Differential effects of chronic hypoxia and intermittent hypocapnic and eucapnic hypoxia on pulmonary vasoreactivity


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Intermittent hypoxia (IH) resulting from sleep apnea can lead to pulmonary hypertension (PH) and right heart failure, similar to chronic sustained hypoxia (CH). Supplemental CO2, however, attenuates hypoxic PH. We therefore hypothesized that, similar to CH, IH elicits PH and associated increases in arterial endothelial nitric oxide synthase (eNOS) expression, ionomycin-dependent vasodilation, and receptor-mediated pulmonary vasoconstriction. We further hypothesized that supplemental CO2 inhibits these responses to IH. To test these hypotheses, we measured eNOS expression by Western blot in intrapulmonary arteries from CH (2 wk, 0.5 atm), hypocapnic IH (H-IH) (3 min cycles of 5% O2/air flush, 7 h/day, 2 wk), and eucapnic IH (E-IH) (3 min cycles of 5% O2, 5% CO2/air flush, 7 h/day, 2 wk) rats and their respective controls. Furthermore, vasodilatory responses to the calcium ionophore ionomycin and vasoconstrictor responses to the thromboxane mimetic U-46619 were measured in isolated saline-perfused lungs from each group. Hematocrit, arterial wall thickness, and right ventricle-to-total ventricle weight ratios were additionally assessed as indexes of polycythemia, arterial remodeling, and PH, respectively. Consistent with our hypotheses, E-IH resulted in attenuated polycythemia, arterial remodeling, RV hypertrophy, and eNOS upregulation compared with H-IH. However, in contrast to CH, neither H-IH nor E-IH increased ionomycin-dependent vasodilation. Furthermore, H-IH and E-IH similarly augmented U-46619-induced pulmonary vasoconstriction but to a lesser degree than CH. We conclude that maintenance of eucapnia decreases IH-induced PH and prevents HI-dependent increases in arterial eNOS. In contrast, increases in pulmonary vasoconstrictor reactivity following H-IH are unaltered by exposure to supplemental CO2.

Exposure to chronic hypoxia (CH) resulting from high altitude or chronic obstructive pulmonary disorders also results in pulmonary hypertension, right ventricular hypertrophy, and right heart failure. These responses to CH are generally thought to be a function of increases in pulmonary vascular resistance resulting from polycythemia, arterial remodeling, and vasoconstriction. Studies from our laboratory and others have demonstrated that animals exposed to CH develop pulmonary hypertension and alterations in pulmonary vasoreactivity, including enhanced vasoconstrictor responsiveness to receptor-mediated agonists, as well as a compensatory upregulation of pulmonary arterial endothelial nitric oxide synthase (eNOS) and a consequent increase in endothelium-derived nitric oxide-dependent vasodilation (23, 32, 33, 37, 40, 42). However, whether IH mediates similar changes in pulmonary vasoreactivity and eNOS expression remains to be established. Furthermore, considering that continuous CO2 supplementation blunts the pulmonary hypertensive response to CH (21, 31), it is possible that intermittent hypercapnia associated with SA provides a protective mechanism to limit the severity of IH-induced pulmonary hypertension.

Based on these observations, we hypothesized that similar to CH, IH induces pulmonary hypertension and associated increases in arterial eNOS expression, enhanced ionomycin-dependent vasodilation, and receptor-mediated vasoconstriction and that supplemental CO2 attenuates these responses to IH. These hypotheses were tested in lungs from CH rats (2 wk at 0.5 atm), hypocapnic IH (H-IH) rats (3 min cycles of 5% O2/air flush, 7 h/day, 2 wk) eucapnic IH (E-IH) rats (3 min cycles of 5% O2, 5% CO2/air flush, 7 h/day, 2 wk) (1, 20), or their respective control groups. Our findings indicate that, both CH and H-IH rats demonstrate right ventricular hypertrophy, polycythemia, pulmonary arterial remodeling, increased vascular eNOS expression, and enhanced vasoconstriction to U-46619. However, despite this upregulation of arterial eNOS, lungs from H-IH rats did not exhibit the increased ionomycin-dependent vasodilation characteristic of CH. Finally, we found that maintenance of eucapnia by provision of supplemental CO2 attenuated IH-induced polycythemia, vascular remodeling, right ventricular hypertrophy, and associated induction of arterial eNOS, but it did not alter IH-dependent increases in vasoconstrictor reactivity to U-46619.

