Relative contribution of Rho kinase and protein kinase C to myogenic tone in rat cerebral arteries in hypertension

Yagna P. R. Jarajapu1,2 and Harm J. Knot1,2,3

1Department of Pharmacology and Therapeutics, University of Florida College of Medicine, Gainesville, Florida; 2Wake Forest Institute of Regenerative Medicine, Wake Forest University Baptist Medical Center, Winston-Salem, North Carolina; and 3Medical Physiology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

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J jarajapu, Yagna P. R., and Harm J. Knot. Relative contribution of Rho kinase and protein kinase C to myogenic tone in rat cerebral arteries in hypertension. Am J Physiol Heart Circ Physiol 289: H1917–H1922, 2005. First published June 24, 2005; doi:10.1152/ajpheart.01012.2004.—Arterial smooth muscle constriction in response to pressure, i.e., myogenic tone, may involve calcium-dependent and calcium-sensitization mechanisms. Calcium sensitization in vascular smooth muscle is regulated by kinases such as PKC and Rho kinase, and activity of these kinases is known to be altered in cardiovascular disorders. In the present study, we evaluated the relative contribution of PKC and Rho kinase to myogenic tone in cerebral arteries in hypertension. Myogenic tone and arterial wall calcium in Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR) were measured simultaneously, and the effect of PKC and Rho kinase inhibitors on myogenic tone was evaluated. SHR arteries showed significantly greater myogenic tone than WKY arteries. Pressure/wall tension-arterial wall calcium curves showed a hyperbolic relation in WKY rats, but the curves for SHR arteries were parabolic. Myogenic tone was decreased by the Rho kinase inhibitors Y-27632 and HA-1077, with a significantly greater effect in SHR than in WKY arteries. Reduction in myogenic tone produced by the PKC inhibitor bisindolylmaleimide I in WKY and SHR arteries was significantly less than that produced by Rho kinase inhibition. The pressure-dependent increase in myogenic tone was significantly decreased by Y-27632, and the decrease was markedly greater than that produced by bisindolylmaleimide I in SHR arteries. In WKY arteries, the pressure-dependent increase in myogenic tone was decreased to a similar extent by Y-27632 and bisindolylmaleimide I. These results suggest greater myogenic tone with increased calcium sensitization in SHR arteries, largely because of Rho kinase activation, with a minor contribution of PKC activation.

spontaneously hypertensive rats; cerebral arteries; myogenic tone

VASCULAR SMOOTH MUSCLE CONTRACTION is regulated by electro-mechanical and pharmacomechanical coupling mechanisms, resulting in increased intracellular calcium with and without membrane depolarization, respectively. Calcium imaging combined with force/tension measurements has shown evidence of the dissociation between force and intracellular calcium in intact pressurized arteries. The extent of myosin light chain (MLC) phosphorylation or force of contraction induced by an agonist is greater than that caused by equal amounts of calcium increased by depolarization, a phenomenon termed calcium sensitization (26).

Different studies proposed that, in vascular smooth muscle, calcium sensitization produced by contractile agonists involves decreased MLC phosphatase activity mediated by protein kinase C (PKC) or RhoA-dependent kinase, i.e., Rho kinase. PKC modulates calcium sensitivity through downregulation of MLC phosphatase or phosphorylation of caldesmon and calponin (10, 17), whereas Rho kinase inhibits MLC phosphatase activity by phosphorylation of the regulatory subunit of the enzyme (6). Reduced MLC phosphatase activity leads to increased MLC phosphorylation without a change in intracellular calcium or MLC kinase activity.

Hypertension, a risk factor for the incidence of stroke (4), is characterized by increased vascular smooth muscle contraction, increased peripheral vascular resistance, and structural remodeling of arteries, with arterial hyper- and hyporesponsiveness to endogenous vasoconstrictors and vasodilators, respectively. Cerebral resistance arteries, by virtue of their ability to constrict in response to intraluminal pressure, i.e., myogenic tone, protect the brain from the potential hemorrhage associated with hypertension. Autoregulation in cerebral arteries may operate at a higher level in hypertension (27, 28), presumably because of increased myogenic tone.

