Analysis of pulmonary vasodilator responses to the Rho-kinase inhibitor fasudil in the anesthetized rat

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Badejo AM Jr, Dhaliwal JS, Casey DB, Gallen TB, Greco AJ, Kadowitz PJ. Analysis of pulmonary vasodilator responses to the Rho-kinase inhibitor fasudil in the anesthetized rat. Am J Physiol Lung Cell Mol Physiol 295: L828–L836, 2008. First published August 8, 2008; doi:10.1152/ajplung.00402.2008.—The small GTP-binding protein Rho and its downstream effector, Rho-kinase, are important regulators of vasoconstrictor tone. Rho-kinase is upregulated in experimental models of pulmonary hypertension, and Rho-kinase inhibitors decrease pulmonary arterial pressure in rodents with monocrotaline and chronic hypoxia-induced pulmonary hypertension. However, less is known about responses to fasudil when pulmonary vascular resistance is elevated on an acute basis by vasoconstrictor agents and ventilatory hypoxia. In the present study, intravenous injections of fasudil reversed pulmonary hypertensive responses to intravenous infusion of the thromboxane receptor agonist, U-46619 and ventilation with a 10% O2 gas mixture and inhibited pulmonary vasoconstrictor responses to intravenous injections of angiotensin II, BAY K 8644, and U-46619 without prior exposure to agonists, which can upregulate Rho-kinase activity. The calcium channel blocker isradipine and fasudil had similar effects and in small doses had additive effects in blunting vasoconstrictor responses, suggesting parallel and series mechanisms in the lung. When pulmonary vascular resistance was increased with U-46619, fasudil produced similar decreases in pulmonary and systemic arterial pressure, whereas isradipine produced greater decreases in systemic arterial pressure. The hypoxic pressor response was enhanced by 5–10 mg/kg iv nitro-L-arginine methyl ester (l-NAME), and fasudil or isradipine reversed the pulmonary hypertensive response to hypoxia in control and in l-NAME-treated animals, suggesting that the response is mediated by Rho-kinase and L-type Ca2+ channels. These results suggest that Rho-kinase is constitutively active in regulating baseline tone and vasoconstrictor responses in the lung under physiological conditions and that Rho-kinase inhibition attenuates pulmonary vasoconstrictor responses to agents that act by different mechanisms without prior exposure to the agonist.

Rho-kinase pathway; Ca2+ sensitization; pulmonary vascular bed; U-46619; isradipine; hypoxia; nitro-L-arginine methyl ester

The small GTP-binding protein Rho and its downstream effector Rho-kinase play an important role in the regulation of vascular smooth muscle tone (3, 4, 6, 18, 20, 28, 34, 36). It has been hypothesized that the Rho-kinase system is constitutively active in regulating vasoconstrictor tone and that upregulation of this pathway occurs in a variety of cardiovascular diseases (8, 19, 21, 22, 32, 33, 35). Rho-kinase has been shown to be a potential therapeutic target in a number of cardiovascular diseases including pulmonary hypertension (1, 2, 10, 12, 15, 16, 26, 27, 29, 38). It has been reported that Rho-kinase is upregulated in monocrotaline and chronic hypoxia-induced pulmonary hypertension and that Rho-kinase inhibitors have a beneficial effect in the treatment of pulmonary hypertension in rodent models and in patients (1, 2, 10, 12, 15, 16, 26, 29, 38). It has also been reported that chronic hypoxia induces Rho-kinase-dependent myogenic tone in small pulmonary arteries from the rat (7). Although it has been reported that fasudil, a selective Rho-kinase inhibitor, decreases pulmonary arterial pressure when pulmonary vascular resistance is increased by chronic hypoxia, monocrotaline, or nitro-L-arginine methyl ester (l-NAME), less is known about the responses to the Rho-kinase inhibitor when pulmonary vascular resistance is increased on an acute basis by vasoconstrictor agents or hypoxia in a physiological setting (9, 10, 30). The present study was therefore undertaken to investigate responses to fasudil when pulmonary vascular resistance was increased with U-46619 and ventilation with a 10% O2–90% N2 gas mixture. The effects of fasudil on pulmonary vasoconstrictor responses to intravenous injections of angiotensin II, the calcium channel opener BAY K 8644, and the thromboxane mimic U-46619 were also investigated in the rat, and pulmonary vasodilator responses to fasudil and the calcium channel blocking agent isradipine were compared. The results of these studies show that fasudil reverses U-46619 and hypoxia-induced pulmonary hypertension and inhibits pulmonary vasoconstrictor responses to angiotensin II, BAY K 8644 and U-46619 without prior exposure to the agonists, which can upregulate Rho-kinase activity. These data suggest that Rho-kinase is constitutively active and can play a role in the regulation of baseline tone and vasoconstrictor responses in the pulmonary vascular bed of the rat under physiological conditions.