METHODS

All protocols and surgical procedures employed in this study were reviewed and approved by the Institutional Animal Care and Use Committee. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Committee of the University of New Mexico Health Sciences Center (Albuquerque, NM). Male Sprague-Dawley rats (~250–350 g; Harlan Industries) were used for all experiments. Animals were maintained on a 12:12-h light-dark cycle.

**Experimental Groups**

*CH exposure.* Animals designated for exposure to CH were housed in a hypobaric chamber with barometric pressure maintained at ~380 Torr for 2 wk. The chamber was opened three times per week to provide animals with fresh food, water, and clean bedding. On the day of experimentation, rats were removed from the hypobaric chamber and immediately placed in Plexiglas chambers continuously flushed with a 12% O2–88% N2 gas mixture to reproduce inspired PO2 (~70 Torr) within the hypobaric chamber. Control animals were housed at ambient barometric pressure (~630 Torr).

*I-H and E-IH exposure.* H-IH, E-IH, and sham control rats were housed in Plexiglas chambers with free access to food and water and exposed to either IH or air-air sham cycling for 7 h/day for 2 wk as described previously (1, 20). During exposure, the atmosphere was controlled by a constant flow of gas through the boxes. For H-IH and E-IH treatment, the atmosphere alternated every 90 s between room air and hypoxic (nadir inspired O2 fraction = 5%) or hypoxic/hypercapnic air (nadir inspired O2 fraction = 5%; peak inspired CO2 fraction = 5%). Our laboratory has previously demonstrated systemic hypertension characteristic of SA in this model of E-IH (1, 20). For sham exposure, the inflow gas was always room air but the solenoid switches and inlets reproduced the noise and airflow disturbances of the IH protocol.

**Assessment of In Vivo Arterial Blood Gases**

To measure arterial blood gases, rats were anesthetized with isoflurane, and arterial lines were surgically inserted in the abdominal aorta via the femoral arteria. Lines were tunneled subcutaneously to the back of the neck where they were threaded through a plastic harness into a metal spring. The line and spring exited the cage through a small hole in the Plexiglas lid and were attached to a plastic swivel to allow the animal freedom of movement within the cage. The line was filled with saline containing heparin (10 U/ml) and flushed daily. After 7 days of recovery, three arterial blood samples were drawn to measure arterial blood gases using aABL5 blood-gas analyzer: the first before the H-IH or E-IH exposure, the second during the nadir or the most hypoxic portion of the cycling period (~5% O2 and ~5% CO2), and the third at the peak of the air flush (~20% O2 and ~0% CO2). These groups of rats were not carried out to 2 wk of treatment for further study.

**Assessment of Hematocrit and Right Ventricular Weight**

Blood samples were obtained by direct cardiac puncture at the time of lung isolation for measurement of hematocrit. Right ventricular hypertrophy was assessed as an index of pulmonary hypertension, as previously described (32, 37). Briefly, after isolation of the heart, the atria and major vessels were removed from the ventricles. The right ventricle was dissected from the left ventricle and septum, and each was cleaned of blood and weighed. The degree of right ventricular hypertrophy is expressed as the ratio of right ventricle to total ventricle weight and as the ratio of right ventricle to body weight.

