Role of Rho-kinase signaling and endothelial dysfunction in modulating blood flow distribution in pulmonary hypertension

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1Department of Physiology, University of Otago, Dunedin, New Zealand; 2Department of Physiology and Monash Centre for Synchrotron Science, Monash University, Melbourne, Australia; 3Department of Cardiac Physiology and 4Department of Pathology, National Cerebral and Cardiovascular Center Research Institute, Suita, Osaka, Japan; 5Department of Pathology, National Cerebral and Cardiovascular Center Research Institute, Suita, Osaka, Japan; and 6Japan Synchrotron Radiation Research Institute, Hyogo, Japan

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Schwenke DO, Pearson JT, Sonobe T, Ishibashi-Ueda H, Shimouchi A, Kangawa K, Umetani K, Shirai M. Role of Rho-kinase signaling and endothelial dysfunction in modulating blood flow distribution in pulmonary hypertension. J Appl Physiol 110: 901-908, 2011. First published January 6, 2011; doi:10.1152/japplphysiol.01318.2010.—Rho-kinase-mediated vasoconstriction and endothelial dysfunction are considered two primary instigators of pulmonary arterial hypertension (PAH). However, their contribution to the adverse changes in pulmonary blood flow distribution associated with PAH has not been addressed. This study utilizes synchrotron radiation microangiography to assess the specific role, and contribution of, Rho-kinase-mediated vasoconstriction and endothelial dysfunction in PAH. Male adult Sprague-Dawley rats were injected with saline (Cont-rats) or monocrotaline (MCT-rats) 3 wk before microangiography was performed on the left lung. We assessed dynamic changes in vessel internal diameter (ID) in response to L-NAME was accentuated in MCT-rats, but only in the 200- to 300-μm vessels. Moreover the vasoconstrictor response to L-NAME was accentuated in MCT-rats primarily in vessels with an ID < 200 μm. Moreover the vasoconstrictor response to L-NAME was accentuated in MCT-rats, but only in the 200- to 300-μm vessels. These results highlight the importance of Rho-kinase-mediated control and endothelial control of pulmonary vascular tone in PAH. Indeed, an effective therapeutic strategy for treating PAH should target both the smooth muscle Rho-kinase and endothelial pathways.

pulmonary microvessels; vasoconstriction; synchrotron radiation microangiography; rat

PULMONARY ARTERIAL HYPERTENSION (PAH) is a rare adverse disease associated with several lung pathologies, and in no uncertain terms, PAH is life-threatening. Although some advances in the treatment of pulmonary disorders have been made in recent decades, there is still no effective cure. The reason for this extremely poor prognosis is due to, in part, our lack of understanding regarding the mechanisms that regulate pulmonary blood flow distribution in pulmonary hypertension. Nevertheless, over the last decade, it has become apparent that dysfunction of the pulmonary vascular endothelium may play a critical role in the cascade of events that ultimately culminate in the development of PAH (6, 32).

The onset of PAH is characterized by endothelial and smooth muscle cell proliferation (11, 32), sustained pulmonary vasoconstriction (26, 28), vascular smooth muscle remodeling (17), and rarefaction, i.e., a decrease in the total number of vessels in the lung (13, 24), all of which contribute to a sustained increase in pulmonary artery pressure (PAP). These structural deformities ultimately increase the workload of the right ventricle, enhancing the risk of heart failure and, ultimately, mortality.

The pulmonary endothelium modulates vascular tone by releasing various vasoactive mediators, such as nitric oxide (NO), endothelin-1 (ET-1), and prostacyclin, in response to hemodynamic influences (6). These vasoactive mediators activate specific signaling pathways within the smooth muscle cell to modulate vascular tone (15). In recent years, the RhoA/Rho-kinase signaling pathway has received considerable attention, primarily using animal models of PAH, for having a central role in a diverse range of cellular functions, in particular sustaining vascular smooth muscle contraction by augmenting agonist-induced Ca2+ sensitization for myosin phosphorylation (1, 14, 15).

During the pathogenesis of PAH, the ensuing endothelial dysfunction has been associated with impaired NO bioavailability (28, 30). In addition, overactivation of the Rho-kinase signaling pathway plays a pivotal role for sustained vasoconstriction in PAH (1, 17). Ultimately a combination of Rho-kinase-mediated vasoconstriction and endothelial dysfunction are likely to contribute to the sustained increase in vascular resistance, although the literature seems divided as to whether the endothelium (6, 18, 20) or smooth muscle Rho-kinase (15, 23, 43) is chiefly responsible. Moreover, it has not yet been established which region(s) of the pulmonary circulation are most susceptible to developing endothelial dysfunction and/or Rho-kinase-mediated sustained vasoconstriction.

