed in bicarbonate-buffered physiological saline equilibrated with 95% O₂ and 5% CO₂ at room temperature. Basilar artery segments were trimmed to 3-mm lengths and, using two stainless steel wires inserted into the lumen of each segment, were mounted in a tissue bath at 37.5°C at optimum tension (350 mg) as described previously. The tissue bath solution was of the following millimolar composition: 144.2 Na⁺, 4.9 K⁺, 1.6 Ca²⁺, 1.2 Mg²⁺, 126.7 Cl⁻, 25 HCO₃⁻, 1.99 SO₄²⁻, and 11.1 glucose, gassed with 95% O₂ and 5% CO₂. Changes in isometric tension (L), a measure of wall stiffness or resistance to passive stretch, was measured 1 hour after setup. The minimum wire separation to produce a just-discernible increase in T was considered Lₒ. Segments were then stretched in 0.1-mm increments four times after equilibration to the preceding stretch. Subscripts indicate the incremental stretching of segments from rabbits injected with blood (SAH) or saline (C).

The occurrence of spontaneous contractions (if any) during the experiment were noted. We recorded the contraction in response to 10⁻⁹ to 10⁻⁵ M serotonin (5-HT) and the dilation in response to 10⁻⁷ to 10⁻⁴ M acetylcholine (ACh). After the application of 10⁻⁶ M cimetidine, we tested the effect of 10⁻⁷ to 10⁻⁴ M histamine on the segments’ ability to develop tone (contractility). In the presence of 10⁻⁴ M prazosin, 17 and 89 mM KCl was added to the tissue bath solution and we recorded the contraction in response to each concentration. After restoration of normal K⁺ concentrations, we recorded the contraction in response to 10⁻⁷ to 10⁻³ M norepinephrine (NE). Transmural electrical nerve stimulation was delivered from a Grass SD-9 stimulator wired via a low-impedance voltage follower to platinum wire electrodes positioned parallel to the basilar artery segment approximately 0.5–1.0 mm from its outer surface. The stimulation voltage was that which caused a just-discernible response in the presence of 3x10⁻³ M tetrodotoxin. Square-wave monophasic pulse trains were delivered at 4, 8, and 16 Hz, with a pulse duration of 0.3 msec.

Data from rabbits that had been injected with blood were compared with data from sham-injected controls using Student’s unpaired or paired t test; \( p<0.05 \) was considered to indicate a significant difference. Results are reported as mean±SEM.

Results

The T/L curves of the basilar artery segments were significantly displaced upward at day 3 (Figure 1); this trend progressed to day 9. The \( T_{4\,SAH}/T_{4\,C} \) (as an index of stiffness) increased progressively over the 9 days (Figure 2, top), first reaching significance at day 3, when mean \( T_{4\,SAH} \) was 159.8% of \( T_{4\,C} \). The \( T_{4\,SAH}/T_{4\,C} \) ratio correlated positively with days after SAH \((r=0.8, p<0.0005; \) Figure 2, top). The highest mean \( T_{4\,SAH}/T_{4\,C} \) ratio (269.8) was recorded at day 9.

At day 1 and 2, large transient spontaneous increases in wall tone were recorded upon changing the tissue bath solution (Figure 3). The basilar artery segment exhibiting the greatest increase in tone upon changing the solution (up to 45% of maximum contractility) came from a rabbit in which this artery had a diameter 65.7% of baseline on angiography. The increases in tone were reversed by the administration of 10⁻⁵ M papaverine.

Maximum contractility was considered to be that elicited by 10⁻⁴ M histamine. The addition of other agonists to the tissue bath solution in the presence of histamine caused no additional contraction of the basilar artery segment (data not shown). Contractility was markedly reduced at day 3 and declined progressively to day 9. The mean±SEM reduction in contractility from days 6 to 9 was 52.3±3%. Contractility was correlated negatively with days after SAH \((r=0.74, p<0.0005)\).
At days 1, 2, and 3 dose–response curves for 5-HT and NE could be executed adequately because all basilar artery segments contracted. Between days 4 and 9 the mean percentages of segments that failed to contract in response to 5-HT and NE were 47.8% and 26%, respectively. Contraction of the segments in response to 5-HT at doses of up to $10^{-5}$ M did not ever reach tissue maximum. At days 1, 2, and 5 contraction in response to $10^{-5}$ M 5-HT was 174.2%, 172.7%, and 171.7% of control when unresponding segments were excluded (Figure 4). At these three days the mean concentrations of 5-HT to cause 50% inhibition of maximum contractility were significantly increased (2.4, 4.1, and 9.8 times control, respectively; data not shown). From days 6 to 9 contraction in response to $10^{-5}$ M 5-HT was reduced. Contraction in response to 89 mM K+ was significantly and consistently reduced at each day (data not shown). The mean±SEM reduction was 41.3±3.59%. Contraction of the segments in response to $10^{-2}$ M NE was invariably less than tissue maximum and was reduced at days 4 and 7 (data not shown). During the remaining days, no significant alterations in response to NE were recorded.

