Alterations of mechanical properties in canine basilar arteries after subarachnoid hemorrhage

PHYO KIM, M.D., THORALF M. SUNDT, JR., M.D., AND PAUL M. VANHOUTTE, M.D., PH.D.
Department of Physiology and Biophysics and Department of Neurologic Surgery, Mayo Clinic, Rochester, Minnesota

The purpose of this study was to examine the hypotheses that structural stiffening of the arterial wall contributes to chronic cerebral vasospasm, and that alteration in properties of smooth muscle takes place after subarachnoid hemorrhage (SAH). Subarachnoid hemorrhage and subsequent chronic vasospasm were induced in dogs by two cisternal injections of autologous blood (on Day 0 and Day 2). Vasospasm was confirmed by angiography performed on Day 0 and Day 7. Animals in the control group underwent angiography only. On Day 8, the mechanical properties of the basilar arteries were studied in vitro. Passive compliance, measured under total inhibition of spontaneous myogenic tone with diltiazem (10^{-4} M) plus papaverine (10^{-4} M) was smaller in the SAH group. The length-contraction curve was shifted to the left and the optimum length for maximum contraction (L_{max}) was significantly shorter in the spastic blood vessels. The spontaneous myogenic tone was augmented in the SAH group, resulting in an increase in resting tension at each length. By contrast, the maximum contractions in response to KCl and uridine 5'-triphosphate were markedly reduced in the SAH group, without changes in sensitivity to these agents. These differences in mechanical properties were observed in rings both with and without endothelium. The results indicate that, in chronic vasospasm, stiffening of the noncontractile component of the vasculature takes place as well as alterations in the contractile component, both of which presumably contribute to the shift in resting length-tension relationship and length-contraction relationship of the artery. The decreased distensibility, the increase in resting tension, and the shortening of the L_{max} all favor a smaller diameter of the artery after SAH, possibly contributing to vasospasm.

KEY WORDS • morphology • vasospasm • subarachnoid hemorrhage • basilar artery • dog

MORPHOLOGICAL studies in patients and in experimental models of chronic vasospasm after subarachnoid hemorrhage (SAH) report structural changes in the wall of the cerebral arteries. The changes include myonecrosis and fibrosis of the medial layer, inflammatory changes in the adventitia, and proliferative changes in the subintimal layer. Angiographic narrowing of the arteries in cerebral vasospasm may be the result of these structural changes, which would explain why this condition is resistant to attempted pharmacological intervention. Previous studies have shown that endothelium-dependent relaxations are abolished in the canine basilar arteries during chronic vasospasm but that the release of endothelium-derived relaxing factor was maintained. By contrast, endothelium-dependent contractions, most of them mediated by cyclooxygenase products, were maintained. Therefore, it appears that the impairment in endothelium-dependent relaxation lies with a reduced ability of the smooth muscle to relax.

Little is known about the actual changes in mechanical properties of the smooth muscle/arterial wall in chronic vasospasm. Nagasawa, et al., measured the distensibility of the passive component of canine basilar artery in Ca^{2+}-free solution after SAH. In that study, the distensibility (determined from the diameter and the intraluminal pressure of isolated segments of the arteries) was increased in the SAH group; a reduction of the collagen/elastin ratio was demonstrated histologically. Bevan, et al., studied the mechanical properties of cerebral arteries in monkeys after SAH induced by puncture of the internal carotid artery, and observed a decrease in the passive distensibility in arterial rings in the presence of NaNO_2 or MnCl_2; they proposed that the main cause of chronic vasospasm is the increased rigidity of the vessel wall. The present study was under-
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Materials and Methods

Animal Model

Materials in the present study were obtained in part from the animals used in a separate previously published study. Sixteen mongrel dogs of either sex, each weighing between 15 and 25 kg, were used. The animals were randomly divided into two groups (SAH and control groups). In both groups, a transfemoral angiogram of the basilar artery was performed using iothalamate meglumine (Conray 60%, 10 cc injected at a rate of 7 cc/sec) under general anesthesia (thiopental 15 mg/kg and pentobarbital 15 to 25 mg/kg, administered intravenously) and controlled ventilation. In the SAH group, 6 ml of autologous venous blood was injected percutaneously into the cisterna magna subsequent to the angiogram. The cisternal injection of the venous blood was repeated 2 days later (Day 2), also under general anesthesia. In both groups, the angiogram was repeated on Day 7 to confirm the presence of vasospasm. During the two angiography procedures, arterial blood gas levels were monitored to safeguard against vasoconstriction secondary to hypocapnia. On Day 8, the animals were sacrificed with sodium pentobarbital (30 mg/kg injected intravenously) followed by exsanguination. The brain and cervical spinal cord were dissected free and, under a microscope, the basilar artery was separated from the brain stem and subarachnoid clot was removed. Details of the surgical and angiographic procedures have been described elsewhere. The dimensions of the basilar artery were measured on a computer-connected digitizing table after magnification × 4. The procedures and handling of the animals were reviewed and approved by the Institutional Animal Care and Use Committee of the Mayo Foundation.

