The small guanosine triphosphatase Rho and its target, Rho kinase, play important roles in both blood pressure regulation and vascular smooth muscle contraction. Rho is activated by agonists of receptors coupled to cell membrane G protein, such as angiotensin II and phenylephrine. Once Rho is activated, it translocates to the cell membrane where it, in turn, activates Rho kinase. Activated Rho kinase phosphorylates myosin light chain phosphatase, which is then inhibited. This sequence stimulates vascular smooth muscle contraction, stress fiber formation, and cell migration. In this way, Rho and Rho kinase activation have important effects on several cardiovascular diseases. Currently available substances that specifically inhibit this signaling pathway could offer clinical benefits in several cardiovascular, as well as non-cardiovascular, diseases, such as arterial hypertension, pulmonary hypertension, cerebral or coronary spasm, post-angioplasty restenosis, and erectile dysfunction.

Key words: Rho, Rho kinase, Small G proteins, Arterial hypertension, Cardiovascular remodeling.

INTRODUCTION

Arterial hypertension is a cardiovascular disorder characterized by altered vascular tone and increased vascular contractility. It is accompanied by proliferation and migration of vascular smooth muscle cells and differing degrees of inflammation of the arterial wall, processes that together constitute vascular remodeling. Proliferation, migration of vascular smooth muscle cells from the media to the intima, and inflammation of the arterial wall participate in a number of vascular disorders, including for example, the progression of atherosclerosis, restenosis, rejection of bypass grafts, and renal failure. In addition to cardiac remodeling, arterial hypertension is accompanied by hypertrophy of cardiac myocytes, hyper-
plasia of fibroblasts, and alterations of the extracellular matrix.

Of the main complications of arterial hypertension, those associated with the development of atherosclerosis are the least affected by current antihypertensive treatment. A metaanalysis of the effects of antihypertensive treatment revealed a 48% reduction in the incidence of cerebrovascular accidents compared with only a 16% reduction in the incidence of heart disease, figures that are markedly lower than would have been predicted from available epidemiological studies. This finding can be interpreted in a number of ways. One of these is that development of atherosclerosis and the associated complications are not effectively prevented by current antihypertensive treatment. These findings are consistent with the lack of evidence to support lowering of arterial blood pressure as an effective means of reducing atherosclerosis or proliferation of vascular smooth muscle cells, and provide a strong incentive to investigate other mechanisms of vascular damage and remodeling with greater potential for treatment and prevention.

It has recently been shown that activation of the Rho/Rho kinase signal transduction pathway is one of the principal mechanisms of vasoconstriction in arterial hypertension and that this pathway has novel therapeutic potential. Studies have suggested that the small guanosine triphosphatase (GTPase) Rho and its target Rho kinase play a crucial role in the regulation of arterial blood pressure. In vitro studies have demonstrated that the activated form of Rho kinase regulates vascular smooth muscle contraction via phosphorylation of the myosin light chain, sensitization of contractile proteins to Ca++, and the formation of stress fibers. Furthermore, Rho kinase may also be regulated by various components of the cytoskeleton that play an essential role in the mechanotransduction of flow and pressure in the blood vessels. In addition to a role in the pathogenesis of atherosclerosis, Rho kinase participates in the organization of the actin cytoskeleton, in the processes of cell adhesion and motility, in cytokinesis, and in gene expression. Prolonged treatment with fasudil (a specific inhibitor of Rho kinase) has been shown to reduce the development of coronary vascular lesions such as medial thickening and perivascular fibrosis in spontaneously hypertensive rats.