The vasodilatory response to ACh was evaluated after attaining a half-maximal contraction with histamine; $10^{-4}$ M ACh completely relaxed all control basilar artery segments to baseline (Figure 5). There was an increasing impairment of relaxation in response to ACh as early as day 1 (93.9±1.4%, data not shown). At day 2, the segments relaxed to a maximum of only 52±5.8% of baseline in response to $10^{-4}$ M ACh (Figure 5). A level of reduction in relaxation not significantly different from this value was maintained until day 9 (Figure 5). The concentration of ACh to cause 50% inhibition of maximum contraction remained the same from day 1 through day 9 (data not shown).

Transmural electrical stimulation of the sympathetic nerves produced a frequency-dependent contraction (data not shown). The responses of segments from rabbits with SAH were variable and lacked...
FIGURE 3. Recordings of wall tone from rabbit basilar artery segments 2 days after injection of autologous blood (top) or saline (bottom). In rabbit with experimental subarachnoid hemorrhage, large and long-standing spontaneous increases in wall tone developed immediately after changing tissue bath solution (W). Segment relaxed upon administration of $10^{-5}$ M papaverine (PPV).

frequency dependence. The mean±SEM equilibrium response to transmural sympathetic nerve stimulation at 16 Hz was 38.07±4.12% for days 1–4 and 19.55±4.75% for days 5–9 (data not shown).

In Figure 2, bottom, the relation between artery wall stiffness and contractility is shown. The two parameters correlated negatively; the stiffer the artery, the less the contractility.

The papaverine-insensitive diameter of rabbit basilar arteries determined by angiography is directly related to loss of contractility (reduction in maximum response to $10^{-4}$ M histamine) (Figure 6, top) and to wall stiffness (Figure 6, bottom) of segments taken from the same artery. We emphasize that corre-

sponding data for a particular segment are plotted in these graphs. The magnitude of the vasodilator-insensitive component of arterial narrowing is highly significantly related to both artery wall stiffness and loss of contractility.

Discussion

Cerebrovasospasm in humans is usually delayed and long-lasting, reversing only slowly and is generally refractory to treatment with pharmacologic vasodilators. Recently, several models in large animals have been developed that appear to create a vascular state similar to clinical SAH. We have developed a model of cerebrovasospasm in the basilar artery in a small and inexpensive animal, the rabbit. Diffuse cerebrovasospasm was seen on angiography commencing at day 1 and continuing for 9 days; progressive increases in refractoriness to intraarterial papaverine were observed commencing at days 3–5 and increasing to represent 63% of the total narrowing at day 9. In another model, we found that a decrease in vessel wall distensibility and a loss of contractility were the dominant features of segments taken from narrowed arteries 6 days after SAH in monkeys; the greatest narrowing occurred in those vessels that had the least capacity to contract. The measurements we made in this experiment in rabbits are similar to those we made previously in monkeys.

Previous investigators have reported changes similar to those that we found in monkeys and have now determined in rabbits. Nagasawa et al measured the elastic properties of dog cerebral arteries after the intracisternal injection of autologous blood; after 2 days the vessels were more distensible, but they became progressively stiffer by day 7. Kim et al found in their canine model of chronic cerebrovasospasm that the compliance and optimum length of dog basilar arteries were reduced 8 days after SAH.
We found significant increase in the collagen content, measured in only two rabbits as the hydroxyproline/leucine ratio, 9 days after SAH (unpublished observations).

