Calcium sensitivity of vasospastic basilar artery after experimental subarachnoid hemorrhage

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Arteries that develop vasospasm after subarachnoid hemorrhage (SAH) may have altered contractility and compliance. Whether these changes are due to alterations in the smooth muscle cells or the arterial wall extracellular matrix is unknown. This study elucidated the location of such changes and determined the calcium sensitivity of vasospastic arteries.

Dogs were placed under general anesthesia and underwent creation of SAH using the double-hemorrhage model. Vasospasm was assessed by angiography performed before and 4, 7, or 21 days after SAH. Basilar arteries were excised from SAH or control dogs (n = 8–52 arterial rings from 2–9 dogs per measurement) and studied under isometric tension in vitro before and after permeabilization of smooth muscle with α-toxin. Endothelium was removed from all arteries. Vasospastic arteries demonstrated significantly reduced contractility to KCl with a shift in the EC50 toward reduced sensitivity to KCl 4 and 7 days after SAH (P < 0.05, ANOVA). There was reduced compliance that persisted after permeabilization (P < 0.05, ANOVA). Calcium sensitivity was decreased during vasospasm 4 and 7 days after SAH, as assessed in permeabilized arteries and in those contracted with BAY K 8644 in the presence of different concentrations of extracellular calcium (P < 0.05, ANOVA). Depolymerization of actin with cytochalasin D abolished contractions to KCl but failed to alter arterial compliance. In conclusion, it is shown for the first time that calcium sensitivity is decreased during vasospasm after SAH in dogs, suggesting that other mechanisms are involved in maintaining the contraction. Reduced compliance seems to be due to an alteration in the arterial wall extracellular matrix rather than the smooth muscle cells themselves because it cannot be alleviated by depolymerization of smooth muscle actin.

cerebral vasospasm; smooth muscle; intracellular calcium concentration; BAY K 8644

CEREBRAL VASOSPASM remains a leading cause of morbidity and mortality after aneurysmal subarachnoid hemorrhage (SAH). An important question about the pathogenesis of vasospasm is the extent to which the arterial narrowing is reversible and due to active smooth muscle contraction. Prior investigations have produced conflicting results with some studies indicating that vasospasm is completely reversible with adequate doses of pharmacological vasodilators (22, 29) and others suggesting that vasospasm is due to a temporarily irreversible structural change in the arterial wall (44). The latter process would suggest the presence of some degree of arterial remodeling (31). Interpretation of prior studies is compromised by lack of documentation that vasospasm actually occurred as well as the use of models that do not produce severe delayed vasospasm (5) or that use systemic arteries in which the perivascular inflammatory and fibrotic reaction is markedly different from that occurring intracranially (13, 32). Furthermore, investigations that have been done to elucidate the pathogenesis of coronary artery spasm, such as measurements of Ca2+ sensitivity, and to rule out the presence of actin-based, nonsmooth muscle contractions have never been conducted on arteries with vasospasm after SAH.

The purpose of this investigation, therefore, was to determine whether vasospasm is associated with an alteration in Ca2+ sensitivity of the contractile apparatus and to determine the extent to which nonmuscle-based arterial diameter reduction contributes to vasospasm. The dog double-hemorrhage model of SAH was chosen because this model best reproduces vasospasm as it occurs in humans and because it is more practical and simple to use than the nonhuman primate model (24). Assessment of Ca2+ sensitivity may be done by permeabilizing the smooth muscle cells with Triton X-100, saponin, β-escin, or staphylococcal α-toxin. The advantage of the latter is that the pores in the cell membrane formed by this toxin are small (2–3 nm) and permit passage only of low molecular mass compounds (<4 kDa) (16). This maintains as best as possible the intracellular environment so that an accurate assessment of contraction in response to alterations in intracellular Ca2+ concentration ([Ca2+]i) can be obtained with little disruption of intracellular signaling pathways as possible (1).

