Experimental Cerebral Vasospasm. Part 2.

Contractility of Spastic Arterial Wall

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SUMMARY We studied the mechanical properties of canine basilar arteries subjected to experimental subarachnoid hemorrhage (SAH). Smooth muscle contractility was determined from pressure-diameter curves obtained after subjecting the basilar arteries to three different conditions: Krebs-Ringer solution (KRS), Krebs-Ringer solution containing serotonin (5HT), and saline solution.

Pressure-diameter curves obtained in KRS and 5HT are biphasic and have sharp flexions that yield flexion points. The pressure level at the flexion point increases as vasospasm increases. Strong constriction is retained up to that pressure above which the constriction is released abruptly. These data suggest that increasing the intraluminal pressure dilates the spastic artery nonlinearly and that induced hypertension could relieve the cerebral ischemia caused by vasospasm if blood pressure were maintained above the flexion point. The contractile response of spastic arterial wall to serotonin remains unchanged after SAH although the spastic constriction increases progressively and becomes maximal seven days after SAH. The lesser the arterial wall stiffness, the more efficiently it constricts. This means that the diminution of arterial stiffness observed after SAH might be one of the factors promoting the development of vasospasm.

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ALTHOUGH THE PHENOMENON of cerebral vasospasm following the rupture of an aneurysm is well recognized and has been described in many publications, there have been few studies on the mechanical properties of arterial walls subjected to subarachnoid hemorrhage (SAH). There is a controversy as to whether the contractile response of a spastic arterial wall to vasoconstrictors increases as compared to a normal wall.1, 2 While it has been demonstrated in the intracranial and extracranial arteries that the change in connective tissue contents (collagen and elastin) produced in the processes of aging and systemic hypertension alters the contractility of walls,3, 4 there is little information on the correlation that may exist between the connective tissue contents and the contractility of arterial walls subjected to SAH.

In a previous paper, we demonstrated that the vasospasm is attributable to the constriction of vascular smooth muscle and hence is reversible, and that the passive mechanical properties of vascular walls observed under the relaxed condition of the smooth mus-
cle correlate well with the ratio of collagen to elastin contents.3

In this study, isobaric constriction and isometric contraction induced by serotonin were measured in canine basilar arteries subjected to experimental SAH in order to determine the contractility of the spastic cerebral vessel and the effects that the passive elastic properties of the wall may have on cerebrovascular contractility.

Materials and Methods

Experimental Procedures

A total of 35 adult mongrel dogs, weighing from 8 to 12 kg, were used in this study, and were divided into 6 groups: control group (10 dogs) with no treatment and treated groups (25 dogs) in which 3 ml of autogenous fresh arterial blood was injected into the cisterna magna with an exchange of the same amount of cerebrospinal fluid. After a certain period of time following the blood injection, a clivectomy was performed under anesthesia with sodium pentobarbital (25 mg/kg, i.v.) to obtain segments of the basilar artery with the branches ligated and severed. The treated dogs were divided into five groups according to the periods of time elapsed after the treatment: 2, 4, 7, 14 and 28 days. Each treated group was designated as 2-day, 4-day, 7-day, 14-day and 28-day groups, respectively.

Each arterial segment was mounted horizontally at its in vivo axial length in a tissue bath which contained Krebs-Ringer solution (KRS) kept at 37°C and oxygenated with 95% O2-5% CO2. The segment was inflated with the solution from a reservoir under air pressure using the testing apparatus reported elsewhere.5,6 Intraluminal pressure and external diameter were measured respectively, by a strain gauge manometer and a specially designed displacement transducer.7

