The Role of the RhoA/rho-kinase Pathway in Pulmonary Hypertension

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Abstract: The small GTP-binding protein, RhoA, and its downstream effector protein, rho-kinase, have been implicated in the pathogenesis of a number of cardiovascular diseases. The activation of rho-kinase is involved in the development of increased vascular tone, endothelial dysfunction, inflammation, and restenosis, and that the inhibition of rho-kinase has been shown to have a beneficial effect in a variety of cardiovascular disorders. It is our hypothesis that rho-kinase inhibitors promote vasodilation independent of the mechanism that increases vasoconstrictor tone and moreover, the RhoA/rho-kinase pathway has a role in the regulation of smooth muscle tone under physiological conditions. The objective of this review is to improve our current understanding of the role of RhoA/rho-kinase pathway in the regulation of vasoconstrictor tone and the use of rho-kinase inhibitors in the treatment of cardiovascular disorders with an emphasis on pulmonary hypertension.

Key Words: Intracellular signaling peptides and proteins, protein kinases, RhoA/rho-kinase pathway, muscle, smooth, vascular, pulmonary hypertension, pulmonary vascular bed, systemic vascular bed, signal transduction, inhibitors/pharmacology, cardiovascular diseases; fasudil, HA-1077, Y-27632.

INTRODUCTION

The endothelium regulates vascular function. A healthy endothelium maintains normal vascular function by promoting a vasodilator, antiatherogenic state, whereas an activated or dysfunctional endothelium promotes a vasoconstrictor, proatherogenic diseased state [1]. Vascular smooth muscle (VSM) cells coordinate the delivery of blood to meet the metabolic demands of the organs in the body through regional distribution of blood flow by regulating vasoconstrictor tone [2]. The major impact of VSM control of vascular resistance is that an increased contractility, and/or impaired relaxation, would predispose tissue to ischemia and end-organ failure [2]. Increased vascular tone is due to an excess presence of vasoconstrictors (norepinephrine, angiotensin II, endothelin-1) and/or a decreased presence of vasodilators (nitric oxide (NO), prostacyclin, endothelial-derived hyperpolarizing factor) [3-6], resulting in a state of endothelial dysfunction that is believed to be an early component of the hypertensive process [6, 7].

VSM contraction occurs by Ca^{2+}-dependent, as well as by Ca^{2+}-independent mechanisms [8-10]. An initial increase in intracellular Ca^{2+} levels (Ca^{2+}-dependent, initial or trigger phase) leads to an initial transient increase in VSM tone, but cytosolic Ca^{2+} [$(Ca^{2+})_i$] levels subsequently return to near baseline levels [2]. However, VSM has the ability to maintain a contractile response in the presence of submaximal $(Ca^{2+})_i$ levels (a Ca^{2+}-independent or maintenance phase) [11-14]. Since the discovery of this Ca^{2+}-independent phase of vasoconstrictor tone [11-13], there have been numerous investigations examining the intracellular signaling pathway that regulates calcium sensitization, myosin activity, and VSM tone. Moreover, alterations in this Ca^{2+}-independent signaling pathway may play a major role in VSM dysfunction that is seen in a number of cardiovascular diseases (CVD) and other disorders (Table 1).

Ca^{2+}-Dependent and Ca^{2+}-Independent Mechanisms

In the VSM, a rise in $(Ca^{2+})_i$ originates from two linked sources, the extracellular compartment and intracellular stores (Fig. 1). VSM contraction is activated by neurotransmitters (norepinephrine, epinephrine, serotonin), hormones (angiotensin II, thromboxane A_2, endothelin-1), or by myogenic mechanisms (transmural pressure, stretch responses) that involves calcium entry and release [2, 15, 16]. These mechanisms act through activation of calcium channels in the VSM membrane and activation of store-operated calcium channels following depletion of calcium from within the sarcoplasmic reticulum (Fig. 1) [16-18]. However, an important downstream and intracellular regulatory sequence includes changes in myosin light chain kinase (MLCK) activity (Fig. 1) [16, 17]. This enzyme is activated by a $(Ca^{2+})_i$/calmodulin complex and phosphorylates the 20-kDa myosin regulatory light chain (MLC-P) at either serine-19 or tyrosine 18 by MLCK and results in activation of myosin ATPase activity, that induces VSM contraction (Fig. 1) [15, 16].