We used two experimental indexes to reflect functional damage to the basilar artery wall. Our first index is the loss of contractility, and the second is an increase in artery wall stiffness. In the monkey model, changes in this second index could explain the narrowing found on angiography. In rabbits, both these indexes increased following SAH after a latency of several days and both could be correlated with pathophysiologically significant papaverine-insensitive narrowing seen on angiography. The extent of vessel wall damage, expressed as the loss of contractility (the most important role of the cerebral arteries) was 30% in a monkey model on day 6 and approximately 50% in our rabbit model from days 6 to 9. In this regard the maximum responses of the cerebral arteries to 5-HT, NE, and K⁺ were decreased 7 days after large-volume SAH in monkeys and dogs. On the other hand, the responses to 5-HT, NE, and K⁺ were increased temporarily in cat and rabbit basilar arteries after small (1-2 ml) injections of blood. These authors also recorded increased sensitivity of the basilar artery to these agonists. Explanations of these observation and discrepancies can only be speculative.

Figure 6. Graph of relation between in vivo papaverine-insensitive diameter of rabbit basilar artery (top) and loss of contractility (reduction in maximum contraction in response to $10^{-4}$ M histamine) ($r=0.79485$, $p<0.0005$) and (bottom) artery wall stiffness (ratio of force developed after fourth incremental stretching of segment from experimental and control rabbits) ($r=0.83316$, $p<0.0005$). n=5–7 for each group.
Our finding of depressed neurogenic vasoconstriction is also consistent with artery wall damage and is in agreement with reduced neuroconstrictor and neuromodulatory mechanisms in monkeys. Ultrastructural changes, such as disintegration and disappearance of vesicles in the adrenergic terminals and an increase in the maximal binding of norepinephrine to adrenergic receptors, might reflect the basis of impaired and abnormal adventitial adrenergic nerve function. Loss of catecholamine fluorescence after SAH was demonstrated in basilar arteries of rats, rabbits, and cats. Based on these data, denervation hypersensitivity was proposed as a factor contributing to cerebrovasospasm. However, in the basilar artery of rabbits and cats it was not possible to demonstrate increased sensitivity to histamine, 5-HT, NE, or K⁺ after chemical or surgical sympathetic denervation. The extent of the change in sensitivity with acute denervation is small and could not by itself account for the arterial narrowing. We recorded no consistent changes in sensitivity to histamine or NE in this study. There was an increase in the sensitivity to 5-HT, but the physiological significance of this increase is uncertain. Postsynaptic denervation hypersensitivity is usually thought to affect all postsynaptic responses.

Changes in endothelium function might contribute to cerebrovasospasm. Hemoglobin inhibits endothelium-derived relaxing factor (EDRF) in cerebral as well as systemic arteries. However, the significance of this inhibition is uncertain. If inhibition of EDRF release is important, then the implicit assumption is that there is a normal high baseline level of EDRF release, but there is no evidence of this. Endothelium-dependent vasorelaxation was temporarily impaired 4 days after SAH in rabbits. Kim et al reported a positive correlation between in vivo endothelium-dependent vasorelaxation and the degree of in vivo vasospasm in a dog model and have shown that it is not the luminal release of EDRF but probably the mechanism of transferring EDRF to the smooth muscle cell layer that is influenced. In our rabbit model, the highest degree of in vivo vasoconstriction was seen when endothelium-dependent vasorelaxation was impaired least. Nevertheless, loss of endothelial function might enhance the vasoconstrictor influences of substances released from the clot, such as serotonin (as has been observed in rabbits) and thrombin (which also has an endo-
clot, such as serotonin (as has been observed in
surfaces of arteries taken during surgery 9 and 21 days after SAH has been reported. Type V collagen occurs in healing wounds along with myofibroblasts and disappears after several weeks. Chronic arterial narrowing seems to be a dynamic process that, through mechanisms of remodeling wall texture, reverses after several weeks. A recent report of angioplasty for dilating narrowed arteries indicates that structural changes are a main feature of cerebrovasospasm. Recent clinical and experimental observations by Chyatte and colleagues support the damage hypothesis. Monkeys pretreated with diltiazem were also protected, presumably due to minimization of calcium entry and prevention of calcium overload. High concentrations of clot-originating spasmodens are known to be toxic to cerebral artery walls. Thus, it seems possible that damage to the vessel wall is a major event in the evolution of chronic cerebrovasospasm.

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