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Review Article

Rho/Rho-kinase as a novel therapeutic target in the treatment of cardiovascular diseases

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CONTENTS

Abstract

The small GTP-binding protein, Rho, whose proper membrane localization and function are dependent on isoprenylation, plays an important role in mediating cellular signals in the vascular wall. Rho/Rho-kinase has recently been the object of intensive investigations. Available data suggest that the Rho/Rho-kinase system may be involved in vascular smooth muscle contraction, vascular remodeling, hemostasis and development of arteriosclerosis at multiple steps. In addition, at least some of the pleiotropic effects of the 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors can be explained via the Rho/Rho-kinase pathway. An understanding of Rho/Rho-kinase might provide the means for developing therapeutic strategies for the treatment and prevention of cardiovascular disease. Further elucidation of the role of Rho/Rho-kinase in the circulatory system will help to identify novel therapeutic targets for the effective treatment of cardiovascular diseases such as atherosclerotic heart disease and hypertension.

Introduction

Small GTP-binding proteins (G-proteins) are monomeric G-proteins which are key molecules on diverse cell functions. The G-proteins are inactive in their guanosine diphosphate (GDP)-bound form and active in their guanosine triphosphate (GTP)-bound form (Fig. 1) (1, 2). At present, more than 100 small G-proteins have been identified in eukaryotes from yeast to humans, and they comprise a superfamily. The Rho gene was discovered as a homolog of the Ras gene in *Aplysia* in 1985 (3). On the basis of structure and function, three subfamilies of Rho superfamily proteins have been identified, which include Rho, Rac and Cdc42. Many cell functions, including maintenance of morphology, aggregation, motility, membrane ruffling, smooth muscle contraction, cytokinesis in mammals and bud formation in yeast are regulated through the dynamic reorganization of actin filaments. It has long been known that Ca^{2+} is a key regulator of the cytoskeleton (4), and evidence is now accumulating that the Rho superfamily is another important regulator of these actin-dependent cell functions. Rho/Rac/Cdc42 proteins cooperate and regulate actin polymerization. Rho proteins regulate stress fiber formation, while Rac proteins regulate ruffling and lamellipodia formation, and Cdc42 regulates filopodium formation (5-9).

Recent data indicate that the Rho superfamily proteins are involved in events beyond cytoskeletal regulation (10-14), for example, they regulate the organization

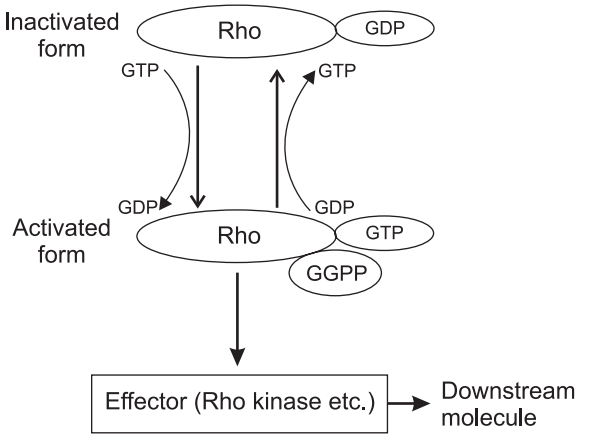

Fig. 1. Effectors transduce activated-Rho signal to a downstream molecule. Biological activity of Rho depends on isoprenylation by geranylgeranylpyrophosphate (GGPP).

of actin and other functions through effectors, which transduce the activated Rho signal to a downstream molecule. Rho-kinase is a serine/threonine kinase and an effector of Rho that was identified as a Rho binding protein from bovine brain (15, 16). Rho-kinase consists of two isoforms, Rho-kinase/ROCKα/ROCK 2 and ROCKβ/ROCK 1 (17, 18). An understanding of Rho/Rho-kinase is not only the basis of the cellular and molecular mechanism in physiological and pathophysiological states, but might also provide a means for developing therapeutic strategies for the treatment and prevention of cardiovascular disease. Rho/Rho-kinase has recently been investigated intensively. This review addresses Rho/Rho-kinase-related regulation of cardiovascular disease at the cellular and molecular levels. Information obtained from experimental studies is summarized in Table I.

