Pressure-dependent myogenic constriction of cerebral arteries occurs independently of voltage-dependent activation

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Lagaud, G., N. Gaudreau, E. D. W. Moore, C. van Breemen, and I. Laher. Pressure-dependent myogenic constriction of cerebral arteries occurs independently of voltage-dependent activation. Am J Physiol Heart Circ Physiol 283: H2187–H2195, 2002. First published August 15, 2002; 10.1152/ajpheart.00554.2002.—Pressure-induced decreases in arterial diameter are accompanied by membrane depolarization and Ca2+ entry via voltage-gated Ca2+ channels. Recent evidence also suggests the involvement of Ca2+ sensitization of the contractile proteins. Both PKC and Rho kinase are candidate second messengers for the mediation of the sensitization process. We investigated the signaling pathways of pressure-induced decreases in rat cerebral artery diameter in vessels that were depolarized with a 60 mM potassium-physiological salt solution (KPSS). Arteries were mounted on a pressure myograph, and pressure-induced contractions were recorded. In some experiments simultaneous changes in intracellular Ca2+ concentration ([Ca2+]i) were recorded by using fura 2 fluorescence photometry. Pressure increases induced constriction with significant changes in [Ca2+]i at high pressures (60–100 mmHg). The ratio of the change in diameter to change in [Ca2+]i was greater for pressure-induced constriction compared with constriction produced by depolarization with 60 mM KPSS, suggesting that in addition to increases in [Ca2+]i, enhanced myofilament Ca2+ sensitivity occurs during pressure-induced decreases in arterial diameter. Depolarizing the membrane with 60 mM KPSS increased [Ca2+]i via a Ca2+ influx pathway insensitive to PKC inhibition. Cerebral arteries were able to maintain their diameters in the continued presence of 60 mM KPSS. Pressure-induced constriction under these conditions was not associated with further increases in Ca2+ but was abolished by selective inhibitors of PLC, PKC, and Rho kinase. We report for the first time that in rat cerebral arteries, pressure-induced decreases in arterial diameter are not only due to increases in voltage-gated Ca2+ influx but also to accompanying increases in myofilament sensitivity to Ca2+ mediated by PKC/Rho kinase activation.

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IN ORGANS such as the brain, heart, and kidneys, blood flow is autoregulated over a range of physiological pressures such that the total blood flow remains nearly constant (3, 8, 39a, 43). Thus, at higher distending pressures, the wall tension is greater, and this serves to regulate a diameter that is appropriate to maintain a near-constant blood flow. This is the myogenic response, first reported in 1902 by Bayliss (2), that describes the ability of arteries to constrict in response to increases in pressures and is now known to occur independently of neural, humoral, or endothelial influences. There is currently great interest in the mechanism of pressure-induced arterial constriction because small arteries produce tone independently of other vasomotor influences (e.g., endothelial factors, pharmacological agents, neurogenic substances, and metabolites) (3).

It is universally accepted that pressure-induced constriction of small arteries is associated with membrane depolarization (4, 9), leading to Ca2+ entry through L-type voltage-gated Ca2+ channels (VGCC) (38) and possibly nonspecific cation or transient receptor potential channels (9, 50). Changes in membrane potential and subsequent entry of Ca2+ are thought to be necessary for pressure-induced constriction (27). An equally attractive hypothesis is the pressure-dependent formation of HETEs, which are potent inhibitors of Ca2+-activated (KCa) channels extending the depolarization of the vascular wall (19).

Several investigators have also suggested a role for membrane-derived lipid products in the regulation of myogenic tone. Evidence supporting a role for PKC in vascular myogenic tone was first established using pharmacological agents (29) and later through direct measures of diacyl glycerol, the natural activator of PKC (37). Pressure-induced activation of PKC has been proposed to maintain constriction without additional increases in Ca2+ entry or myosin light chain phosphorylation (21, 31). Recent evidence indicates that this Ca2+ sensitization occurs through intracellular pathways linked with Rho kinase (52).