After incubating the segment in the KRS for 30 minutes at an intraluminal pressure of 100 mmHg, a pressure-diameter curve was recorded during inflation of the arterial segment from 0 mmHg to 250 mmHg. This relation was considered to represent the elastic properties of the arterial wall in KRS. After the intraluminal pressure was returned to 100 mmHg, serotonin (5HT) was added to the solution in a concentration of 10−5 M. When the peak contraction had been reached, a second pressure-diameter curve was recorded from 0 mmHg to 250 mmHg and was assumed to represent the active elastic properties of the segment in 5HT. After the intraluminal pressure was returned again to 100 mmHg, the bath was drained and rinsed with a saline solution and then incubated in the solution for at least 30 minutes. The pressure-diameter curve obtained in this solution by the same procedures as carried out previously represents the passive elastic properties of the arterial segment. Our preliminary study showed that at this concentration of serotonin the maximum contraction can be obtained both in the control and the spastic basilar arteries. No difference was observed between the curves obtained in the pure saline solution and in the saline solution mixed with a metabolic inhibitor, which indicates that a blood vessel has little smooth muscle tone in the pure saline solution. After these mechanical experiments, the segment was removed from the bath, lightly blotted on filter paper, and then weighed.

Data Analysis

For the evaluation of the mechanical properties of blood vessels from their pressure-diameter curves, we calculated tangential mid-wall stress, σ, and tangential mid-wall strain, εm, to normalize the force-displacement relations of walls with different cross-sectional areas under different conditions of smooth muscle tone (fig. 1). These parameters are defined by the following equations:

\[ \sigma = \frac{P_R}{R_0 - R_i}, \quad (1) \]

and

\[ \varepsilon_m = \frac{[(R_o + R_i)/2] - (r_o + r_i)/2 - 1}{2}, \quad (2) \]

where \( P \) is the intraluminal pressure, \( R_o \) the external wall radius, \( R_i \) the internal wall radius, \( r_o \) and \( r_i \) the external and internal radii at 0 mmHg under the passive condition of smooth muscle, respectively. The internal wall radius was calculated from the external wall radius, in vivo axial strain, and volume of the segment assuming the wall density to be 1.06 g/cm3.10

Diameter response, \( \frac{(\Delta D_{m/D_m})_{KRS}}{(\Delta D_{m/D_m})_{SS}} \) and \( \frac{(\Delta D_{m/D_m})_{5HT}}{(\Delta D_{m/D_m})_{SS}} \), were calculated to express the isobaric constrictions of a blood vessel under the active conditions in KRS and 5HT at a given pressure level, respectively, and were defined as:

![Figure 1](https://stroke.ahajournals.org/lookup/suppl/doi:10.1161/01.STR.14.4.850/-/DC1/figures/1.png)

**Figure 1.** Schematic representation of the methods utilized to evaluate the contractility of control and treated arteries from their pressure-diameter curves. Pressure-midwall diameter (Pi-Dm) and stress-mid-wall strain (σ-εm) curves of a basilar artery from the 7-day group depicted under different conditions of smooth muscle in saline solution (SS), Krebs-Ringer solution (KRS) and Krebs-Ringer solution containing serotonin (5HT) are represented by solid, broken and dotted lines, respectively. Diameter response, \( \frac{(\Delta D_{m/D_m})_{KRS}}{(\Delta D_{m/D_m})_{SS}} \), stands for isobaric constriction at a given pressure and active stress, \( \Delta \sigma \), for isometric contraction at a given strain.
The mid-wall diameters under the active conditions in KRS and 5HT, and under the passive condition in the saline solution, respectively. Active stress, $\Delta \sigma_{KRS}$ and $\Delta \sigma_{5HT}$, were calculated to express the isometric contractions of a blood vessel at a given strain level and were defined as:

$$\Delta \sigma_{KRS} = \sigma_{KRS} - \sigma_{SS},$$

and

$$\Delta \sigma_{5HT} = \sigma_{5HT} - \sigma_{SS},$$

where $\sigma_{KRS}$, $\sigma_{5HT}$ and $\sigma_{SS}$ are the wall stresses developed at a given strain under three different conditions. The details of the experimental procedures and data analysis employed in this study have been described previously. $^{5, 6, 8}$

