Rho Kinases in Cardiovascular Physiology and Pathophysiology

Gervaise Loirand, Patrice Guérin, Pierre Pacaud

Abstract—Rho kinases (ROCKs) are the first and the best-characterized effectors of the small G-protein RhoA. In addition to their effect on actin organization, or through this effect, ROCKs have been found to regulate a wide range of fundamental cell functions such as contraction, motility, proliferation, and apoptosis. Abnormal activation of the RhoA/ROCK pathway has been observed in major cardiovascular disorders such as atherosclerosis, restenosis, hypertension, pulmonary hypertension, and cardiac hypertrophy. This review, based on recent molecular, cellular, and animal studies, focuses on the current understanding of ROCK signaling and its roles in cardiovascular physiology and pathophysiology. (Circ Res. 2006;98:322-334.)

Key Words: Rho kinase ■ cardiovascular diseases ■ Rho-GTP–binding proteins ■ signal transduction

RhoA is one of the best-known members of the Rho protein family that, in addition to its effect on actin organization or through this effect, regulate a wide range of fundamental cell functions such as contraction, motility, proliferation, and apoptosis.1 RhoA acts as a molecular switch that cycles between an inactive GDP-bound and an active GTP-bound conformation interacting with downstream targets (effectors) to elicit cellular responses. Rho kinases (ROCKs) are the first and the best-characterized RhoA effectors. However, ROCKs can be considered more generally as Rho effectors because they also bind other Rho proteins such as RhoB and RhoC.2 Since their discovery in 1996, ROCKs have been extensively studied, leading to the publication of >1300 articles, many of which focus on ROCK functions in the cardiovascular system. The interest for ROCKs in the heart and vessels has been further reinforced by the observation that the beneficial cardiovascular effects of statins result, at least in part, from the inhibition of ROCKs.3 Indeed, by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A reductase, statins reduce cholesterol synthesis but also prevent the formation of geranylgeranylpyrophosphate required for membrane translocation and activation of RhoA, the main upstream activator of ROCKs. In this review, we describe the current understanding of ROCK signaling and its roles in cardiovascular physiology and pathophysiology.

ROCK Isoform Structure and Expression

ROCKs are serine/threonine kinases with a molecular mass of ≈160 kDa. They are expressed in invertebrates (C. elegans, Drosophila, and mosquito) and in vertebrates (zebrafish, Xenopus, chicken, bovine, mouse, rat, and human). Two isoforms of ROCK encoded by two different genes have been identified: ROCK-1 (ROCK I, P160-ROCK, or ROK

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ROCK-2 (ROCK II or ROKα). Human ROCK-1 and ROCK-2 genes are located on chromosome 18 (18q11.1) and chromosome 2 (2p24), respectively. ROCK sequences comprise a kinase domain located at the amino terminus of the protein, followed by a coiled-coil region containing the Rho-binding domain (RBD) and a pleckstrin-homology (PH) domain with a cysteine-rich domain (Figure 1). ROCK-1 and ROCK-2 are highly homologous, with an overall amino acid sequence identity of 65%. The identity in the RBD is 58% and approaches 92% in the kinase domain.6

ROCK-1 and ROCK-2 are ubiquitously expressed, with a preferential expression of ROCK-2 mRNA in brain and skeletal muscle.6,7 Both ROCK-1 and ROCK-2 are expressed in vascular smooth muscle and in heart.8

Although regulation of ROCK expression has not been extensively analyzed, some studies have reported changes in ROCK expression. Both ROCK-1 and ROCK-2 mRNAs and proteins are upregulated through protein kinase C- and nuclear factor κB (NF-κB)–dependent pathways by angiogenesis (angiogenesis) via all type 1 receptor stimulation and by interleukin-1B.9 Upregulation of ROCK was also described in vivo in coronary artery of mice receiving continuous AII administration.9 In vitro, the stimulatory effect of AII on the enzyme activity of ROCK, lacking the C-terminal portion of the protein (RBD and PH domains) are constitutively active, whereas C-terminal portions of ROCKs expressed in cells act as dominant negatives.10 It has thus been suggested that the C-terminal region of ROCKs is a negative regulatory region, responsible for autoinhibition of the kinase activity in resting cells, probably through interaction with the catalytic domain of ROCKs (Figure 2).17 In addition to this self-associative interaction, oligomerization (dimerization) also influences the kinase activity of ROCKs by regulating its affinity for ATP.18 Binding of active GTP-bound form of RhoA to RBD stimulates the phosphotransferase activity of ROCKs by disrupting the interaction between the catalytic and the inhibitory C-terminal region of the enzyme (Figure 2). However, the stimulatory effect of GTP-RhoA on the enzyme activity of ROCKs is limited to a 1.5- to 2-fold increase.19 Lipid messengers such as arachidonic acid (AA) or sphingosine phosphorylcholine (SPC) are able to efficiently stimulate ROCK activity (5- to 6-fold increase) independently of RhoA.19,20 AA, and presumably SPC, interact with the negative regulatory region of ROCK, possibly the PH domain, thus disrupting its inhibitory action on the catalytic activity of ROCK (Figure 2).17 ROCKs are also activated by cleavage of the inhibitory C-terminal region, which results in the release of a truncated, active form of the kinase in the cells. ROCK-1 is cleaved by caspase-3 at the cleavage site DETD during apoptosis (Figure 2).21 This consensus sequence for caspase-3 cleavage is not present in ROCK-2.21 However, the proapoptotic protease granzyme B cleaved the C terminus of ROCK-2 at IGLD, thus removing an inhibitory domain similar to that deleted in ROCK-1 by caspase-3 cleavage (Figure 2).22 The consensus cleavage sequence for granzyme B is missing in ROCK-1.22