MATERIALS AND METHODS

Animal model. Twenty-seven mongrel dogs weighing 15–25 kg underwent baseline cerebral angiography and then were randomly assigned to serve as controls (n = 6) or to undergo creation of SAH by injection of 0.5 ml/kg autologous, nonheparinized, arterial blood into the cisterna magna on the day of angiography (day 0) and then again 2 days later (day 2). SAH dogs underwent repeat angiography on days 4 (n = 7), 7 (n = 11), or 21 (n = 5), after which they were euthanized. Control dogs underwent baseline angiography, followed by injection of 0.9% NaCl (0.5 ml/kg) into the cisterna magna. The injection was repeated on day 2, and angiography and euthanasia were done on day 7. Methods for angiography, creation of SAH, monitoring of physiological parameters (blood pressure, heart rate, body temperature, and arterial carbon dioxide and oxygen concentrations), and standardization of angiography have been described (2, 21). All procedures were performed with the animals under general anesthesia...
induced by sedation with intravenous injection of pentothal sodium (15 mg/kg), followed by tracheal intubation and ventilation on oxygen and 1–2% isoflurane. Animals were euthanized under general anesthesia by exsanguination and transcardiac perfusion with 8 liters ice-cold PBS (pH 7.4) at physiological intraluminal pressure. This volume of perfusate completely cleared intraluminal blood and rapidly cooled the brain. After perfusion, the brain was excised and placed in 4°C PBS. The arachnoid membrane surrounding the basilar artery was removed under an operating microscope. The endothelial layer was removed by passing an angiography guidewire of the same diameter as the artery through the arterial lumen and then flushing the artery with PBS. Endothelial removal was confirmed by lack of immunohistochemical staining with CD31 of arterial cross sections with the endothelium removed, compared with immunoreactivity present in arteries without endothelial removal, as well as by preliminary pharmacological studies demonstrating selective loss of endothelium-dependent relaxations to adenosine triphosphate in endothelial-denuded segments. All procedures on animals were performed under general anesthesia with endotracheal intubation and ventilation using sterile techniques and were approved by the Institutional Animal Care and Use Committee.

Isometric tension recording. The excised basilar artery was cut into rings (3.5 mm long) as measured under an operating microscope with a graduated ocular. Rings were mounted on metal hooks immersed in tissue baths on a novel tension recording system, designed and constructed by one of the authors (J. Young). The hooks were connected to transducer arms via vertical bars with the distance between the hooks controlled by a micrometer. The baths contained Krebs-Henseleit buffer containing (in mmol/l) 123 NaCl, 5.9 KCl, 2.5 CaCl2, 1.2 MgSO4, 25 NaHCO3, 1.2 KH2PO4, and 11 glucose, bubbled with 95% O2-5% CO2 (pH 7.4). Bath temperature was controlled thermostatically at 37°C. Force was measured by force measurement beams (Advanced Custom Sensors, model 6000-c25). Acquired data were sampled at 2 Hz using custom software (J. Young, University of Chicago), implemented using Igor and NiDAQ Tools 1.5 (Wavemetrics, Lake Oswego, OR), running on a personal computer.

The diameter of the basilar artery in vivo was calculated by measurement of arterial diameters on angiograms that were corrected for magnification using a radiopaque marker in each radiograph and measurement beams (Advanced Custom Sensors, model 6000-c25). The diameter of the basilar artery in vivo was calculated by measurement of arterial diameters on angiograms that were corrected for magnification using a radiopaque marker in each radiograph and measurement beams (Advanced Custom Sensors, model 6000-c25). Acquired data were sampled at 2 Hz using custom software (J. Young, University of Chicago), implemented using Igor and NiDAQ Tools 1.5 (Wavemetrics, Lake Oswego, OR), running on a personal computer.

Arterial contractility and Ca2+ sensitivity. The cross-sectional area of control arteries at baseline (ANOVA). SAH was associated with significant reduction in basilar artery diameter on days 4, 7, and 21 with no pairwise differences between any of the times after SAH (day 4, 53 ± 5% decrease, n = 7; day 7, 59 ± 7% decrease, n = 9; and day 21, 31 ± 10% decrease, n = 5, P < 0.05, paired t-tests, Fig. 1). Animals euthanized on day 21 had angiography on day 7, at which time they had severe vasospasm (58 ± 6% decrease, P < 0.05, paired t-test). Control dogs did not develop vasospasm (day 7, 98 ± 3% of baseline, not significant). There were no significant differences in physiological parameters between groups at each time or within groups over time (data not shown).