However, it is also known that the relationship between the amount of $(Ca^{2+})_i$ increase and the degree of contraction does not always correlate [8, 9, 19-21]. VSM also has the ability to maintain a contractile response in the presence of low $(Ca^{2+})_i$ levels, an observation subsequently labeled as "Ca^{2+}-sensitization of the contractile apparatus" or "an increase in the Ca^{2+}-sensitivity" [9, 16, 22]. As a result, VSM contraction is subjected to regulation by the intracellular Ca^{2+} signal and by intracellular alterations in Ca^{2+}-sensitivity (Fig. 2).

Numerous etiologies are involved in the development of cardiovascular diseases and other disorders (Table 1) due to the required number of intermediate steps between receptor activation, signal transduction, and VSM contraction [2]. Moreover, a number of intracellular protein kinases have been identified as key determinants controlling VSM tone and that kinase inhibition is currently being used in the treatment of CVD [8, 9, 19-21, 23, 24]. One of these protein kinases, the RhoA/rho-kinase pathway, has recently attracted considerable attention for its role in the pathogenesis of CVD and other disorders, including pulmonary hypertension (PH) (Table 1).

Pulmonary Hypertension

PH is a heterogeneous group of disorders characterized by a sustained increase in pulmonary arterial pressure of greater than 25 mm Hg at rest or greater than 30 mm Hg with exercise [6, 25-27]. PH develops as a sporadic disease (idiopathic), as an inherited disorder (familial), or in association with certain conditions or risk factors (e.g., collagen vascular diseases, portal hypertension, human immunodeficiency virus infection, ingestion of drugs, toxins, or dietary products, congenital systemic-to-pulmonary shunts, or in persistent fetal circulation) [28-33]. PH ultimately results in right ventricular failure and death [6, 34-36]. Unfortunately, early diagnosis of PH is often delayed due to the similarity, or overlap, of clinical symptoms

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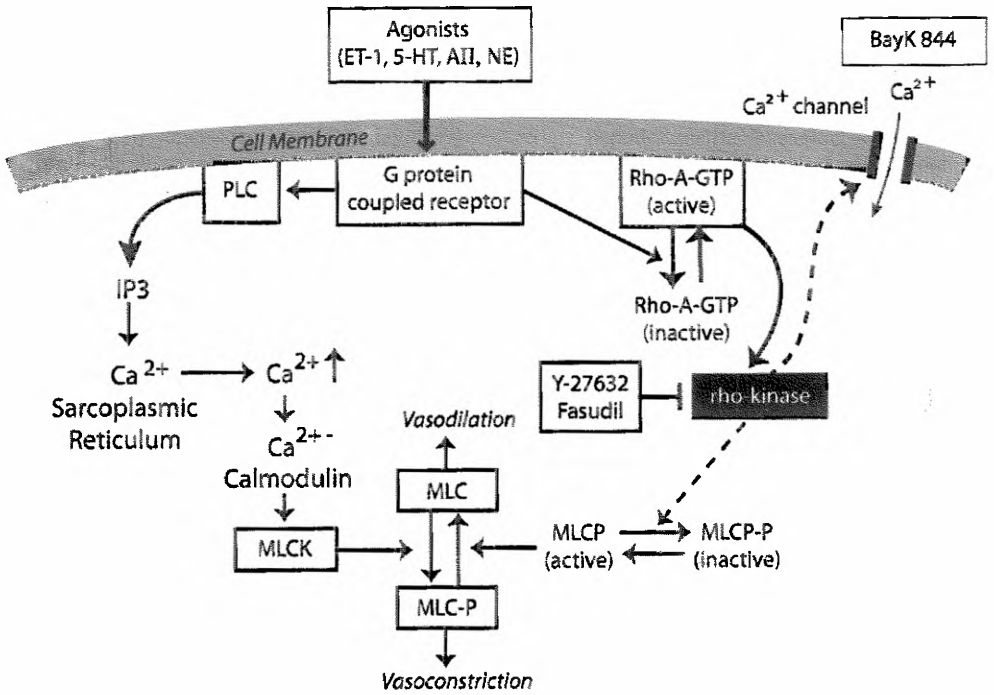

Fig. (1). Modified from [87].