One of the limitations in identifying specific segments of the pulmonary circulation prone to pathological changes has been the inability to clearly visualize the specific vessels (ID < 200 μm) within the pulmonary vascular bed. More standard methods of assessing the vascular anatomy of the diseased lung necessitate removal of the lung from the animal (i.e., in vitro) for angiography. Conventional angiography methods have considerable limitations in visualizing the vessels that are most...
susceptible to pathological changes (i.e., ID < 200 μm). We have previously demonstrated the validity and accuracy of synchrotron radiation (SR) microangiography for visualizing the distribution of pulmonary blood vessels (ID > 80 μm) in a closed-chest rat model with PAH (34, 36). Moreover, SR is effective for visualizing dynamic and regional changes in vessel caliber, such as those changes associated with endothelium-dependent and -independent vasodilation.

To date, some studies using animal models have reported the significance of vascular Rho-kinase hypersensitivity for modulating PAP and pulmonary vascular resistance in PAH (9, 28, 29). However, it remains unclear what effect Rho-kinase inhibition has on the adverse structural changes in pulmonary blood flow distribution associated with PAH. This objective can successfully be achieved by visualizing the pulmonary circulation using SR microangiography.

Accordingly, this study aimed to determine the contribution of the Rho-kinase signaling pathway for the sustained increase in PAP and, importantly, the adverse change in pulmonary blood flow distribution in an animal model of PAH. We also aimed to assess segmental differences in pulmonary endothelium integrity for modulating the pulmonary vasculature and, thus, vessel caliber size, with specific emphasis on the NO pathway.

METHODS

All experiments were approved by the local Animal Ethics Committee of SPRING-8 and were conducted in accordance with the guidelines of the Physiological Society of Japan.

Animals and Surgical Preparation

Experiments were conducted on 21 male Sprague-Dawley rats (10 wk old; body wt ~290–350 g). Three weeks before experimentation rats received a subcutaneous injection of either monocrotaline (0.5 ml, n = 5) or MCT rats (n = 6) were used for testing fasudil (10 mg/kg iv), a Rho-kinase inhibitor that interrupts signal transduction for contraction within the vascular smooth muscle. Pulmonary angiograms were recorded after maximal hemodynamic responses were attained, ~20 min following fasudil administration. The maximal effects of fasudil are evident after 15–20 min and are maintained for >120 min.

Experimental protocol 1: role of the Rho-kinase pathway in modulating pulmonary vessel diameter. Separate groups of Cont rats (n = 5) and MCT rats (n = 6) were administered fasudil (3.0 mg·kg⁻¹·min⁻¹ for 5 min iv) to assess endothelium-dependent vasodilation, 2) the NO donor sodium nitroprusside (SNP, 5 μg·kg⁻¹·min⁻¹ for 5 min iv) to assess endothelium-independent vasodilation, and 3) the NO synthase inhibitor, Nω-nitro-L-arginine methyl ester (L-NAME, 50 mg/kg iv). Lung microangiography was performed after the 5th minute of ACh and SNP infusion and 20 min following the bolus dose of L-NAME. At least 10–15 min was required for all cardiovascular variables to return to baseline values following ACh and SNP drug interventions.

The doses of all pharmacological agents used in this study are based on well-documented reports in the literature as well as our own preliminary experiments.

Morphometric Analysis

Following the completion of each experiment, rats were euthanized via anesthetic overdose, and the heart was excised. The atria were removed and the right ventricle wall separated from the left ventricle and septum. Tissues were blotted and weighed and normalized to 100 g body wt. Right and left ventricular weights were expressed as the ratio of the RV to the left ventricle + septum weight (WRV/WLV + septum Fulton’s ratio).

Data Acquisition and Analysis

The RVP and ABP signals were detected using separate Deltran pressure transducers (Utah Medical Products), and the signals were relayed to Powerlab bridge amplifiers (ML117, AD Instruments, Japan) and then continuously sampled at 500 Hz with eight-channel MacLab/8s interface hardware system (AD Instruments) and recorded on a Macintosh Power Book G4 using Chart (v. 5.0.1, AD Instruments). HR was derived from the arterial systolic peaks.