Rho/Rho-kinase and hypertension

Several lines of evidence indicate that Rho/Rho-kinase may play an important physiological role in arteriosclerosis. Abnormal smooth muscle relaxation may be one of the major causes of disease states such as hypertension. Vascular tone is regulated by cytoplasmic free Ca^{2+} concentrations. Agonists activate Ca^{2+} release from sarcoplasmic reticulum, followed by enhancement of the binding of Ca^{2+} to calmodulin. The Ca^{2+}-calmodulin complex activates myosin light chain (MLC) kinase. As a result, MLC kinase increases MLC phosphorylation of myosin II. Phosphorylated MLC causes smooth muscle contraction. Rho is involved in the GTPγS-induced enhancement of Ca^{2+}-dependent MLC phosphorylation in aortic smooth muscle cells. Rho plays an important regulatory role in agonist-induced MLC phosphorylation and contraction (19). The Rho-kinase inhibitor, (+)-(*R*)-*trans*-4-(1-aminoethyl)-*N*-(4-pyridyl)cyclohexanecarboxamide dihydrochloride (Y-27632), inhibits agonist-induced vascular contraction by inhibiting the Ca^{2+} sensitization mechanism. Y-27632 decreases blood pressure in hypertensive rat models (20). Rho-kinase activity contributes substantially to cerebral artery tone *in vivo*, and this effect is augmented in the cerebral circulation during chronic hypertension (21). Fasudil (1-(5-isoquinolinesulfonyl)-homopiperazine, HA-1077) ameliorates forearm blood flow in patients with hypertension (22). Fasudil is a non-specific inhibitor against protein kinase C (PKC), protein kinase A, Ca^{2+}/calmodulin-dependent protein kinase II and Rho-kinase. Fasudil has an antispastic effect and suppresses the spasms of cerebral arteries after subarachnoidal hemorrhage. Fasudil has been improved to (*S*)-(+)-2-methyl-1-[(4-methyl-5-isoquinoline)sulfonyl]-homopiperazine (H-1152P), which is a more selective inhibitor of Rho-kinase (23). Inhibition of Rho/Rho-kinase system in smooth muscle cell causes vascular relaxation. This process might be useful therapeutically for the treatment of hypertension (10, 24).

Table I: Expected effects of Rho/Rho-kinase inhibitors on vascular wall cells.

Endothelial cells
eNOS expression and activity ↑
Plasminogen activator inhibitor-1 expression ↓
Tissue-type plasminogen activator expression ↑
Tissue factor ↓
Leukocyte adhesion to endothelial cell ↓
Smooth muscle cells
Relaxation (tonus) ↑
Migration and proliferation ↓
iNOS expression ↑
Apoptosis ↑
Adipocytes
Plasminogen activator inhibitor-1 expression ↑

Rho/Rho-kinase and vascular remodeling

Rho may be involved in vascular remodeling. Mechanical stress induces DNA synthesis and expression of proto-oncogene in vascular smooth muscle cells. Inhibition of Rho/Rho-kinase suppresses ERK activation and DNA synthesis induced by the mechanical stress. Cytochalasin D, which selectively disrupts the network of actin filaments, markedly inhibited stress-induced ERK activation. These results suggest that Rho/Rho-kinase modulates signal transduction via the actin network (25). RhoA protein and activity are increased in vessels from hypertensive rats, and activation of RhoA decreases expression of the cyclin-dependent kinase inhibitor $p27^{Kip1}$ (26). DNA synthesis and migration stimulated by thrombin in vascular smooth muscle cells are decreased by inhibition of Rho/Rho-kinase (27). Use of a Rho-kinase inhibitor and gene transfer of dominant negative Rho-kinase suppresses constrictive remodeling induced by IL-1beta and neointimal formation after balloon injury in pigs (28-31). Rho/Rho-kinase is involved in smooth muscle cell proliferation. Inhibition of Rho/Rho-kinase may ameliorate the vascular remodeling derived from maladaptation.