Methods

The experimental protocols used in these studies were approved by the Animal Care Committee of the Tulane University Medical School, and all procedures were conducted in accordance with institutional guidelines. For these experiments, adult male Sprague-Dawley rats (Charles River) weighing 260–400 g were anesthetized with Inactin (100 mg/kg ip) and placed in a supine position on a catheterization table. Supplemental doses of Inactin were administered intravenously to maintain a uniform level of anesthesia. Body temperature was maintained by a heating lamp. The trachea was cannulated with a short segment of PE 240 tubing to maintain a patent airway, and the animals spontaneously breathed room air. A femoral artery was catheterized with PE 50 tubing for the measurement of systemic arterial pressure, and the left jugular and femoral veins were catheterized with PE 50 tubing for intravenous injections or infusions of...
drugs and fluids. For measurement of pulmonary arterial pressure, a specially designed single lumen 3F catheter with a radio-opaque marker and curved tip was passed from the right jugular vein into the right ventricle and main pulmonary artery under fluoroscopic guidance (Picker Surveyor), as described previously (9, 13). Pulmonary and systemic arterial pressures were measured with NAMIC Perceptor transducers (Boston Scientific), digitized by a BIOPAC MP100 data acquisition system, and stored on a Dell PC. Cardiac output was measured by the thermodilution technique. A known volume (0.2 ml) of indicator 0.9% NaCl at room temperature was injected into the jugular vein catheter with its tip near the right atrium. Changes in blood temperature were measured by a 1.5-Fr thermistor microprobe (Columbus Instruments) positioned in the aortic arch from the left carotid artery.

In the first set of experiments, changes in pulmonary and systemic arterial pressure and cardiac output in response to intravenous injections of graded doses of fasudil (HA-1077, LC Laboratories), 1-(5-isooquinolinesulfonyl)homopiperazine, and isradipine (Sigma-Aldrich) were investigated under control baseline conditions. The order of injection of the various doses of the agents was randomized, and sufficient time (30–60 min for fasudil and isradipine) was permitted between injections for parameters to return toward baseline values.

In the second set of experiments, changes in pulmonary and systemic arterial pressure in response to intravenous injections of fasudil and isradipine were measured when pulmonary arterial pressure was increased to a high steady level by intravenous infusion of U-46619. In these experiments, U-46619 infusion was started at a high rate and then reduced to 160–240 ng/min to maintain pulmonary arterial pressure at ~30 mmHg. U-46619 (Cayman) was dissolved in 95% ethanol and diluted in 0.9% sodium chloride solution. Isradipine was dissolved in 0.9% sodium chloride and 0.1% Tween 80 (Sigma-Aldrich), fasudil was dissolved in 0.9% saline, and solutions were prepared daily or every other day.

The effect of ventilatory hypoxia on the response to fasudil and isradipine was investigated, and the animals breathed a 10% O2-90% N2 gas mixture from a plastic hood placed over the end of the endotracheal tube. The period of ventilation with hypoxic gas lasted 4–6 min and could be repeated a number of times, and the effect of treatment with 5–10 mg/kg iv L-NAME (Sigma-Aldrich) on the response to hypoxia was also investigated. Arterial PO2, PCO2, and pH were measured with a Radiometer (Copenhagen, Denmark) NPT7 series analyzer blood gas analyzer from a 0.2-ml blood sample from the femoral artery catheter.