In addition to the organ/tissue distribution of ROCK isoforms, several studies have analyzed the subcellular localization of ROCKs. ROCKs are essentially distributed in the cytoplasm but are partially translocated to peripheral membrane on RhoA activation.4–6 However, although the main fraction of ROCKs is soluble, ROCKs have also been found located at the cleavage furrow during cytokinesis,11 at stress fiber12 and at vimentin intermediate filament network.13 The mechanisms responsible for the subcellular localization of ROCKs are still unclear.

To investigate in vivo distribution/function of ROCK isoforms, ROCK-1– and ROCK-2–knockout mice have been generated recently.14,15 Loss of ROCK-1 results in the eyelids open at birth and omphalocele phenotype in mice,14 whereas loss of ROCK-2 results in placental dysfunction leading to intrauterine growth retardation and fetal death.15 However, in both groups of knockout animals, mice that survive develop normally and are fertile. These observations suggest that ROCK-1 and ROCK-2 function in a redundant manner and indicate a possibility that each is able to compensate functionally for the loss of the other in most systems, except some tissues, such as placenta. The cardiovascular phenotype of ROCK-1 and ROCK-2 knockout mice has not been analyzed.

### Regulation of ROCK Activity

The kinase domain of ROCKs is localized in the N-terminal region of the protein sequence. Truncated forms of ROCKs lacking the C-terminal portion of the protein (RBD and PH domains) are constitutively active, whereas C-terminal portions of ROCKs expressed in cells act as dominant negatives.10 It has thus been suggested that the C-terminal region of ROCKs is a negative regulatory region, responsible for autoinhibition of the kinase activity in resting cells, probably through interaction with the catalytic domain of ROCKs (Figure 2).17 In addition to this self-associative interaction, oligomerization (dimerization) also influences the kinase activity of ROCKs by regulating its affinity for ATP.18 Binding of active GTP-bound form of RhoA to RBD stimulates the phosphotransferase activity of ROCKs by disrupting the interaction between the catalytic and the inhibitory C-terminal region of the enzyme (Figure 2). However, the stimulatory effect of GTP-RhoA on the enzyme activity of ROCKs is limited to a 1.5- to 2-fold increase.19 Lipid messengers such as arachidonic acid (AA) or sphingosine phosphorylcholine (SPC) are able to efficiently stimulate ROCK activity (5- to 6-fold increase) independently of RhoA.19,20 AA, and presumably SPC, interact with the negative regulatory region of ROCK, possibly the PH domain, thus disrupting its inhibitory action on the catalytic activity of ROCK (Figure 2).17 ROCKs are also activated by cleavage of the inhibitory C-terminal region, which results in the release of a truncated, active form of the kinase in the cells. ROCK-1 is cleaved by caspase-3 at the cleavage site DETD during apoptosis (Figure 2).21 This consensus sequence for caspase-3 cleavage is not present in ROCK-2.21 However, the proapoptotic protease granzyme B cleaved the C terminus of ROCK-2 at IGLD, thus removing an inhibitory domain similar to that deleted in ROCK-1 by caspase-3 cleavage (Figure 2).22 The consensus cleavage sequence for granzyme B is missing in ROCK-1.22

In addition to these positive regulations, negative control of the kinase activity of ROCKs has also been described. The small G-protein RhoE binds to the N-terminal region of ROCK-1 (amino acids 1–420) containing the kinase domain (Figure 2).23 RhoE binding to ROCK-1 inhibits its activity and prevents RhoA binding to RBD.23 Two other small G-proteins, Gem and Rad, have been shown to bind and inhibit ROCK function, but their mechanism of action is not defined.24
ROCK Substrates

