Rosiglitazone attenuates hypoxia-induced pulmonary arterial remodeling

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PULMONARY HYPERTENSION (PH) is a disease characterized by increased pulmonary arterial (PA) pressure with concomitant increases in pulmonary vascular resistance (PVR), frequently leading to right ventricular (RV) failure and death (56). The chronic elevation of PVR has been attributed to a combination of vasoconstriction and progressive structural remodeling of the pulmonary arterial tree. Characteristic features of this vascular remodeling include vessel wall thickening as a result of resident vessel wall cell proliferation, migration, and exces-
studies are even more compelling. Oral administration of synthetic PPARγ ligands inhibits neointimal hyperplasia in balloon-injured rat and rabbit arterial wall (7, 38, 42). PPARγ agonists also reduce the size of atherosclerotic lesions in LDL receptor-deficient (11) and apolipoprotein E-deficient mice (9). In human subjects with type 2 diabetes, TZDs slow the progression of intimal-medial thickening (40, 41). Overall, these data indicate that PPARγ agonists inhibit SMC proliferation and migration and prevent pathological vascular remodeling in the systemic vasculature.

Recently, Matsuda, et al. (45) reported that the TZDs pioglitazone and troglitazone inhibit the development or PH and attenuate PA remodeling in monocrotaline-treated rats. Pioglitazone and troglitazone inhibit the development or PH and the systemic vasculature. These results indicated that PPARγ agonists have the same beneficial impact on the pulmonary arterial bed as they do in the systemic circulation. However, the physiological and biochemical mechanisms underlying these beneficial effects were not explored.

Given these considerations, we hypothesized that PPARγ agonists might also prevent pulmonary arterial (PA) remodeling and the development of PH in response to chronic hypoxia. We found that the PPARγ agonist rosiglitazone (ROSI) had little effect on PH and had moderate effects on RV hypertrophy following exposure of rats to chronic hypoxia. However, remodeling of the PA wall was almost entirely attenuated by ROSI. ROSI blocked proliferation of cells in the media and adventitia of the vessel wall but did not affect cell death. ROSI also decreased collagen and elastin deposition in the vessel wall and reduced collagen and elastin production by PA SMCs in vitro. ROSI also increased matrix metalloproteinase (MMP)-2 protein levels in cultured SMCs and MMP-2 activity in lung lysates. Finally, we noted that hypoxia induced the appearance of cells expressing the cell surface marker c-Kit, which is found on hematopoietic progenitor cells and cells of the promyelocytic lineage. ROSI blocked the appearance of these cells even in lung tissue from animals exposed to chronic hypoxic conditions. We conclude that although ROSI prevents vascular remodeling in response to hypoxia, it fails to attenuate the development of PH. This may be due to the inability of ROSI to modulate other prohypertensive processes including vasoconstriction, polycythemia, and increases in cardiac output.

METHODS

Materials. Male and female Wistar-Kyoto rats were purchased from Harlan (Indianapolis, IN). Polyclonal antibodies to c-Kit (CD117) and proliferating cell nuclear antigen (PCNA) were purchased from Dako Cytomation (Carpenteria, CA). Polyclonal antibodies to α-smooth muscle (α-SM) actin, Fasudil (HA-1077), and peroxidase-Schiff staining reagents were purchased from Sigma (St. Louis, MO). Picrosirius red stain was obtained from Electron Microscopy Sciences (Hatfield, PA). Polyclonal antibodies to total and phospho-Akt and p70 S6 kinase were obtained from Cell Sciences (Hatfield, PA). Polyclonal antibodies to c-Kit and Fasudil (HA-1077) were purchased from BD Biosciences (San Jose, CA). Novex gelatin zymogram mini-gels and reagents were purchased from Invitrogen (Carlsbad, CA). Diet 5008 (6.5% fat, 3.31 kcal/g) impregnated with 0.005% ROSI (Avandia; Glaxo Smith Kline, Research Triangle Park, NC) was prepared by Test Diet (Richmond, IN).