In Vitro Studies

Rings about 4 mm long were cut from the basilar arteries. The endothelium was removed from some rings by gentle mechanical rubbing of the intimal surface with a stainless steel wire. To confirm removal of the endothelium, the rings were fixed at the end of the experiments and examined histologically with hematoxylin and eosin staining. The removal allowed good preservation of the layers below the internal elastic lamina (Fig. 1). The rings were connected to an isometric force transducer with two triangular stainless steel wire stirrups (0.035 in. in diameter) inserted in the lumen and interlocked at the base to prevent bending. They were suspended with solid stainless steel rods (approximately 0.5 mm in diameter) in a 37°C organ chamber filled with modified Krebs-Ringer bicarbonate buffer.

Fig. 1. Light micrographs of basilar artery rings from a subarachnoid hemorrhage group dog after completion of an organ chamber experiment: rings with endothelium (A: × 640, B: × 100); and rings without endothelium (C: × 640, D: × 100). Note that the internal elastic lamina and the layers below including the adventitia are well maintained in the rings without endothelium. H & E.
solution (composition (mM): NaCl 118.3, KCl 4.7, CaCl₂ 2.5, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 25.0, calcium ethylenediaminetetra-acetic acid 0.026, and glucose 11.1) bubbled with a 95% O₂/5% CO₂ gas mixture. The rings of the basilar artery were equilibrated for 30 minutes at the exact length where the tension started to increase measurably, which is referred to as the initial length.

In rings where compliance of the noncontractile component was measured, the length-tension relationship was examined in the presence of a supramaximum dose of the calcium antagonist diltiazem (10⁻⁴ M) and papaverine (10⁻⁴ M). Under this condition, no myogenic tone was observed. Preliminary experiments demonstrated that the combination of these drugs abolished contractions in response to KC1 (10⁻⁸ to 10⁻⁴ M) and norepinephrine (10⁻⁹ to 10⁻⁴ M). Contractions in response to prostaglandin F₂α (10⁻⁹ to 10⁻⁴ M) and uridine 5'-triphosphate (UTP: 10⁻⁸ to 10⁻⁶ M) were inhibited by more than 95%. The rings were stretched progressively in 0.4-mm increments, and each time the rings were allowed at least 30 minutes to reach plateau tension. Compliance (the inverse of the incremental elastic modulus)¹⁸ was calculated by dividing the difference in length between the two points (mm) by the difference in the resting tension (gm) at these two points. Thus, compliance (mm/gm) = (L - L') / (T - T'), where T = the tension observed at length L and T' = the tension observed at length L'. Unless otherwise mentioned, the difference of the length L and L' was taken as 0.4 mm.

In rings where myogenic activity was measured, the length for maximal active contraction (Lₘₐₓ) was determined using a standard concentration of uridine 5'-triphosphate (10⁻⁵ M).¹² The rings were stretched in increments of 0.2 mm and measured with the aid of a micrometer connected to the transducer. After each increment of length, the rings were equilibrated for 30 minutes and allowed to reach a stable tension, which was measured as the resting tension at that length. Responses to UTP were measured as the contraction from the resting tension. Concentration-response curves in the response to UTP (10⁻⁹ to 10⁻³ M) and KC1 (5 to 60 mM) were obtained at the optimum point.

**Drugs**

The following drugs were used: diltiazem hydrochloride, papaverine hydrochloride, and UTP. Drug concentrations are expressed as molar concentration in the original bath solution.

**Data Analysis**

The data are expressed as means ± standard error of means. Student's unpaired t-test was used for statistical comparisons. P values smaller than 0.05 were considered statistically significant.

**Results**

**Angiographic Measurement of Vasospasm**

In the SAH group the mean cross-sectional area of the basilar artery measured angiographically on Day 7 was 34% ± 5.5% of that on Day 0 in eight dogs; in the control group the ratio was 97% ± 9.2% in eight dogs (p < 0.005).

