Hypertension and RhoA/Rho-Kinase Signaling in the Vasculature

Highlights From the Recent Literature

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Abstract—Under normal conditions, contractile activity in vascular smooth muscle is initiated by either receptor activation (norepinephrine, angiotensin II, etc.) or by a stretch-activated mechanism. After this activation, several signaling pathways can initiate a Ca\(^{2+}\)-calmodulin interaction to stimulate phosphorylation of the light chain of myosin. Ca\(^{2+}\) sensitization of the contractile proteins is signaled by the RhoA/Rho-kinase pathway to inhibit the dephosphorylation of the light chain by myosin phosphatase thereby maintaining force generation. In opposition to force generation, NO is released from endothelial cells and causes vasodilation through inhibition of the RhoA/Rho-kinase signaling pathway. This brief review will highlight recent studies demonstrating a role for the RhoA/Rho-kinase signaling pathway in the increased vasoconstriction characteristic of hypertension. (*Hypertension. 2004;44:796-799.*)

**Key Words:** vasculature ■ signal transduction ■ muscle, smooth ■ nitric oxide ■ vasoconstriction

Increased peripheral vascular resistance causes elevated arterial pressure in hypertension. Arterial wall thickening, increased vasoconstriction, and reduced vasodilation contribute to this increased peripheral resistance. Multiple regulatory processes (neural, humoral, etc.) and complex cell signaling pathways modulate vascular smooth muscle cell (VSMC) contraction, relaxation, and growth. Under normal conditions, these regulatory processes maintain vessel wall integrity and do not contribute to pathological increases in blood pressure. Remodeling of the vasculature in hypertension involves rearrangement of cellular and extracellular components and has been reviewed extensively.\(^1\)\(^-\)\(^6\) The present review highlights recent developments in the understanding of cellular and molecular mechanisms underlying increased vasoconstriction in hypertension with an emphasis on the RhoA/Rho-kinase signaling pathway in vascular smooth muscle.

**Contractile Mechanism**

Vascular smooth muscle contraction is principally regulated by receptor and mechanical (stretch) activation of the contractile proteins.\(^7\) Depolarization of the plasma membrane can also trigger contraction. For contraction to occur, myosin light chain (MLC) kinase must phosphorylate the light chain of myosin, enabling the cycling of myosin cross-bridges with actin.\(^7\)\(^-\)\(^9\) Thus, contractile activity is determined primarily by the phosphorylation state of the light chain of myosin.\(^8\)\(^,\)\(^9\) In VSMCs of some blood vessels, phosphorylation of the light chain of myosin is maintained at a low level in the absence of external stimuli (ie, no receptor or mechanical activation). This activity results in what is known as myogenic tone. Recent work suggests that 20-HETE may play a signaling role in the myogenic response of pig coronary arteries. Randriamboavonjy et al\(^10\) observed that 20-HETE elicited contraction and phosphorylation of the light chain of myosin. Both of these activities were inhibited by Y-27632, a Rho-kinase inhibitor (see below for description of signaling pathway).

**Ca\(^{2+}\)-Dependent Contraction of Vascular Smooth Muscle**

Contraction of vascular smooth muscle is initiated by a Ca\(^{2+}\)-mediated change in the thick filaments.\(^8\)\(^,\)\(^9\)\(^,\)\(^11\) In response to specific stimuli, the intracellular concentration of Ca\(^{2+}\) increases, and this Ca\(^{2+}\) combines with calmodulin. The Ca\(^{2+}\)-calmodulin complex activates MLC kinase to phosphorylate the light chain of myosin. Cytosolic Ca\(^{2+}\) is increased by 2 mechanisms: (1) Ca\(^{2+}\) release from the sarcoplasmic reticulum; and (2) Ca\(^{2+}\) entry from the extracellular space through Ca\(^{2+}\) channels (receptor-operated Ca\(^{2+}\) channels). Agonists (norepinephrine, angiotensin II, endothelin, etc.) binding to specific receptors and coupled to a heterotrimeric G-protein stimulate phospholipase C activity. This enzyme catalyzes formation of 2 second messengers, inositol trisphosphate (IP\(_3\)) and diacylglycerol (DG), from the membrane lipid phosphatidylinositol 4,5-bisphosphate. Binding of IP\(_3\) to receptors on the sarcoplasmic reticulum results in release of Ca\(^{2+}\) into the cytosol. DG, along with Ca\(^{2+}\), activates protein kinase C (PKC). PKC promotes contraction by phosphorylation of L-type Ca\(^{2+}\) channels or other proteins that regulate cross-bridge cycling. Finally, voltage-operated
Ca$^{2+}$ channels in the membrane also open in response to membrane depolarization brought on by VSMC stretch.