The effect of intravenous injection of fasudil on increases in pulmonary arterial pressure in response to intravenous injections of U-46619, angiotensin II, and BAY K 8644 was investigated. Angiotensin II (Sigma-Aldrich) was dissolved in 0.9% saline, and BAY K 8644 (Tocris) was dissolved in a 0.9% saline and 0.1% Tween 80 solution (Sigma-Aldrich). In these experiments, both a pretreatment and reversal (posttreatment) protocol were followed. With the pretreatment protocol, fasudil (3 mg/kg) or isradipine (0.1 mg/kg) was injected intravenously 5–10 min before intravenous injections of U-46619, angiotensin II, or BAY K 8644. In the reversal protocol, U-46619, angiotensin II, or BAY K 8644 were injected before and 5–10 min after intravenous injection of fasudil (3 mg/kg iv) or isradipine (0.1 mg/kg iv).

Fig. 1. Bar graphs comparing changes in pulmonary and systemic arterial pressure, cardiac output, and pulmonary and systemic vascular resistance in response to intravenous injections of fasudil under baseline conditions and during infusion of U-46619. Bottom, right bar graphs show pulmonary arterial pressure in the control period, during infusion of U-46619 and after injection of fasudil (3 mg/kg iv). *P < 0.05 compared with control.
To quantify lung Rho-kinase activity, protein expression of the total and phosphorylated (p) ERM proteins (ezrin, radixin, and moesin), a substrate for Rho-kinase, were analyzed by Western blotting of total protein extracts from the lung of rats subjected to U-46619, angiotensin II infusion, and l-NAME administration and from control rats. Membranes were blocked with 5% milk for 1 h, washed, and then incubated overnight at 4°C with antibodies directed against pERM and ERM (1:1,000; Cell Signaling Technology, Danvers, MA) in 5% milk. The membranes were again washed and incubated with horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (1:5,000) for 1 h at room temperature. The protein bands were visualized with an ECL detection kit (KPL, Gaithersburg, MD) after exposure to X-ray film. The extent of ERM phosphorylation was normalized to total ERM (1).

The data are expressed as means ± SE. For comparison of decreases in systemic and pulmonary arterial pressures during U-46619 infusion and with l-NAME treatment, the decreases in pressure were expressed as percent decrease to normalize values. The relationship between decreases in pulmonary arterial pressure and baseline pressure was analyzed by least squares regression. Total pulmonary vascular resistance was calculated by dividing mean pulmonary arterial pressure by the cardiac output and is referred to as pulmonary vascular resistance in the manuscript. Left ventricular end-diastolic pressure was measured as an index of left atrial pressure in some experiments and was not changed by intravenous injections of fasudil. Systemic vascular resistance was calculated by dividing mean systemic arterial pressure by the cardiac output. The data were analyzed by paired or group t-tests or an ANOVA with Dunnett’s post hoc test. The criterion for statistical significance was P < 0.05.

RESULTS

Responses to fasudil and isradipine. Under baseline conditions, intravenous injections of fasudil or isradipine caused small decreases in pulmonary arterial pressure, larger dose-dependent decreases in systemic arterial pressure, and no change or increases in cardiac output (Figs. 1 and 2). The Rho-kinase inhibitor and calcium entry blocking agent decreased pulmonary and systemic vascular resistance (Figs. 1 and 2). Responses to fasudil and isradipine were compared when pulmonary vascular resistance was increased by U-46619. The intravenous infusion of U-46619 increased pulmonary arterial pressure with little effect on systemic arterial pressure and decreased cardiac output (Table 1). Pulmonary vascular resis-
tance was increased by 112%, whereas systemic vascular resistance was increased by 39%. During infusion of U-46619, when tone in the pulmonary vascular bed was at a high steady level, intravenous injections of fasudil or isradipine caused dose-dependent decreases in pulmonary and systemic arterial pressures, increases in cardiac output except at the highest dose of isradipine, and decreases in pulmonary and systemic vascular resistances (Figs. 1 and 2). The reversal of the increase in pulmonary arterial pressure produced by U-46619 by fasudil or isradipine is shown in the bottom right of Figs. 1 and 2.

Pulmonary arterial pressure was increased significantly by U-46619 infusion. The intravenous injection of 3 mg/kg fasudil (Fig. 1) or 0.1 mg/kg isradipine (Fig. 2) decreased pulmonary arterial pressure to values not significantly different than baseline pressure ($P < 0.05$, ANOVA and Dunnett’s test).