**Results**

**Pressure-diameter Curve and Flexion Point**

Examples of pressure-diameter curves of a basilar artery are shown in figure 2. The solid curve of each group obtained in the saline solution is convex toward the diameter axis, indicating that the arterial wall becomes stiffer with elevation of pressure when the smooth muscle is relaxed. The activation of smooth muscle caused constriction and made the pressure-diameter curves shift toward the pressure axis. The curves of 4-day and 7-day groups obtained in KRS are biphasic and have sharp flexions at intraluminal pressures of 40 and 20 mmHg, respectively. The wall is fairly stiff below the flexion point and becomes distensible after the intraluminal pressure exceeds the flexion point. The curve of each group observed in 5HT has a flexion point at higher intraluminal pressure than that in KRS, below which pressure the wall manifests very little distension with the increase in pressure.

![Figure 2](image-url)  
**Figure 2.** Examples of pressure-diameter curves of a basilar artery obtained from control and treated groups. Arrows indicate flexion points.

The pressure levels at which the flexion points appear in KRS and 5HT are summarized in figure 3. This pressure observed in 5HT increases with time, reaches the maximum value of 220 mmHg on the 7th day after treatment, and then falls to around the control value on the 14th and 28th days. In the case of KRS, flexion points appear just in the 4-day and 7-day groups and their pressure levels are much lower than in the case of 5HT.

**Diameter Response and Active Stress**

Figure 4 summarizes the relations between the diameter response caused by 5HT and intraluminal pressure in the control and treated groups. Pressure dependence of the diameter response observed in the control, 2-day, 14-day and 28-day groups is somewhat different from that in the 4-day and 7-day groups. The diameter responses in the former 4 groups are maximal below 100 mmHg and decrease monotonously with the
pressure elevation. The 4-day and 7-day arteries, on the other hand, retain their strong constriction up to a pressure level of approximately 200 mmHg, since they have their flexion points in such high pressure range. After exceeding these points, their diameter responses decrease rather rapidly.

Figure 5 shows the changes in diameter response at 100 mmHg developed by KRS and 5HT. Each diameter response first increases with time and reaches a maximum value on the 7th day and then decreases rapidly, recovering to the control value on the 28th day after the treatment. The changes in the diameter response with time are rather paralleled to each other.

Figure 6 exhibits the maximum active stress induced by 5HT and the active stress by KRS at the same strain as the former is developed. These two stresses change with time in a manner similar to the diameter response, being maximum in the 7-day group and returning to the control values in the 14-day and/or 28-day groups.

The maximum active stresses developed by 5HT are plotted against maximum diameter responses in figure 7. The 7-day artery has the highest maximum active stress and maximum diameter response. Although there is no significant difference in the maximum active stresses in the control, 2-day, 14-day and 28-day groups, the 2-day artery yields a significantly greater maximum diameter response than those in the other 3 groups.

**Discussion**

Kuwayama et al.\(^1\) and White et al.\(^2\) have reported successful production of the cerebral vasospasm in the dog by the same method used in this study. Pressure-diameter curves obtained in the KRS, 5HT and saline solution were considered to represent the elastic properties of an arterial wall under three different conditions of smooth muscle contraction, that is, under the vasospastic, maximally contracted, and fully relaxed conditions. Although vasospasm was detected in KRS in the present in vitro experiment, the diameter response observed, i.e. isobaric constriction, was smaller than that measured angiographically by Kuwayama. The reason for this difference has been discussed previously.\(^5\)

Significant increases in the diameter response and active stress developed by 5HT were observed 7 days after experimental SAH (fig. 5 and 6). To evaluate the contractility of treated arterial walls, the differences in the active stress as well as in the diameter response between two conditions in the KRS and 5HT are plotted against the period after the treatment in figure 8. No significant change with time can be detected in these two values. These results imply that the contractile response of spastic arterial walls to serotonin remains unchanged after SAH although the contractile capacity of the wall itself increases with the advance of cerebral vasospasm shown in figures 5 and 6. Toda et al.\(^1\) and Lobato et al.\(^2\) have measured isometric tension to investigate the contractility of spastic arterial walls. Toda demonstrated the decreased contractility of walls and attributed the result to their impaired metabolism, while Lobato documented the increased contractility and ascribed the hypersensitivity of walls to vasoconstrictors. In those studies they did not consider the contraction retained in the spastic arterial wall before
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Differences in active stress, \( \Delta \sigma \), as well as in diameter response, \( \Delta Dm/Dm \), between two conditions in Krebs-Ringer solution (KRS) and Krebs-Ringer solution containing serotonin (5HT).