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different from that of rings from dogs 4, 7, or 21 days after SAH (control, 0.28 ± 0.03 μm²; day 4, 0.23 ± 0.03 μm²; day 7, 0.24 ± 0.04 μm²; and day 21, 0.28 ± 0.04 μm²; n = 5 cross sections from 4–7 dogs, P > 0.05, ANOVA). Thus no correction for changes in cross-sectional area needed to be applied. When baseline stretch of arterial rings was set to correspond to diameter in vivo, there was a significant difference in tension even at baseline with tension in control arteries significantly greater than in all other groups (P < 0.05, ANOVA, Fig. 2A).

Contractions of arteries to KCl were significantly reduced at all times after SAH compared with control (n = 39 rings from 5 dogs) with no difference in the contractions between days 4 (n = 29 rings from 7 dogs), 7 (n = 52 rings from 8 dogs), and 21 (n = 29 rings from 4 dogs, P < 0.05, ANOVA, Fig. 2A). Contractions of arteries 4 days after SAH were significantly greater than those 7 and 21 days after SAH for [KCl] (≥30 mmol/l; P < 0.05, Tukey’s test). Because baseline tension differed between groups, the contractions were normalized to the maximal contraction achieved. This showed that the concentration-contraction curves were very similar, but there were small but significant increases in the EC50 for contraction 4 and 7 days after SAH compared with control and day 21 (P < 0.05, ANOVA, Fig. 2B). This suggests that arteries 4 and 7 days

Fig. 1. Percent reduction in angiographic arterial diameters by day after subarachnoid hemorrhage (SAH). There was significant reduction in basilar artery diameter days 4 (n = 7), 7 (n = 9), and 21 (n = 5) after SAH with no significant differences between times (values are means ± SE; P values are as indicated, paired t-tests and ANOVA).

Fig. 2. Contractions to KCl in unpermeabilized rings without (A) and with (B) normalization to maximal contraction to KCl and contractions to Ca2+ in permeabilized rings without (C) and with (D) normalization. A: contractions to KCl were reduced at all times after SAH compared with control (control, n = 39 rings from 5 dogs; day 4, n = 29 rings from 7 dogs; day 7, n = 52 rings from 8 dogs; and day 21, n = 29 rings from 4 dogs; values are means ± SE, P < 0.05, ANOVA). Notation of P values is omitted on graphs for clarity. Contractions of arteries 4 days after SAH were significantly greater than those 7 and 21 days after SAH for [KCl] (≥30 mmol/l; P < 0.05, Tukey’s test). B: when contractions were normalized to maximal contraction achieved, curves were very similar, but there were small but significant increases in the EC50 for contraction 4 and 7 days after SAH compared with control and day 21 (P < 0.05, ANOVA). C: contractions of permeabilized arteries to Ca2+ also varied significantly for each [Ca2+] (control, n = 22 rings from 6 dogs; day 4, n = 19 rings from 4 dogs; day 7, n = 17 rings from 5 dogs; and day 21, n = 16 rings from 4 dogs; P < 0.05, ANOVA). There were significant pairwise differences between control and days 4 and 7 for [Ca2+] (0.05 and 0.1 μmol/l, respectively) and between control and days 7 and 21 for all [Ca2+] (≥0.1 μmol/l; P < 0.01, Tukey’s test). Contractions at day 4 also were higher than days 7 and 21 at [Ca2+] ≥0.5 μmol/l (P < 0.05, Tukey’s test). D: when contractions to Ca2+ were normalized to maximal contraction achieved and EC50 values were calculated, significant variance was noted, but the only pairwise difference was between days 7 and 21 (P < 0.05, Tukey’s test).
after SAH have lower permeability to K⁺, which is consistent with our group’s (10) electrophysiological studies showing decreased K⁺ currents in these arteries.