The consensus sequence of ROCK phosphorylation site is RXXS/T or RXS/T. ROCKs seem to require the basic amino acid such as Arg (R) close to its phosphorylation site. More than 15 ROCK substrates have been identified (Table 1). For a large portion of ROCK substrates, the functional consequence of ROCK-mediated phosphorylation is related to actin filament formation and organization and cytoskeleton rearrangements (Table 1). An important subset of ROCK targets, including the myosin phosphatase target subunit (MYPT-1), CPI-17, the 20-kDa myosin light chain (MLC), and calponin plays key roles in smooth muscle cell contraction (see “ROCKs and vascular smooth muscle cell contraction” below; Figure 3). MYPT-1 is the major effector of ROCK-mediated Ca\(^{2+}\) sensitization of the contraction in smooth muscle. However, the relative contribution of ROCK-mediated phosphorylation of MLC, CPI-17, and calponin to ROCK-dependent contraction of smooth muscle remains to be determined.

The cardiac troponin has been identified recently as a ROCK substrate. Phosphorylation of troponin by ROCK leads to inhibition of tension generation in cardiac myocytes. Phosphatase and tensin homologue (PTEN) is also a newly identified ROCK substrate. PTEN is a phosphatase that dephosphorylates both proteins and phosphoinositide substrates and has important roles in the regulation of intracellular signaling, in particular, the phosphatidylinositol 3-kinase (PI3-kinase)/Akt pathway, involved in the regulation of cell growth, protein synthesis, transcriptional regulation, and cell survival. The phosphorylation of PTEN by ROCK stimulates its phosphatase activity. Reduction of ROCK-mediated PTEN phosphorylation could thus be responsible for the stimulation of Akt signaling induced by ROCK inhibitors in endothelial cells.

Other ROCK targets such as Tau, microtubule-associated protein 2, and collapsin response mediator protein 2 are not described in detail here.

In addition, although ROCK-mediated phosphorylation has not been firmly demonstrated, interaction of ROCKs with other proteins suggests that additional targets exist. Active ROCK interacts with and phosphorylates the insulin receptor substrate-1 (IRS-1) in vascular smooth muscle cells, leading to inhibition of both insulin-induced IRS-1 tyrosine phosphorylation and PI3-kinase activation. In vascular smooth muscle cells from hypertensive rats, the ROCK/IRS-1 asso-
Cytoskeleton regulating proteins

Adducin
Increased aducin/F-actin interaction
Assembly of spectrin/F-actin network; increase of cell motility
28

ERM
Decrease of intra- or intermolecular head-to-tail association of ERM
Actin filament/membrane interaction; microvilli formation
27

MARCKS
?
Cytoskeletal rearrangement
29, 30

NHE1
Stimulation of its Na+/H+ exchanger activity
Actin stress fiber formation
29

EF-1 alpha
Inhibition of its binding to F-actin
Increase of actin stress fiber formation?
29

LIM-kinases 1 and 2
Stimulation of kinase activity
Actin polymerization (through phosphorylation and inactivation of cofilin); Coordination of microtubule destabilization and actin formation
29, 30

RhoE
Increase RhoE stability
Potentialization of the inhibitory effect of RhoE on actin stress fiber formation and Ras-induced transformation
29

Intermediate filaments

GFAP
Inhibition of its filament formation
Regulation of cytokinesis
29, 30

NF-L
Inhibition of its filament formation
?
29

Desmin
Inhibition of its filament formation
?
29

Vimentin
Inhibition of its filament formation
Regulation of cytokinesis
26

Contraction regulating proteins

MYPT-1
Inhibition of MLCP activity
Ca2+ sensitization of smooth muscle contraction/stress fiber formation
31

CPI-17
Inhibition of MLCP activity
Ca2+ sensitization of smooth muscle contraction
32

MLC
Stimulation of actomyosin ATPase activity
Ca2+ sensitization of smooth muscle contraction/stress fiber formation
25

Calponin
Inhibition of calponin binding to actin
Ca2+ sensitization of smooth muscle contraction
33

Tropomodulin
Inhibition of actomyosin ATPase activity
Inhibition of tension generation of cardiac myocytes
34

Microtubule regulating proteins

Tau
Reduction of its activity
Regulation of microtubule dynamics
30

MAP 2
?
30

Neuronal proteins

CRMP-2
?
Growth cone collapse
29, 30

Signaling proteins

PTEN
Stimulation of phosphatase activity
Decrease of intracellular PtdIns(3,4,5)P3 level; tumor suppression
35

ERM indicates ezrin, radixin, moesin; MARCKS, myristoylated alanine-rich C-kinase substrate; NHE1, Na+/H+ exchanger; EF-1α, elongation factor 1-α; GFAP, glial fibrillary acidic protein; NF-L, neurofilaments; MAP 2, microtubule-associated protein 2; CRMP-2, collapsin response mediator protein 2.

