RhoA and resistance artery remodeling

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Loirand, Gervaise, Malvyne Rolli-Derkinderen, and Pierre Pacaud. RhoA and resistance artery remodeling. Am J Physiol Heart Circ Physiol 288: H1051–H1056, 2005; doi:10.1152/ajpheart.00710.2004.—Resistance arteries are able to adapt to physiological and pathophysiologic stimuli to maintain adequate perfusion according to the metabolic demand of the tissue. Although vasomotor control allows rapid adaptation of lumen diameter, vascular remodeling constitutes an active process that occurs in response to long-term alterations of hemodynamic parameters. Unfortunately, this initially adaptive process contributes to the pathology of vascular diseases. Recent studies have demonstrated the participation of Rho protein signaling pathways in several cardiovascular pathologies including hypertension, coronary artery spasm, effort angina, atherosclerosis, and restenosis. Functional analyses have further revealed that RhoA-dependent pathways are involved in excessive contraction, migration, and proliferation associated with arterial diseases. The present review focuses on the role of Rho proteins, in particular RhoA, in vascular smooth muscle cells and the involvement of Rho-dependent signaling pathways in resistance artery remodeling, more particularly in relation to hypertension.

signal transduction; smooth muscle; vascular diseases

RHE PROTEINS

The small G proteins of the Rho (Ras homolog) family comprise 20 members in mammals (10). The Rho protein family is subdivided into three major classes: Rho, consisting of RhoA, RhoB, and RhoC; Rac, consisting of Rac1, Rac2, Rac3, and RhoG; and Cdc42, consisting of Cdc42, TC10, TCL, Wrch1, and Chp/Wrch2. Most studies rely on RhoA, Rac1, and Cdc42 proteins as prototypes. Their best-characterized function is the regulation of actin dynamics (57, 58). Studies performed in many different cell types with constitutively
active or dominant-negative forms of Rho proteins have shown that RhoA regulates the assembly of actin stress fibers (contractile actomyosin filaments) (57) whereas Rac and Cdc42 regulate the polymerization of actin to form specific peripheral structures: lamellipodia for Rac (58) and filopodial protrusions for Cdc42 (50, 58). RhoA, Rac, and Cdc42 are also involved in the assembly of integrin-based matrix adhesion complexes such as focal adhesions (50, 58). In addition to their effect on actin organization, or through this effect, Rho proteins regulate a wide range of fundamental biological functions such as contraction, motility, adhesion, cell shape and polarity, and gene transcription (16, 81). Rho proteins act as molecular switches that cycle between an active GTP-bound and an inactive GDP-bound conformation. In their GTP-bound state, they interact with downstream targets (effectors) to elicit cellular responses. This cycle is under the direct control of three groups of regulatory proteins (16, 81). Classically, in the inactive GDP-bound form, the Rho protein is locked in the cytosol by guanine dissociation inhibitors (GDIs) that block the cycle (Fig. 1). The guanine nucleotide exchange factors (GEFs) catalyze the exchange of GDP for GTP to activate the Rho protein (66, 90). Activation is then turned off by GTPase-activating proteins (GAPs) that enhance the intrinsic GTPase activity of the Rho protein, leading to the hydrolysis of GTP to GDP (46). All Rho proteins are prenylated at their carboxy terminus (18, 68, 86). This prenylation is involved in translocation of the active GTP-bound form of the Rho protein to the membrane, where it interacts with its effector proteins to generate downstream responses (86). The Rho protein cycle is controlled by various signals such as G protein-coupled receptor, tyrosine kinase receptor, and cytokine receptor activation or cell adhesion and integrin clustering, which essentially regulate the activity of GEFs (9, 10, 15, 24, 33, 69). More than 50 GEFs, 70 GAPs, 4 GDIs, and over 60 targets have so far been identified for the Rho proteins in mammals (16), supporting the complexity and the importance of Rho protein signaling in the regulation of cell functions.

TOOLS AND METHODS FOR ANALYSIS OF RHO-DEPENDENT SIGNALING PATHWAYS

The discovery of functional roles of Rho-dependent signaling pathways is tightly related to the development of new research tools and methods. The main classic approaches used to analyze Rho protein-dependent signaling are described here, with a special focus on RhoA and its main target, Rho kinase.