Calcium sensitivity was tested in two ways. Responses of endothelium-denuded control and SAH rings to A23187 and to increasing concentrations of BAY K 8644 were determined in the presence of different concentrations of extracellular Ca²⁺ (Fig. 3). There were no contractions to A23187. First, the lowest concentration of BAY K 8644 that produced the maximal contraction in Krebs-Henseleit buffer was determined and found to be 5 μmol/l for both control and SAH rings (data not shown). Arterial rings were then contracted in response to BAY K 8644 in bath solutions containing different concentrations of Ca²⁺, and it was noted that the absolute magnitude of contractions of SAH arteries was significantly lower than those of control arteries SAH (control, n = 8 rings from 2 dogs; and day 7, n = 7 rings from 2 dogs; P < 0.05, unpaired t-tests, Fig. 3A). After normalization of contractions to the maximal contraction obtained with KCl (60 mmol/l), there was a significant decrease in the sensitivity of contraction to increasing extracellular Ca²⁺ in the presence of a concentration of BAY K 8644 (5 μmol/l) in arteries from dogs 7 days after SAH (P < 0.05, unpaired t-tests, Fig. 3B).

In the second method, artery rings were permeabilized with α-toxin, and contractions from diameter in vivo to increasing [Ca²⁺] were assessed. A similar change was noted in SAH arteries in that baseline tension was lower compared with control arteries, although this was not statistically significant.

The absolute magnitude of contractions to [Ca²⁺] was decreased at all times after SAH (control, n = 22 rings from 6 dogs; day 4, n = 19 rings from 4 dogs; day 7, n = 17 rings from 5 dogs; and day 21, n = 16 rings from 4 dogs, P < 0.05, ANOVA, Fig. 2C). There were significant pairwise differences between control and days 4 and 7 for [Ca²⁺] (0.05 and 0.1 μmol/l, respectively) and between control and days 7 and 21 for all [Ca²⁺] (≥0.1 μmol/l; P < 0.01, Tukey’s test). Contractions at day 4 also were higher than days 7 and 21 at [Ca²⁺] ≥0.5 μmol/l (P < 0.05, Tukey’s test). When contractions were normalized to the maximum contraction to Ca²⁺ and EC₅₀ values were calculated, significant variance was noted with the curve shifted right, indicating reduced Ca²⁺ sensitivity 4 and 7 days after SAH, and shifted left 21 days after SAH. The only pairwise difference was between days 7 and 21 (P < 0.05, Tukey’s test, Fig. 2D).

To determine whether differences in stretch at baseline affected the magnitude of the evoked contractions, contractions to KCl (60 mmol/l) or Ca²⁺ (1 μmol/l) were assessed for arteries at different times after SAH at the same baseline stretch (Fig. 3). Baseline stretch (0.70 mm) was equivalent to a slightly greater diameter than in vivo for SAH arteries and less than in vivo for control arteries. The concentrations of KCl and Ca²⁺ were chosen as those that produced contractions that were not statistically different between groups for arteries stretched to their diameter in vivo (Fig. 2, A and C). When stretched to the same baseline length, there was significant variation in contraction to KCl, with contraction on day 4 greater than in controls and on days 7 and 21 (P < 0.05, ANOVA with Tukey’s test). These measurements were repeated for permeabilized arteries exposed to Ca²⁺, and there were no significant differences between groups, although the same trend is evident with lower contraction on day 7 compared with other days (Fig. 4).

Arterial compliance. There was decreased compliance in arteries 4, 7, and 21 days after SAH that was present even under conditions of nominal [Ca²⁺] in permeabilized arteries (Fig. 5, A and B). For intact rings under conditions of inhibition of all smooth muscle tone with nicardipine (10 μmol/l) and papaverine (10 μmol/l), there was significant variation in tension at stretch >0.75 mm (P < 0.005, ANOVA) with significantly greater tension in arteries at longer times after SAH (control, n = 38 rings from 5 dogs; day 4, n = 24 rings from 6 dogs; day 7, n = 30 rings from 5 dogs; and day 21, n = 29 from 4 dogs). When rings were permeabilized, the tension did not significantly change in control rings or in those at any time after SAH for a given stretch, except for control rings...
where there was a decrease in tension at lengths of 1.25 and 1.75 mm (P < 0.05, t-test, Fig. 5, B and C). Otherwise, the findings were identical to those in nonpermeabilized arteries with a decrease in compliance after SAH that became progressively greater with time (control, n = 21 rings from 5 dogs; day 4, n = 19 rings from 4 dogs; day 7, n = 20 rings from 5 dogs; and day 21, n = 29 from 4 dogs; P < 0.05, ANOVA, Fig. 5, B and C). This suggests that the decrease in compliance is not due to Ca2+-mediated contractile processes in the arterial wall.