Animal care and treatment. All animal procedures were performed with approval and in accordance with the guidelines of the University of Colorado Health Sciences Center for Laboratory Animal Care. Male and female Wistar-Kyoto rats (6 wk old) were randomized to normoxic (Nx; 1,600 m, 630 mmHg) or hypobaric hypoxia (Hx; 5,500 m, 410 mmHg) and treated with and without the PPARγ agonist ROSI at 5 mg·kg⁻¹·day⁻¹ for 3 wk (n = 6 animals/treatment group). To test whether ROSI could reverse remodeling, some animals were maintained under normoxic and hypoxic conditions for 21 days and received ROSI for the last 7 days. Fresh food [Diet 5008 with or without (control Chow) ROSI], water, and clean cages with fresh bedding were provided every other day. Light was maintained on a 12-h cycle, and humidity was 40–45% with a temperature of 25–27°C. The animals were monitored daily, and weight was checked once a week. At the end of 3 wk, the rats were anesthetized and catheterized and the following measurements were obtained: body weight, hematocrit, mean PA pressure, cardiac output, total pulmonary resistance, mean PA pressure in response to inhaled nitric oxide (NO; 80 ppm) or intravenously delivered of Fasudil. After the rats were killed, RV hypertrophy and PA wall morphology data were collected.

Hemodynamic measurements and tissue procurement. Rats received a combination of ketamine (75 mg/kg) and xylazine (6 mg/kg) intraperitoneally as anesthetic. The ventral neck area and the area dorsally between the scapulae were shaved and the areas scrubbed with betadine solution. A 2-cm incision was made over the right ventral neck area to expose the internal jugular vein. A polyvinyl (PV-1) catheter was threaded into the pulmonary artery via the right internal jugular vein. A catheter pressure tracing was transduced and monitored with an oscilloscope, and the location of the tip was identified by the characteristic shapes of the PA pressure waveforms. The catheter was then securely sutured into place. An additional catheter was inserted into the jugular vein for drug infusion and venous return. The right carotid artery was isolated, and a catheter was inserted 4 cm into the artery and securely sutured into place for measurements of blood gases and cardiac output. All catheters were routed, exteriorized, and sutured in place. Rats were placed in a 3-in. wide, 3-in. high, and 7-in. long Plexiglas box with gas delivery ports for the delivery of air or air mixed with NO. Sampling of the gas inside the box over time showed that complete gas exchange within the box occurred within 2 min. The implanted catheters were attached to pressure transducers and/or cardiac output equipment for hemodynamic measurements. Cardiac output was determined using a standard dye dilution method. Total pulmonary resistance was calculated by dividing the mean PA pressure by cardiac output (50). All measurements were made while the animals were anesthetized.

Following hemodynamic measurements, the anesthetized animals were treated with the RhoA/Rho kinase inhibitor Fasudil (10 mg/kg IV) to dilate the vasculature before fixation. The rats were then euthanized by exsanguination. Lungs were fixed with 4% paraformaldehyde in PBS containing 5 mM EDTA by airway inflation at 30 cmH₂O pressure. Pulmonary arteries were perfused with 4% paraformaldehyde-PBS-5 mM EDTA (to maintain vasodilation) at a pressure similar to that measured in vivo. The heart and lungs were then removed en bloc. Wet RV weights were measured and used to calculate the ratio of RV weight to total body weight. Lung tissue was fixed overnight in 4% paraformaldehyde and embedded in paraffin.

Immunostaining and TUNEL staining. Five-micrometer sections of paraffin-embedded lung tissue were depar-
affinized with Hemo-D and rehydrated in a graded ethanol-water series. Sections were subjected to antigen retrieval in citrate buffer in a microwaveable pressure cooker for 20 min. Sections were blocked with PBS containing 5% horse serum for 30 min at room temperature. The sections were incubated overnight in PBS-5% FBS at 4°C with the primary antibodies as indicated. The sections were then washed and incubated with the indicated Alexa Fluor-conjugated secondary antibodies for 1 h at room temperature. TUNEL staining was performed using ApoAlert DNA fragmentation assay kits according to the manufacturer’s directions.

Microscopy and lung morphometric analysis. Five-micrometer sections of fixed lung or heart tissue were subjected to hematoxylin and eosin or pentachrome staining by the University of Colorado Health Sciences Center Histology Core Laboratory. Periodic acid-Schiff staining of heart sections or picrosirius red staining of lung sections was performed as directed by the supplier’s instructions. Distal pulmonary vessels (outside diameter 10–50 μm) were assessed by a blinded observer for degrees of circumferential α-SM actin-positive staining indicative of muscularization. Vessels smaller than 10 μm were considered capillaries and excluded from further consideration. Proximal vessels (outside diameter 50–250 μm) were analyzed for medial wall thickness at four points around the vessel circumference and for lumen diameter along two axes.