**Ca$^{2+}$ Sensitization Mechanism and Contraction of Vascular Smooth Muscle**

In addition to the Ca$^{2+}$-dependent activation of MLC kinase, the state of myosin phosphorylation is further regulated by MLC phosphatase. This enzyme dephosphorylates the light chain of myosin to promote smooth muscle relaxation. There are 3 subunits of MLC phosphatase: a 37-kDa catalytic subunit, a 20-kDa subunit, and a 110- to 130-kDa myosin-binding subunit. The myosin-binding subunit, when phosphorylated, inhibits MLC phosphatase, allowing the light chain of myosin to remain phosphorylated, thereby promoting contraction. Rho-kinase, a serine/threonine kinase, phosphorylates the myosin-binding subunit of MLC phosphatase, inhibiting its activity and thereby promoting the phosphorylated state of the MLC and contraction. Pharmacological inhibitors of Rho-kinase, such as fasudil and Y-27632, block its activity by competing with the ATP-binding site on the enzyme. Rho-kinase inhibition induces relaxation of isolated segments of blood vessels contracted to many different agonists and lowers blood pressure in hypertensive animal models.

The precise cellular mechanism coupling activation of VSMCs to the RhoA/Rho-kinase signaling pathway is unknown. Currently, it is thought that membrane receptors activate a heterotrimeric G-protein that is coupled to RhoA/Rho-kinase signaling via guanine nucleotide exchange factors (RhoGEFs). Because RhoGEFs facilitate activation of RhoA, they regulate signaling duration and intensity via heterotrimeric G-protein receptor coupling. There are >70 RhoGEFs in the human genome, and 3 RhoGEFs have been identified in vascular smooth muscle: PDZ-RhoGEF, LARG (leukemia-associated RhoGEF), and p115RhoGEF.

Several recent studies suggest a role for additional regulators of MLC kinase and MLC phosphatase. Calmodulin-dependent protein kinase II relaxes smooth muscle by decreasing sensitivity of MLC kinase for Ca$^{2+}$. Additionally, MLC phosphatase activity is stimulated by telokin (a 16-kDa protein) and is inhibited by a downstream mediator of protein kinase C, CPI-17.

**Increased Vasoconstriction and RhoA/Rho-Kinase Signaling in Hypertension**

Alterations in activity of RhoA/Rho-kinase signaling have been proposed to contribute to the increased peripheral vascular resistance in hypertension. Using Y-27632 to inhibit Rho-kinase, Uehata et al. have demonstrated a role for Rho-kinase specific to receptor-mediated contraction. Additionally, they were the first to demonstrate a role for RhoA and Rho-kinase in various animal models of hypertension. After oral administration of Y-27632, blood pressure was measured at selected time intervals in several models of experimental hypertension, including spontaneously hypertensive rats (SHR) and deoxycorticosterone acetate (DOCA)-salt and renal hypertensive rats. In all groups of hypertensive rats, treatment with Y-27632 caused a decrease in systolic blood pressure. In contrast, the blood pressure in normotensive rats showed only a small transient decrease. A recent study also demonstrated that the expression level of membranous RhoA and phosphorylation of the target proteins of Rho-kinase was significantly greater in N-nitro-L-arginine methyl ester (l-NAME)–treated rats than in normotensive rats. These findings suggested a significantly greater contribution in vivo of RhoA/Rho-kinase–mediated Ca$^{2+}$ sensitization to the regulation of blood pressure in hypertensive animals.

Recently, several studies have addressed changes in the RhoA/Rho-kinase pathway in isolated vascular segments from hypertensive animals. Asano and Nomura compared the inhibitory effects of Y-27632 on contractile responses in small and large mesenteric arteries from SHR and normotensive rats. The arteries were contracted to a plateau phase with norepinephrine and subsequently treated with the Rho-kinase inhibitor. The Rho-kinase inhibitor caused relaxation in arteries from both strains, and the magnitude was greater in arteries from SHR. It was concluded that RhoA/Rho-kinase signaling played a greater role in maintaining the plateau phase of contraction in arteries from SHR than in those from normotensive rats. Interestingly, in both rat strains, the large mesenteric artery relaxed more to the Rho-kinase inhibitor than the small resistance artery. Thus, it appears that contractile responses in the large arteries may have a greater contribution from RhoA/Rho-kinase signaling pathway than the small arteries.