Signal transduction mechanisms involved in development of myogenic tone have not been completely elucidated. Different studies showed evidence for involvement of depolarization-induced calcium influx (15), activation of phospholipase C (20), and calcium sensitization (16, 18, 30). Mechanisms underlying calcium sensitization in pressure-induced activation may involve PKC (7, 9) as well as the RhoA/Rho kinase pathway (19, 21). The arterial hyperreactivity to agonists and pressure stimulus in hypertension may involve calcium-dependent and calcium-sensitizing mechanisms. In this study, we evaluated the contribution of two different calcium-sensitization mechanisms to the myogenic tone of cerebral arteries in hypertension.

The focus of the present study was to evaluate 1) myogenic tone and wall calcium in cerebral arteries from spontaneously hypertensive rats (SHR) and normotensive control Wistar-Kyoto (WKY) rats and 2) the relative contribution of PKC and Rho kinase pathways in the myogenic tone of cerebral arteries from SHR and WKY rats. Effects of the structurally different Rho kinase inhibitors Y-27632 and HA-1077 and the structurally different PKC inhibitors chelerythrine chloride and bisindolylmaleimide I on myogenic tone were evaluated.

METHODS

SHR and WKY normotensive controls, 25–30 wk of age, were used in the present study (Charles River). Systolic blood pressure was measured by the tail cuff method. Animal procedures have been the costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Drugs, chemicals, and solutions. Y-27632 was purchased from Tocris Cookson (Ellisville, MO), chelerythrine chloride from Calbiochem (San Diego, CA), HA-1077 and bisindolylmaleimide I from Sigma (St. Louis, MO), and fura 2-AM from Molecular Probes (Eugene, OR). Stock solutions (10 mmol/l) of Y-27632 and HA-1077 were prepared in distilled water, and stock solutions of chelerythrine chloride and bisindolylmaleimide I were prepared in DMSO. The composition of PCSF was as follows (mmol/l): 120 NaCl, 3 KCl, 24 NaHCO3, 1.2 NaH2PO4·H2O, 2.5 CaCl2, 1.2 MgSO4·7H2O, and 4 glucose. PCSF with 16 and 60 mmol/l KCl was prepared by replacing NaCl with an equimolar quantity of KCl. Calcium-free PCSF was prepared by replacing CaCl2 with an equimolar quantity of MgSO4·7H2O with 2 mmol/l EGTA.

RESULTS

Systolic blood pressure was significantly higher in arteries from SHR than in arteries from age-matched WKY rats: 184 ± 2 (n = 19) and 128 ± 2 (n = 20) mmHg (P < 0.05). All arteries developed myogenic tone at 70 mmHg; myogenic tone was 30 ± 1% in WKY rats (n = 20) and 37 ± 1% in SHR (P < 0.01, n = 19), active diameter = 93 ± 3 μm in WKY rats and 85 ± 3 μm in SHR, and passive diameter = 153 ± 11 μm in WKY rats and 128 ± 6 μm in SHR. No significant difference was observed in passive diameter between the two groups. Elevation of potassium to 60 mmol/l KCl increased constriction, with no difference between strains: 184 ± 7% in WKY rats (n = 8) and 165 ± 9% in SHR (n = 10). Similarly, arterial wall calcium (fura-2 ratio) increased to a similar extent in response to 60 mmol/l KCl: 2.14 ± 0.10 in WKY rats (n = 6) and 2.12 ± 0.06 in SHR (n = 4).

Pressure-dependent changes in myogenic tone and arterial wall calcium. Figure 1 shows the myogenic tone developed in WKY and SHR arteries at intraluminal pressures of 10–199 mmHg. In arteries from both strains, 10-mmHg increments in intravascular pressure increased myogenic tone up to 60 mmHg, and myogenic tone was maintained at almost the same level at up to ~150 mmHg. Myogenic tone then tended to decrease or arteries tended to lose resistance and dilate gradually (Fig. 1) at >150 mmHg. Pressure-myogenic tone curves in WKY and SHR arteries were significantly different (P < 0.01, two-way ANOVA).