Microscopy was performed on a Nikon TE2000-U inverted epifluorescent microscope. Bright-field, phase-contrast, and fluorescent digital deconvolution images were captured to a personal computer with either a Spot RT/KE monochrome camera or Spot Insight color camera (Diagnostic Imaging, Sterling Heights, MI). Images were analyzed and processed with MetaMorph 6.1 software (Molecular Devices, Sunnyvale, CA).

PA SMC isolation and cell culture. Five hundred-micrometer PAs were recovered from adult rat lungs. Segments of the PAs were cut open and mechanically stripped of adventitia and endothelium. The segments were then placed lumen side down into individual wells of a six-well plate. Tissue explants were maintained in complete DMEM supplemented with 200 U/ml penicillin, 0.2 mg/ml streptomycin, and 10% FCS. Although these vessels are larger than those evaluated by morphometric analysis (50–250 μm), techniques to isolate cells from smaller vessels have not been developed.

Since our goal was to obtain pure subpopulations of SMCs, we selectively isolated individual cell colonies with a distinct, although uniform, morphological appearance from primary culture by using cloning cylinders. Expression of SMC-specific markers (α-SM actin and SM-myosin heavy chains) in each isolated cell subpopulation was selected for further experimentation. Individual cell colonies growing from tissue explants in primary culture were isolated by placing cloning cylinders (5–10 mm in diameter, greased on the bottom) over each cell colony of interest. Cells within the ring were trypsinized and transferred to a 24-multilwell plate for expansion. Cells were passaged in DMEM containing 10% FCS. During experiments, the cells were placed in sealed Plexiglas chambers filled with either normoxic (80% nitrogen, 20% oxygen) or hypoxic (97% nitrogen, 3% oxygen) gas mixtures. The gas mixture in each chamber was replaced every 24 h.

Fig. 1. Rosiglitazone (ROSI) attenuates and reverses hypoxia-induced pulmonary arterial (PA) remodeling. Adult male and female rats were fed untreated or ROSI-impregnated chow and maintained under normoxic or hypoxic conditions for 21 days (A). An additional group of animals was maintained under hypoxic conditions without ROSI for 14 days, followed by hypoxic conditions with ROSI for an additional 7 days (B). Representative bright-field photomicrographs of hematoxylin and eosin-stained lung sections show that ROSI inhibited (A) and reversed (B) hypoxia-induced PA remodeling. Bar, 100 μm. C: morphometric analysis confirms that ROSI blocked (solid bars) or reversed (open bar) hypoxia-induced PA wall thickening compared with control (hatched bars). Data are average values obtained from at least 30 vessels with diameters between 50 and 250 μm from 6 animals per group; error bars represent SD. *P ≤ 0.05.
All studies were carried out using cells at passages 1–8. Cell cultures were tested for mycoplasma contamination with the use of a Gen-Probe Mycoplasma T. C. rapid detection system (San Diego, CA).

Lung and SMC lysates, Western blots, and zymograms. For Western blots, lysates from whole lung or heart (39) or from cultured SMCs (24) were prepared as previously described. After correcting for protein concentrations, cell lysates were mixed with an equal volume of Laemmli SDS loading buffer, resolved on 10% polyacrylamide-SDS gels, and transferred to polyvinylidene difluoride membranes. The blots were blocked with PBS containing 5% dry milk and 0.1% Tween 20 and then treated with antibodies that recognize the target proteins indicated overnight at 4°C. The blots were washed and subsequently treated with appropriate secondary antibodies conjugated to horseradish peroxidase. After the blots were washed, specific immune complexes were visualized with SuperSignal West Pico chemiluminescent substrate.

For zymography, portions of whole lung were homogenized in PBS without protease inhibitors. Equal amounts of lysate protein were mixed with an equal volume of SDS loading buffer (without reducing agent) and immediately resolved on 10% polyacrylamide-gelatin Novex Zymogram minigels. MMP activity was detected using matched reagents according to the manufacturer’s directions.