In rats made hypertensive by treatment with an inhibitor of NO synthase, t-NAME, Seko et al. observed that Y-27632 lowered blood pressure. Protein levels of RhoA, Rho-kinase, and several other components of this signaling pathway in aorta from t-NAME hypertensive rats were not significantly different from normotensive values. However, the level of active RhoA (translocated to the membrane) was increased in aorta from t-NAME hypertensive rats. This same pattern of no change in expression levels and increased active RhoA was also observed in stroke-prone SHR (SHRSP), renal hypertensive rats, and mineralocorticoid hypertensive rats. However, an angiotensin II receptor antagonist selectively and significantly reduced RhoA activation in vascular smooth muscle from SHRSP animals.

Studies from our laboratory indicate that there is an increase in activity of the Rho/Rho-kinase pathway in blood vessels of mineralocorticoid hypertensive rats. In our initial studies, relaxation in response to inhibition of Rho-kinase with Y-27632 was measured in isolated mesenteric arteries (endothelium removed) from DOCA-salt hypertensive and normotensive rats. After contraction with serotonin, the addition of Y-27632 resulted in a concentration-dependent increase in activity of the Rho/Rho-kinase pathway in blood vessels of mineralocorticoid hypertensive rats. After contraction with serotonin, the addition of Y-27632 resulted in a concentration-dependent decrease in isometric tension, which was greater in arteries from hypertensive rats than in arteries from normotensive rats.

Recent studies by Wehrwein et al. indicate that myogenic tone in aortic segments isolated from mineralocorticoid hypertensive rats is also inhibited by Y-27632. Aortic segments from normotensive rats did not develop myogenic tone. A recent report from our laboratory suggests that the activation of RhoA is a consequence of hypertension. mRNA expression levels of 3 regulators of G-protein signaling (RGS) domain containing RhoGEFs, positive regulators...
of RhoA were not significantly different at 4 weeks of age between SHRSP and Wistar-Kyoto (WKY) animals with similar systolic blood pressure. However, the expression levels of these RhoGEFs were significantly higher in SHR animals at 12 weeks of age when systolic blood pressure was higher in SHRSP (187 ± 5 mm Hg) and WKY rats (126 ± 2 mm Hg). Therefore, we suggest that the activation of RhoA is a consequence rather than a cause of hypertension.

We have also examined the relaxation response to Y-27632 in superior mesenteric arteries isolated from rats after 13 days of angiotensin II–induced hypertension. After contraction induced with phenylephrine, mesenteric arteries (no endothelium) relaxed in a concentration-dependent fashion in response to Y-27632. The relaxation of arteries from hypertensive rats demonstrated a leftward shift in the concentration response curve to Y-27632 compared with normotensive values, suggesting that vessels from the angiotensin II–induced hypertensive animals have an increased sensitivity to Rho-kinase inhibition.

A role for enhanced activity of RhoA/Rho-kinase signaling in clinical hypertension has also been demonstrated. Masumoto et al. measured forearm blood flow using plethysmography in normotensive and hypertensive subjects during intra-arterial infusion of graded doses of a Rho-kinase inhibitor (fasudil) or sodium nitroprusside. Resting forearm vascular resistance was higher in the hypertensive subjects. Fasudil increased forearm blood flow, and the magnitude of the fall in vascular resistance was greater in hypertensive subjects. In contrast, forearm vasodilator responses evoked by sodium nitroprusside in hypertensive subjects were comparable to normotensive values. These results demonstrate that enhanced RhoA/Rho-kinase signaling may be involved in the increased peripheral vascular resistance in clinical hypertension. Interestingly, cigarette smoking, a risk factor for cardiovascular disease, has been shown to activate RhoA/Rho-kinase signaling in the forearm vasculature of humans.

As noted above, RhoGEFs may couple heterotrimeric G-protein activation to RhoA/Rho-kinase signaling. A recent study by Ying et al. demonstrated higher mRNA expression of RGS domain containing RhoGEFs in aortic segments from adult SHRSP compared with normotensive values. In young SHRSP, before systolic blood pressure had increased to levels found in adulthood, mRNA expression levels of the 3 RhoGEFs were not increased above normotensive values. Thus, it appears that overexpression of these regulatory proteins may be an adaptive response in the vasculature attributable to increased blood pressure.

Another possible mechanism to link receptor occupation, heterotrimeric G-protein activation, and RhoA/Rho-kinase signaling is through generation of reactive oxygen species (ROS). Jin et al. observed that Y-27632 relaxed rat aortic segments made to contract in response to experimental protocols that generate ROS. Mechanistically, it was observed that ROS increased migration of RhoA to the plasma membrane, where it was activated. Because oxidative stress characterizes many models of hypertension, it may be that ROS-dependent activation of the RhoA/Rho-kinase pathway contributes to increased peripheral vascular resistance. Together, these studies in experimental and clinical hypertension implicate that the contribution of the RhoA/Rho-kinase pathway to the mediation of constrictor activity is augmented in the vasculature of hypertensive animals.