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In this study, we describe the interaction of membrane potential and lipid mediators in the regulation of myogenic tone in small cerebral arteries and examine the hypothesis that pressure-activated PKC regulation of tone occurs independently of changes in membrane potential. There are several studies, as reviewed recently (9), that clearly demonstrate increased Ca\(^{2+}\) sensitivity in mesenteric (48) and skeletal (8, 47, 54) arterioles. A steep relationship between increases in extracellular K\(^+\) and the membrane potential regulates pressure-induced constriction of cerebral arteries (27), for which the most widely held explanation is that pressure depolarizes the smooth muscle leading to an increased intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]), (4, 18). This is thought to load the sarcoplasmic reticulum leading to spontaneous release of Ca\(^{2+}\) sparks that activate K\(_{Ca}\) channels (27) producing membrane hyperpolarization. In this study, the regulation of pressure-induced constriction was investigated under conditions in which changes in the activity of K\(_{Ca}\) channels were prevented by pretreating cerebral arteries with a solution containing 60 mM K\(^+\) (60 mM KPSS). At this concentration of external K\(^+\), the membrane potential of smooth muscle cells in rat cerebral arteries is approximately −21 mV, and further changes in voltage-gated Ca\(^{2+}\) are unlikely (27). We report for the first time that cerebral arteries are able to maintain their diameters under conditions that would preclude additional changes in voltage-gated Ca\(^{2+}\) entry and that pressure-induced vasoconstriction under such conditions is sensitive to inhibitors of PLC, PKC, and Rho kinase.

**METHODS**

**Vessel isolation and cannulation.** Male Sprague-Dawley rats (200–300 g) were anesthetized with intraperitoneal injections of pentobarbital sodium (Somnotol, 30 mg/kg) and heparin (Hepalean, 500 U/kg) and then killed by decapitation. The brain was removed and transferred to a dissection chamber warmed the PSS to 37°C. Distal middle cerebral arteries (diameter 100–200 \(\mu\)m) were dissected from surrounding connective tissue and transferred to the experimental chamber of an arteriograph filled with oxygenated PSS at 37°C.

Each vessel was tied onto a proximal glass microcannula with a tip diameter of 60–80 \(\mu\)m by using single strands (20 \(\mu\)m) of 4-0 braided nylon suture; the perfusion pressure was then gently raised to clear the vessel of blood. The distal end of the artery was then similarly mounted to the outflow microcannula. After several minutes of perfusion, the distal outflow cannula was closed, and the transmural pressure was slowly increased to 80 mmHg by using an electronic pressure servosystem (Living Systems Instrumentation, Burlington, VT). Thus pressure-induced constrictions were recorded under conditions of no flow.

The PSS in the vessel chamber was continuously recirculated by superfusion around the pressurized artery at a flow of 20–25 ml/min passing through an external reservoir that was bubbled with a gas mixture of 95% O\(_2\)-5% CO\(_2\). Others have used a gassing mixture of 10% O\(_2\)-5% CO\(_2\)-85% \(\mathrm{N}_2\) (6, 7) and report pressure-constriction curves that are similar to those reported here with a 95% O\(_2\)-5% CO\(_2\) mixture where we obtain ~30–35% maximal pressure-induced constriction (see RESULTS). A heating pump connected to a glass heat exchanger warmed the PSS to 37°C, and a pH microprobe was positioned in the chamber to monitor pH, which was maintained at 7.4 ± 0.04 by adjustment of the reservoir gassing rate.

The arteriograph, containing a cannulated pressurized artery, was placed on the stage of an inverted microscope and allowed to equilibrate for 60 min. Arterial dimensions were measured by using a video system, which produced continuous measurements of luminal diameter and wall thickness. Pressure-induced cerebral artery constriction developed spontaneously and consistently during equilibration, resulting in significantly reduced luminal diameter. Once attained, it remained stable for hours unless perturbed by changes in transmural pressure or the addition of vasoactive compounds (45).