Fig. (1): Schematic drawing showing the interactions of receptor operated calcium channels and store-operated calcium channels and of rho-kinase in activation of myosin light chains in vascular smooth muscle. Myosin light chain (MLC) phosphorylation (MLC-P) and vascular smooth muscle (VSM) cell contraction are primarily determined by the balance between MLC kinase (MLCK) that induces VSM contraction, and MLC phosphatase (MLCP) that induces VSM relaxation. Increases in intracellular Ca^{2+} concentrations, Ca^{2+} entry (Ca^{2+} channels) and from receptor-operated calcium channels (G protein coupled receptor due to agonist interactions with ET-1, endothelin-1; 5-HT, serotonin; AII, angiotensin II; or NE, norepinephrine) with subsequent Ca^{2+} release from the sarcoplasmic reticulum (SR), increase intracellular Ca^{2+}-Calmodulin complex that stimulates MLCK and induces VSM contraction. In the resting state, RhoA/GDP exists in the cytosol. However, with activation of certain trimeric G proteins, RhoA/GDP migrates and is exchanged to RhoA/GTP (active) on the membrane of the VSM cell where it interacts with rho-kinase to initiate increased phosphorylation of MLCP (active) to MLCP-P (inactive). MLCP opposes the action of MLCK. MLCP, myosin light chain phosphatase (active); MLCP-P, myosin light chain phosphatase (inactive); PLC, phospholipase C; IP3, inositol triphosphate.

Table 1. Animal and Human Investigations into the Role of the RhoA/rho-kinase Pathway in Cardiovascular Diseases and in Other Disorders (Animal Studies : Human Studies)

Role of the RhoA/rho-kinase Pathway			
Vascular Smooth Muscle Contraction	Arteriosclerotic Diseases	Other Smooth Muscle Disorders	Other Disorders
Coronary vasospasm [93-100] : [101-107]	Angina [97, 108-112] : [101, 103, 105-107, 113, 114]	Bronchial asthma [90, 115-127] : [128-141]	Osteoporosis : [142]
Cerebral vasospasm [95, 143-154] : [76]	Myocardial infarction [112, 155-161] : [106, 114, 162-167]	Glaucoma [168-171] : [172-176]	Erectile dysfunction [177-186] : [187-190]
Systemic hypertension [191-195] : [196-199]	Restenosis [200-206] : [207]	Cerebral injury : [163, 208-217]	Renal disease [86, 218-230] : [231-240]
Pulmonary hypertension [51, 52, 67, 69, 86, 193, 241-254] : [48, 49]	Stroke [152, 155, 255-268] : [210, 213, 216, 269-271]		Cancer [272-288] : [231, 289-325]
Sudden death : [326]	Heart failure [67, 158, 327-336] : [337-339]		Insulin resistance [340-351] : [271]
	Cardiac allograft vasculopathy [352] : [73, 353, 354]		Hypercholesterolemia [355]: [356]
	Vein graft disease [357-360] : [354, 361-363]		

as observed in other, more common CVD [6, 32, 37-41]. Studies in animal models of PH and in patients with PH suggest that small GTPase, RhoA, and its downstream effector, rho-kinase, may play a key role in the pathogenesis of PH [42-49]. Most of our knowledge about the cardiovascular effects of rho-kinase inhibition has come from human observations, in-vitro studies, and chronic injury models such as chronic hypoxia- and monocrotaline- induced PH (Table 1) and we review these studies in the following sections.