All imaged vessel branches were counted. Vessels were categorized according to ID: 100–200 μm, 200–300 μm, and 300–500 μm. The ID of 94 vessels, comprising four branching generations, was measured in 10 Cont-rats, and the ID of 78 vessels was measured in 11 MCT-rats. The ID of individual vessels was measured under basal conditions and then in response to each of the experimental conditions.

Image Analysis

The computer-imaging program Image Pro-Plus (ver. 4.1, Media Cybernetics) was used to enhance contrast and the clarity of angiogram images [see Schwenke et al. (35) for a full description]. The line-profile function of Image Pro-Plus was used as an accurate method for measuring the ID of individual vessels (34, 36). A 50-μm-thick tungsten filament, which had been placed directly across the corner of the detector’s window, appeared in all recorded images and was subsequently used as a reference for calculating vessel diameter (μm), assuming negligible magnification.

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Statistical Analysis

All statistical analyses were conducted using Statview (v5.01, SAS Institute, Cary, NC). All results are presented as means ± standard error of the mean (SE). One-way ANOVA (factorial) was used to test for significant differences in 1) vessel caliber under basal conditions compared with each of the experimental conditions (e.g., ACh, SNP, etc.); and 2) values for Cont-rats compared with MCT-rats. Where statistical significance was reached, post hoc analyses were incorporated using the paired or unpaired t-test with the Dunnett’s correction for comparisons. A P value ≤ 0.05 was predetermined as the level of significance for all statistical analysis.

RESULTS

Baseline

MCT induced pulmonary arterial hypertension (see Table 1), evident by our observation that systolic RVP (sRVP) of MCT-rats was ~82% higher than that of Cont-rats (P < 0.01). The adverse increase in PAP resulted in the development of right ventricular hypertrophy in MCT-rats [e.g., RV/(LV + Sep) ratio of 0.52, cf. 0.33 in Cont-rats; Table 1]. Mean ABP (MABP) and HR were not significantly different between Cont-rats and MCT-rats.

This study utilized SR to visualize blood flow distribution within the pulmonary microvessels of both Cont-rats and MCT-rats. The baseline angiograms presented in Fig. 1 highlight the difference in the branching patterns for Cont-rats and MCT-rats, ranging from the axial artery to the 4th generation of branching similar to our first report. The total number of vessel branches visible within each baseline image (i.e., 9.5 mm imaging window) was counted. As illustrated in Fig. 2, there were fewer radiopaque vessels primarily of the 4th branching generation for MCT-rats (16 ± 1 vessels; P < 0.05; n = 11) compared with Cont-rats (25 ± 1 vessels, P < 0.05; n = 10).

Rho-Kinase Inhibition (Fasudil)

The Rho-kinase inhibitor, fasudil (10 mg/kg), caused pulmonary vasodilation in control rats (ID 100–200 μm), as well as in MCT-rats (ID 100–300 μm; P < 0.05) (Fig. 3). In addition, unlike any other agent tested, fasudil also promoted the recruitment and (re)perfusion of vessels that were Table 1. Baseline hemodynamic data and heart weights of rats pretreated with a subcutaneous injection of either monocrotaline or vehicle

<table>
<thead>
<tr>
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<th>Control (n=11)</th>
<th>MCT (n=10)</th>
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<tbody>
<tr>
<td><strong>Baseline hemodynamic data</strong></td>
<td></td>
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</tr>
<tr>
<td>BW, g</td>
<td>325 ± 6</td>
<td>287 ± 5†</td>
</tr>
<tr>
<td>sRVP, mmHg</td>
<td>28.3 ± 1.1</td>
<td>51.6 ± 5.0†</td>
</tr>
<tr>
<td>MABP, mmHg</td>
<td>113 ± 4</td>
<td>121 ± 8</td>
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<tr>
<td>HR, beats/min</td>
<td>395 ± 13</td>
<td>413 ± 7</td>
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<tr>
<td>Heart weight data</td>
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<tr>
<td>(Heart weight/100 g BW), mg</td>
<td>260 ± 7</td>
<td>306 ± 10*</td>
</tr>
<tr>
<td>(RV/100 g), mg</td>
<td>64 ± 2</td>
<td>105 ± 7†</td>
</tr>
<tr>
<td>(LV + septum)/100 g), mg</td>
<td>196 ± 6</td>
<td>201 ± 5</td>
</tr>
<tr>
<td>RV/(LV + septum)</td>
<td>0.33 ± 0.01</td>
<td>0.52 ± 0.03†</td>
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Data are presented as means ± SE; n = 11 monocrotaline-treated rats (MCT-rats) and n = 10 vehicle-treated (control) rats (Cont-rats). BW, body weight; sRVP, systolic right ventricular pressure; MABP, mean arterial pressure; HR, heart rate; RV, right ventricle; LV, left ventricle. Significant difference between Cont-rats and MCT-rats (*P < 0.01; †P < 0.001).
†Significant difference between Cont-rats and MCT-rats (branching generation only) the administration of fasudil (10 mg/kg iv).