Cardiomyocyte hypertrophy. Five-micrometer sections of paraffin-embedded hearts were stained with periodate-Schiff dye according to the manufacturer’s directions.
date-Schiff reagents to highlight cardiomyocyte borders. Bright-field images of regions of heart containing transverse-sectioned cardiomyocytes were captured to a computer as described above. The diameter of approximately round cardiomyocytes containing visible nuclei was determined in two perpendicular directions by using MetaMorph software and was averaged. The diameters of at least 100 cardiomyocytes were measured from each animal.

Statistics. Statistical analysis was performed using the Super ANOVA software program (Abacus Concepts, Berkeley, CA). Comparisons were performed using two-way analysis of variance followed by the Scheffe’s multiple comparison test for individual comparisons within and between groups of data points. Data were considered statistically significant with a \( P \) value \(< 0.05\).

RESULTS

ROSI attenuates pulmonary arterial remodeling and RV hypertrophy in response to hypoxia. There was no statistical difference between the normoxic (Nx)-control and Nx-ROSI or hypoxic (Hx)-control and Hx-ROSI-treated groups in body weight at the end of 3 wk, although the ROSI-treated animals tended to be heavier (data not shown). The animals subjected to chronic hypoxia for 3 wk demonstrated the expected polycythemia; however, there was no statistical difference between the hematocrit of Hx-control and Hx-ROSI-treated groups (data not shown).

Examination of hematoxylin and eosin-stained lung tissue sections demonstrated a profound increase in PA wall thickness in Hx-control compared with Nx-control animals (Fig. 1A). However, ROSI treatment for the entire 21-day period completely blocked PA remodeling. We also investigated whether PPAR\( \gamma \) agonist treatment could reverse hypoxia-induced PA remodeling. For these experiments, animals were exposed to hypoxic conditions for 14 days in the absence of ROSI. ROSI treatment was then conducted for next 7 days with continued exposure to hypobaric hypoxic conditions. As

![Fig. 4. ROSI inhibits hypoxia-induced proliferation of cells in the PA wall. Adult male rats were fed a normal diet or chow impregnated with ROSI and maintained under normoxic or hypoxic conditions for 21 days. Five-micrometer sections of lung tissue were subjected to immunohistochemical staining for PCNA, \( \alpha \)-SM actin, and 4–6-diamidino-2-phenylindole (DAPI; nuclei). A: representative fluorescent digital deconvolution photomicrographs show that hypoxia-induced proliferation in the vessel wall was attenuated by ROSI. Bars, 100 \( \mu \)m. B: morphometric quantitation of proliferating (PCNA positive) cells vs. total cell number (DAPI) in the vessel wall confirms that hypoxia increased proliferation and ROSI decreased proliferation within the PA wall. Data are average values obtained from 25 vessels measured per animal, from \( n = 6 \) animals per group; error bars represent SD. \(* P \leq 0.05\). C: representative fluorescent digital deconvolution photomicrographs show proliferation of both actin-negative and actin-positive cells in the PA wall of hypoxic control rats.](http://ajplung.physiology.org/)}
demonstrated in Fig. 1B, substantial PA remodeling was already evident after 14 days of hypoxic exposure. However, in the animals treated with ROSI for the last 7 days of hypoxic exposure, remodeling was reduced to near normal levels. Morphometric analysis (vessel wall thickness/lumen radius) confirmed a marked decrease in proximal and distal PA vessel wall (50- to 250-μm-diameter vessels) remodeling compared with untreated Nx- and Hx-controls (Fig. 1C). Morphometric analysis also demonstrated that starting ROSI after the PA remodeling had been established could reverse the PA remodeling to near normal levels. Similar results were obtained with both male and female rats. To ensure that these differences were not due to differences in vascular tone, we treated the animals with the vasorelaxant Fasudil (RhoA/Rho kinase inhibitor) immediately before lung removal and inflated and perfused the lungs with buffers containing 5 mM EDTA. This attenuation also was readily evident in the lack of distal muscularization of the small PA arterioles within the distal lung parenchyma (Fig. 2). Late ROSI treatment also diminished muscularization of distal arterioles but was much less effective than ROSI treatment initiated at the onset of hypoxic exposure.