Hypoxic Mediated Pulmonary Vasoconstriction

Pulmonary vasoconstriction is believed to be an early component of PH and is due to an imbalance of endogenous vasoconstrictors to vasodilators, with calcium ions playing a primary role in regulating VSM contraction [17, 25, 44, 50-55]. Hypoxia can induce PH in part by inhibiting oxygen-sensitive, voltage-gated potassium channels in pulmonary artery smooth muscle cells [56, 57]. Hypoxia has also been shown to increase Rho-kinase expression and activity, as well as significantly decrease eNOS mRNA and inhibit endothelial NO synthase (eNOS) activity [53]. Moreover, several mechanisms mediate Ca^{2+}-sensitization through inhibition of MLCP *via* activation of RhoA/rho-kinase (Fig. 1) [58, 59]. Hypoxia has been shown to activate rho-kinase signaling in pulmonary arteries, in small pulmonary resistance vessels, and in cultured pulmonary arterial smooth muscle cells from rats, and that rho-kinase has been hypothesized to have an

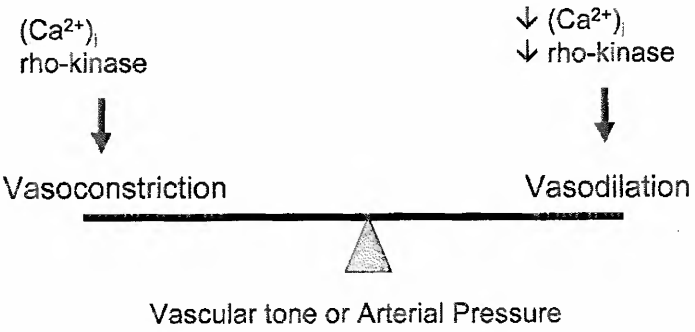

Fig. (2). Activation of rho-kinase or an increase in cytosolic Ca^{2+} $(Ca^{2+})_i$ leads to vasoconstriction, whereas a decrease in $(Ca^{2+})_i$ or a decrease in rho-kinase activity leads to vasodilation.

important role in mediating the sustained phase of acute hypoxic pulmonary vasoconstriction (HPV) [60, 61]. Chronic hypoxia-induced augmentation of RhoA- and rho-kinase- induced pulmonary vascular smooth muscle Ca^{2+}-sensitization were observed to be associated with elevated RhoA and rho-kinase activity and increased rho-kinase expression [62]. Moreover, inhibition of rho-kinase abolishes the sustained phase of hypoxic vasoconstriction in rat pulmonary arteries and in perfused lungs [60, 61] possibly through activation of MLCP with resultant decreased phosphorylation of myosin light chains (Fig. 1) [61]. Increases in eNOS protein have been observed in rho-kinase inhibitor-treated lungs during exposure to hypoxia suggesting that an upregulation of eNOS is involved in the attenuation of HPV [63]. Moreover, it has been shown that an increased sensitivity to NO has been observed in pulmonary arteries exposed to chronic hypoxia [64, 65], and that enhanced endothelial-derived NO reactivity following long-term hypoxia may be a function of elevated eNOS expression [63, 66-69].