Fig. 1. Myogenic tone developed in posterior cerebral arteries from Wistar-Kyoto (WKY, n = 6) normotensive rats and spontaneously hypertensive rats (SHR, n = 6) at intraluminal pressures of 10–199 mmHg. Pressure-myogenic tone curves were significantly different (P < 0.01, two-way ANOVA).
Elevation of intraluminal pressure from 10 to 150 mmHg increased arterial wall calcium in WKY rats; this increase was maintained at almost the same level from 150 to 199 mmHg (Fig. 2A). At low (≤30 mmHg) pressure, arterial wall calcium was significantly greater in SHR than in WKY rats (P < 0.05, Student’s t-test at 10, 20, and 30 mmHg). At 50–150 mmHg, where active myogenic tone was observed, the wall calcium of SHR arteries was maintained at nearly the same level, but further elevation of pressure actually resulted in a decrease (Fig. 2A). Pressure-arterial wall calcium curves in WKY and SHR were significantly different (P < 0.001, two-way ANOVA). Because wall tension may represent the stimulus that triggers myogenic tone, we explored the relation between wall tension and arterial wall calcium.

Effect of PKC inhibitors on myogenic tone in cerebral arteries. A concentration-dependent decrease in myogenic tone was not observed with chelerythrine chloride (10 nmol/l–3 μmol/l) in WKY and SHR arteries (Fig. 3A). At 3 μmol/l, the maximum decrease in myogenic tone was 12 ± 5% (n = 4) and 35 ± 11% (n = 5) in WKY and SHR arteries (not significantly different). Bisindolylmaleimide I (10 nmol/l–3 μmol/l) produced a concentration-dependent decrease in myo-

Fig. 2. A: pressure-arterial wall calcium relationship in posterior cerebral arteries from WKY rats and SHR at intraluminal pressures of 10–199 mmHg. Fura 2 ratios are a measure of arterial wall calcium and expressed as percentage of response to 60 mmol/l KCl. Pressure-arterial wall calcium curves in WKY and SHR arteries were significantly different (P < 0.001, two-way ANOVA). B: data in A plotted by replacing pressures with wall tension developed at different pressures. Curves show hyperbolic (WKY) and parabolic (SHR) relationships between wall tension and arterial wall calcium.

Fig. 3. Effect of PKC inhibitors chelerythrine chloride (A) and bisindolylmaleimide I (B) on myogenic tone of posterior cerebral arteries from WKY rats and SHR at intraluminal pressure of 70 mmHg. Maximum decrease observed in SHR arteries by bisindolylmaleimide I was significantly different (P < 0.05, n = 5) from that observed in WKY arteries. C: decrease in myogenic tone produced by 3 μmol/l bisindolylmaleimide I at intraluminal pressure of 10–150 mmHg in WKY and SHR arteries. Decrease is significantly greater in SHR than in WKY arteries (n = 5, P < 0.05, two-way ANOVA).
genic tone in SHR arteries; in WKY arteries, myogenic tone was affected only at 3 μmol/l (Fig. 3B). The maximum decrease in myogenic tone was significantly higher in SHR than in WKY arteries: 51 ± 9% vs. 24 ± 8% (n = 5, P < 0.05). At 10 μmol/l, chelerythrine chloride and bisindolylmaleimide I irreversibly abolished myogenic tone in WKY and SHR arteries.

The pressure-dependent increase in myogenic tone in WKY and SHR arteries was examined in the absence and presence of 3 μmol/l bisindolylmaleimide I (Fig. 3C). Development of myogenic tone was decreased by 10–20% at 40–150 mmHg in the presence of this PKC inhibitor in WKY and SHR arteries. Myogenic tone was more sensitive to this PKC inhibitor in SHR than in WKY arteries, and the decrease in myogenic tone observed at different pressure points in SHR arteries was significantly higher (P < 0.01, two-way ANOVA; Fig. 3C).

Effect of Rho kinase inhibitors on myogenic tone in cerebral arteries. Y-27632 concentration dependently (1 nmol/l–3 μmol/l) reduced myogenic tone in WKY and SHR arteries pressurized at 70 mmHg. The maximum decrease in myogenic tone was significantly greater in SHR than in WKY arteries: 80 ± 8% vs. 45 ± 3% (n = 5, P < 0.004; Fig. 4A). Similar results were observed with HA-1077. Myogenic tone was decreased concentration dependently by HA-1077 (1 nmol/l–30 μmol/l), with a maximum decrease of 73 ± 6% (n = 5) in SHR arteries, which is significantly higher (P < 0.001) than that observed in WKY arteries (35 ± 5%, n = 5; Fig. 4B). Effects of Y-27632 and HA-1077 in these arteries were readily reversible.