RV hypertrophy (RV weight divided by total body weight) was significantly reduced in the Hx-ROSI rats compared with Hx-controls, indicating that ROSI treatment attenuated RV hypertrophy in response to PH (Fig. 3A). The inhibition of RV hypertrophy was due, at least in part, to the ability of ROSI to block hypertrophy of individual cardiomyocytes (Fig. 3B). Cardiomyocyte hypertrophy has been linked to increased activation of Akt (16, 47) and p70 S6 kinase (51) signaling pathways in response to various stimuli. We found that hypoxia significantly increased Akt activation (phospho-Akt levels) but had no effect on p70 S6 kinase activity in heart lysates (Fig. 3C). However, cardiac phospho-Akt levels were markedly reduced in Hx-ROSI rats.

**ROSI regulates proliferation, but not cell death, in the hypoxic PA wall.** To begin to delineate the mechanisms by which ROSI attenuates PA remodeling in response to hypoxia, we examined the impact of ROSI on proliferation and apoptosis in PAs of lung tissue sections. Using immunostaining for PCNA as a marker for proliferating cells, we detected numerous proliferating cells in the thickened PA walls of Hx-control animals (Fig. 4, A and B). These proliferating cells were largely detected in the adventitial layer of the PA wall and did not stain for α-SM actin (Fig. 4C). However, PCNA-positive cells were virtually absent in PA walls from Hx-ROSI animals, suggesting that ROSI blocks proliferation of cells in the PA wall. ROSI might also prevent PA wall thickening by increasing

![Fig. 5. No significant differences in apoptosis in the PA wall were observed with ROSI and/or hypoxia treatments.](image-url)

**A:** Sections of paraformaldehyde-fixed lung tissue were subjected to TdT-mediated dUTP nick end labeling (TUNEL) and DAPI (nuclei) staining. Representative fluorescent digital deconvolution photomicrographs show no significant differences in apoptosis among treatment groups. Bars, 100 μm. **B:** Morphometric quantitation of TUNEL-positive cells vs. total cell number (DAPI) in the vessel wall. Data confirm no significant differences in apoptosis in the PA wall under any of the conditions.
apoptosis of vessel wall cells. We assessed apoptosis in lung sections by TUNEL staining and found no significant differences under any condition (Fig. 5, A and B).

**ROSI regulates extracellular matrix metabolism.** Another potential mechanism by which TZDs might reduce PA wall remodeling could be by inhibiting the synthesis and deposition of extracellular matrix (ECM) or by increasing the breakdown of the ECM. To test this hypothesis, we measured collagen deposition in the PA wall by pentachrome and picrosirius red staining, and elastin deposition by pentachrome staining. We noted substantial amounts of collagen (yellow material in pentachrome-stained sections, red material in picrosirius red-stained sections) in the media and adventitia of vessels from Hx-control animals (Fig. 6A). Likewise, the number of elastin fibers (gray fibers in pentachrome-stained sections) was elevated in Hx-control vessel walls. However, collagen and elastin staining in Hx-ROSI or Hx-late ROSI animals were reduced to levels comparable to those observed in Nx-control animals.

Similar results were obtained with cultured PA SMCs. Pentachrome staining revealed a marked increase in intracellular and extracellular elastin production in cells exposed to hypoxia but not in cells exposed to hypoxia and treated with ROSI (Fig. 6B). Likewise, picrosirius red staining revealed increased collagen synthesis in hypoxic SMCs but not in cells exposed to hypoxia and treated with ROSI (Fig. 6, B and C).

The impact of ROSI on certain MMPs also was examined. In SMCs, ROSI reduced expression of MMP-9, either alone or in