Monocrotaline Mediated Pulmonary Vasoconstriction

Subcutaneous injection of a pyrrolizidine alkaloid, monocrotaline, in rats can result in the development of severe PH, with associated remodeling of the lung vasculature, increased mortality rate, and is used as an experimental model to study PH [70, 71]. The role of the RhoA/rho-kinase pathway has been examined in this experimental model [70]. Animals received a subcutaneous injection of monocrotaline, which resulted in the development of severe PH, right ventricular hypertrophy, and pulmonary vascular lesions in 3 weeks and these events were associated with a high mortality rate [70]. In some experiments, the simultaneous administration of fasudil with monocrotaline strikingly reduced the development of PH, right ventricular hypertrophy, pulmonary vascular lesions, and markedly improved the survival rate [70]. Moreover, a suppression of VSM proliferation and macrophage infiltration enhanced VSM apoptosis, and amelioration of endothelial dysfunction and of VSM hypercontraction were also observed [70]. In another series of experiments, animals were allowed to develop monocrotaline-induced PH and were then treated with fasudil. In these animals with established PH, the administration of fasudil produced a reduction in right ventricular systolic pressure as well as a reduction in systemic arterial pressure and also an improved survival rate [70]. These results indicate that rho-kinase is upregulated under these conditions and is involved in the pathogenesis of PH but that treatment with a rho-kinase inhibitor has pharmacologic benefit [70]. Similar hemodynamic findings were observed in another experimental model of established monocrotaline-induced PH in rats, where the oral administration of fasudil in progressive doses, was able to produce a selective reduction in pulmonary arterial pressure without causing significant reductions in systemic arterial pressure (fasudil, 10 mg/kg) [72]. However, at the highest dose studied (fasudil, 30 mg/kg), both pulmonary and systemic arterial pressures were reduced [72]. These data suggest that rho-kinase activity was upregulated in pulmonary arteries, but not in the systemic vascular bed following administration of monocrotaline, in that the 10 mg/kg dose of fasudil inhibited only rho-kinase activity in pulmonary arteries without an effect in the aorta and suggest that fasudil can produce a dose-dependent, selective vasodilatation of the pulmonary vascular bed in this model of experimental PH [72].

High-Flow Mediated Pulmonary Hypertension

The role of the RhoA/rho-kinase pathway was investigated in the pathogenesis of high blood-flow induced PH model in rats [51]. In this study, the animals underwent a carotid artery/jugular vein shunt procedure producing high pulmonary blood flow that results in both acute and chronic elevation of right ventricular systolic pressure, significant pulmonary artery medial wall thickening, right ventricular hypertrophy, and an increase in RhoA and rho-kinase activity [51]. Following treatment with fasudil, lower pulmonary artery systolic pressure, suppression of pulmonary artery smooth muscle cell proliferation, attenuation of pulmonary artery medial wall thickening, and inhibition of right ventricular hypertrophy were observed [51]. Moreover, a significant suppression of rho-kinase activity, but not RhoA activity, was observed [51]. These data suggest that the RhoA/rho-kinase pathway is associated with both the acute pulmonary arterial hypertension and in the chronic pulmonary vascular remodeling seen in high flow-induced PH, and that the rho-kinase inhibitor, fasudil, could improve the hemodynamic and vascular changes seen in this experimental form of PH [51].

Additional Studies of RhoA/rho-kinase Pathway

RhoA has been shown to modulate Ca^{2+}-independent contraction in permeabilitized VSM cells and with the development of first-generation rho-kinase inhibitors, the role of this pathway can be investigated in intact vascular preparations [11, 73, 74]. One of the best characterized rho-kinase inhibitors, Y-27632 a pyridine derivative, selectively targets p160/rho-kinase from the family of RhoA-associated protein kinases [75]. Studies have shown that Y-27632 binds to and inhibits this rho-kinase with a nearly 200-fold greater affinity when compared to other protein kinases, such as protein kinase C, or cAMP-dependent protein kinase [75]. This protein kinase inhibitor as well as another rho-kinase inhibitor, fasudil, which was developed as a clinical agent to suppress the development of cerebral vasospasm following subarachnoid hemorrhage in humans [76], were used to investigate the physiologic role of the RhoA/rho-kinase pathway under baseline conditions and during periods of enhanced vasoconstrictor tone induced with the thromboxane A_2 mimic, U-46619, under conditions of reduced endothelial NO production, and during acute hypoxia in an intact chest rat model [77]. It is our hypothesis that rho-kinase inhibition promotes vasodilation that is independent of the mechanism that increases intracellular Ca^{2+} and increases vasoconstrictor tone (Fig. 3). The purpose of this review is to discuss the role of rho-kinase in the regulation of vasoconstrictor function in the pulmonary vascular bed.