ROCK Functions in Vascular Smooth Muscle Cells

A large body of evidence has now been obtained regarding the important functions of ROCKs in vascular physiology, particularly in vascular smooth muscle cells. The major part of available data regarding ROCK-dependent functions in vascular smooth muscle cells has been obtained by the use both in vitro and in vivo of the pharmacological ROCK inhibitors fasudil (AT877 and HA-1077), hydroxyfasudil, and Y-27632. However, like all pharmacological agents, these inhibitors have only a relative specificity. Therefore, it is important to mention that the involvement of ROCKs in a particular function has been firmly established only when pharmacological data are supplemented by molecular analyses. ROCK is recognized as a major regulator of cell...
contraction but has also been demonstrated to be critical in controlling migration, proliferation, cell apoptosis/survival, gene transcription, and differentiation (Figure 3).

**ROCKs and Vascular Smooth Muscle Cell Contraction**

The major regulatory mechanism of smooth muscle contraction is phosphorylation/dephosphorylation of MLC. MLC is phosphorylated by the Ca\(^{2+}\)-calmodulin–activated MLC kinase (MLCK) and dephosphorylated by the Ca\(^{2+}\)-independent MLC phosphatase (MLCP). Thus, a rise in cytosolic Ca\(^{2+}\) concentration produces smooth muscle contraction by activation of MLCK and consequent phosphorylation of MLC (Figure 4). However, it is now well established that MLC phosphorylation and tension can be induced independently of change in cytosolic Ca\(^{2+}\) concentration. Agonists (noradrenaline, endothelin, thromboxane, etc.) that bind to G-protein–coupled receptors produce contraction by increasing both the cytosolic Ca\(^{2+}\) concentration and the Ca\(^{2+}\) sensitivity of the contractile proteins, which is responsible for the tonic component of vascular smooth muscle cell contraction in various vascular beds, including pulmonary artery, mesenteric artery, and portal vein.

ROCK activity is recognized as the major regulator of the Ca\(^{2+}\) sensitization of the contractile proteins, which is responsible for the tonic component of vascular smooth muscle cell contraction in various vascular beds, including pulmonary artery, mesenteric artery, and portal vein. It has recently been shown that ROCK activity is also involved in the myogenic tone. In pressurized small arteries, the use of ROCK inhibitors has revealed that Rho–ROCK pathway is active in the absence of vasoconstrictors, keeping the vessels in a state of high calcium sensitivity and basal tone.

**ROCKs 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 pathologic stimuli. 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). 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. Recently, RhoA/ROCK signaling has been demonstrated to be a critical mechanism for controlling smooth muscle differentiation through the regulation of SRF-dependent transcription.

Expression of a constitutively active RhoA mutant in vascular smooth muscle cells increases the activity of smooth muscle–specific promoters, whereas inhibition of Rho by C3 transferase or inhibition of ROCKs by Y-27632 decreases the expression of smooth muscle differentiation marker genes. Changes in RhoA/ROCKs expression or activity could thus underlie vascular smooth muscle cell phenotype alterations.

**ROCKs and Vascular Smooth Muscle Cell Proliferation**

ROCK inhibition by Y-27632 suppresses the platelet-derived growth factor (PDGF)-BB–induced activation of extracellular-regulated kinase 1/2 (ERK1/2) and proliferation of vascular smooth muscle cells, indicating the participation of ROCK in PDGF-induced smooth muscle cell proliferation. Similarly, vascular smooth muscle cell proliferation induced by thrombin and urotensin-II are inhibited by ROCK blockers, suggesting a role for ROCKs in G-protein–coupled receptor–stimulated cell proliferation. ROCKs may also play an important role in All-induced vascular hypertrophy. The cyclin-dependent kinase inhibitor p27\(^{Kip1}\) plays a crucial role in cell proliferation. ROCK activation downregulates p27\(^{Kip1}\) expression, leading to the acceleration of cell cycle progression. The antiproliferative effect of ROCK inhibitors has been ascribed to upregulation of p27\(^{Kip1}\) expression. An additional mechanism through which ROCK may regulate smooth muscle cell proliferation involves ERK1/2. In vascular smooth muscle cells, ROCK inhibitors have been found to suppress PDGF-BB–induced ERK1/2 activation but have no effect on ERK1/2 activation induced by serotonin. However, the nuclear translocation of ERK1/2...
activated by serotonin is inhibited by treatment with the ROCK inhibitor Y-27632. Although it has been initially described that nuclear translocation of ERK1/2 depends on the actin cytoskeleton organization, it seems that the inhibitory effect of ROCK inhibitor on ERK1/2 translocation does not result from its action on actin cytoskeleton organization.

Opposite data that do not reveal a substantial role for RhoA/ROCK in the regulation of vascular smooth muscle cell proliferation have also been reported. Although inhibition of RhoA blocks PDGF-induced migration, it has no effect on PDGF-induced proliferation of human vascular smooth muscle cells. These data are also supported by in vivo studies showing that ROCK inhibitors, fasudil, and Y-27632 do not affect vascular smooth muscle cell proliferation induced by balloon injury.