The administration of vasoconstrictors, and this limits the significance of their findings.

Under active smooth muscle condition in KRS or 5HT, pressure-diameter curves have flexion points, and shift toward the pressure axis compared with those obtained under passive smooth muscle condition in saline solution (figs. 2 and 3). The strong vasoconstriction, accompanied by little change in the vascular diameter with pressure elevation, is retained up to the pressure level at the flexion point, above which the constriction is released abruptly (fig. 4). Although there have been many studies on the mechanical properties of arterial walls, the existence of flexion point over the intraluminal pressure of 100 mmHg is documented only in canine saphenous and rabbit ear arteries\(^1\) as well as human vertebral artery subjected to SAH.\(^8\) To explain the mode and mechanism of vasospasm from biomechanical viewpoints, we attach great importance to the flexion point, and explained its appearance by a possible mechanical interaction between connective tissue and contracted smooth muscle.\(^8\)

Under physiological conditions it is generally accepted that arterioles reduce their lumen caliber and hence increase the peripheral resistance greatly with an increase in intraluminal pressure, while the larger arteries are hardly or only slightly contracted by that stimulus.\(^16,17\) With cerebral vasospasm, it has been suggested that it is the spastic main trunk of the cerebral artery that determines the regional cerebral blood flow. An increase in blood pressure not only accelerates the flow velocity through the constricted vessel\(^18\) but also expands it and hence improves the ischemic deficit both in humans\(^19,20\) and experimental animals.\(^21,22\) The results obtained in this study imply that increasing the intraluminal pressure dilates a spastic artery nonlinearly (fig. 2), and that induced hypertension could improve the cerebral ischemia of vasospasm if the blood pressure were maintained above the flexion point.

Both isobaric constriction (diameter response) and isometric contraction (active stress) caused by serotonin were measured in this study. Figure 7 shows that there is not a good correlation between the maximum values of these two parameters. Similar results have been observed in aged and hypertensive rats by Cox, who ascribed them to changes in the passive elastic properties of walls which are eventually determined by the quality and/or quantity of connective tissues.\(^3,4\) We measured the incremental elastic modulus of the canine basilar arteries subjected to experimental SAH under the fully relaxed condition of smooth muscle in saline solution to evaluate the passive elastic properties inherent to their wall materials.\(^5\) The passive elastic

![Graph](image)

**Figure 8.** Differences in active stress, \( \Delta \sigma \), as well as in diameter response, \( \Delta Dm/Dm \), between two conditions in Krebs-Ringer solution (KRS) and Krebs-Ringer solution containing serotonin (5HT).

![Graph](image)

**Figure 9.** Ratio of maximum diameter response to maximum active stress versus incremental elastic modulus, \( E_{inc} \), at 100 mmHg obtained under the relaxed condition of smooth muscle.
properties are changed greatly by SAH and the treated arterial walls have significantly lower moduli than the control ones, having a minimum value in the 2-day group. The elastic modulus correlated well with the ratio of collagen to elastin contents through the post-treated period. The ratios of maximum diameter responses to maximum active stresses were calculated to express the efficacy of constriction of arterial walls since diameter change is a primary consideration with respect to vasospasm. They were plotted against the incremental elastic moduli to establish the effects of passive elastic properties on the contractility of walls subjected to SAH (fig. 9). A rather good correlation was obtained between these two parameters, indicating that the lesser the stiffness of arterial wall, the more efficiently it constricts. These results indicate that the decreased stiffness of arterial wall subjected to SAH might be one of the factors promoting vasospasm.

References
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