Comparison of pulmonary and systemic responses. The relative decreases in pulmonary and systemic arterial pressures in response to fasudil and isradipine were compared under baseline and elevated tone conditions, and these data are summarized in Fig. 3. When responses are expressed as percent decrease to normalize values in the pulmonary and systemic vascular beds under baseline conditions, the percent decreases in systemic arterial pressure are significantly greater than percent decreases in pulmonary arterial pressure in response to both agents (Fig. 3). However, when responses are compared during infusion of U-46619, which produced a greater increase in pulmonary than systemic vascular resistance, the percent decreases in systemic and pulmonary arterial pressure in response to fasudil were similar, whereas the percent decreases in systemic arterial pressure were greater than the percent decreases in pulmonary arterial pressure in response to isradipine (Fig. 3).

The relationship between pulmonary arterial pressure and decreases in pulmonary arterial pressure in response to fasudil and isradipine when pulmonary arterial pressure was increased with U-46619 was examined, and these data are shown in Fig. 4. There was a significant correlation between the decreases in

### Table 1. Effect of U-46619 infusion on systemic and pulmonary arterial pressure, cardiac output, and heart rate

<table>
<thead>
<tr>
<th></th>
<th>Systemic Arterial Pressure, mmHg</th>
<th>Pulmonary Arterial Pressure, mmHg</th>
<th>Cardiac Output, ml/min</th>
<th>Heart Rate, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>97 ± 1.6</td>
<td>18 ± 0.3</td>
<td>110 ± 3.1</td>
<td>320 ± 6</td>
</tr>
<tr>
<td>U-46619 infusion</td>
<td>102 ± 2.0*</td>
<td>30 ± 0.4*</td>
<td>83 ± 3.1*</td>
<td>313 ± 6</td>
</tr>
</tbody>
</table>

$n = 54–69$

Values are means ± SE. *$P < 0.05$ compared with control.
vasodilator responses to fasudil

Fig. 4. Relationship between decreases in pulmonary arterial pressure in response to intravenous injection of 3 mg/kg fasudil and pulmonary arterial pressure (A) and decreases in pulmonary arterial pressure in response to intravenous injection of 0.1 mg/kg isradipine and pulmonary arterial pressure (B) when pulmonary arterial pressure was increased with an infusion of U-46619. *n* = number of experiments.

pulmonary arterial pressure in response to intravenous injections of fasudil (3 mg/kg) or isradipine (0.1 mg/kg) and the level of pulmonary arterial pressure (Fig. 4).

Responses to fasudil, isradipine, and hypoxia. Responses to fasudil and isradipine were investigated when the animals breathed 10% O2-90% N2 gas mixture, and these data are summarized in Fig. 5. Ventilation with the hypoxic gas decreased arterial PO2 and produced a significant increase in pulmonary arterial pressure, a significant decrease in systemic arterial pressure, and a significant decrease in cardiac output, which subsequently returned to baseline value (Table 2 and Fig. 5A). The increase in pulmonary arterial pressure in response to ventilation with 10% O2 gas mixture was reversed by the intravenous injection of fasudil (1 mg/kg) or isradipine (0.03 mg/kg) (Fig. 5A). Lower doses of fasudil and isradipine were used in these experiments than in L-NAME experiments because hypoxia decreases systemic arterial pressure, and systemic pressure was higher in L-NAME-treated animals.

The effect of L-NAME on the response to hypoxia was investigated, and these data are summarized in Table 3 and Fig. 5B. The administration of 5–10 mg/kg iv L-NAME caused a significant increase in pulmonary and systemic arterial pressures and a significant decrease in cardiac output (Table 3). When L-NAME-treated animals breathed the hypoxic gas, the increase in pulmonary arterial pressure was significantly greater than the decrease observed in control animals breathing hypoxic gas (Fig. 5). The intravenous injection of fasudil (3 mg/kg) or isradipine (0.1 mg/kg) produced a significant decrease in pulmonary arterial pressure in L-NAME-treated animals breathing the hypoxic gas mixture (Fig. 5B). The increase in pulmonary arterial pressure in response to ventilation with the 10% O2 gas mixture was significant in control and in L-NAME-treated animals. Pulmonary arterial pressure was not significantly different before and after intravenous injection of fasudil or isradipine (which reversed the pressor response) in control or in L-NAME-treated animals (*P* < 0.05, ANOVA and Dunnett’s test).