To further define the basis for the decreased compliance, nonpermeabilized arterial rings were treated with the actin depolymerizing agent cytochalasin D (10 μmol/l). Addition of cytochalasin D caused a significant decrease in resting tension in both control arteries and arteries 7 days after SAH when they were set at a stretch of 1.25 mm. This is compatible with presence of myogenic tone under these conditions (arteries: control, n = 8 rings from 2 dogs; and day 7, n = 12 rings from 2 dogs; P < 0.05, t-test, Fig. 6A). The remaining tension, however, was significantly greater in SAH arteries compared with controls. Cytochalasin D decreased or abolished contractions to KCl (60 mmol/l) in both sets of arteries, confirming that actin was depolymerized. Finally, compliance curves showed that significantly lower compliance persisted in arteries 7 days after SAH compared with controls (control, n = 11 rings from 3 dogs; and day 7, n = 15 rings from 3 dogs; P < 0.05, ANOVA, Fig. 6B). In conjunction with the data on compliance in permeabilized arteries, this proves that the decrease in compliance must be due to a change in a nonactin-based process in the arteries, which would likely be outside the smooth muscle cell in the extracellular matrix.

**DISCUSSION**

There are several new findings reported here. It is documented for the first time that the Ca2+ sensitivity of vascular smooth muscle is reduced during vasospasm 4 and 7 days after SAH in dogs and that it is increased 21 days after SAH when

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Fig. 4. Contractions of arteries to KCl (60 mmol/l) or to Ca2+ (1 μmol/l) in permeabilized arteries when baseline stretch of all arteries was set to 0.70 mm. There was significant variation in contraction to KCl with contraction on day 4 greater than in controls and on day 21 (control, n = 22 rings from 6 dogs; day 4, n = 19 rings from 4 dogs; day 7, n = 17 rings from 5 dogs; and day 21, n = 16 rings from 4 dogs; P < 0.05, ANOVA, Fig. 6A). There was no significant variation in contraction to Ca2+ although the same trend is evident with lower contraction on day 7.

Fig. 5. Compliance of rings before and after permeabilization. A: for intact rings under conditions of inhibition of all smooth muscle tone, there was significant variation in tension at stretch >0.75 mm (P < 0.005, ANOVA) with significantly greater tension in arteries at longer times after SAH (control, n = 38 rings from 5 dogs; day 4, n = 24 rings from 6 dogs; day 7, n = 30 rings from 5 dogs; and day 21, n = 29 from 4 dogs). B: in permeabilized rings, findings were identical to those in nonpermeabilized arteries with a decrease in compliance after SAH that became progressively greater with time and that was not altered by permeabilization (control, n = 21 rings from 5 dogs; day 4, n = 19 rings from 4 dogs; day 7, n = 20 rings from 5 dogs; and day 21, n = 29 from 4 dogs; P < 0.05, ANOVA). C: compliance before and after permeabilization at a stretch of 1.25 mm shows that tension decreased significantly in control rings after permeabilization but not in rings after SAH (P < 0.05, t-test) and that tension was higher than in control rings at all times after SAH (P < 0.005, ANOVA).
vasospasm is resolving. Second, there is a decrease in the sensitivity of vascular smooth muscle to contraction to KCl at the same times. Third, there is a decrease in compliance of rings before and after addition of cytochalasin D (10 μmol/l), showing that there was significantly lower compliance in arteries 7 days after SAH compared with controls (control, n = 11 rings from 3 dogs; and day 7, n = 15 rings from 3 dogs; P < 0.05, ANOVA).