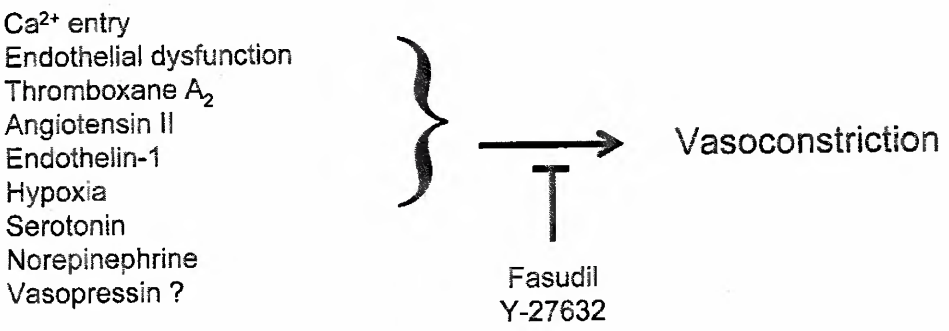

Fig. (3). It is our hypothesis that rho-kinase inhibitors promote vasodilation independent of the mechanism that increases vasoconstrictor tone.

Responses under Baseline and Enhanced Tone Conditions

It has been reported that the rho-kinase inhibitor, fasudil, inhibited hypoxia and potassium chloride-induced contraction in rat pulmonary arterial VSM cells and inhibited responses to potassium chloride in isolated perfused rat lungs, suggesting that Ca^{2+} entry was impaired [78]. In a recent study, it was demonstrated that fasudil has significant vasodilator activity in the pulmonary and systemic vascular beds of the rat under baseline conditions suggesting that the rho-kinase pathway is constitutively active under normal physiologic conditions [74]. The non-selective NO synthase (NOS) inhibitor, L-NAME, produced large increases in total pulmonary and systemic vascular resistance that could be reversed by fasudil, by a calcium channel blocker, isradipine, and by an NO donor. Following treatment with the NOS inhibitor, pulmonary and systemic vasodilator responses to fasudil were increased, and pulmonary vasodilator responses became dose dependent. Moreover, when decreases in pressure in response to fasudil were compared on a percent-decrease basis to normalized values, decreases in pulmonary arterial pressure in response to lower doses of fasudil were greater than the decreases in systemic arterial pressure after L-NAME treatment, whereas a different pattern of response was observed with the calcium channel blocker, isradipine [74]. These data are consistent with the results in the fetal lamb pulmonary circulation and in the isolated hypoxia-exposed perfused rat lung in which vasoconstrictor responses to another NOS inhibitor, L-NA, were prevented or reversed by Y-27632 or by fasudil [52, 55]. The results with the NOS inhibitor and Y-27632 indicate that NO plays an important role in the maintenance of baseline tone and in modulating the response to hypoxia in the pulmonary vascular bed and the observations in other studies [74, 77]. The results of experiments in the intact chest rat suggest that the RhoA/rho-kinase pathway is involved in the normal physiological regulation of tone in both the pulmonary and systemic vascular beds, and that rho-kinase, or increased Ca^{2+} entry, can mediate the increase in vasoconstrictor tone observed when NO synthesis is inhibited in the intact chest preparation [74]. The explanation for the difference in results with L-NAME in the intact-chest rat and in isolated perfused rat lung preparations may involve differences in blood flow, which is much lower in the isolated lung [57, 74, 77-79].