The observation that fasudil or isradipine were capable of reversing increases in pulmonary arterial pressure in response to U-46619 and hypoxia could be interpreted to suggest that calcium entry and Rho-kinase may act as parallel pathways to mediate vasoconstriction. To determine if these pathways could also interact in series responses to lower doses of these agents when given separately and when injected together were investigated. The intravenous injection of lower doses of fasudil or isradipine produced smaller decreases in pulmonary arterial pressure when given separately than when given together (Fig. 5C). The intravenous injection of combined smaller doses of fasudil and isradipine produced decreases in pulmonary arterial pressure that were similar to responses to larger doses of fasudil or isradipine when given alone in U-46619-infused animals (*P* < 0.05, ANOVA and Dunnett’s test).

Effect of fasudil and isradipine on pulmonary pressor responses to U-46619, angiotensin II, and BAY K 8644. The effect of fasudil or isradipine on increases in pulmonary arterial pressure in response to intravenous injections of U-46619, angiotensin II, and BAY K 8644 was investigated in pretreatment (paired) and posttreatment (unpaired) experiments, and these data are summarized in Fig. 6. The intravenous injections of U-46619, angiotensin II, and BAY K 8644 increased pulmonary arterial pressure, and the increases in pulmonary arterial pressure in response to U-46619, angiotensin II, and BAY K 8644 were decreased significantly when given after intravenous injection of fasudil (3 mg/kg) or isradipine 0.3 mg/kg iv (Fig. 6). When administered 5–10 min before the injection of the vasoconstrictor agents in another group of animals, the intravenous injection of fasudil (3 mg/kg) or isradipine (0.3 mg/kg) also significantly attenuated the increases in pulmonary arterial pressure in response to intravenous injections of U-46619, angiotensin II, or BAY K 8644 (Fig. 6).

Lung Rho-kinase activity. The activity of Rho-kinase in lung tissue was analyzed by Western blotting of protein extracts from the lung of rats subjected to angiotensin II infusion (200–1,000 ng/min) for 15–120 min, BAY K 8644 (10–100 μg/kg) for 15–30 min, U-46619 infusion for 30–120 min, or injection of L-NAME (50–100 mg/kg iv) and from control rats receiving saline infusion for phosphorylated and total ERM, and these data are summarized in Fig. 7. Activated Rho-kinase has been shown to directly phosphorylate COOH-terminal threonine residues of the ERM proteins to regulate their function (1), and relative Rho-kinase activity was determined as a
measure of the ratio of phosphorylated to total ERM (1, 16). Lungs from the control group had a basal level of Rho-kinase activity, with a pERM-to-ERM ratio of 0.94. In all three treatment groups, this ratio was significantly increased (L-NAME, 2.39; U-46619, 2.32; angiotensin II, 2.23, BAY K 8644, 3.11), indicating increased activation of Rho-kinase following administration of the vasoconstrictor agonists or L-NAME (Fig. 7). The increase in Rho-kinase activity could be observed in as little as 15 min or at periods up to 2 h (for U-46619 infusion) after treatment was initiated. Protein bands are shown for three animals in each treatment group; however, up to six animals were studied in each group, and the bands shown are representative of the group (Fig. 7).

**DISCUSSION**

New findings in this study are that fasudil and isradipine reversed U-46619 and hypoxia-induced pulmonary hypertension and attenuated pulmonary pressor responses to intravenous injections of angiotensin II, BAY K 8644, and U-46619, suggesting a role for Ca\(^{2+}\)/H\(^{+}\) sensitization and Ca\(^{2+}\)/H\(^{+}\) influx in mediating responses to the vasoconstrictor agents. Although it is known that fasudil decreases pulmonary arterial pressure in rodents with monocrotaline, chronic hypoxia, and L-NAME...
induced pulmonary hypertension, less is known about responses to the Rho-kinase inhibitor under physiological conditions when tone is increased on an acute basis (1, 2, 9, 10, 12, 15, 16, 27, 29, 30).