**Passive Length-Tension Relationship**

The passive length-tension relationship was measured in the presence of diltiazem (10⁻⁴ M) and papaverine (10⁻⁴ M). The compliance calculated from the data...
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FIG. 4. A: Length-contraction relationship to uridine 5'-triphosphate (UTP, 10⁻⁵ M) during progressive stepwise stretch in rings with endothelium. Open bars represent data from the control group and shaded bars represent those from the subarachnoid hemorrhage group. Initially, the rings were equilibrated for 30 minutes at a length where the tension started to increase measurably, which is referred to as the initial length. B: Resting length-tension relationship in rings with endothelium in the control (open bars) and in the subarachnoid hemorrhage groups (shaded bars). The asterisks indicate a significant difference in tension between the two groups (p < 0.05, Student’s unpaired t-test). Data in A and B are shown as means ± standard error of the means of recordings made in 32 rings (four rings from each of eight different animals).

showed significant decreases at +0.8 mm and +1.2 mm in the SAH group (Fig. 2); the compliance was comparable between the two groups at +1.6, +2.0, and +2.4 mm. In rings without endothelium, the difference in passive compliance between the two groups was more pronounced (Fig. 3). After removal of the endothelium, a significant increase in passive compliance was observed in the control group, but not in the SAH group.

Optimum Length

In the control group, L_max (as determined in response to UTP) was +1.19 ± 0.04 mm (from the initial length in 32 samples, four rings from each of eight different animals) in rings with endothelium. In the SAH group the maximum contraction was reached at a significantly shorter length (Fig. 4A). The L_max in rings with endothelium was +0.83 ± 0.03 mm (from the initial length in 32 samples, four rings from each of eight different animals). In the rings without endothelium, the L_max in response to UTP was +1.44 ± 0.05 mm in the control group and +0.83 ± 0.04 mm in the SAH group (32 samples, four rings from each of eight different animals). The difference between the two groups was statistically significant. In the control group an increase in L_max (1.19 ± 0.04 mm vs. 1.44 ± 0.05 mm) was observed after removal of endothelium but not in the SAH group.

FIG. 5. Maximum contraction in the presence of KCl in rings with and without endothelium in the control (open bars) and subarachnoid hemorrhage (SAH) groups (shaded bars). Measurements were made at optimum length in each ring. Data are presented as means ± standard error of the means for 32 rings (four rings from each of eight different animals). The asterisks indicate a significant difference between the two groups (p < 0.05, Student’s unpaired t-test).

Resting Tension

The resting tension at lengths ranging from +0.6 mm to +1.2 mm was significantly larger in the SAH group in rings both with (Fig. 4B) and without endothelium (Table 1). When comparing rings with and without endothelium, the resting tension became significantly smaller after endothelium removal in both groups (Fig. 4B and Table 1).

Contractions to KCl

The maximum contraction in response to KCl was significantly smaller in the SAH group than in the control group in rings both with and without endothelium (Fig. 5). The concentration-response curves in rings both with and without endothelium reached a plateau at 40 mEq, where contractions were compared (Fig. 6).

Contractions to Uridine 5'-Triphosphate

The mean contraction in response to UTP (10⁻⁵ M), obtained at the L_max of each ring, was significantly smaller in the SAH group than in the control group in rings both with and without endothelium (Fig. 7). The Concentration-response relationship to UTP, expressed as a percentage of the maximum contraction to KCl in each ring, was not significantly different between the two groups in rings with endothelium (Fig. 8). In rings without endothelium, the proportion to the maximum contraction in response to KCl in each ring was augmented in the SAH group (Fig. 8).

Discussion

The present study indicates that a significant stiffening of the arterial wall takes place following chronic cerebral vasospasm in the dog. Compliance of the non-contractile component, measured in the presence of
P. Kim, T. M. Sundt, Jr., and P. M. Vanhoutte

TABLE 1
Resting tension and contractions to UTP in rings without endothelium *

<table>
<thead>
<tr>
<th>Parameter &amp; Group</th>
<th>Length of Stretch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+0.4 mm</td>
</tr>
<tr>
<td>resting tension (gm) control</td>
<td>0.47 ± 0.08</td>
</tr>
<tr>
<td>SAH</td>
<td>0.7 ± 0.095</td>
</tr>
<tr>
<td>(24 rings)</td>
<td></td>
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<tr>
<td>contraction to UTP (gm) control</td>
<td>3.27 ± 0.62</td>
</tr>
<tr>
<td>SAH</td>
<td>1.89 ± 0.22</td>
</tr>
<tr>
<td>(20 rings)</td>
<td></td>
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</table>

* Resting tension and contraction in response to uridine 5'-triphosphate (UTP, 10⁻⁵ M) in rings without endothelium during progressive stretch of arterial rings from control and subarachnoid hemorrhage (SAH) group dogs. Unless otherwise specified the number of ring recordings were 32 (four rings from each of eight different animals).
† Significant difference between the control and SAH group.