**Experimental procedure.** After development of pressure-induced constriction, intravascular pressure was decreased to 10 mmHg and then raised in 20-mmHg steps to 120 mmHg while corresponding changes in vessel diameter were measured. At each step, the diameter was monitored for 5–10 min until a stable diameter was achieved. The protocol was repeated, and the results were averaged. After the study of the relationship between transmural pressure and the absence of any compounds, transmural pressure was lowered to 20 mmHg, a maneuver that places the vessel below the lower limit of the pressure range for myogenic tone. Luminal diameter was allowed to stabilize for 15–20 min before an activator (indolactam, 1 \(\mu\)M) or inhibitor of PKC (calphostin C, 1 \(\mu\)M) or PLC (U-73122, 1 \(\mu\)M) was added to the superfusing buffer.

The effects of U-73122 (1 \(\mu\)M), calphostin C (1 \(\mu\)M), and Rho kinase inhibitor Y-27632 (1 \(\mu\)M) on the pressure-diameter responses were examined under conditions where further changes in membrane potential are prevented with 60 mM KPSS. These enzyme inhibitors were used at concentrations described by others and us (1, 13, 14, 20, 24, 32-34, 40, 41, 52) as selective for their intracellular targets in isolated arterial preparations. Inhibitors were added to the superfusing buffer and allowed to circulate for 20 min until a new steady-state diameter was reached. At the conclusion of each experiment, the superfusion solution was changed to a Ca\(^{2+}\)-free PSS that contained 2 mM EGTA and no CaCl\(_2\). Vessels were incubated for 20 min and the pressure steps repeated to obtain the “passive” diameter of each vessel to calculate the percent constriction.

**Measurement of [Ca\(^{2+}\)]\(_i\), and arterial diameter.** Arteries were loaded with fura 2, a Ca\(^{2+}\)-sensitive fluorescent dye. Fura 2-AM (10 \(\mu\)l of 1 mM stock solution) was premixed with an equal volume of a 25% solution of pluronic acid in DMSO and then diluted in 5 ml PSS to yield a final concentration of 2 \(\mu\)M. The cannulated middle cerebral artery was incubated in the fura 2-AM-PSS loading solution for 1 h at room temperature, followed by a washout period of 30 min at 37°C.

A 75-W xenon arc was used for excitation. The emission was recorded at 510 nm, whereas the excitation wavelength was alternated between 340 and 380 nm using a diffraction grating. Felix quantitative ratio fluorescence software was used to convert the emitted fluorescence to relative measures of [Ca\(^{2+}\)]\(_i\). (Photon Technology International; Monmouth Junction, NJ). The measurements in each vessel were normalized to the maximum recorded in response to 60 mM KPSS.

**Expression of results and statistical analysis.** At each pressure, the diameter was expressed as a percent decrease from the “passive” diameter or percent constriction = 100% × (D\(_{Ca-free}\) – D\(_{PSS}\)/D\(_{Ca-free}\)), where D is the arterial diameter in Ca\(^{2+}\)-free PSS or in PSS. All results are expressed as...
means ± SE of n experiments. One vessel was taken from each animal. Statistical evaluation was done by ANOVA followed by Newman-Keuls tests. Means were considered significantly different when P < 0.05.

Drugs and solutions. The ionic composition of the PSS was (in mM) 119 NaCl, 4.7 KCl, 1.18 KH2PO4, 24 NaHCO3, 1.17 MgSO4·7H2O, 1.6 CaCl, 5.5 glucose, and 0.026 EDTA. Acetylcholine chloride, bradykinin, calphostin C, and indolactam were purchased from Sigma (Ontario, Canada). A solution rich in K+ (60 mM KPSS) was made by isosmotic substitution for Na+. U-73122 was purchased from Research Biochemicals International (Boston, MA). Stock solutions were diluted in deionized water (NANOpure). Indomethacin was prepared in DMSO. The effects of DMSO were tested and did not alter the pressure-diameter or fluorescence changes. Constrictor responses of arteries loaded with fura 2-AM were not different from control segments that were not incubated with the dye.