Endothelium-Dependent Vasodilation: Responses to ACh

The administration of exogenous NO (SNP at 5 \(\mu\)g·kg\(^{-1}\)·min\(^{-1}\) for 5 min) caused significant pulmonary vasodilation, of similar magnitude for both Cont-rats (\(n = 5\)) and MCT-rats (\(n = 5\)), evident by an increase in the ID of vessels <300 \(\mu\)m (for MCT-rats) and/or <200 \(\mu\)m (for Cont-rats) (Fig. 3). Consequently, sRVP decreased in response to SNP in MCT-rats (18% decrease, \(P < 0.01\)), but less so in Cont-rats (14% decrease, NS) (Fig. 4).

Importantly, in Cont-rats the magnitude of response to SNP (vessel ID and sRVP) was similar to that of ACh, whereas in MCT rats, the magnitude of response to ACh appears “blunted” compared with that observed for SNP (Fig. 3). SNP also caused a significant decrease in MABP in both Cont-rats and MCT-rats (43% and 32% decrease, respectively), although HR was not altered (Fig. 4).

Responses to L-NAME

The inhibition of endogenous eNOS, using L-NAME (50 mg/kg), caused severe pulmonary vasoconstriction, as highlighted in the angiographs of Fig. 5. In addition, smaller vessels (<150 \(\mu\)m) were often no longer visible following L-NAME administration (see white circles of Fig. 5), due to the potent vasoconstriction preventing adequate perfusion of the vessel with iodinated contrast agent. In Cont-rats the magnitude of constriction increased as vessel caliber decreased (Fig. 3). In MCT-rats, although the magnitude of constriction was similar to Cont-rats for the 100–200 \(\mu\)m vessels, it was significantly exacerbated for the 200- to 300-\(\mu\)m vessels (\(P < 0.001\); Fig. 3). Consequently, the accentuated pulmonary vasoconstriction likely contributed to a greater increase in sRVP in MCT rats compared with Cont-rats (Fig. 4). L-NAME also caused an increase, albeit not significant, in systemic MABP in both Cont-rats (23% increase) and MCT-rats (15% increase), and a decrease in HR [4% (not significant) and 11% decrease (\(P < 0.05\)), respectively] (Fig. 4).

**Discussion**

The primary results of this study highlight that in a monocrotaline model of PAH, there is 1) restoration/reperfusion of...
blood flow to previously occluded vessels, thus lowering PAP, following inhibition of Rho-kinase-mediated vasoconstriction; 2) adverse functional changes in endothelial modulation of pulmonary vessel caliber; and 3) a region-specific (vessel ID 200–300 μm) enhanced role of NOS.

By utilizing the high definition achieved with SR microangiography, we have consistently shown a significant reduction in the number of perfused vessels (3rd to 4th branching generation) following the development of PAH, regardless of etiology, within a closed-chest rat model (34–36). Based on the early work of Reid and colleagues (13, 24) it had become generally accepted that vascular remodeling and vessel rarefaction were principally responsible for the sustained increase in vascular resistance.

Rho-Kinase-Mediated Control of Vascular Smooth Muscle

Over the last decade the paradigm of vessel rarefaction during PAH has been disputed, and although vascular remodeling is clearly evident in PAH (37), an increasing number of studies using animal models of PAH support the concept that enhanced Rho-kinase-mediated pulmonary vasoconstriction is the primary factor for the sustained increase in vascular resistance (9, 23, 28, 29, 42). Crossno et al. (8) recently showed that the therapeutic prevention of medial thickening (using rosiglitazone) associated with chronic hypoxia did not necessarily lead to a decrease in PAP, because of the inability to repress sustained Rho-kinase-mediated vasoconstriction.