THE RHO/RHO KINASE SIGNAL TRANSDUCTION PATHWAY

The small GTPase superfamily contains more than 100 structurally related proteins that undergo conformational changes and alter their subcellular localization in a manner that is dependent on guanine nucleotides. Small GTPases are active when bound to GTP and inactive when bound to GDP. In their activated state, they bind effectors that, in turn, regulate a large number of biological processes. They are controlled by various classes of regulatory proteins. Figure 1 shows the cycle of activation and inactivation of the small GTPases. With few exceptions, their activation is mediated by guanine nucleotide exchange factors (GEFs). These factors displace the GDP dissociation inhibitor (GDI), releasing the isoprenylated residue that anchors the small GTPase to the plasma membrane, and catalyzing the exchange of GDP for GTP. GTP binding induces a conformational change in the small GTPase that activates it and allows it to bind effectors. GTPase activating protein (GAP) stimulates the intrinsic GTPase activity of the small GTPase. GTP hydrolysis returns the small GTPase to its inactive state bound to GDP and GDI.
tion inhibitor (GDI) and unmask the isoprene group that anchors the small GTPase to the plasma membrane, as well as catalyzing the exchange of GDP for GTP. GTP binding induces a conformational change in the small GTPase that activates it and allows it to bind effectors. GTPase activating proteins (GAP) stimulate the intrinsic hydrolysis of GTP and lead to the rapid conversion of small GTPases to their inactive state bound to GDP and GDI.

Based on structural and functional relationships, the small GTPase superfamily is subdivided into 5 families: Ras, Rho, Rab, Arf, and Ran (Figure 2).7 Members of the Ras family include the 3 classical isoforms H-Ras, K-Ras, and N-Ras, and are primarily involved in the regulation of cell proliferation and differentiation. The classical isoforms of Ras also regulate apoptosis. As shown in Figure 2, all of these effects are likely to be regulated by the extracellular regulated kinases (ERK), a subfamily of the mitogen-activated protein kinases (MAPK), or phosphatidylinositol-3-kinase (PI3K).3

RhoA has opposing actions to RhoB and inhibits RhoA-mediated gene expression. When Rho kinase is activated by RhoA it phosphorylates myosin light chain phosphatase (MLCP; Figure 3), thereby inhibiting it and favoring the contraction of vascular smooth muscle cells, the formation of stress fibers, and cell migration. The availability of Y-27632, a selective inhibitor of Rho kinase12 (Figure 4), as well as dominant negative forms of the enzyme, have been useful in elucidating the roles of this pathway.
The processes regulated by the different families of small GTPases are extremely diverse. In fact, almost no area of cell biology or biomedical science in which a role for these proteins has not been identified. Furthermore, there are a large number of interactions between members of the Ras and Rho families. The signal transduction pathways regulated by members of the Rho family play an important role in a number of pathologic conditions, including cancer, inflammation, bacterial infection, and arterial hypertension.13-15 Ras also controls the generation of reactive oxygen species (ROS), both in leukocytes and nonhematopoietic cells.16 These GTPases act as molecular controls in a wide variety of cell processes, among which the best characterized is the regulation of the actin cytoskeleton. Some actions of G-protein-coupled receptors are mediated by small GTPases like Rho. It has been demonstrated that agonists of G-protein-coupled receptors such as angiotensin II, lysophosphatidic acid, carbachol, and phenylephrine increase the concentration of Rho at the membrane and reduce its levels in the cytosol, indicating translocation and activation of Rho.17 The Rho signal transduction pathway participates in various pathophysiologic processes in the cardiovascular system.18-21 The majority of these processes are directly or indirectly associated with arterial hypertension and its associated complications (Table 1).

INTERACTION BETWEEN ANGIOTENSIN II AND RHO IN THE CARDIOVASCULAR SYSTEM

Vascular remodeling occurs during normal development and participates in various physiologic processes. However, structural changes to the vasculature can be pathologic as well as adaptive and can lead to the development of arterial hypertension, atherosclerosis, and diseased venous bypass grafts. Angiotensin II contributes to vascular remodeling through the activation of signaling cascades that promote vasoconstriction, cell proliferation, and inflammation. The cytoskeleton also participates in adaptive structural responses of the vasculature. The cytoskeleton mediates vasoactive responses and transduces both mechanical stimuli and pharmacologic signals. Some of the cytoskeletal changes that occur in vascular remodeling, specifically in vascular smooth muscle cells, are induced by angiotensin II. In fact, Rho has been directly linked to pathologic vascular remodeling through a series of lines of evidence that demonstrate that angiotensin II activates the Rho/Rho kinase pathway and regulates the cytoskeleton.22 Figure 2 summarizes the angiotensin II-mediated regulation of...
α has been shown that the activity of the skeletal muscle is also suppressed by pretreatment with exoenzyme C3.