Although it is known that Y-27632 decreases pulmonary arterial pressure in rodents with monocrotaline- and chronic hypoxia- induced PH and that the response to acute hypoxia is reversed [55, 60, 61, 63, 78, 80-87], less is known about responses to this rho-kinase inhibitor when tone is increased on an acute basis with vasoconstrictor agonists. Activation of the thromboxane receptor to induce VSM contraction has been suggested to occur through activation of rho-kinase [88]. Moreover, responses to Y-27632 have not been compared in the pulmonary and systemic vascular beds in an intact animal preparation [55, 70, 80, 89]. In experiments in the intact chest rat, the administration of Y-27632 decreased pulmonary and systemic arterial pressure in a dose related manner. Inasmuch as cardiac output was not decreased and left ventricular end-diastolic pressure, as a measure of left atrial pressure, was unchanged, the decreases in pressure reflect decreases in pulmonary and systemic vascular resistances. The decreases in pulmonary arterial pressure were modest under baseline conditions reflecting the low level of tone in the pulmonary vascular bed and were similar to responses to other vasodilator agents under baseline tone conditions. However, when baseline tone was increased with an U-46619 infusion, the iv injections of Y-27632 produced larger dose-dependent decreases in pulmonary arterial pressure. These data are in agreement with the observations that the vasoconstrictor effect of thromboxane receptor activation is dependent upon rho-kinase activation [90]. Studies have examined the role of thromboxane induced vascular resistance in an isolated lung preparation in the rat, and following rho-kinase inhibition with Y-27632, attenuated pulmonary vasoconstrictor responses to the thromboxane A_2 mimic, U-46619, were observed [91]. These studies are in agreement with other studies showing that Y-27632 and fasudil caused complete relaxation of U-46619-induced vasoconstriction in human internal mammary arteries [73], whereas Y-27632 could only partially inhibit U-46619-induced contractions in bovine middle cerebral arteries [92].

Additionally, the effects of acute hypoxia on the pulmonary vascular bed and the response to Y-27632 during acute hypoxia were also investigated in the intact chest rat model. Ventilation with the 10% O_2/90% N_2 gas mixture increased pulmonary arterial pressure. Systemic arterial pressure and cardiac output were decreased and cardiac output returned back towards control values during the period of hypoxia. The administration of the potent rho-kinase inhibitor, Y-27632, reversed the pulmonary hypertensive response to acute ventilatory hypoxia. Moreover, prior administration of Y-27632 before ventilation with the hypoxic gas mixture prevented the increase in pulmonary arterial pressure in response to hypoxic gas ventilation. The decreases in pulmonary and systemic arterial pressure in response to Y-27632 and to fasudil were compared in U-46619-infused animals and show that the dose response curves for fasudil are approximately one half log unit to the right of the curves for Y-27632, indicating greater potency for Y-27632 and that both agents have similar efficacy and similar effects on the pulmonary and systemic vascular beds in the rat (unpublished observations).

New information from recent studies is that administration of the rho-kinase inhibitor, Y-27632, has vasodilator activity in the pulmonary and systemic vascular beds under normal physiologic conditions. Moreover, pulmonary vasodilator responses to Y-27632 were enhanced when baseline tone is increased by either the thromboxane A_2 agonist, U-46619, or by the non-selective NOS inhibitor, L-NAME, and that the rho-kinase inhibitor prevents or reverses the response to acute hypoxia. The results with the NOS inhibitor indicate that NO plays an important role in the maintenance of baseline tone and in modulating the response to hypoxia in the pulmonary vascular bed. Comparison of the relative decreases in pulmonary and systemic arterial pressure under elevated tone conditions indicates that the rho-kinase inhibitor has similar vasodilator activity in the pulmonary and systemic vascular beds. The observation that Y-27632 promoted vasodilation under resting and enhanced tone conditions provide support for the hypothesis that the rho-kinase Ca^{2+}-