**Rho protein inhibitors and activators.** Bacterial toxins and exoenzymes targeting Rho proteins are commonly used to analyze the role of these signaling proteins (3, 7, 8). The C3 exoenzyme from *Clostridium botulinum* ADP ribosylates the Asn41 residue of Rho but not Rac or Cdc42 (7). ADP-ribosylation leads to inactivation of Rho proteins. The C3 transferase does not easily enter cells and therefore could be used in cell culture but not in intact tissues. To facilitate the penetration of the C3 transferase, chimeric proteins such as DC3B, which utilizes the B fragment of diphtheria toxin (4, 19), and Tat-C3, which utilizes the protein transduction domain of the human immunodeficiency virus Tat protein (48, 63), have been used successfully in whole tissues. Less specifically, toxin B from *Clostridium difficile* glycosylates and prevents activation of Rho proteins as well as Rac and Cdc42 (40).

The role of Rho proteins has also been analyzed by the use of activating toxins. The exotoxin cytoxic necrotizing factor 1 of *Escherichia coli* is taken up by endocytosis into host cells and deamidates Gln63 of Rho and Gln61 of Rac and Cdc42 (2). Deamidation of the Gln residues leads to inhibition of the intrinsic GTPase activity and therefore to constitutive activation of Rho, Rac, and Cdc42 (17).

**Measurement of Rho activity.** Activation of Rho protein is associated with the exchange of GDP for GTP and the translocation of the active GTP-bound Rho from the cytosol to the membrane (19, 37). Rho activation can therefore be assessed by both measurement of the amount of cellular GTP-Rho and detection of the Rho protein in membrane fractions by Western blot analysis. These approaches are commonly used for the assessment of RhoA activity (19, 22, 61, 62). The method used for measuring the amount of GTP-RhoA is based on the specific binding of GTP-RhoA (but not GDP-RhoA) to the Rho binding domain (RBD) of RhoA effectors (56). The RBD of rhotekin (56) and mDia (32) are used to precipitate active GTP-RhoA from protein extracts from cells or tissues.

**Rho kinase inhibitors.** Cell-permeant Rho kinase inhibitors, in particular Y-27632 and HA-1077 (14, 60, 79), are largely used to analyze the functional roles of RhoA/Rho kinase activation. These compounds are not completely specific for Rho kinase but are ~100-fold more active on Rho kinase than other kinases such as conventional protein kinase C (14). A

![Fig. 1. RhoA protein cycle as an example of Rho protein function.](image-url)
main advantage of these inhibitors is that they can be used in vivo, by oral administration in animal models, allowing assessment of Rho kinase function at integrated levels.

RHO PROTEINS IN VASCULAR SMOOTH MUSCLE CELLS

A large body of evidence has now been obtained regarding the important functions of Rho proteins in vascular physiology under normal conditions (42, 74, 83). In vascular smooth muscle cells, direct measurements of the amount of GTP-bound RhoA or translocation of RhoA to the membrane have demonstrated that several agonists of G protein-coupled receptors including thrombin, thromboxane A2, endothelin, carbachol, angiotensin II, α-adrenergic agonists, sphingolipids, and extracellular nucleotides stimulate RhoA activity (69, 75).

RhoA and vascular smooth muscle cell contraction. RhoA is now recognized as the major regulator of the Ca2+ sensitization of the contractile proteins, which is responsible for the tonic component of vascular smooth muscle cell contraction. The mechanism of RhoA-mediated Ca2+ sensitization has been the subject of recent reviews and is not discussed in detail here (54, 75). RhoA-dependent, vasoconstrictor-induced Ca2+ sensitization is mediated by the RhoA effector Rho kinase, which phosphorylates and inhibits the activity of the myosin light chain phosphatase. In addition, it was shown recently by transfection of the dominant-negative form of RhoA or Rho kinase that the RhoA-dependent signaling pathway is involved in myogenic tone (6). In pressurized small arteries the use of Rho kinase inhibitor has revealed that the Rho-Rho kinase pathway is active in the absence of vasoconstrictors, keeping the vessels in a state of high Ca2+ sensitivity and basal tone (36, 67, 82). In addition to this role in the regulation of contraction, a large number of in vitro studies performed on arterial smooth muscle cells in culture have also revealed a major contribution of RhoA in the control of differentiation, migration, and proliferation.