RESULTS

Myogenic tone in rat cerebral resistance arteries. Cerebral arteries developed graded pressure-induced constrictions over a pressure range of 40–100 mmHg. Figure 1A shows a representative steady-state response of middle cerebral arteries (mean resting diameter: 169.5 ± 7.7 μm; n = 15) to increases in intraluminal pressure. Pressure-induced constriction of middle cerebral arteries is associated with small changes in the 340- to 380-nm (340/380) fluorescence ratio of fura 2 from 1.60 ± 0.31 at 20 mmHg to 2.57 ± 1.79 at 60 mmHg (Fig. 1B). Significant changes in the 340/380 fluorescence ratio occurs at 60 (34.6 ± 4.1%; n = 24) and 100 mmHg (43.5 ± 5.8%; n = 24) compared with responses at lower transmural pressures (10 and 20 mmHg). However, no significant changes in the 340/380 ratios occurred when intraluminal pressure was increased further from 60 to 100 mmHg. Removal of the endothelium or pretreatment of vessels with L-NAME (200 μM) and indomethacin (10 μM) did not change pressure-induced constriction or the 340/380 fluorescence ratio (data not shown).

Comparison of changes in diameter and [Ca2+], induced by pressure and 60 mM KPSS. Figure 2A shows that changes of transmural pressure from 20 to 80 mmHg in rat cerebral arteries caused a change in the 340/380 fluorescence ratio (from 1.02 ± 0.31 to 1.47 ± 0.17; n = 7), which is associated with a gradual constriction (from 2.8 ± 1.6% to 36.3 ± 2.9%; n = 7) (Fig. 2B). When intraluminal pressure was below the lower limit of the pressure range for myogenic tone (20 mmHg), application of 60 mM K-PSS caused a significant elevation of the 340/380 fluorescence ratio from 1.82 ± 1.02 to 4.25 ± 0.72 (n = 6) (Fig. 2C). This was accompanied by a vasoconstriction (46.3 ± 9.3%; n = 6; Fig. 2D).

The constrictions caused by 80 mmHg and 60 mM KPSS are of a similar magnitude (P > 0.05). However, 60 mM KPSS-induced constriction was accompanied by a significantly greater increase in the 340/380 fluorescence ratio than that produced by the 80-mmHg pressure-induced constriction (Fig. 2, A and C). The ratio of percent constriction over change in the 340/380 fluorescence ratio is significantly higher for pressure-induced constriction (at 80 mmHg) than for depolarization-induced tone (60 mM KPSS) (Fig. 2E), indicating that pressure-induced constriction had a lower Ca2+ requirement. These results suggest that pressure-induced constriction is associated with enhanced Ca2+ sensitivity of the myofilaments.

Effect of indolactam (1 μM), U-73122 (1 μM), and calphostin C (1 μM) on myogenic tone. The effects of pharmacologically modulating PKC activity in cerebral arteries are summarized in Fig. 3. Exogenous activation of PKC with indolactam (1 μM) reduced the vessel diameter significantly greater than pressure-induced constriction at 60 mmHg (P < 0.05). Inhibition of PKC with calphostin C (1 μM) abolished the vasoconstrictor effect of indolactam while not altering the constriction to 60 mM KPSS. The PLC inhibitor U-73122 (1 μM) inhibited pressure-induced constriction at 60 mmHg from 31.3 ± 1.4% to 11.5 ± 0.2% (n = 5). However, a VGCC blocker, nifedipine (1 μM), abolished both pressure (60 mmHg)- and depolarization (60 mM KPSS)-induced constriction (data not shown).

Effect of 60 mM KPSS on myogenic tone and [Ca2+]. A series of experiments were designed to determine the primary events in pressure-induced constriction in ce-
rebral arteries (Fig. 4, A–C). Step increases of transmural pressure (20–120 mmHg) in the presence of 60 mM KPSS (depolarized cerebral arteries) produced constriction (Fig. 4B), allowing arterial diameter to be maintained as the pressure was raised. Others have reported that the membrane potential of smooth muscle cells in the cerebral arteries incubated with 60 mM KPSS is approximately $-21 \text{ mV}$ and that further changes in membrane potential are unlikely under these conditions (27).