Collectively, these observations show that the role of ROCKs in the control of vascular smooth muscle cell proliferation is not fully elucidated. Considering the unexpected result that both smooth muscle cell differentiation and proliferation are positively regulated by ROCKs, it is obvious that further analyses are required, in particular, the examination of a potential differential involvement of ROCKs depending on the differentiation status of smooth muscle cells.

ROCKs and Vascular Smooth Muscle Cell Migration

Pharmacological blockade of ROCK activity or transfection of a dominant-negative form of ROCK inhibits vascular smooth muscle cell migration induced by PDGF and lyso-
phosphatidic acid through both MLC phosphorylation-dependent and -independent pathways.\textsuperscript{58,65} Similarly, UTP- and thrombin-induced smooth muscle cell migration is also blocked by ROCK inhibition.\textsuperscript{66,67} Migration induced by activation of the urokinase-type plasminogen activator receptor involved RhoA/ROCK activity in human vascular smooth muscle cells.\textsuperscript{68} ROCK inhibition also blocks the migration of vascular smooth muscle cells in 3D collagen matrix.\textsuperscript{69}

Surprisingly, ROCK inhibition by fasudil was also found to increase cell motility of differentiated aortic smooth muscle cells, suggesting that downregulation of ROCK activity induced cell motility.\textsuperscript{70} These conflicting data suggest again that the role of ROCKs in vascular smooth muscle cell migration is not firmly established and could also depend on the differentiation status of smooth muscle cells.

**ROCKs in Endothelial Cells**

ROCK-dependent regulation of actin cytoskeleton organization and cell contractility is involved in the regulation of endothelial permeability. Thrombin-induced endothelial barrier disruption involved microtubule disassembly and is mediated by phosphorylation and activation of MYPT-1 and LIM-kinase 1 by ROCK.\textsuperscript{71,72} Again, through modulation of the actin cytoskeleton organization, RhoA/ROCK signaling promotes endothelial migration in response to vascular endothelial growth factor, sphingosine-1-phosphate, and shear stress.\textsuperscript{73} Therefore, by mediating an increase in endothelial permeability and migration, ROCK activation appears to be a key event in the initiation of angiogenic process.

Activation of ROCKs in endothelial cells also participates in the regulation of gene expression. ROCKs positively regulate the expression of endothelial tissue factor, intercellular adhesion molecule-1, and plasminogen activator inhibitor-1 (PAI-1).\textsuperscript{74–76}

Through multiple mechanisms, ROCK negatively regulates NO production by endothelial cells. Activation of RhoA/ROCK decreases endothelial NO synthase (eNOS) expression by reducing eNOS mRNA stability.\textsuperscript{77} Consequently, ROCK inhibitors or statins upregulate eNOS expression.\textsuperscript{78,79} ROCKs also negatively regulate eNOS function via a tonic inhibitory effect on PI3-kinase/Akt pathway\textsuperscript{80} and possibly by stimulation of arginase activity.\textsuperscript{81}

**ROCKs in Cardiac Cells**

ROCK activity regulates major morphogenetic events during embryonic development including formation of the heart.\textsuperscript{81} ROCK-I and -II transcripts are enriched in cardiac mesoderm. Treatment of neurulating embryos with the ROCK inhibitor Y27632 causes severe cardiac developmental defects. The embryos exposed to Y27632 form two laterally positioned beating hearts, indicating that ROCKs regulate the migration of the cardiac precursors to the ventral midline and fusion of the bilateral heart primordia.\textsuperscript{82} Moreover, ROCK inhibition induces early expression of cardiac α-actin, a marker of cardiomyocyte differentiation, coincident with the upregulated expression of the transcription factors SRF and GATA-4.\textsuperscript{83} Thus, ROCKs also regulate the myocardial differentiation. In cultured murine embryos, inhibition of ROCKs decreases cell proliferation in the heart but does not modify programmed cell death, suggesting that ROCK activity is not involved in cardiomyocyte apoptosis but regulates cardiomyocyte division during heart development.\textsuperscript{84} This effect is mediated through the regulation of expression of cell cycle proteins, cyclin D3, CDK6, and p27\textsuperscript{Kip1} in cardiomyocytes. ROCKs also play a role in endocardial cell differentiation and migration.\textsuperscript{85} ROCK-1 and ROCK-2 are found in the endocardial cushions during development. In cultured endocardial cushions, inhibition of ROCKs prevents the epithelial–mesenchymal transition and cell migration.\textsuperscript{86} Because endocardial cushions play an important role in cardiac septation, it is likely that ROCK-dependent differentiation and migration of endocardial cells is critical for normal heart development.