RhoA and vascular smooth muscle cell differentiation. In contrast to the majority of differentiated cells, smooth muscle cells retain the capacity to modulate their phenotype and to proliferate in response to a variety of extracellular and intracellular signals and pathological stimuli (23). Smooth muscle cell differentiation is marked by the coordinated expression of several smooth muscle-specific contractile and cytoskeletal genes regulated directly by serum response factor (SRF) (45, 53). In vascular diseases, this SRF-dependent program of smooth muscle cell differentiation is compromised and the normal contractile smooth muscle phenotype is subverted to one of growth and excess matrix production (45). Recently, RhoA signaling was demonstrated to be a critical mechanism for controlling smooth muscle differentiation through the regulation of SRF-dependent transcription (39, 42). Expression of constitutively active RhoA mutant increases the activity of smooth muscle-specific promoters, whereas inhibition of RhoA by C3 transferase decreases the expression of smooth muscle differentiation marker genes (42). Moreover, expression and activity of RhoA have been shown to be high in contractile smooth muscle cells, whereas the synthetic phenotype was associated with low RhoA expression (87). Change in RhoA expression or activity could thus underlie vascular smooth muscle cell phenotype alterations. It has been suggested that the loss of RhoA expression observed in pulmonary artery from rats with chronic pulmonary hypertension could be involved in pulmonary artery smooth muscle cell dedifferentiation and pulmonary artery remodeling (64).

RhoA and vascular smooth muscle cell migration. Direct inhibition of Rho by C3 transferase, or pharmacological blockade of Rho kinase activity, inhibits vascular smooth muscle cell migration induced by PDGF and lysophosphatidic acid through both myosin light chain phosphorylation-dependent and -independent pathways (1). Similarly, UDP- and thrombin-induced smooth muscle cell migration is also blocked by RhoA and Rho kinase inhibition (11, 71). In human arterial smooth muscle cells, overexpression of N-cadherin induces a significant upregulation of RhoA activity (5). Accordingly, the down-regulation of N-cadherin associated with restenosis stimulates migration of smooth muscle cells by RhoA deactivation (5). Migration induced by activation of the urokinase-type plasminogen activator receptor involves RhoA/Rho kinase and Rac1 activities in human vascular smooth muscle cells (31). The mechanisms by which RhoA regulates vascular smooth muscle cell migration are not fully understood but involve the assembly of focal adhesion complexes. PDGF-induced vascular smooth muscle cell migration is associated with RhoA-dependent phosphorylation of three focal adhesion-associated proteins: focal adhesion kinase, tensin, and paxillin (38).

RhoA and vascular smooth muscle cell proliferation. Whereas the farnesylated Ras protein can promote cell cycle progression via activation of the mitogen-activated protein kinase pathway (28), geranylgeranylated RhoA causes cellular proliferation, possibly through destabilizing p27Kip1 protein (25). Interestingly, inhibition of isoprenylation of small G proteins by statins leads to inhibition of vascular smooth muscle proliferation that is reversed by geranylgeranyl pyrophosphate but not farnesyl pyrophosphate (37). Indeed, direct inhibition of Rho by C3 transferase or by a dominant-negative Rho mutant increases p27Kip1 and inhibits Rb hyperphosphorylation and smooth muscle cell proliferation after PDGF stimulation (37). These findings indicate that Rho mediates PDGF-induced smooth muscle cell proliferation. Similar experiments have shown that vascular smooth muscle cell proliferation induced by thrombin and urotensin II is dependent and/or inhibited by Rho kinase blockers, suggesting a role for RhoA in G protein-coupled receptor-stimulated cell proliferation (63, 70). Rho and Rho kinase may also play an important role in angiotensin II-induced vascular hypertrophy (88). However, opposite data that do not reveal a substantial role for RhoA in the regulation of vascular smooth muscle cell proliferation have also been reported (38). Although C3 inhibits PDGF-induced migration, it has no effect on PDGF-induced proliferation of human vascular smooth muscle cells. In addition, in two in vivo studies Rho kinase inhibition did not affect smooth muscle cell proliferation after balloon injury in rat arteries (49, 73). The inhibitory effect of Rho kinase blockers has been ascribed to stimulation of apoptosis, although the mechanism of RhoA/Rho kinase-mediated regulation of apoptosis was not determined. Collectively, these observations show that the role of RhoA in the control of vascular smooth muscle cell proliferation is not fully elucidated. Further analyses are required, in particular to examine a potential differential involvement of RhoA depending on the differentiation status of smooth muscle cells.