The classic model of smooth muscle contraction posits that an increase in [Ca^{2+}], binds calmodulin, which then activates myosin light chain kinase (34). Myosin light chain kinase phosphorylates the 20-kDa myosin light chain, activating actomyosin ATPase activity and inducing cross-bridge cycling and contraction. If this were the only process regulating contraction, then there should be a good correlation among increased [Ca^{2+}], myosin light chain phosphorylation, and contraction. This is not the case, however, and it is well known that, for instance, [Ca^{2+}],-force relations are different for KCl and agonist-induced contractions (12). Other examples are that relaxation can be induced by cyclic nucleotides in the absence of a decrease in [Ca^{2+}], and that tonic smooth muscle contraction is associated with persistent force but with a decrease in [Ca^{2+}]. The variation in contraction in response to the same [Ca^{2+}], is a variation in the Ca^{2+} sensitivity of contraction (33). This has not been previously measured in vasospastic smooth muscle. The decrease in sensitivity observed here suggests that the contraction during vasospasm is less dependent on increased [Ca^{2+}], than in control arteries. Because this decreased sensitivity persisted after arteries were removed from the animals, there must be a persistent change in signal transduction pathways involved in Ca^{2+} sensitivity. Another method to investigate this hypothesis might be to assess the relaxation pattern of the rings after the immediate stretch.

Our results are the opposite of what has been suggested to occur during vasospasm (25, 36, 45). The Ca^{2+} sensitivity of vascular smooth muscle has been suggested to be regulated by Rho kinase, protein kinase C, CPI-17 (myosin light chain phosphatase inhibitor protein), and myosin phosphatase (34). Oxyhemoglobin, a putative cause of vasospasm, induced translocation of Rho to the smooth muscle membrane of rabbit cerebrovascular smooth muscle cells (45). Contraction of these arteries to oxyhemoglobin was inhibited by Y-27632, an inhibitor of Rho kinase. These contractions also were inhibited by the protein kinase C inhibitor Ro-32–0432, which also inhibited translocation of protein kinase C types α and ε. The mRNA for Rho A and Rho kinases α and β were increased in the rat basilar artery after SAH during vasospasm (25). Sato and colleagues (36) reported that in the dog basilar artery, rendered vasospastic by the same method used in our experiments, Rho kinase was activated and that vasospasm was reversed partially by Y-27632. None of these studies actually measured Ca^{2+} sensitivity. With a consideration of the complexity of the regulation of smooth muscle contraction, it is not surprising that changes in single kinases that are involved and the use of pharmacological agents, which may have nonspecific effects, would not necessarily correlate with actual Ca^{2+} sensitivity. More study is needed to define the basis for the changes in Ca^{2+} sensitivity reported here and their overall importance in vasospasm.

Prior studies have reported that the magnitude of contraction of vasospastic arteries may be increased or decreased. Some reports cannot be assessed because whether or not vasospasm actually occurred in the arteries being studied was not documented (27, 28, 42). In general, reports of increased magnitude of contraction of vasospastic arteries after SAH are limited to the study of SAH models where the assessment is carried out very early after SAH, before the clinically relevant phase of vasospasm occurs or in models where vasospasm is mild or absent (38, 42, 46). In models where moderate to severe vasospasm develops and when the arteries are assessed during vasospasm, contractility is almost always reduced (11, 18, 35). This was shown in detailed time-course studies (43) in rabbits and is consistent with the decrease in contractility observed in dog arteries during severe vasospasm in this study. The decrease in contractile force and persistent change in arterial diameter that occurs makes it difficult to compare pharmacological responses of normal and vasospastic arteries because in