Phenylalanine in cardiac myocytes, an effect that was found to increase incorporation of tritiated thymidine, probably due to the activation of RhoA. This effect is partially inhibited by pretreatment with Y-27632, an inhibitor of Rho. These findings suggest that Rho participates in the angiotensin II-mediated activation of ERK proteins in cardiac myocytes. 

Bradykinins and the Rho Signal Transduction Pathway

Angiotensin II, Vascular Inflammation, and RhoA

It has been postulated that Rho kinase could participate in atherogenesis, based on studies of the role of this pathway in vascular inflammation. Monocyte chemoattractant protein-1 (MCP-1) is a chemokine that regulates monocyte recruitment and participates in atherogenesis. Rho kinase regulates the angiotensin II-induced production of MCP-1, independently of ERK activation. In vascular smooth muscle cells, this MCP-1 production is blocked by botulinum exotoxin C3. Overexpression of a dominant-negative Rho kinase or the use of Y-27632 significantly inhibits the angiotensin II-dependent expression of MCP-1.

Bradykinins and the Rho Signal Transduction Pathway

Few studies have assessed the possible interaction of bradykinins with the Rho signaling pathway, and even fewer in relation to cardiovascular remodeling. It has been shown in A549 epithelial cells that bradykinin stimulates the activation of NF-κB and the synthesis of interleukin-1β, and that RhoA is both necessary and sufficient to mediate this effect.

TABLE 1. Cardiovascular Processes Involving Rho and Rho Kinase

<table>
<thead>
<tr>
<th>Process</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascular sensitization to calcium through phosphorylation of actin and c-fos promoters</td>
<td>Increase in DNA synthesis and reexpression of fetal genes and increased protein synthesis</td>
</tr>
<tr>
<td>Cardiac Myocyte Hypertrophy</td>
<td>Angiotensin II evokes a variety of hypertrophic responses in cardiac myocytes, including activation of various protein kinases, reexpression of fetal genes, and increased protein synthesis.</td>
</tr>
<tr>
<td>Hypertrophy and Hyperplasia of Vascular Smooth Muscle Cells</td>
<td>Rho and Rho kinase, as well as c-fos, play a key role in the hypertrophic changes induced in vascular smooth muscle cells by angiotensin II. In this cell type, Y-27632 abolishes both mRNA and protein expression of c-fos in response to angiotensin II. Mechanotransduction in vascular smooth muscle cells depends on the presence of intact actin filaments. Furthermore, Rho is activated by stretching, and the Rho/Rho kinase pathway mediates stretch-induced ERK activation and vascular hyperplasia.</td>
</tr>
</tbody>
</table>


Cardiovascular Processes Involving Rho and Rho Kinase

Vascular sensitization to calcium through phosphorylation of the myosin light chain, causing arterial vasoconstriction, increased vascular resistance, and arterial hypertension. Activation of ERK protein kinases and arterial contraction induced by angiotensin II in resistance vessels. Contribution to central arterial tone in chronic arterial hypertension.

Mediation of the effects of thrombin on the proliferation and migration of vascular smooth muscle cells expressed as plasminogen activator-1 mRNA and perivascular and left ventricular myocardial fibrosis in arterial hypertension induced by chronic administration of angiotensin II. Early inflammation of the coronary arterial wall and late coronary remodeling in experimental arterial hypertension produced by chronic inhibition of endothelial nitric oxide synthase.

THE ROLE OF RHO MODULATION IN CARDIOVASCULAR REMODELING

Nitric oxide induces vasodilation through cyclic GMP. A recent study demonstrated that protein kinase G phosphorylates RhoA and inhibits its activity. In addition, sodium nitroprusside reverses the RhoA translocation induced by phenylephrine, indicating inhibition of RhoA activity. Figure 3 summarizes the mechanisms through which RhoA has been observed to cause vasoconstriction in arterial hypertension.

Few studies have addressed the role played by this signal transduction pathway in arterial hypertension in humans. In a recent study in hypertensive patients, fasudil, a specific inhibitor of Rho kinase used in the treatment of cerebrovascular spasm in subarachnoid hemorrhage, was seen to induce a stronger vasodilatory response in the forearm of hypertensive than control subjects, while the response to sodium nitroprusside was similar in both patient groups. This result constitutes the first evidence that the Rho/Rho kinase pathway participates in the pathogenesis of increased systemic vascular resistance in hypertensive patients.