The results of the present study show that fasudil decreases pulmonary and systemic arterial pressures and vascular resistances under baseline conditions, suggesting that Rho-kinase-mediated Ca\(^{2+}\)/H\(^{+}\) sensitization has a constitutive role in regulating baseline tone in the pulmonary and systemic vascular beds. The observation that the decrease in systemic arterial pressure is greater may reflect the lower level of baseline tone in the pulmonary vascular bed or greater constitutive activity of Rho-kinase in the systemic vascular bed. The present results show that pulmonary vasodilator responses to fasudil are enhanced when baseline tone is increased with U-46619 or hypoxia. These results suggest that Rho-kinase-mediated Ca\(^{2+}\)/H\(^{+}\) sensitization plays a role in mediating the response to the thromboxane receptor agonist and hypoxia in the pulmonary vascular bed of the rat.

In addition to decreasing pulmonary arterial pressure to baseline value when pulmonary vascular resistance was elevated with U-46619 or hypoxia, the injection of fasudil before injection of U-46619, angiotensin II, or BAY K 8644 attenuated the increase in pulmonary arterial pressure in response to intravenous injection of the vasoconstrictor agents, which have been shown to upregulate Rho-kinase activity in smooth muscle (28, 29). The present results show that treatment with U-46619, angiotensin II, l-NAME, and BAY K 8644 that increased pulmonary arterial pressure increase phosphorylated ERM in lung tissue. The results of experiments showing that fasudil attenuated pulmonary pressor responses without prior exposure to the agonist suggest that the Rho-kinase inhibitor can attenuate vasoconstrictor responses without previous exposure to a stimulus for upregulation of Rho-kinase. It is also possible that pretreatment with fasudil prevented the upregulation of Rho-kinase by the vasoconstrictor agents in these experiments. These data suggest that inhibition of Rho-kinase can modify pulmonary vasoconstrictor responses and provide evidence in support of a constitutive role for the Rho-kinase system in the regulation of vasoconstrictor responses in the pulmonary vascular bed.

### Table 3. Effect of l-NAME on systemic and pulmonary arterial pressure, cardiac output, and blood gas values

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>l-NAME</th>
<th>%90 N(_2) -10% O(_2) Gas + l-NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic arterial pressure, mmHg</td>
<td>104±5</td>
<td>139±4*</td>
<td>99±6</td>
</tr>
<tr>
<td>Pulmonary arterial pressure, mmHg</td>
<td>17±1</td>
<td>26±1*</td>
<td>34±1*</td>
</tr>
<tr>
<td>Cardiac output, ml/min</td>
<td>120±3</td>
<td>96±5*</td>
<td>77±5*</td>
</tr>
<tr>
<td>pH</td>
<td>7.40±0.01</td>
<td>7.39±0.03</td>
<td>7.45±0.03</td>
</tr>
<tr>
<td>P(_{O_2})</td>
<td>80±2</td>
<td>78±5</td>
<td>41±2*</td>
</tr>
<tr>
<td>P(_{C0_2})</td>
<td>51±2</td>
<td>47±3</td>
<td>35±2*</td>
</tr>
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</table>

Values are means ± SE. n = 5–11. *P < 0.05 compared with control. l-NAME, nitro-l-arginine methyl ester.