Thus simultaneous recordings of diameter and Ca$^{2+}$ in Fig. 4A and B, show that pretreatment of cerebral arteries with 60 mM KPSS induced a constriction (48.8 ± 4.2%, n = 7 at 20 mmHg) associated with a transient increase in the 340/380 fluorescence ratio followed by a plateau phase. Step-wise increases of transmural pressure in the presence of 60 mM KPSS produced vasoconstriction without any significant changes in the 340/380 fluorescence ratio. These results suggest that in addition to increases in Ca$^{2+}$, other mechanisms may be important for maintenance of pressure-induced constriction. Data summarized in Fig. 4C are normalized to 60 mM KPSS and show that 60 mM KPSS caused a significant increase in the 340/380 fluorescence ratio at 20 mmHg (88.3 ± 3.2%, n = 6) compared with responses to pressures of 60 mmHg (54.4% ± 7.3%, n = 6) and 100 mmHg (48.6 ± 8.1%, n = 6). The pressure-induced constriction caused by 60 and 100 mmHg was accompanied by similar increases in the 340/380 fluorescence ratio (Fig. 4C).

Effect of U-73122 (1 µM), calphostin C (1 µM), and Rho kinase inhibitor Y-27632 (1 µM) in depolarized cerebral resistance arteries. Pharmacological inhibitors were used to investigate the mechanisms involved in pressure-induced vasoconstriction under conditions where changes in membrane potential are unlikely to occur. Figure 5, A and B, shows the effects of a PLC inhibitor, U-73122 (1 µM), on depolarized cerebral arteries. In the presence of U-73122 (1 µM), application of 60 mM KPSS resulted in a transient elevation of the 340/380 fluorescence ratio. This change in the fura 2
ratio was accompanied by vasoconstriction (Fig. 5B). Further increases of transmural pressure resulted in vasodilatation (Fig. 5B). Data in Fig. 5C summarize the effects of 60 mM KPSS in the presence of calphostin C (1 μM). Increases in transmural pressure in the presence of 60 mM KPSS and calphostin resulted in vasodilatation and decreases in the fura 2 ratio. These data show that calphostin C, in the presence of a 60 mM KPSS depolarizing solution, abolishes the ability of arteries to constrict in response to increases in transmural pressure.

We have previously shown that pressure-induced constriction in rat cerebral arteries is mediated by PKC (40, 41). We used depolarized cerebral arteries to determine whether the ability of arteries to maintain their diameters in the absence of additional pressure-induced depolarization was sensitive to a PKC inhibitor. Figure 6, A and B, shows the effects of a PKC inhibitor, calphostin C, on arteries placed in a depolarizing solution. In the presence of calphostin C (1 μM), application of 60 mM KPSS resulted in a significant elevation of the 340/380 fluorescence ratio (Fig. 6A). This change in the fura 2 ratio was accompanied by arterial constriction (Fig. 6B). Step increases in transmural pressure under these conditions resulted in an inability to regulate pressure-induced constriction (Fig. 6B). Data in Fig. 6C summarize the effects of 60 mM KPSS in the presence of U-73122 (1 μM). Increases in transmural pressure in the presence of 60 mM KPSS and U-73122 resulted in vasodilatation and decreases in the fura 2 ratio. These data show that calphostin C, in the presence of a 60 mM KPSS depolarizing solution, abolishes the ability of arteries to constrict in response to increases in transmural pressure.

A commonly used inhibitor of Rho kinase, Y-27632, was used to examine whether the constriction to pressure in a depolarizing solution was mediated by Rho kinase. These data are shown in Fig. 7. In the presence of Y-27632 (1 μM), application of 60 mM KPSS increased the 340/380 fluorescence ratio (Fig. 7A). This change in the fluorescence ratio was accompanied by cerebral artery constriction (Fig. 7B). Increases of transmural pressure in the presence of 60 mM KPSS...(continued)
and Y-27632 resulted in a loss of pressure-induced constriction (Fig. 7B). The summarized data in Fig. 7C show that 60 mM KPSS increases the 340/380 fluorescence ratio and that in the presence of this depolarizing solution and Y-27632, increasing the transmural pressure to 60 and 100 mmHg resulted in vasodilation and reduction in the fura 2 fluorescence ratio. Thus pressure-induced constriction in depolarized cerebral arteries occurs without increases in the fura 2 ratio and is likely PKC and Rho kinase mediated.