Rho and hemostasis

Blood coagulation is an important step in the development of cardiovascular diseases. Thrombin, the multifunctional enzyme generated in the context of vascular injury from the circulating prothrombin, is implicated in atherosclerosis and its complications. Thrombin decreases endothelial nitric oxide synthase (eNOS) mRNA in human umbilical vein endothelial cells, but inhibition of Rho/Rho-kinase prevents the downregulation of eNOS expression by thrombin (32). Thrombin also upregulates tissue factor, which plays a pivotal role in thrombus formation in acute coronary syndromes. Inhibition of Rho-kinase decreases tissue factor expression (33). Plasminogen activator inhibitor-1 (PAI-1) is an important regulator of fibrinolysis, and upregulation of PAI-1 levels

in plasma is an important risk factor for myocardial infarction and deep vein thrombosis. Angiotensin II (Ang-II)-induced PAI-1 mRNA upregulation in the rat left ventricle was inhibited by Y-27632. In addition, increased RhoA protein, Rho-kinase and *c-fos* gene expression, and MLC phosphorylation were suppressed by Y-27632 (34). Ang-II increases PAI-1 expression in vascular smooth muscle cells and dominant-negative form of Rho-kinase or Y-27632 also completely prevented PAI-1 induction by Ang-II (35). Furthermore, Y-27632 increases PAI-1 mRNA expression and protein synthesis in 3T3-L1 mouse adipocytes (36). The effect of Rho-kinase inhibition may be cell- or organ-specific and dependent upon the agonists used.

Rho and HMG-CoA reductase inhibitors

Posttranslational isoprenylation of the Rho proteins is required for plasma membrane localization and biological function of Rho (Fig. 1). 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (HRIs) have recently been shown to prevent the development of atherosclerosis. Large clinical trials have demonstrated the favorable effect of HRIs in the primary and secondary prevention of coronary heart disease (37, 38). Clinical benefits result from combined actions on various components of the atherosclerotic lesions. By inhibiting L-mevalonic acid synthesis, HRIs prevent the synthesis of isoprenoid intermediates of the cholesterol biosynthetic pathway such as farnesylpyrophosphate and geranylgeranylpyrophosphate. The membrane association of Rho and its biological activity was inhibited by lovastatin, an HRI. Membrane attachment and biological activity of RhoA depend on isoprenylation (39). HRIs exert therapeutic effects other than simply lowering plasma cholesterol. Rho may be a major target of statin treatment (40).

Rho and inflammation

Recent studies revealed that inflammation has a major impact on the initiation and development of arteriosclerosis. Treatment of vascular smooth muscle cells with HRIs produced significantly higher amounts of IL-1β-evoked NO and inducible nitric oxide synthase (iNOS) protein compared with controls. Similarly, toxin B from *Clostridium difficile* and C3 ADP-ribosyl transferase toxin from *Clostridium botulinium* (C3 exotoxin) that specifically inactivate Rho proteins increased NOS products (NO and citrulline) and iNOS expression. Toxin B increased the activity of iNOS promoter-reporter construct in vascular smooth muscle cells. Both toxins enhanced IL-1β-stimulated iNOS expression and NO production. These data demonstrate that inhibition of Rho induces iNOS and suggest a role for Rho protein in IL-1β-stimulated NO production in smooth muscle cells (41). Treatment with C3 exotoxin was also associated with superinduction of iNOS in pulmonary artery smooth muscle cells, suggesting an inhibitory role for Rho (42). Atorvastatin affects cytokine-induced iNOS expression in native endothelial and smooth muscle cells (43). Inhibition of Rho/Rho-kinase may aggravate iNOS production. On the other hand, long-term oral treatment with fasudil inhibited macrophage accumulation and migration, and coronary lesion formation in porcine coronary arteries *in vivo* (44). Whether inhibition of Rho/Rho-kinase is beneficial or exerts unfavorable effects in inflammatory responses in arteriosclerosis is controversial.

Conclusions

Rho/Rho-kinase inhibitors are under intensive investigation and development. However, the evaluation for clinical use is far from being complete. Further studies are clearly needed. The actions of Rho inhibitors indicate that Rho has a major role in arteriosclerosis, although the responsible mechanisms may involve as yet unidentified functions. The Rho/Rho-kinase system is involved in the contractility of vascular smooth muscle cells, and inhibition of Rho/Rho-kinase may be an important target of hypertension treatment. Recent evidence suggests that the Rho/Rho-kinase system may be involved at multiple steps in the development of arteriosclerosis, including vascular remodeling, coagulation and fibrinolysis. In addition, at least some of the pleiotropic effects of HRIs can be explained via the Rho/Rho-kinase pathway. Further elucidation of the role of Rho/Rho-kinase in the circulatory system will help to identify novel therapeutic targets for the effective treatment of cardiovascular diseases.

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