Fig. 6. Bar graph comparing increases in pulmonary arterial pressure in response to intravenous injections of U-46619, angiotensin II (ANG II), and BAY K 8644 in paired treatment (A) (experiments in which the agonists were given before and after fasudil or isradipine) and unpaired (B) (pretreatment experiments in which the agonists were only given after fasudil or isradipine). n = number of experiments. *The response is significantly different than control.
Fasudil has been reported to inhibit calcium entry through store-operated and voltage-operated channels in rat pulmonary arterial smooth muscle cells and to attenuate pulmonary vasoconstrictor responses to depolarizing potassium solution in isolated rat lungs (30, 36). In the present study, both fasudil and isradipine decreased pulmonary and systemic vascular resistance. However, the calcium channel blocker caused a greater fall in systemic arterial pressure. Fasudil and isradipine had similar inhibitory effects on responses to intravenous injections of the vasoconstrictor agents suggesting that inhibition of calcium entry through voltage-dependent channels and Rho-kinase inhibition can attenuate pulmonary hypertensive responses. The observation that fasudil and isradipine reverse pulmonary hypertensive responses to U-46619 and hypoxia and are capable of decreasing pulmonary arterial pressure to baseline value may be interpreted to suggest that Rho-kinase-mediated Ca\(^{2+}\) sensitization and calcium entry are parallel mechanisms. The finding that responses to lower doses of fasudil and isradipine are additive and are similar when combined to responses to large doses of these agents suggest that the Rho-kinase and calcium entry pathways can also in some situations act in series. These conclusions about parallel and series mechanisms are highly speculative and may not take into account other factors such as the relationship between Ca\(^{2+}\) entry and Rho-kinase activity.

It has been reported that fasudil can selectively decrease pulmonary arterial pressure in rodent models and humans with pulmonary hypertension when Rho-kinase is upregulated (1, 16). It has also been shown that fasudil inhalation selectively decreases pulmonary arterial pressure (26). These data suggest that fasudil can produce a selective effect on the pulmonary vascular bed when Rho-kinase is upregulated on a chronic basis by a pathophysiological process or when the agent is delivered to lung in a selective manner by way of the airways (1, 12, 15, 16, 26, 29).

The observation that isradipine decreases pulmonary vascular resistance when baseline tone is increased with U-46619 is consistent with the hypothesis that calcium entry is involved in the mediation of the pulmonary vasoconstrictor response to U-46619. The pulmonary response to U-46619 can be inhibited with thromboxane receptor blocking agents and is not dependent on platelet aggregation or changes in bronchomotor tone, and this agent has greater effect on the pulmonary than systemic vascular bed (13, 14, 17, 24, 25). The observation that isradipine has substantial pulmonary vasodilator activity is consistent with studies showing that calcium entry blockers are useful in the treatment of the early phase of primary pulmonary hypertension (31).

The Rho-kinase inhibitor Y-27632 has been shown to inhibit acute hypoxic pulmonary vasoconstriction in both conscious normal and chronically hypoxic rats (27). The results of the present study showing that the response to hypoxia is reversed by fasudil are consistent with previous studies with Y-27632 (27) and are consistent with studies with chronic hypoxia in the isolated rat lung (27).

The administration of L-NAME in low doses increased pulmonary arterial pressure and enhanced the response to hypoxia. The intravenous injection of fasudil or isradipine reversed the pulmonary hypertensive response to hypoxia in L-NAME-treated animals. The dose of L-NAME used in this study is lower than doses used in our previous study and provides support for the hypothesis that constitutive release of NO from the endothelium plays an important role in the maintenance of low baseline tone and in modulating the pulmonary vasoconstrictor response to hypoxia in the intact rat and is consistent with results of previous studies (9, 11). The observation that the response to hypoxia can be reversed by fasudil or isradipine in L-NAME-treated rats suggests that calcium entry and/or sensitization may be involved in the vasoconstrictor response and is consistent with previous data (27).

In summary, the results of the present study show that fasudil and isradipine reversed U-46619 and hypoxia-induced pulmonary hypertension and attenuated pulmonary vasoconstrictor responses to intravenous injections of angiotensin II, BAY K 8644, and U-46619. Pulmonary vasodilator responses to fasudil and isradipine were dependent on the existing level of baseline tone, and the inhibitory effect of fasudil could be demonstrated without prior exposure to agonists, which can...
upregulate Rho-kinase activity in the lung. When pulmonary vascular resistance was increased with U-46619, fasudil and isradipine had similar vasodilator activity in the pulmonary vascular bed, whereas isradipine produced greater decreases in systemic arterial pressure suggesting that fasudil may have less systemic effects when used to treat pulmonary hypertension. The response to ventilatory hypoxia was increased by L-NAME-treated animals, suggesting that the response was mediated by Rho-kinase and L-type calcium channels. The results of these studies suggest that Rho-kinase is constitutively active in regulating baseline tone and vasoconstrictor responses in the pulmonary vascular bed of the rat under physiological conditions.

REFERENCES