THE RELATIONSHIP BETWEEN RHO SIGNALING AND ATHEROSCLEROSIS

There are various interactions between Rho signaling and the structure and function of the endothelium in both normal and pathologic conditions. These interactions participate in the development of arterial hypertension and are associated with the progression of atherosclerosis in various arteries.

The integrity of the endothelium depends on intercellular adhesions mediated by vascular-endothelial (VE) cadherin that, in turn, depend on connections to the actin cytoskeleton. Rho controls cytoskeletal dynamics and cadherin function in epithelial and endothelial cells. The function of these cadherins is also regulated by ROS, which control the pathophysiological changes associated with inflammation and endothelial damage, and with migration of endothelial cells and angiogenesis.

Endothelial dysfunction is characterized by an altered endothelial response that favors the processes of vasoconstriction, cell adhesion, and coagulation. The statins, inhibitors of HMG-CoA reductase, are useful for reversing endothelial dysfunction, an effect that appears to be independent of the reduction of serum cholesterol concentration. Rho-dependent activation of preproendothelin-1 expression is inhibited by statins. Thus, control of vascular tone and proliferation mediated by endothelin-1 is regulated at multiple levels, and Rho signaling may play a significant role.

Rho modulates the permeability of endothelial monolayers and regulates actin filament assembly, contractile activity due to myosin, and the distribution of the calcium dependent cell adhesion molecule VE cadherin. Rho and the contractile processes mediated by RhoA do not participate in the increased permeability induced by bradykinin and platelet activating factor in intact microvessels. The regulation of the endothelial cell barrier is absolutely dependent on components of the cytoskeleton. Factors that cause edema induce contraction of this barrier through actomyosin, associated with phosphorylation of myosin light chain and reorganization of filaments. Alteration of this structural assembly increases dysfunction of the endothelial barrier by activating specific signaling pathways that “talk” to networks of microfilaments and increase the Rho-mediated contractility of the endothelial barrier. The migration of endothelial cells induced by vascular endothelial growth factor (VEGF) is a critical step in angiogenesis, and requires the participation of Rac, another small GTPase.

THE RELATIONSHIP BETWEEN RHO SIGNALING AND VASCULAR INFLAMMATION, ADHESION MOLECULES, AND PROCOAGULATION IN ARTERIAL HYPERTENSION

Inflammation is crucial to the pathogenesis of atherosclerosis and plays an important role in the vascular complications associated with arterial hypertension. Transmigration of monocytes into the subendothelial space, via integrin activation and chemotaxis, is the initial step in atherosclerotic plaque formation and inflammation. Integrins are activated by ERK, while p38-MAPK and Rho control chemotaxis mediated by MCP-1. Rho and Rho kinase are upstream of p38-MAPK in MCP-1 signaling. Thus, ERK and p38-MAPK regulate distinct signal cascades that lead to integrin activation and chemotaxis induced by MCP-1.

In addition to regulating fibrinolytic activity, plasminogen activator inhibitor type I (PAI-1) also plays a role in the pathogenesis of atherosclerosis and arterial hypertension. Angiotensin II, acting through the type 1 angiotensin II receptor, upregulates protein and mRNA expression of PAI-1 in vascular smooth muscle cells. Overexpression of a dominant-negative Rho kinase or the use of Y-27632 completely blocks the induction of PAI-1 expression by angiotensin II, without affecting ERK activation. These observations indicate that activation of the ERK and Rho kinase pathways is necessary for the induction of PAI-1 expression by angiotensin II. Rho kinase could be a novel target through which to inhibit the effects of angiotensin II in the treatment of arterial hypertension and atherosclerosis.