The pressure-dependent increase in myogenic tone in WKY and SHR arteries (Fig. 4C) was examined in the absence and presence of 1 μmol/l Y-27632. Development of myogenic tone was decreased with an increase in pressure in WKY and SHR arteries at 20–150 mmHg, with the latter showing higher sensitivity. The decrease in development of myogenic tone in WKY arteries was <10% throughout the pressure range examined. In SHR arteries, the decrease in development of myogenic tone was 15–25% at 10–150 mmHg, which was significantly higher than that observed in WKY arteries (P < 0.001, two-way ANOVA; Fig. 4C). Pressure-myogenic tone curves in SHR arteries in the presence of Y-27632 were significantly different from those in the presence of bisindolylmaleimide I (P < 0.05, 2-way ANOVA).

DISCUSSION

The present study confirms that myogenic tone in rat cerebral arteries operates at a higher level in hypertension. We further provide the first evidence for dissociation of the calcium-myogenic tone relation, implying increased calcium sensitization in cerebral arteries in hypertension. Furthermore, this study provides evidence for a greater involvement and contribution of the RhoA/Rho kinase pathway than of PKC in the elevated myogenic tone of cerebral arteries in hypertension.

Pressure-mediated autoregulation in cerebral arteries in hypertension. The present study shows clear differences in the pressure-dependent constriction of cerebral arteries from normotensive and hypertensive rats at 10–199 mmHg. Higher myogenic tone was observed in hypertensive arteries at 10–130 mmHg than in normotensive arteries. In agreement with the present study, Smeda et al. (24) showed increased myo-

![Fig. 4. Effect of Rho kinase inhibitors Y-27632 (A) and HA-1077 (B) on myogenic tone of posterior cerebral arteries from WKY rats and SHR at intraluminal pressure of 70 mmHg. Maximum decrease induced by both inhibitors was significantly higher in SHR than in WKY arteries (P < 0.01, n = 5). C: decrease in myogenic tone produced by 1 μmol/l Y-27632 at 10–150 mmHg intraluminal pressure in WKY and SHR arteries. Decrease was significantly greater in SHR than in WKY arteries (n = 5, P < 0.001, two-way ANOVA).](http://ajpheart.physiology.org/10.220.32.246)
genic tone in hypertensive cerebral arteries compared with normotensive arteries with intraluminal pressures ≤140 mmHg. Studies by Dunn et al. (5) in middle cerebral arteries from hypertensive Brattleboro rats showed similar results. Findings from the present study support the notion that cerebral arterial autoregulation operates at a higher level of perfusion pressure in hypertension, which might be a consequence of altered signal transduction mechanisms involved in the production of myogenic tone. Loss of autoregulation predisposes for hypertensive encephalopathy and hemorrhagic stroke (25).

Arterial wall calcium-myogenic tone relation in cerebral arteries in hypertension. This is the first study to evaluate the relation between arterial wall calcium and myogenic tone over a wide range of pressures in cerebral arteries from WKY rats and SHR. In WKY rats, a defined hyperbolic relation was observed between the increase in pressure (or wall tension) and arterial wall calcium. The hyperbolic curve in the SHR arteries suggests deviation of the calcium-myogenic tone relation in hypertension with increased tone compared with WKY rats at lower or similar levels of arterial wall calcium. These observations suggest increased calcium sensitization as a possible underlying mechanism in the increased myogenic tone of hypertensive arteries, although studies with permeabilized arteries to quantify the extent of calcium sensitization would provide direct evidence for this conclusion.

As observed in WKY arteries in this study, we reported a similar relation in the same arteries from Sprague-Dawley rats (15) between intravascular pressure and membrane potential depolarization and between pressure and arterial wall calcium. These findings imply that increases in intravascular pressure cause depolarization, leading to increased cytosolic calcium in the arterial wall. Earlier studies showed evidence of an increased depolarization of cerebral arteries as a function of pressure (8, 32) in arteries from SHR compared with WKY rats, which should result in enhanced calcium influx via voltage-dependent calcium channels. Although these electrical changes may explain the initial higher levels of calcium in SHR (at <40 mmHg) than in WKY arteries, they do not explain the lower levels of calcium at higher pressures in SHR than in WKY arteries, despite higher myogenic tone. Dissociation of the calcium-myogenic tone relation in SHR arteries could only be attributed to a process referred to as calcium sensitization (26, 29).