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Changes in arterial structures typically involve smooth muscle cell growth, dedifferentiation, and migration. The roles of RhoA in the regulation of all these cell functions (see above) provide the functional basis for the involvement of RhoA in arterial remodeling.

RHOA AND VASCULAR REMODELING ASSOCIATED WITH HYPERTENSION

Direct measurements of the amount of active GTP-bound RhoA in arteries from several animal models of hypertension have revealed an increased RhoA activity (72). In addition, Rho kinase inhibitors normalized arterial pressure in these models (79). These observations have thus revealed the importance of the RhoA/Rho kinase signaling pathway in the vascular hyperreactivity associated with hypertension. Several studies have therefore examined the potential role of this signaling pathway in pathological arterial remodeling. Long-term blockade of Rho kinase suppresses vascular lesion formation such as medial hypertrophy and perivascular fibrosis in small coronary artery from spontaneously hypertensive rats (47). Similar observations have been made in the rat model of hypertension induced by chronic inhibition of NO synthesis (30). In both models, the activity of the RhoA/Rho kinase pathway is found to be increased. Because the inhibition of angiotensin II type 1 receptor prevents upregulation of RhoA/Rho kinase activity, it has been suggested that an increase in angiotensin II activity participates in the activation of RhoA in hypertension rats (30). This is in agreement with another report showing that, in vivo, long-term infusion of angiotensin II increases the activity of RhoA/Rho kinase, increases medial thickness, and promotes perivascular fibrosis in coronary arteries (26). Both angiotensin II-induced coronary hypertrophy and fibrosis are inhibited by Rho kinase inhibitor. This effect of Rho kinase inhibition is associated with a marked reduction of angiotensin II-induced superoxide anion production (26), angiotensin II-induced monocyte chemoattractant protein-1, and plasminogen activator inhibitor type 1 expression (20, 77).

Although angiotensin II seems to participate substantially in the activation of RhoA in hypertensive vascular disease, a potential role of the increased arterial pressure cannot be excluded. In hypertension mechanical strain on the vessel wall is increased, and it has been shown that mechanical stress such as hydrostatic pressure and stretch stimulates vascular smooth muscle cell proliferation (27, 41). In addition, mechanical strain potentiates the mitogenic activity of angiotensin II in vascular smooth muscle cells (76). Indeed, mechanical stretch induces RhoA activation in vascular smooth muscle cells, and stretch-induced extracellular signal-regulated kinase activation and vascular smooth muscle cell growth are inhibited by C3 exoenzyme or Rho kinase inhibition (51, 89).

Together, these recent data point out a substantial role of RhoA and Rho kinase in arterial remodeling associated with hypertension and show that different upstream signals can converge toward RhoA activation in hypertensive vascular diseases. It should be mentioned that, on the other hand, flow reduction or elevation leads to the downregulation of RhoA and Rho kinase gene expression in small mesenteric arteries (85). Inhibition of Rho kinase potentiates the inward hypertrophic remodeling in response to reduced flow, suggesting that the decrease in RhoA expression is involved in the low-flow-induced remodeling. According to the role of RhoA in the control of smooth muscle differentiation, it can be speculated that these changes in RhoA and Rho kinase gene expression are required for a transition of the cells from a stable to a dynamic, remodeling-prone state.

In conclusion, the RhoA-dependent signaling pathway is recognized as an essential regulator of vascular reactivity and arterial remodeling. However, although arterial remodeling in hypertension involves an increase in RhoA/Rho kinase activity, remodeling induced by flow alteration is associated with a downregulation of RhoA and Rho kinase. These observations thus suggest that the regulation of RhoA signaling and the functional consequences of alterations in this major signaling pathway are certainly more complex than previously thought. Probably, optimal steady-state RhoA expression and activity are needed to maintain “normal” smooth muscle cell functions and differentiation and arterial wall structure integrity. Clearly, more studies are now needed to understand how this complex signaling pathway regulates smooth cell functions under physiological conditions as well as in association with vascular diseases.

GRANTS

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REFERENCES


The small GTP-binding protein rho regulates the...