**Vascular Morphometry**

Effects of CH, H-IH, and E-IH on pulmonary arterial remodeling were compared by quantitative morphometric analyses of arterial cross sections in lungs from each group of rats as our laboratory have described previously (32–34). Lungs were isolated from rats using established procedures (32, 33, 37). Animals were anesthetized with pentobarbital sodium (200 mg/kg ip). After cannulation of the trachea with a 17-gauge needle stub, the lungs were ventilated using a Harvard positive pressure rodent ventilator (model 683) at a frequency of 55 breaths/min and a tidal volume of 2.5 ml with a warmed and humidified gas mixture (6% CO2–21% O2–balance N2). Peak inspiratory pressure was set at 9 cmH2O, and positive end-expiratory pressure was maintained at 3 cmH2O. After a median sternotomy, heparin (100 U in 0.1 ml) was injected directly into the right ventricle, and the pulmonary artery cannulated with a 13-gauge needle stub. The preparation was immediately perfused at 0.8 ml/min by a Masterflex micropump (model 7524-10) with physiological saline solution (PSS) containing (in mM) 129.8 NaCl, 5.4 KCl, 0.5 NaH2PO4, 0.83 MgSO4, 19 NaHCO3, 1.8 CaCl2, and 5.5 glucose with 4% bovine serum albumin (wt/vol) added as a colloid. Papaverine (10−4 M) was also included in the PSS to maintain the vasculature in a dilated state during subsequent fixation. The left ventricle was cannulated with a plastic tube (4 mm outer diameter), and the heart and lungs were removed en bloc and suspended in a humidified chamber maintained at 38°C. The perfusion rate was gradually increased to 60 ml/min−1·kg body wt−1. Perfusate was pumped through a water-jacketed bubble trap maintained at 38°C before entering the pulmonary circulation. Experiments were performed with lungs in one of three conditions, achieved by elevating the perfusate reservoir until venous pressure (Pv) was ~12 mmHg. Previous work from our laboratory suggests that maximal recruitment and thus maximal vascular surface area is achieved at this flow and Pv (8). Pulmonary arterial pressure (Pa) and Pw were measured via side ports in the arterial and venous lines using Spectramed model P23 XL pressure transducers and recorded on a Gould RS 3400 chart recorder. The vasculature was initially washed with 250 ml of PSS, followed by 250 ml of fixative (0.1 M phosphate-buffered saline with 4% paraformaldehyde, 0.1% gluteraldehyde, and 10−4 M papaverine). Lungs were additionally inflated with fixative via the trachea to a pressure of 25 cmH2O during perfusion. The trachea was ligated with 2-0 silk, the arterial and venous lines simultaneously clamped, and the lungs immersed in fixative. A transverse section (2–3 mm thick) of tissue from the left lobe was removed and rinsed in phosphate-buffered saline. Sections were dehydrated in increasing concentrations of ethanol, cleared in xylene, and mounted in paraffin.

Transverse sections of the left lung were cut (4 µm thick) and mounted onto Superfrost Plus slides (Fisher Scientific). Sections were stained for elastin (Sigma Accustain Elastic Stain kit), and arteries were identified by the presence of an internal elastic lamina (32–34). Vessels were examined with a ×40 objective on a Nikon Optiphot microscope, and images were generated with a digital charge-coupled device camera (Photometrics CoolSNAP) and processed with MetaMorph software (Universal Imaging). Measurements were performed on 39–88 arteries (<50 µm outer diameter) and 7–55 arteries (50–100 µm) per rat from 4–5 rats/group. These measurements included medial circumference, assessed from the outer margin of the external elastic lamina, and luminal (14) diameter and were made using a blinded analysis.

**Western Blotting for eNOS**

Pulmonary arterial eNOS expression was compared between control and CH rats and between sham, H-IH, and E-IH rats by Western blotting as described previously (34, 44). Intrapulmonary arteries from pentobarbital sodium-anesthetized (200 mg/kg ip) rats were dissected from accompanying airways and surrounding lung tissue and snap-frozen in liquid N2. Each sample was homogenized on ice in 10 mM Tris-HCl buffer (pH 7.4) containing 255 mM sucrose, 2 mM EDTA, 12 µM leupeptin, 1 µM pepstatin A, 0.3 µM aprotinin, and 2 mM phenylmethylsulfonyl fluoride (all from Sigma). Homogenates were centrifuged at 1,500 g at 4°C for 10 min to remove tissue debris. Protein concentrations of samples were determined by the Bradford method (Bio-Rad Protein Assay). Tissue sample proteins from control/CH groups and sham/H-IH/E-IH groups were resolved by SDS-PAGE with 7.5% acrylamide on separate gels. In addition to samples, each gel included a molecular mass (Bio-Rad) standard. The separated