In rat fibroblasts, inhibition of PKC or RhoA selectively inhibits induction of c-fos by transforming...
growth factor β1 (TGF-β1), indicating possible roles for both PKC and RhoA in the induction of c-fos by TGF-β1.40

Rho participates in signaling through chemotactic receptors that initiate rapid adhesion in leukocytes.41 The interactions of endothelial ICAM-1 and VCAM-1 with their ligands are involved in the differential regulation of distinct steps in diapedesis by modulating the balance of active and inactive forms of small GTPases.42 In endothelial cells of the aorta, the statin cerivastatin blocks lipopolysaccharide-induced expression of ICAM-1 mRNA. Cotreatment with geranylgeranyl pyrophosphate reverses the effect of the statin, indicating that this effect is caused by inhibition of Rho activity.43

Rho mediates the assembly of focal adhesions through integrins and actin stress fibers. Genetic inhibition of RhoA in human endothelial cells has demonstrated a requirement for Rho in the assembly of stable adhesions with monocytes through clustering of the receptors E-selectin, ICAM-1, and VCAM-1, which recognize ligands present on monocytes.44

The endothelium of the cerebral blood vessels, which constitutes the blood-brain barrier, controls the adhesion and migration of lymphocytes into the brain. The cell adhesion molecule ICAM-1 participates in the extravasation, morphological changes, and gene regulation associated with the migration of lymphocytes across the blood-brain barrier through activation of Rho and phosphorylation of the endothelial actin cytoskeleton.45,46 In the brain, the signaling pathway mediated by ICAM-1 is a central element in facilitating lymphocyte migration. Rho proteins, which require posttranslational prenylation to be functionally active, are essential components of this signaling cascade. It has been suggested that the signal transduction systems of cerebral endothelial cells, particularly Rho proteins, could be attractive pharmacological targets through which to recruit leukocytes to the central nervous system.47

It has been observed in experiments with human T lymphocytes that Rho and Rho kinase regulate the tyrosine kinase PYK2 by controlling F-actin-mediated control of the integrin lymphocyte function-associated antigen-1, highlighting a novel form of modulating the activity of this cytoplasmic tyrosine kinase. In addition, Rho regulates the chemokine-induced polarization and migration of lymphocytes, in which cdc42 plays a key role.48 Anti-thrombin directly inhibits chemokine-induced migration of monocytes and lymphocytes via the effects of its heparin binding site by activating PKC and Rho.49

Disequilibrium between nitric oxide and endothelin-1 plays an important role in cardiovascular diseases. Thrombin exerts powerful effects on endothelial function. A study has addressed the molecular mechanisms through which thrombin regulates the expression of endothelial nitric oxide synthase (eNOS) and endothelin converting enzyme-1 (ECE-1) in human endothelial cells. Incubation of human umbilical cord endothelial cells with thrombin caused a marked downregulation of eNOS expression and increased the levels of ECE-1 protein in a concentration dependent manner. Thrombin also downregulated and upregulated mRNA expression of eNOS and ECE-1, respectively. The effects of thrombin on eNOS and ECE-1 were found to be mediated by the Rho and ERK pathways, respectively.50

NADH/NAD(P)H Oxidases, Reactive Oxygen Species, and Rho

The NADH/NAD(P)H oxidase pathway is one of the main producers of ROS in the vascular system. Superoxide (O2-) anions are generated that can react with nitric oxide to form the peroxynitrite anion, which lacks the relaxant activity of nitric oxide on the smooth muscle. Angiotensin II induces activation of NADH/NAD(P)H oxidases in vascular smooth muscle cells, leading to increased production of O2·- and inactivation of nitric oxide. In phagocytes, the NAD(P)H oxidase system that generates O2·- is regulated by small G proteins related to Rac.51

In the vascular smooth muscle cells of the rat aorta, inhibition of Gβγ with pertussis toxin and inhibition of NADH/NAD(P)H oxidase reduces stimulation of p21-activated kinase (PAK) induced by lysophosphatidic acid (LPA). Thus, in these cells, LPA activation of PAK is mediated by Gβγ and is dependent on Src. In addition, activation of PAK by LPA requires generation of ROS.52

Tissue factor activates the extrinsic pathway of coagulation, which leads to thrombin formation. Thrombin induces expression of tissue factor mRNA in vascular smooth muscle cells and, consequently, contributes to prolonged procoagulant activity and increased thrombogenicity at sites of vascular lesion. NAD(P)H oxidase participates in the redox-sensitive induction of tissue factor mRNA expression and the surface procoagulant activity activated by thrombin. This response is mediated by the NAD(P)H oxidase-dependent activation of MAPK, and by the PKC/kinase B pathway. Given that active tissue factor promotes the formation of thrombin, NAD(P)H oxidase could play a crucial role in perpetuating thrombotic effects in lesioned vessel walls.53