Role of PKC and Rho kinase in increased myogenic tone in hypertension. The second focus in this study was to evaluate the relative importance of PKC and Rho kinase in increased myogenic tone. The potential limitation is the choice of a PKC inhibitor with higher selectivity. The inhibitors used in the present study, chelerythrine chloride and bisindolylmaleimide I, inhibit PKC in nanomolar concentrations (IC50 = 600 and 10 nmol/l for chelerythrine chloride and bisindolylmaleimide I, respectively). In the present study, nonspecific effects were observed at >3 μmol/l. Neither inhibitor significantly affected myogenic tone in nanomolar concentrations in WKY and SHR arteries. The maximum decrease in myogenic tone produced by bisindolylmaleimide I was significantly higher in SHR than in WKY arteries. Because the effect of PKC inhibitors was small in arteries with established myogenic tone, we also evaluated the effect of PKC inhibition by bisindolylmaleimide I in development of myogenic tone with gradual increases in intraluminal pressure. In these experiments, the decrease in myogenic tone was <20%, with SHR arteries showing significantly higher sensitivity.

Earlier studies in arteries from normotensive rats that provided evidence for pressure-induced activation of PKC used PKC activators (7) and inhibitors (1, 14, 18). Studies with activators can provide evidence only for a permissive role of PKC, rather than pressure-induced activation of PKC. Different PKC inhibitors show concentration-dependent nonspecific effects in functional studies. Chelerythrine chloride at 10 μmol/l completely reduced myogenic tone in rat skeletal muscle arterioles, whereas no effect was observed at lower concentrations (1, 18). In afferent arterioles, this inhibitor showed similar blockade of constriction by phorbol ester at 1 and 10 μmol/l, whereas sensitivity of myogenic tone was different at these two concentrations (14), indicating that the effect of higher concentrations on myogenic tone was not necessarily due to PKC inhibition. In the present study, <10 μmol/l did not affect myogenic tone, whereas 10 μmol/l showed nonspecific effects. Furthermore, in cell-based bioassays, chelerythrine chloride did not inhibit PKC, whereas bisindolylmaleimide I derivatives were potent and selective inhibitors (3). In the present study, bisindolylmaleimide I showed a minimal effect on myogenic tone in WKY arteries but a significantly greater effect in SHR arteries, suggesting a minor role for PKC in basal myogenic tone that is increased in hypertension. The effects of PKC inhibition were significantly less than those obtained with Rho kinase inhibitors (see below).

Y-27632 and HA-1077 have been used in different studies, and they have been considered selective inhibitors of Rho kinase I and II; IC50 values for these two compounds for Rho kinase II inhibition were 0.8 nmol/l and 1.9 μmol/l, respectively, with 10- to 50-fold selectivity over other kinases (3, 29). In the present study, these two inhibitors produced a concentration-dependent decrease in myogenic tone in WKY and SHR arteries, with a significantly greater decrease in SHR arteries. The decrease in pressure-dependent development of myogenic tone by Y-27632 in WKY arteries is minimal and similar to that produced by bisindolylmaleimide I, but in SHR arteries the decrease is significantly greater (~25%) and is also greater than that produced by bisindolylmaleimide I in SHR arteries. These results suggest that in normotensive arteries the Rho kinase-mediated calcium sensitization is involved in maintenance of basal myogenic tone in cerebral arteries and, to a minor extent, in pressure-dependent development. In hypertension, pressure-dependent activation of this enzyme is greater, with an increased contribution to the maintenance of myogenic tone. The increased calcium sensitization observed in SHR arteries is largely due to an increased Rho kinase activation with a minor contribution from PKC.

Our results are consistent with findings from earlier studies. Y-27632 produced a persistent fall in blood pressure in SHR, renal hypertensive rats, and deoxycorticosterone acetate-salt rats, whereas its effects in WKY rats were minimal or transient (29). Wire-mounted serotonin-precontracted mesenteric arteries from mineralocorticoid-hypertensive rats showed greater relaxation to Y-27632 (31). Increased Rho kinase activation, but not PKC, was also shown by a cranial window approach in cerebral arteries from hypertensive rats (2, 12) and by a biochemical approach in aorta in different rat models of hypertension (23). Evidence for an increased Rho kinase activation in coronary and cerebral vasospasm was also reported (13,
22). The present study illustrates the importance of Rho kinase in the elevated pressure-induced autoregulation of blood flow in hypertension and identifies the RhoA/Rho kinase pathway as a possible therapeutic target in treatment of hypertension-related cerebrovascular diseases.

REFERENCES