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**Fig. 6.** ROSI decreases collagen and elastin levels in the PA wall and in cultured PA smooth muscle cells (SMCs). A: adult male rats were fed a normal diet or chow impregnated with ROSI and maintained under normoxic or hypoxic conditions for 21 days. Five-micrometer sections of fixed lung tissue were subjected to pentachrome or picrosirius red staining as indicated. Representative bright-field photomicrographs show that ROSI inhibited hypoxia-induced collagen (picrosirius) and elastin (pentachrome) deposition in the arterial wall. B: PA SMCs were maintained under normoxic (21% O2) or hypoxic (3% O2) conditions with and without ROSI for 72 h. Cells were then fixed and subjected to pentachrome and picrosirius red staining. Representative bright-field photomicrographs show that ROSI blocked hypoxia-induced collagen and elastin deposition in cultured SMCs. C: PA SMCs were subjected to hypoxia or treated with PDGF, with and without ROSI. Collagen synthesis was quantitated by staining the cells with picrosirius red followed by extraction of the dye. The absorbance of the extracts was measured and averaged for each treatment. Data show that ROSI blocked collagen synthesis in SMCs exposed to hypoxia or PDGF. Data are average values from 3 separate experiments with each treatment performed in triplicate; error bars represent SD. *P ≤ 0.05.
SMCs were maintained under normoxic (21% O\textsubscript{2}) or hypoxic (3% O\textsubscript{2}) combination with exposure to hypoxic conditions (Fig. 7). ROSI decreases matrix metalloproteinase (MMP)-9 levels and increases MMP-2 levels in whole lung lysates and isolated PA SMCs. Western blotting in whole cell lysates from adult male rats fed a normal diet or chow impregnated with ROSI and maintained under normoxic or hypoxic conditions for 21 days. A representative zymogram is shown, with the positions of MMP-9 and MMP-2 indicated at right of blot. Data show that ROSI stimulated expression of MMP-2 even in cells exposed to hypoxia. ROSI had no effect on MMP-9 levels. Blot is representative of 3 separate experiments. Norm, normoxic; Hypox, hypoxic.

**DISCUSSION**

In this study we have shown that the PPAR\textgamma agonist ROSI inhibits remodeling of the PA wall and muscularization of distal arterioles normally observed in chronic hypoxic PH. More impressive was the observation that ROSI reversed remodeling due to prior hypoxic exposure. The decreased pressure as result of inhaled NO at 80 ppm in both the Hx-control and Hx-ROSI animals, but this was not statistically significant (Fig. 10). However, when animals were subsequently treated with the RhoA/Rho kinase inhibitor Fasudil (10 mg/kg iv) (20, 48), mean PA pressures fell to near normal levels within 1–2 min. ROSI fails to block the development of hypoxic PH. PA catheterization performed on both the Hx-control and Hx-ROSI groups demonstrated no attenuation of hypoxic PH in male or female rats (Fig. 9A). Measurements of cardiac output output demonstrated an increase in both ROSI-treated groups (Fig. 9B), but this was not statistically significant. Total pulmonary resistance was widely variable but generally lower in the ROSI-treated groups (Fig. 9C). To determine whether the elevated PA pressures were due to sustained vasoconstriction, some animals received inhaled NO during the hemodynamic assessments. In general, there was a modest decrease in mean PA pressure as result of inhaled NO at 80 ppm in both the Hx-control and Hx-ROSI animals, but this was not statistically significant (Fig. 10). However, when animals were subsequently treated with the RhoA/Rho kinase inhibitor Fasudil (10 mg/kg iv) (20, 48), mean PA pressures fell to near normal levels within 1–2 min.

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and tissue types. Our results indicate that ROSI has a similar impact on PA remodeling and cells that make up the PA wall. It is interesting, however, that ROSI had little or no effect on the development of hypoxic PH. TZDs have been long recognized to exhibit antihypertensive effects in the systemic circulation (7, 18, 52). These effects are largely due to the negative impact of TZDs on angiotensin II receptor signaling (22, 30, 60) and RhoA/Rho kinase activation (62), which normally promote vasoconstriction. TZDs also block the production of endothelin-1 and other vasoactive peptides by ECs (23) and decrease the generation of oxidative stress/reactive oxygen species (25). In our studies, Rho kinase-mediated vasoconstriction (20, 48) appeared to be the predominant cause of hypoxia-induced PA pressure elevation, since acute inhibition of Rho kinase with Fasudil normalized PA pressures. The inability of ROSI to repress Rho kinase-mediated vasoconstriction in the pulmonary circulation given that TZDs inhibit RhoA/Rho kinase activation and block vasoconstriction in the systemic vasculature remains a mystery.