Phagocytes produce O2·- through the reduction of molecular oxygen by the NAD(P)H oxidase complex. This complex is formed from a membrane-bound flavocytochrome and 3 cytosolic proteins, one of which is a dimer of Rac1 p21 or Rac2 p21 and the Rho- guanine nucleotide dissociation inhibitor (RhoGDI). It has been reported, that following dissociation of RhoGDI, Rac1 p21 bound to GDP is the physiologic activator of...
The role of the Rho kinase p160-ROCK in the production of O$_2^{-}$ by human polymorphonuclear leukocytes, and in the aggregation and adhesion of this cell type has been investigated under physiologic conditions using the selective p160-ROCK inhibitor Y-27632. Here it was observed that production of O$_2^{-}$ stimulated by phorbol ester was inhibited by Y-27632 in a concentration-dependent manner, indicating that p160-ROCK participated in this process and in the aggregation of human polymorphonuclear leukocytes.

Hydrogen peroxide, a reactive oxygen species, causes pulmonary edema, and increases hydrostatic pressure and vascular permeability. Increased permeability is accompanied by contraction and reorganization of the cytoskeleton in endothelial cells that leads to the formation of intercellular adhesions. The Rho family is also implicated in cell contraction. It has been demonstrated that an inhibitor of Rho kinase blocks the generation of this type of edema in rabbit lung, antagonizing the effects of H$_2$O$_2$. This finding indicates that Rho is involved in H$_2$O$_2$-induced pulmonary edema.

In conclusion, the Rho/Rho kinase signal transduction pathway represents a newly recognized mechanism of vasoconstriction in arterial hypertension, pathologic cardiovascular remodeling, and a number of other cardiovascular and noncardiovascular conditions, and may represent a novel and promising therapeutic target.

**PLEIOTROPIC EFFECTS OF THE STATINS THROUGH THE RHO SIGNALING PATHWAY**

Statins, inhibitors of HMG-CoA reductase, are potent inhibitors of cholesterol biosynthesis. A number of clinical studies have demonstrated beneficial effects of statins in the primary and secondary prevention of heart disease. However, their action seems to extend beyond what would be expected on the basis of hypolipidemic effects alone. Recent experimental and clinical evidence reveals that the pleiotropic effects of statins are independent of their actions on cholesterol and involve an improvement or restoration of endothelial function, increasing plaque stability and reducing oxidative stress and vascular inflammation. A number of the pleiotropic effects of the statins are mediated by their capacity to block the synthesis of important isoprenoid intermediates (farnesyl or geranylgeranyl) that serve as lipid anchors for various intracellular signaling molecules. In particular, the inhibition of small GTPases (Figure 4), whose membrane localization and function depend on the process of isoprenylation (specifically farnesylation or geranylgeranylation), would play an important role in mediating the direct cellular effects of the statins on the vessel wall.

Despite all of the recent findings that have been discussed here, a number of questions remain to be answered in the coming years. It will be important to determine whether inhibition of distinct points in the Rho signaling pathway, once activated, affects the process of vascular remodeling in arterial hypertension or only blood pressure, given the many possible therapeutic targets beyond arterial hypertension (Table 2 and Figure 4).

In conclusion, the Rho/Rho kinase signal transduction pathway represents a newly recognized mechanism of vasoconstriction in arterial hypertension, pathologic cardiovascular remodeling, and a number of other cardiovascular and noncardiovascular conditions, and may represent a novel and promising therapeutic target.

**REFERENCES**


**TABLE 2. Diseases in Which Rho/Rho Kinase Could Represent a Therapeutic Target**

<table>
<thead>
<tr>
<th>Cardiovascular diseases</th>
<th>Noncardiovascular diseases</th>
</tr>
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<tbody>
<tr>
<td>Arterial hypertension</td>
<td>Bronchial asthma</td>
</tr>
<tr>
<td>Cerebral and coronary vasospasm</td>
<td>Premature birth</td>
</tr>
<tr>
<td>Postangioplasty restenosis</td>
<td>Cancer (angiogenesis, cell proliferation, and motility and migration of tumor cells)</td>
</tr>
</tbody>
</table>

Taken from Sekiya et al.58

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Jalil J, et al. Rho/Protein Kinase and Cardiovascular Remodeling