Therefore, in the present study, papaverine was used in addition to diltiazem, on the assumption that its effect is due to inhibition of phosphodiesterase and an increase in the cyclic adenosine monophosphate (cAMP) concentration. Elevation of the intracellular cAMP causes binding of Ca⁺⁺ to microsomal protein and decrease of free Ca⁺⁺, resulting in relaxation of vascular smooth muscle. The combination of the two drugs was chosen to ensure that contraction due to intracellular release of Ca⁺⁺ as well as that due to extracellular Ca⁺⁺ is suppressed. After removal of the endothelium, a significant increase in passive compliance was observed in the control group. This finding has two possible explanations. First, the intimal layer may contribute to the structural rigidity of the arterial wall. Alternatively, the procedure of mechanical removal of endothelium may cause damage and softening of the noncontractile component of the medial or adventitial layers. Morphologically, there was no apparent change in the integrity of the outer two layers. Functionally, the maximum contractions in the presence of KCl were unchanged after endothelial removal and those in the presence of UTP were larger, indicating that there was no damage to the smooth muscle. Therefore, it appears unlikely that the removal of endothelium caused softening of the medial or adventitial layers. The increase in compliance after removal was not observed in the SAH group. The effect was different, probably because the increase in structural rigidity after SAH masked the small decrease due to loss of the intima.

There was a marked shift in the resting length-tension relationship, resulting in an apparent shortening of the arterial wall with chronic vasospasm. Indeed, in the tension range of +0.6 to +1.2 mm from the initial length, resting tension was significantly increased in the SAH group, in rings both with and without endothelium. Assuming that the passive noncontractile component and the active contractile component are connected “in series” in the arterial wall, the leftward shift observed in the resting length-tension relationship is consistent with the increased structural rigidity observed in the present study.
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FIG. 7. Contraction in response to uridine 5'-triphosphate (UTP, 10^{-5} M) in rings with or without endothelium from the control group (open bars) and the subarachnoid hemorrhage (SAH) group (shaded bars). Contractions were measured at optimum length in each ring. Data are expressed as means ± standard error of the means for 32 rings (four rings from each of eight different animals). The asterisks indicate a significant difference between the two groups (p < 0.05, Student's unpaired t-test).

shift of the length-tension curve could be accounted for by possible alterations in each of the two components. The stiffening or decrease in elasticity in the passive component would result in increased stretch in the "series-connected" contractile component at a given apparent total stretch, and thus may have caused the leftward shift of the length-tension relationship. Alternatively, damage to the smooth muscle and alteration of the contractile apparatus itself may have caused changes in length-contraction characteristics, favoring shorter lengths.

The observed shift of the optimum length for active contraction can also be explained by the same two possible mechanisms involving the passive and the active components. The optimum length was shortened by approximately 0.4 mm in the SAH group in rings with endothelium and by 0.6 mm in rings without endothelium, which is more than the apparent shift seen in the resting length-tension curve (about 0.2 mm in rings with endothelium and 0.4 mm in rings without endothelium). Therefore, it appears that, in addition to the effect of stiffening of the passive "in series" component, a change in characteristics of the contractile apparatus itself is contributing to the shift in the optimum length.

Active contractions were markedly reduced following SAH. Thus, contractions to potassium ions, which are mediated by membrane depolarization and Ca^{2+} influx, were almost halved in rings both with and without endothelium at the optimum length. Maximum responses to UTP (which causes contractions by a receptor-mediated mechanism) were also significantly reduced at optimum lengths in rings both with and without endothelium after SAH. However, the concentration-response curve to UTP, if expressed as a percentage of the maximum contraction to KCl, was not significantly altered in rings with endothelium after SAH, and was even shifted in an upward direction in rings without endothelium. These data suggest that the reduction in the absolute response to UTP is not due to a decreased sensitivity at the receptor level, but rather to a reduction in the capability of the contractile apparatus to respond during chronic vasospasm.

A preceding study using the same animal model revealed that endothelium-dependent relaxations were abolished and endothelium-dependent contractions were maintained in proportion to the maximum contractions induced by UTP. Endothelium-independent relaxations to serotonin were diminished. Release of the endothelium-derived relaxing factor itself was unchanged after SAH/vasospasm. The present analysis, focused on the mechanical properties of the spastic arteries, demonstrates that in fact significant changes occur in the characteristics of the active contractions of the smooth muscle as well as in the passive compliance of the noncontractile component of vasculature after SAH. The leftward shift in the length-tension relationship of the smooth muscle and the increased rigidity of the noncontractile component may contribute to the pathogenesis of vasospasm, along with loss of endothelium-dependent relaxations.

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Address reprint requests to: Phyo Kim, M.D., Ph.D., Department of Neurologic Surgery, Mayo Clinic, Rochester, Minnesota 55905.