The physiological role of ROCKs in the cardiac conduction system and ventricular repolarization process has also been assessed. Using Y-27632 in isolated, blood-perfused canine atrioventricular node preparation, it has been shown that ROCK activity functions to moderately facilitate the atrioventricular nodal conduction and slightly delays ventricular repolarization process.\textsuperscript{87}

Recently, ROCK activation has been shown to alter cardiac myofilaments response to Ca\textsuperscript{2+} by a mechanism involving troponin phosphorylation.\textsuperscript{34} ROCK-mediated troponin phosphorylation induces depression of the tension generation and the ATPase rate of cardiac myofilaments. However, the physiological or pathophysiological role of ROCK-dependent troponin phosphorylation remains to be determined.

**ROCKs and Cardiovascular Diseases**

Accumulating evidence indicates a role for ROCKs in the pathogenesis of cardiovascular diseases (Table 2). However, it should be pointed out that most of the data have been obtained in animal models for cardiovascular diseases such as hypertension, restenosis, atherosclerosis, pulmonary hypertension, cerebral vasospasm, vascular aneurysms, myocardial ischemia/reperfusion injury, cardiac hypertrophy, and ventricular remodeling.

**ROCKs and Hypertension**

Systemic hypertension is characterized by a high arterial pressure level resulting from increased vascular resistance attributable to both enhanced contractility and arterial wall remodeling. The ROCK inhibitors Y-27632 and fasudil normalize arterial pressure in animal models of hypertension indicating the importance of the ROCK signaling pathway in the vascular hyper-reactivity associated with hypertension.\textsuperscript{40} Direct measurements of the amount of active GTP-bound RhoA in arteries from several animal models of hypertension have suggested that an increased RhoA activity is responsible for enhanced ROCK activation in this pathological context.\textsuperscript{85} In addition, long-term blockade of ROCK suppresses vascular lesion formation such as medial hypertrophy and perivascular fibrosis in small coronary artery from spontaneously hypertensive rats.\textsuperscript{86} Similar observations have been made in the rat model of hypertension induced by chronic inhibition of NO synthesis.\textsuperscript{87} In both models, the activity of RhoA/ROCK pathway is found to be increased. Because the inhibition of AII type 1 receptor prevents the upregulation of RhoA/ROCK activity, it has been suggested that an increase
in AII activity participates in the activation ROCK in hypertensive rats. This is in agreement with another report showing that in vivo, long-term infusion of AII increases the activity of RhoA and ROCK increases medial thickness and promotes perivascular fibrosis in coronary arteries. Both AII-induced coronary hypertrophy and fibrosis are inhibited by ROCK inhibitors. This effect of ROCK inhibition is associated with a marked reduction of AII-induced superoxide anion production, AII-induced monocyte chemoattractant protein-1, and PAI-1.

Although AII seems to substantially participate in the activation of ROCKs 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 stimulates vascular smooth muscle cell proliferation. Indeed, stretch-induced ERK activation and vascular smooth muscle cell growth are inhibited by ROCK inhibition.

An additional and important role of the ROCK pathway that can account for its involvement in hypertension is the alteration of the expression of genes important in the regulation of arterial tone and structure such as PAI-1 and eNOS. Excessive RhoA/ROCK activity could thus participate in endothelial dysfunction and the decreased NO production associated with arterial diseases.

Together, these recent data point to a substantial role of ROCKs in hypertension and show that different upstream signals can converge toward ROCKs in hypertensive vascular diseases.

**ROCKs and Restenosis**

Restenosis is the renarrowing of an artery that was previously opened generally by angioplasty. Restenosis involves vascular smooth muscle cell migration and proliferation and excessive extracellular matrix production, leading to neointima formation. ROCK blockers have been shown to inhibit neointimal formation after balloon injury in rat and pig. In these models, the effect of ROCK inhibitors has been ascribed either to an antiproliferative effect through downregulation of the cyclin-dependent kinase inhibitor p27 \textsuperscript{kip1} or to stimulation of apoptosis. ROCK inhibition-induced stimulation of apoptosis has recently been correlated with an increased expression of the proapoptotic protein Bax in neointimal smooth muscle cells.

Long-term inhibition of ROCKs also reduces neointimal formation after stent implantation in pig, which is in agreement with the maintained activation of RhoA observed in human arteries after stent implantation. In addition, it has been shown that rapamycin induced a loss of RhoA expression and that the inhibitory action of rapamycin on RhoA/ROCK plays a key role in its antirestenotic effect.

**ROCKs and Atherosclerosis**

Atherosclerosis is the underlying disorder in the majority of patients with cardiovascular disease. Atherosclerosis is a complex process involving inflammatory cells, endothelial dysfunction, smooth muscle cell proliferation, extracellular matrix alteration, and thrombosis.