**DISCUSSION**

An understanding of the cellular basis for pressure-induced constriction of small arteries is an area of increasing interest. There are two generally accepted facets to our knowledge of the underlying mechanisms of pressure-induced constriction of arteries. First, it has an absolute requirement for extracellular Ca\(^{2+}\). Second, activation of lipid mediators such as PKC is involved. We have explored the interaction of these two pathways in the response of arterial diameter to increases in pressure. We report the following observations: 1) that significantly lower Ca\(^{2+}\) levels are required for pressure-induced constriction compared with depolarization-induced constriction from high K\(^+\); 2) pressure-induced constriction is maintained in arteries incubated in a depolarizing (60 mM KPSS) solution; and 3) pressure-induced constriction occurring in a high K\(^+\) (60 mM KPSS) solution is related to activation of PLC, PKC, and Rho kinase, leading to increased Ca\(^{2+}\) sensitivity of the contractile apparatus.

In the rat middle cerebral artery, pressure-induced constriction is associated with relatively small increases in the free [Ca\(^{2+}\)]. Thus Ca\(^{2+}\) sensitivity as measured by the ratio of constriction and the 340/380 fluorescence ratio is significantly higher for pressure-induced constriction than for high K\(^+\) depolarizing-induced constriction (36, 48). These findings suggest that in addition to an elevation of [Ca\(^{2+}\)], pressure-

![Figure 6](http://ajpheart.physiology.org/)

**Fig. 6.** Effect of 60 mM KPSS on the diameter and the 340/380 fluorescence ratio of a rat cerebral artery in the presence of calphostin C (1 μM). Original traces show changes in diameter in response to step increases in pressure (A), corresponding 340/380 fluorescence ratio (B), and normalized 340/380 fluorescence ratio in response to elevation of intraluminal pressure from 20 to 100 mmHg on depolarized arteries (60 mM KPSS) in the presence of calphostin C (1 μM) (C). Values are means ± SE. *P < 0.01, significantly different from the response obtained at 60 mmHg. †P < 0.001, significantly different from the response obtained at 100 mmHg.

![Figure 7](http://ajpheart.physiology.org/)

**Fig. 7.** Effect of 60 mM KPSS on the diameter and the 340/380 fluorescence ratio of a rat cerebral artery in the presence of Y-27632 (1 μM). Original traces showing changes in diameter in response to step increases in pressure (A), corresponding 340/380 fluorescence ratio (B), and normalized 340/380 fluorescence ratio in response to elevation of intraluminal pressure from 20 to 100 mmHg on depolarized arteries (60 mM KPSS) in the presence of Y-27632 (1 μM) (C). The 340/380 fluorescence ratio was normalized to the change in the ratio produced by 60 mM KPSS in the same vessel. Values are means ± SE.
induced constriction is accompanied by increased myofilament Ca$^{2+}$ sensitivity. Inhibition of PLC or PKC (13, 14, 21, 25, 29, 30, 37, 40, 41) or removal of extracellular Ca$^{2+}$ or inhibition of VGCC abolishes pressure-induced constriction in cerebral arteries (27).

Some studies have reported that pressure-induced constriction was solely dependent on pressure-induced activation of Ca$^{2+}$ entry via VGCC, with no role for an increase in myofilament Ca$^{2+}$ sensitivity (4, 27, 38, 35). To determine whether membrane depolarization of cerebral resistance arteries is the sole determinant of pressure-induced constriction, we designed a series of experiments where arteries were incubated with a depolarizing solution (60 mM KPSS) and changes in diameter produced by increased transmural pressure were recorded. We observed that under such conditions, arteries were still able to constric in response to increases in transmural pressure, i.e., arteries maintained a relatively constant diameter with increases in pressure. The fact that pressure-induced constriction still occurred in a 60 mM KPSS depolarizing solution when, presumably, the membrane potential was close to the theoretical K$^+$ equilibrium potential (5, 11, 27) suggests that K$^+$ channel activity alone cannot account for changes in membrane potential that occur during pressure-induced constriction.