**NO Signaling Inhibits RhoA/Rho-Kinase Signaling in Vascular Smooth Muscle**

Endothelium-dependent vasodilation is primarily stimulated by NO released from endothelial cells. NO binds to smooth muscle cell–soluble guanylate cyclase, leading to an increase in cyclic GMP (cGMP) levels and the subsequent activation of cGMP-dependent protein kinase (cGK). NO/cGK signaling decreases intracellular Ca²⁺ via inhibition of L-type Ca²⁺ channels and activation of sarcoplasmic reticulum Ca²⁺ ATPase, and it induces membrane hyperpolarization. NO has other actions, such as activation of ribosyl transferases and nitration of proteins, that could also contribute to vasodilatory effects.

Recent evidence suggests that NO may also induce vasodilation through inhibition of RhoA/Rho-kinase signaling pathway. Studies by Sauzeau et al. demonstrated that sodium nitroprusside and constitutively active cGK inhibited phenylephrine-induced translocation of RhoA from the cytosolic to membrane fraction in rat aorta, indicating NO/cGK-mediated inactivation of RhoA. In a recent study from our laboratory, we examined whether NO endogenously induces the relaxation of rat aorta via inhibition of the RhoA/Rho-kinase signaling pathway. The Rho-kinase inhibitor Y-27632 attenuated the maximal force generation to phenylephrine in endothelium-intact rat aorta. In endothelium-denuded rings, Y-27632 was ineffective at inhibiting the phenylephrine-induced contraction. Additionally, Y-27632 was also less effective at inhibiting the phenylephrine-induced contraction of endothelium-intact rings in the presence of NO synthase or guanylate cyclase inhibition. Exogenous addition of sodium nitroprusside restored the ability of Y-27632 to attenuate phenylephrine-induced contraction. Rho-kinase inactivation was also found to increase the sensitivity of endothelium-denuded aorta to sodium nitroprusside. A recent report also suggests that NO may also alleviate blood pressure by inhibiting stretch-induced mitogen-activated protein activation in mesangial cells through RhoA activation.

An additional report demonstrates that RhoA/Rho-kinase pathway negatively regulates endothelial NO synthase (eNOS) phosphorylation through inhibition of protein kinase B, whereas it downregulates eNOS expression independent of protein kinase B. These data demonstrate that NO inhibits Rho-kinase activity and Rho/Rho-kinase negatively regulates eNOS phosphorylation, supporting the hypothesis that NO-mediated vasodilation occurs partially through inhibition of RhoA/Rho-kinase activity and RhoA/Rho-kinase ability to prevent eNOS phosphorylation.

A recent study by Carter et al. demonstrates that acute and chronic NOS inhibition in intact rats enhances α-2 adrenergic receptor–stimulated RhoA/Rho-kinase in isolated aortic segments. In a series of experiments, these investigators demonstrated that α-2 adrenergic–induced contraction in hypertensive and normotensive thoracic aorta requires Rho-kinase activity. Acute and chronic NOS inhibition augmented...
sensitivity to Y-27632 relaxation in α-2 adrenoreceptor–constricted aorta. Acute NOS inhibition increased Y-27632 sensitivity more than chronic NOS inhibition hypertension. Overall, this study supports the concept that the inhibitory effect of NO on RhoA/Rho-kinase signaling is less active in chronic NOS-inhibited hypertensive aorta.

Summary
Contractile activity in vascular smooth muscle is initiated by a Ca\(^{2+}\)-calmodulin interaction to stimulate phosphorylation of the light chain of myosin. A Ca\(^{2+}\) sensitization of the contractile proteins is signaled by the RhoA/Rho-kinase pathway to inhibit the dephosphorylation of the light chain by myosin phosphatase, maintaining force generation. Several recent studies demonstrate that components of the RhoA/Rho-kinase pathway are increased in hypertension, suggesting that they play a mechanistic role in the elevated peripheral resistance characteristic of this disease state. Finally, recent work supports the hypothesis that NO may also induce vasodilation through inhibition of the novel RhoA/Rho-kinase signaling pathway. Thus, impaired NO signaling in hypertension may lead to enhanced vasoconstriction because of a lack of inhibition of RhoA/Rho-kinase signaling in VSMCs.

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References
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