<table>
<thead>
<tr>
<th>ROCK Targets</th>
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<th>Possible Link to Cardiovascular Disorders</th>
<th>Reference</th>
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<tr>
<td>Smooth muscle cells</td>
<td>MYPT-1 (MLC, CPI 17, calponin)</td>
<td>↑ MLC phosphorylation</td>
<td>Contraction</td>
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<td></td>
<td>?</td>
<td>↑ p27\textsuperscript{kip1}, ↑ ERK1/2</td>
<td>Proliferation</td>
<td>Restenosis, Atherosclerosis, PHT</td>
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<td>Cytoskeleton rearrangements</td>
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<td></td>
<td>PTEN</td>
<td>↓ Bax</td>
<td>↓ Apoptosis</td>
<td>Restenosis</td>
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<td></td>
<td>PTEN</td>
<td>↓ Akt</td>
<td>↑ Apoptosis</td>
<td>Aneurysms</td>
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<td>?</td>
<td>↓ eNOS expression</td>
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<td></td>
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<td>↓ arginase activity</td>
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<td></td>
<td>PTEN</td>
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<td>Cardiomyocytes</td>
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<td></td>
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<td>↓ Bcl-2 and ↓ Akt</td>
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<td></td>
<td>?</td>
<td>Myofibrillar organization, ↑ ERK1/2, ↑ GATA-4</td>
<td>↑ Cell growth</td>
<td>Hypertrophy/remodeling</td>
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<tr>
<td></td>
<td>?</td>
<td>Cyclin D3, CDK6, p27\textsuperscript{kip1}</td>
<td>Proliferation</td>
<td>Hypertrophy/remodeling</td>
</tr>
</tbody>
</table>

Putative targets are indicated in italic.

ICAM indicates intercellular adhesion molecule; HT, hypertension; PHT, pulmonary hypertension; TF, tissue factor.
ROCKs have been shown to be upregulated at inflammatory arteriosclerotic lesions and cause coronary vasospastic responses through inhibition of MLCP in both a porcine model of coronary artery spasm and arteriosclerotic human arteries. Furthermore, long-term inhibition of ROCKs causes a marked regression of coronary arteriosclerosis and disappearance of coronary vasospastic activities in vivo in pig and in the low-density lipoprotein receptor knockout mice model of atherosclerosis. It has been suggested that ROCK activity contributes to the development of early atherosclerosis, possibly through its modulatory activity on NF-κB activation and T lymphocyte proliferation. Thus, these data are in agreement with the previous indirect observation that the anti-inflammatory and antiarteriosclerotic properties of statins are mediated, at least in part, by inhibition of Rho protein isoprenylation, preventing the activation of downstream Rho targets such as ROCKs.

**ROCKs and Pulmonary Hypertension**

Pulmonary hypertension is abnormally high blood pressure in the arteries of the lungs. The pathogenesis of pulmonary hypertension is a multifactorial process that comprises sustained vasoconstriction and structural remodeling of pulmonary arteries leading to reduction of the lumen area of the pulmonary microvasculature and fixed elevation of pulmonary resistance. Reduced endothelium-derived NO production in pulmonary arterial vessels has been implicated in the pathophysiology of hypertension. It has recently been shown in vitro in human pulmonary endothelial cells that hypoxia-induced decrease in eNOS expression is mediated by ROCK. In addition, several studies indicate that activation of the RhoA/ROCK pathway contributes to both vasoconstriction and vascular remodeling associated with pulmonary hypertension. The ROCK inhibitor Y-27632 attenuates acute hypoxia-induced vasoconstriction and reduces the development of chronic hypoxia-induced pulmonary hypertension and vascular remodeling. The ROCK-dependent calcium sensitization in small pulmonary arteries has been shown to be enhanced by chronic hypoxia in rats in association with a 2-fold increase in ROCK expression. The ROCK pathway is also substantially involved in monocrotaline-induced pulmonary hypertension in rats and long-term inhibition of ROCK by orally given or inhaled fasudil, prevents or causes a marked improvement of monocrotaline-induced pulmonary hypertension through multiple mechanisms including inhibition of smooth muscle cell proliferation and increased apoptosis, reduced macrophage infiltration and improvement of endothelium-dependent relaxation. Although the mechanisms leading to the increased RhoA/ROCK activity are not identified, these data indicate that activation of the RhoA/ROCK pathway is involved in the pathogenesis of pulmonary hypertension.