Perhaps one of the most fundamental and novel findings of this study is the observation that acute Rho-kinase inhibition improved pulmonary blood flow distribution in PAH rats, not only by dilating already-perfused vessels (ID > 100 μm), but also by restoring blood flow to vessels that had not previously been perfused (ID < 100 μm). This reversal of sustained vasoconstriction was associated with a beneficial decrease in pulmonary pressure. We have previously described the resolution limitations of SR for accurately measuring vessel ID of pulmonary vessels in vivo (35). Although we cannot accurately detect the border of smaller vessels (<80 μm), so as to measure vessel ID, we are still able to “distinguish” smaller vessels due to the opacity caused by the contrast agent. Therefore, the complete absence of opaqueness in the specified regions of interest of the microangiograms, which later showed vessel perfusion following fasudil administration, suggests that the vessels were completely, or near-completely, closed before Rho-kinase inhibition.

Although previous studies have highlighted the Rho-kinase pathway in sustained vasoconstriction, in both anesthetized rat preparations (3, 7, 10), and in isolated vessel preparations (5, 16, 26, 41), this study shows for the first time the direct transient changes in vessel caliber, indicative of vascular tone, and the subsequent restoration of pulmonary blood flow, in a whole animal model with PAH following Rho-kinase inhibition.

Fig. 4. Hemodynamic responses (% change) to 1) protocol 1 = fasudil (10 mg/kg iv) in Cont-rats and MCT-rats; or 2) protocol 2 = ACh (3.0 μg·kg⁻¹·min⁻¹ for 5 min), SNP (5.0 μg·kg⁻¹·min⁻¹ for 5 min), and L-NAME (50 mg/kg, in a 0.3-ml bolus). *Significant increase/decrease in response to each drug. †Significant difference between Cont-rats and MCT-rats (P < 0.05).

Fig. 5. Microangiogram images showing the branching pattern of small pulmonary arterioles in a MCT-rat (A) and an exceptional vasoconstrictive response to L-NAME (50 mg/kg iv) (B). Pulmonary branches to the 4th generation from the left main axial artery were visible. The tungsten wire in top left corner of each image is a reference of 50-μm diameter. The black arrows point to branches of the pulmonary vessels that have constricted in response to L-NAME. The open white circles in A indicate vessels that constricted so strongly in response to L-NAME that in B the vessels are no longer visibly opaque due to lack of perfusion.
This study, therefore, provides evidence against the paradigm of pulmonary vessel “rarefaction” (i.e., vessel loss) as the primary cause for reduced pulmonary blood flow distribution in PAH. Unfortunately, the lack of cardiac output data, to calculate vascular resistance, prevents us from establishing the relative effectiveness of fasudil for decreasing pulmonary vascular resistance as a result of vessel reperfusion. However, the fact that rarefaction is not necessarily prevalent in PAH provides further insight and hope for developing effective therapeutic strategies for numerous pulmonary diseases evident in society today.

In both normal and PAH rats of this study, fasudil caused dilation in vessels with an ID > 100 μm. In PAH rats, the fact that smaller vessels (ID < 100 μm) were also reperfused suggests that the small resistance vessels may be particularly vulnerable to an enhanced Rho-kinase-mediated vasoconstriction during the development of PAH. This study used an animal, MCT-induced model of PAH that does not necessarily mimic the precise arteriopathy of PAH in humans. Nevertheless, the ability of fasudil to restore blood flow to vessels that had not been adequately perfused in an animal model makes it an attractive candidate for clinical trials of PAH in humans, especially since Rho-kinase inhibition is more effective than other conventional vasodilators, such as NO, at promoting pulmonary vasodilation (23), particularly at the various branching generations of the pulmonary microcirculation.

During the development of PAH, Rho-kinase activation is enhanced and the expression of the GTP-binding protein RhoA is elevated (14, 15, 42). The upstream mechanism(s) for accentuating Rho-kinase activation during the development of PAH is unknown and is likely to be multifactorial (for review, see 27). Moreover, recent evidence indicates that Rho-kinase activation contributes to the onset of endothelial dysfunction (27, 43).

Although our results strongly implicate Rho-kinase-mediated vasoconstriction as the primary cause for the increase in vascular resistance, one significant limitation of SR that we have previously discussed (34, 35) is the inability to assess the integrity of the vascular wall in PAH and, thus, identify the basis for the change in internal vessel dimension and pulmonary blood flow. Indeed, SR is only able to measure the internal diameter of perfused vessels (assuming the vessel contains sufficient contrast medium) and is therefore a straightforward, albeit powerful, method for assessing gross anatomical changes in the pulmonary circulation of the hypertensive lung. Further insight into the structure and/or distribution of vasoactive substances, such as endothelial NO synthase (eNOS), ET-1, or even RhoA/Rho-kinase, would require further immuno/histological analysis, which unfortunately was not performed in this study.