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**Fig. 6.** A: baseline tension of control arteries and day 7 SAH arteries before and after addition of cytochalasin D (10 μmol/l) at baseline stretch of 1.25 mm. There was a significant decrease in tension in both arteries (control, n = 8 rings from 2 dogs; and day 7, n = 12 rings from 2 dogs; P < 0.05, t-test). Cytochalasin D also abolished contractions to KCl (60 mmol/l). B: compliance of rings before and after addition of cytochalasin D (10 μmol/l), showing that there was significantly lower compliance in arteries 7 days after SAH compared with controls (control, n = 11 rings from 3 dogs; and day 7, n = 15 rings from 3 dogs; P < 0.05, ANOVA).
most studies arteries are set to a similar degree of baseline tension (4) but not to a stretch equivalent to the diameter of the artery in vivo, yet it is known that contractile activity varies with stretch (7, 37, 40). Our investigations show that contractions of vasospastic arteries are reduced, particularly more than 7 days after SAH, even when accounting for differences in stretch at baseline.

Prior investigations have also suggested that vasospastic arteries are stiffer than normal arteries (4, 15, 22, 43), but, again, many studies did not account for differences in diameter at baseline or determine the basis for the change. The mechanical properties of arteries, however, depend greatly on the degree of stretch or what would be intraluminal pressure in vivo, as well as on the degree of activation of the smooth muscle (7). Also, in most studies, the endothelium was not removed, so it is not known how this affected contractile responses. The arterial wall may be modeled as intra- and extracellular resistance components with the intracellular resistance in parallel with the smooth muscle that acts to adjust tension in the system (7). The decreased compliance of vasospastic arteries could arise from increased resistance intraor extracellularly and in actin filaments or other structural proteins in the arterial wall. The location of the change has not been determined. The present studies show that the decreased compliance cannot be reversed by permeabilization of the smooth muscle or depolymerization of actin with cytochalasin D. This suggests that the reduced compliance must be due to an alteration in the extracellular matrix or to a fixed, nonactin-based contraction in the smooth muscle. Because there is no known basis for the latter, the alteration must be in the extracellular matrix. The layer(s) of the arterial wall that are altered must be the tunica media and/or adventitia because we removed the endothelium in our studies. Pathological changes, including evidence of fibrosis of the arterial wall that could stiffen vasospastic arteries, have been reported (23, 30), although other studies, including one using quantitative measurements of collagen, have found minimal or no evidence for increases in collagen or other structural proteins in vasospastic arteries (9, 20). Therefore, more detailed analysis of such arteries needs to be done to determine what causes the decreased compliance and the pathogenesis of its development.

Finally, we report that the EC50 for KCl 4 and 7 days after SAH are significantly less sensitive to KCl. One prior investigation (35), using the same model as used here, found no change in the EC50 for KCl 2, 7, and 14 days after SAH. The reason for this discrepancy is not clear, but the shift in EC50 is consistent with and provides functional corroborative data to our electrophysiological studies, indicating that K+ conductance is reduced in vasospastic arteries (10).

Some of the limitations of the methods used here need to be mentioned. First, the Ca2+ sensitivity measured by α-toxin permeabilization may not accurately reflect the true Ca2+ sensitivity because the permeabilization may allow loss of intracellular molecules that influence Ca2+ sensitivity. Other methods to assess Ca2+ sensitivity might be contractions to the Ca2+ ionophore A23187, in the presence of different concentrations of extracellular Ca2+. This method could not be used here because these arteries did not contract to this ionophore. Contractions to BAY K 8644, also in the presence of different concentrations of extracellular Ca2+, were assessed, and decreased sensitivity to Ca2+ was also found, consistent with the results determined by permeabilization. This method, however, assumes equal expression and response of L-type, voltage-gated Ca2+ channels between control and SAH arteries. There is no information available on the expression and function of these channels after SAH. Second, the isometric tension recording technique allows the arteries to shorten longitudinally in vitro (17). If there is differential shortening between control and SAH arteries, then the compliance and contraction measurements again may not reflect the true state in vivo.

In conclusion, this study shows for the first time that vasospastic smooth muscle has reduced sensitivity to Ca2+ and to depolarization with KCl. The reduced compliance of vasospastic arteries was shown to reside in a nonactin, nonsmooth muscle-based process, likely the extracellular matrix. These findings have important implications for the etiology and pathogenesis of vasospasm.

GRANTS

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