Increasing transmural pressure of arteries placed in a depolarizing (60 mM KPSS) solution induced constriction without significant changes in [Ca$^{2+}$]. At this concentration of external [K$^+$], the membrane potential of smooth muscle cells in the cerebral artery wall from rat is approximately −21 mV, and further changes in membrane potential are unlikely (27). We assessed the possible involvement of the PKC-Rho kinase pathway in pressure-induced constriction when the membrane potential was unlikely to change. To this end, we used Y-27632, a selective inhibitor of Rho kinase that is ~200 times more selective for inhibiting Rho kinase than other kinases such as PKC, cAMP-dependent protein kinase, or myosin light chain kinase (46). Our results demonstrate that Rho kinase is likely the effector that mediates Ca$^{2+}$ sensitization of pressure-induced constriction. The redistribution of Rho to the plasma membrane correlates with Ca$^{2+}$ sensitization (5). Furthermore, activation of Rho kinase leads to accumulation of phosphorylated myosin light chains (26). Rho-A has recently been demonstrated to be translocated to the membrane during stretch-induced contraction of rabbit basilar arteries (52). A number of phospholipid-metabolizing enzymes may be regulated through Rho kinase-dependent pathways, including regulation of the supply of phospholipids needed to sustain Ca$^{2+}$ mobilization and presumably PKC signaling by PLC-coupled receptors (53). Taken together, our data indicate that PKC and Rho kinase mediate Ca$^{2+}$ sensitization of pressure-induced constriction in depolarized arteries and are in agreement with the findings of Yeon et al. (52). In the presence of raised [Ca$^{2+}$], caused by membrane depolarization with 60 mM KPSS, arteries passively dilated in response to raised pressure when pretreated with inhibitors of PLC, PKC, or Rho kinase. By using 60 mM KPSS, we clamped the membrane potential near the equilibrium potential for K$^+$, thereby resetting the steady-state [Ca$^{2+}$], and contractile activity. When, in addition, changes in myofilament Ca$^{2+}$ sensitivity are prevented by addition of inhibitors of PLC, PKC, or Rho kinase, the ability of the artery to respond actively to an increase in pressure has been blocked. Under these conditions the artery will behave passively and thus increase its diameter upon an increase of intraluminal pressure.

In addition to PKC and Rho kinase, 20-HETE, the primary cytochrome P-450 metabolite produced in cerebral arteries, is also an important pressure-activated vasoconstrictor (12, 17, 19). 20-HETE inhibits the activity of KC$_{Ca}$ channels, depolarizes cerebral arterial smooth muscle, and thereby potentiates pressure-induced constriction (17, 19). It is unlikely that this pathway contributes significantly to the pressure-induced constriction that occurs in rat cerebral arteries incubated with a depolarizing solution. In nondepolarized arteries where regulation of K$^+$ channel activity is possible, the role of 20-HETE in signaling of pressure-induced constriction in the rat middle cerebral artery was confirmed by the attenuation of constriction by 17-octadecynoic acid, a specific inhibitor of 20-HETE formation (data not shown).

In summary, we demonstrate that pressure-induced constriction in the cerebral artery is mediated by PKC, which modulates intracellular Ca$^{2+}$ sensitivity, possibly through activation of Rho kinase. With placement of the arteries in a depolarizing solution, additional pressure-induced alterations in Ca$^{2+}$ or K$^+$ channel activity were minimized. The pressure-induced constrictions that persisted in arteries placed in a depolarizing solution were sensitive to inhibitors of PLC, PKC, and Rho kinase, suggesting that intracellular mechanisms unrelated to Ca$^{2+}$-dependent activation of cellular pathways may be important in the mechanisms of pressure-induced constriction. Whereas Ca$^{2+}$ entry via voltage-gated ion channels is an essential component of pressure-induced constriction of small arteries, we provide evidence that other intracellular events are able to maintain active constriction in response to pressure changes under conditions where additional Ca$^{2+}$ entry into the cell is minimal. Our findings support an important role for Ca$^{2+}$ sensitization during pressure-induced constriction in rat cerebral arteries.

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