One answer may lie in the ability of troglitazone or pioglitazone to decrease PA pressures in monocrotaline-treated rats (45). Monocrotaline-induced vasoconstriction also can be reduced with Fasudil, indicating that monocrotaline, like hypoxia, promotes Rho kinase-mediated vasoconstriction. This suggests that different TZDs may have diverse effects on RhoA/Rho kinase signaling. However, the differences between our results and those of Matsuda et al. (45) may have other explanations. Strain-specific differences (Wistar-Kyoto in our

Fig. 8. ROSI inhibits the appearance of c-Kit-positive cells in the PA wall in response to chronic hypoxia. Adult male rats were fed a normal diet or chow impregnated with ROSI and maintained under normoxic or hypoxic conditions for 21 days. Five-micrometer fixed lung tissue sections were subjected to immunohistochemical staining for c-Kit and α-SM actin. DAPI was used to locate nuclei. A: representative phase-contrast and fluorescent digital deconvolution photomicrographs show that hypoxia induced the recruitment of c-Kit-positive cells to the arterial adventitia. ROSI blocked the appearance of these cells. Bars, 100 μm. B: morphometric quantitation of c-Kit-positive cells vs. total cell number (DAPI). Results confirm that hypoxia stimulated the appearance of c-Kit-positive cells in the PA wall and that ROSI blocked this phenomenon. Data are average values obtained from 25 measurements per animal, from n = 6 animals per group; error bars represent SD. *P ≤ 0.05. C: representative fluorescent digital deconvolution photomicrographs demonstrate that c-Kit-positive cells did not colocalize with actin-positive regions of the PA wall in hypoxic control animals.
phosphodiesterase activity (5, 6, 29). Our data suggest that RhoA/Rho kinase inhibitors such as Fasudil may be more effective than inhaled NO at promoting pulmonary vasodilation.

A related issue is the development of hypoxic PH (elevated PA pressure) in rats treated with ROSI, which represses PA remodeling. This suggests that the remodeling or vessel wall thickening may not be a necessary factor in the development of the hypertension itself. This concept is supported by studies employing methods to prevent or compensate for fixation method-dependent changes in lumen area. These studies showed that when the pulmonary vascular bed was maximally vasodilated during lung fixation, there was no reduction in vessel luminal area associated with the medial and adventitial thickening (33, 35). Other experiments have demonstrated that angiotensin-converting enzyme inhibitors prevent PA remodeling in rats exposed to chronic hypoxia but do not attenuate the development of PH or RV hypertrophy (10). More recent studies indicate that acute inhibition of RhoA/Rho kinase signaling almost completely reverses PH in rats exposed to chronic hypoxia, although the brief exposure to the inhibitors would not be expected to have any effect on the structural thickening of the PA wall (48). If this is the case in human PH, then agents such as ROSI that prevent or reverse remodeling without affecting vasoconstriction may have little benefit. However, the dissociation of vascular remodeling from PH may merely reflect a feature of the hypoxic rodent models that is not relevant to humans or other large animals. For example, studies employing neonatal calves exposed to chronic hypoxia show pronounced decreases in lumen cross-sectional area due to inward encroachment on the vascular lumen (19), and constrictive andobliterative neointimal lesions are characteristic of human severe idiopathic pulmonary arterial hypertension (PAH). Thus ROSI and the other TZDs may prove useful in preventing or reversing the obliterative neointimal lesions of severe PAH (54). We are currently assessing the impact of

Fig. 10. Effect of inhaled NO or intravenous Fasudil on mean PA pressures. Adult male rats were fed a normal diet or chow impregnated with ROSI and maintained under normoxic or hypoxic conditions for 21 days. Mean PA pressures were allowed to return to initial levels between the 2 treatments. Control animals (hatched bars) received ambient air alone. Fasudil normalized PA pressures were measured while the animals were exposed to NO mixed with air (80 ppm; solid bars) followed by intravenous injection of Fasudil (open bars). PA pressures were allowed to return to initial levels between the 2 treatments. Control animals (hatched bars) received ambient air alone. Fasudil normalized PA pressures were measured while the animals were exposed to NO mixed with air (80 ppm; solid bars) followed by intravenous injection of Fasudil (open bars). PA pressures were allowed to return to initial levels between the 2 treatments. Control animals (hatched bars) received ambient air alone. Fasudil normalized PA pressures were measured while the animals were exposed to NO mixed with air (80 ppm; solid bars) followed by intravenous injection of Fasudil (open bars).
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ROSIGLITAZONE inhibits hypoxic pulmonary arterial remodeling. Our results are consistent with the ability of ROSI and other TZDs to block vascular remodeling in the systemic vasculature but also highlight an important difference in the ability of ROSI to affect vasoconstriction in the pulmonary versus systemic vasculature.

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