**Endothelial Modulation of Vascular Tone**

The endothelium plays a critical role in modulating normal pulmonary vascular tone by releasing various vasoactive mediators such as NO, ET-1, prostacyclin, serotonin, and thromboxane. It is not surprising, therefore, that injury to the endothelium during the onset of PAH has been implicated as a primary catalyst for promoting adverse endothelial and smooth muscle cell proliferation, and sustained vascular vasoconstriction (6).

In this study, we observed that endothelium-dependent vasodilation was impaired in rats with PAH, which is consistent with other reports (2, 18, 20). Moreover, by utilizing SR microangiography, we were also able to quantify the magnitude of dysfunction and further show that it was primarily the small microvessels (ID ~100–200 μm; 3rd to 4th branching generation) that were most susceptible to impairment of the endothelium-mediated vasodilation.

During the development of PAH the expression of eNOS is altered, although it is partially dependent on the etiology with some reports suggesting eNOS is reduced by MCT (18, 22, 31) but elevated in chronic hypoxia (19, 37, 38). Regardless of etiology, and the alteration in eNOS expression, there is compelling evidence that NO bioavailability is impaired in PAH (25, 31, 33) due to an increase in NO degradation, through an increase in oxidative stress (21, 30).

In this study the inhibition of endogenous NO production (using L-NAME) caused pulmonary vasoconstriction in all vessels with an ID < 500 μm, but particularly those vessels with an ID of 100–200 μm. Under normal conditions NO plays a “quiescent” role in maintaining basal pulmonary vasorelaxation (6). However, our results showed the NOS inhibition in control rats caused considerable pulmonary vasoconstriction in the 100- to 200-μm vessels, reflecting a significant role of NO for maintaining vascular tone of the small pulmonary microvessels. Under adverse conditions, such as during the early onset of PAH, the role of NO appears to become critically important (6). Consequently, we observed that the magnitude of constriction in response to L-NAME was accentuated in PAH, but primarily the 200- to 300-μm vessels. Interestingly, these results may suggest that the expression of eNOS was enhanced in the PAH rats of this study, although the increase in eNOS expression may not be uniform throughout the lung, but rather regionally localized to those vessels involved with modulating vascular resistance (ID < 300 μm) (19, 37–39). Alternatively, the regional difference may be due to flow-mediated activation of NOS, which is more apparent in the small arterioles where shear is greatest, especially when such vessels are constricted.

Despite the impaired endothelium-mediated vasodilation reported in this study, we did observe that the vasodilatory sensitivity of the vascular smooth muscle to exogenous NO (SNP) was not impaired in PAH. In contrast, a recent study by Mam et al. (20) reported a reduced NO vascular sensitivity in MCT rats. Discrepancies between studies are, at least to some extent, attributable to the complex and diverse interaction between eNOS gene expression, protein synthesis, and NO production, depending on the etiology of PAH, and the importance of the regulatory role of NO in maintaining vascular tone in PAH (40).

This study also showed that in control rats the vasodilatory responses to ACh and SNP were similar in magnitude and regional variability (greatest dilation at the 100- to 200-μm vessels). These results tend to suggest that NO, among the other endothelial-derived vasodilators stimulated by ACh (e.g., EDHF and prostacyclin), plays a primary role in modulating pulmonary vascular dilatation (4, 12).

In summary, we have utilized SR to assess the role of the endothelium and vascular smooth muscle for modulating pulmonary vascular tone in a rat model, and the adverse changes associated with the pathogenesis of PAH. Specifically, our...
results highlight an enhanced role of endothelial NO for restraining the adverse increase in PAP. In addition, Rho-kinase-mediated vasoconstriction restricts pulmonary blood flow distribution by occluding small microvessels. Hence, inhibition of the Rho-kinase pathway restores pulmonary blood flow and lowers PAP. Based on the results of our study, and other animal studies of PAH, it is anticipated that clinical trials involving Rho-kinase inhibition may provide an effective therapeutic strategy for PAH in humans. However, an effective dose of, say, fasudil would need to be established that was effective at reducing PAP without adversely reducing systemic arterial pressure, as seen in our study. Ultimately, an effective therapeutic strategy for treating PAH would ideally target both endothelial and smooth muscle components.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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