**ROCKs and Cerebral Vasospasm**

Subarachnoid hemorrhage often induces a long-term narrowing of the cerebral artery called cerebral vasospasm responsible for cerebral ischemia. Activation of ROCKs and phosphorylation of MLC and MYPT-1 occur concomitantly during vasospasm induced by subarachnoid hemorrhage in canine basilar artery, suggesting that ROCK activity is involved in the enhancement of cerebral vasospasm by increasing the phosphorylation of MLC, directly or indirectly as a result of the inhibition of MLCP. ROCK inhibition by Y-27632 reduces the vasospasm and simultaneously decreases the phosphorylation of MYPT-1 and MLC. Oxyhemoglobin, considered a major causative component of blood clot responsible for vasospasm, activates ROCKs in cerebrovascular smooth muscle cells.

**ROCKs and Vascular Aneurysms**

Vascular aneurysm is an abnormal widening or ballooning of a portion of an artery related to weakness in the wall of the blood vessel. Atherosclerosis and hypertension favor the development of abdominal aorta aneurysms. In apolipoprotein E-deficient (apoE-KO) mice, AII promotes vascular inflammation and induces abdominal aortic aneurysm formation. Inhibition of ROCK activity results in a reduction of both the incidence and the severity of AII-induced aortic aneurysms in apoE-KO mice. This beneficial effect is ascribed to the inhibition of ROCK-mediated apoptosis and extracellular matrix proteolysis. In contrast to this observation, reduction of neointimal formation after balloon injury by ROCK inhibition has been shown associated with enhanced vascular smooth muscle cell apoptosis. Thus, it is suggested that the role of apoptosis in the pathogenesis of aneurysms may be different from that of other vascular diseases in which links between ROCKs and apoptosis may be regulated differently.

**ROCKs and Cardiac Hypertrophy and Ventricular Remodeling**

Cardiac hypertrophy is a physiological adaptation in response to pressure or volume overload. However, with time, this initial adaptive response becomes maladaptive, switching the heart from a compensated to decompensated state and finally leading to heart failure. Recent cellular and molecular biology studies using ROCK inhibitors have indicated a pivotal role of RhoA/ROCK signaling in many aspects of cardiac function such as
cardiac hypertrophy and ventricular remodeling after myocardial infarction. In the adult rat myocardium, pressure overload induces a rapid activation of ROCK, suggesting that it could play a central role in the coordination of initial mechanisms and adaptative changes triggered by mechanical stress in cardiac myocytes. In Dahl salt-sensitive hypertensive rats, the ventricular hypertrophy and function is significantly ameliorated by ROCK inhibition. It has been suggested that the cardioprotective effect of ROCK inhibition involved upregulation of the downregulated eNOS and the reduction of oxidative stress through the inhibition of NAD(P)H oxidase and lectin-like oxidized low-density lipoprotein receptor-1 expression. Several neurohormonal factors, such as AII, are believed to participate in ventricular hypertrophy and to the transition to heart failure. Long-term inhibition of ROCK by fasudil treatment reduces the AII-induced cardiac myocyte hypertrophy in wild-type as well as in apoE-KO mice. In addition, ROCK inhibition improves cardiac function by preventing AII-induced decrease in ventricular contractility, cardiac output, and cardiac stoke volume. ROCK activity is also involved in the pathogenesis of left ventricular remodeling after myocardial infarction.

In vitro, the assembly of contractile proteins into organized sarcomeric units is one of the prominent features of the neurohormonal factor–induced cardiac myocyte hypertrophic response. In neonatal ventricular myocytes, ROCK activation is one of the key events mediating α1-adrenoceptor activation-induced myofibrillar organization and atrial natriuretic factor (ANF) expression. Similarly, it has been shown by pharmacological inhibition of ROCK activity that ROCKs participate in the increase of ANF production, cell size, protein synthesis, and myofibrillar organization associated to endothelin-1–induced hypertrophic response in cardiac myocytes. Activation of ERK1/2 and of the cardiac transcription factor GATA-4 is identified as downstream nuclear mediators of ROCKs during myocardial hypertrophy.

Clinical Studies

Fasudil, initially described as an intracellular calcium antagonist, has been marked in Japan since 1995 for the treatment of vasospasm after subarachnoid hemorrhage. The first report of a placebo-controlled double-blind trial in 1992 has demonstrated a significant reduction in angiographically revealed vasospasm by intravenous administration of fasudil. Clinical development for this indication is in progress in the United States and Europe. The effect of fasudil was also assessed in patients with stable effort angina pectoris. A multicenter phase II study shows that a 4-week oral treatment with fasudil significantly prolongs the maximum exercise time without any effect on blood pressure and heart rate during exercise. Oral fasudil was well tolerated without any serious adverse reactions. In patients with microvascular angina attributable to coronary microvascular hyperconstriction, fasudil ameliorates myocardial ischemia. In a recent study performed in patients with heart failure, intra-arterial infusion of fasudil reduces the increased forearm vascular resistance in the heart failure group toward the level of the control group.

In patients with severe pulmonary hypertension, pulmonary vascular resistance which was not ameliorated by oxygen in-


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Rho Kinases in Cardiovascular Physiology


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