sensitization pathway is tonically active under physiologic conditions. The data indicate that the relative decreases in pulmonary and systemic arterial pressure under elevated tone conditions are similar and suggest that rho-kinase mediated Ca^{2+}-sensitization plays an important role in both vascular beds and that the highly selective rho-kinase inhibitor, Y-27632, does not have selective pulmonary vasodilatory activity in the intact chest rat under physiologic conditions. Finally, the observation that Y-27632 can promote vasodilation when tone is increased by diverse mechanisms, may in part explain the beneficial effects of rho-kinase inhibition in the treatment of CVD and other disorders that have different etiologies (Table 1). These observations are in contrast with other studies that have shown that the rho-kinase inhibitor, fasudil, has selective vasodilator activity in the rat, but without decreasing systemic arterial pressure [72]. In this study of monocrotaline-induced PH in rats, decreases in the pulmonary arterial pressure were seen in response to fasudil without a decrease in systemic arterial pressure [72]. In another study in an hypoxic-induced PH model, the short-term inhalation of Y-27632 produced decreases in mean pulmonary arterial pressure without reducing mean systemic arterial pressure suggesting selective pulmonary vasodilator activity, whereas in the same study, acute oral administration of Y-27632 produced sustained decreases in both mean pulmonary arterial pressure and in mean systemic arterial pressure [87]. These data suggest that when delivered locally to the lung by aerosol, a selective pulmonary vasodilator response could be achieved [87]. However in studies with hypoxic ventilation, decreases in both pulmonary and systemic arterial pressure were observed and there was no selective effect. Finally, in two human studies, eight female patients in the first study underwent acute evaluation of the right heart catheterization hemodynamic responses to an intravenous infusion of fasudil, 1 mg/min over thirty minutes [48]. Five patients had idiopathic PH and three had collagen vascular disease and all patients had pulmonary arterial pressure greater than 25 mm Hg and a New York Heart Association functional class II or III. The administration of fasudil produced significant decreases in pulmonary vascular resistance (~24%) and small, but significant, decreases in mean pulmonary arterial pressure, and increases in cardiac index [48]. Systemic vascular resistance and systolic systemic arterial pressure were decreased, but that the decrease in the latter was small [48]. In the second human study, nine patients with PH underwent right heart catheterization and were administered fasudil, 30 mg iv over 30 minutes [49]. In this study, significant decreases in pulmonary vascular resistance (~17%) were observed in this group along with observed increases in cardiac output, but without significant changes in systemic arterial pressure [49]. These findings suggest that rho-kinase was upregulated in the lungs of these patients in that pulmonary arterial pressure was decreased in a selective manner [48, 49]. The decreases in systemic arterial pressure are smaller than observed in the intact chest rat, and may be due to the significant increases in cardiac output seen in the human studies with established PH that could offset decreases in systemic arterial pressure [48, 49]. It is also possible that upregulation of rho-kinase in the pulmonary vascular bed could explain the selective effect. Finally, rho-kinase inhibition has been shown to have beneficial effects when investigated in systemic vascular disorders such as coronary vasospasm, cerebral vasospasm, as well as in systemic hypertension and congestive heart failure (Table 1). These systemic studies suggest that this pathway is important in the systemic circulation as well as in the pulmonary circulation and that rho-kinase inhibitors are effective in the treatment of pulmonary and systemic hypertensive disorders.

CONCLUSION

The study of cellular responses to extracellular signals and their transduction mechanisms have provided novel therapeutic approaches for the treatment of CVD and other disorders (Table 1). The first-generation rho-kinase inhibitors, fasudil and Y-27632, have been shown to modulate the RhoA/rho-kinase pathway and that alterations in this pathway play a major role in regulation of vasoconstrictor tone. These findings suggest that rho-kinase mediated Ca^{2+}-sensitization is a constitutively active process that plays a major role in the physiologic regulation of vasoconstrictor tone in the pulmonary and systemic vascular beds and in mediating the pulmonary vasoconstrictor responses. The observation that rho-kinase inhibitors promote vasodilation when tone is increased by diverse mechanisms, including NOS inhibition, may in part explain the beneficial effects of rho-kinase inhibitors in CVD and other disorders including PH in which endothelial dysfunction is present and vasoconstrictor tone is increased by diverse mechanisms which increase $(Ca^{2+})_i$ concentrations in VSM cells. Moreover many studies suggest that the RhoA/rho-kinase pathway has an important role in the early adaptive response of VSM under elevated tone conditions and support our hypothesis that inhibition of rho-kinase will promote vasodilation independent of the mechanism used to increase intracellular Ca^{2+} levels and induce vasoconstriction (Fig. 3).

It is our hypothesis that inhibition of rho-kinase will promote vasodilation independent of the mechanism used to increase intracellular Ca^{2+} levels and induce vasoconstriction.

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