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Isolated Lung Preparation

Lungs were isolated and perfused as described in the *Vascular Morphometry* section above with the exceptions that papaverine was not included in the PSS and meclofenamate (50 μg/ml) (Sigma) was added to eliminate potential complicating influences of endogenous prostaglandins on vascular reactivity. This concentration of meclofenamate is approximately threefold higher than that previously shown to effectively inhibit prostaglandin synthesis in this preparation (10). Following cannulation of the pulmonary artery and left ventricle, the perfusion rate was gradually increased to 30 ml/min/kg body wt). Twenty milliliters of perfusate were washed through the lungs and discarded before recirculation was initiated with the remaining 40 ml. Experiments were performed with lungs in three conditions, achieved by elevating the perfusate reservoir until PV was close to the top of the concentration-response curve for this agonist (37), we were concerned that any potential differences in reactivity at this concentration of ionomycin (100 nM) may not be discernible. Therefore, all subsequent experiments were conducted using a lower concentration of ionomycin (100 nM). Ionomycin was chosen as a non-receptor-mediated vasodilator in this study because interpretation of responses to receptor-mediated agonists could be complicated by possible changes in receptor number or affinity in response to CH, E-IH, and H-IH. Before the first hypoxic stimulus, PO2 values of 10.2 ± 0.3 Torr was achieved, and the vasculature was then diluted with ionomycin. Preliminary experiments conducted using 350 nM ionomycin resulted in rather large vasodilatory responses that were not statistically different between sham (69.1 ± 1.7% reversal of U-46619-induced constriction, n = 3) and H-IH groups (61.2 ± 6.8%, n = 5). Because these responses were close to the top of the concentration-response curve for this agonist (37), we were concerned that any potential differences in reactivity between groups may not be discernible at this concentration. Therefore, all subsequent experiments were conducted using a lower concentration of ionomycin (100 nM). Ionomycin was chosen as a non-receptor-mediated vasodilator in this study because interpretation of responses to receptor-mediated agonists could be complicated by possible changes in receptor number or affinity in response to CH, H-IH, or E-IH. Furthermore, we have previously shown that ionomycin elicits vasodilatory responses in this preparation that are attenuated by nitric oxide synthase inhibition (34, 37) and correlate in magnitude with vascular eNOS protein levels, consistent with a portion of the response being mediated by endothelium-derived nitric oxide (32). Ionomycin was dissolved in anhydrous dimethyl sulfoxide (Sigma) and stored at 4°C.

Vasoconstrictor responses to U-46619. In separate experiments, vasoconstrictor responses to U-46619 (9,11-dideoxy-9α,11α-methanoepoxyprostaglandin F₂₀, Cayman Chemical; 100, 200 nM) were assessed in lungs from each group of rats. U-46619 was employed as a vasoconstrictor stimulus because it provides stable and consistent vasoconstrictor responses in this preparation (32, 35, 37, 38). A stable pressor response to the first dose of U-46619 was allowed to develop before administration of the second dose. U-46619 (Cayman) was dissolved in 95% ethanol and stored at −80°C before use.

Calculations and Statistics

Vascular morphometry. External and luminal arterial diameters were calculated from the medial and luminal circumferences, respectively. Arterial wall thickness was assessed by subtracting luminal radius from external radius and expressed as a percentage of external diameter according to the following formula:

\[
(2 \times \text{wall thickness})/\text{external diameter} \times 100
\]

Isolated lung experiments. Total pulmonary vascular resistance was calculated as the difference between Pa and Pv divided by flow. Vasodilatory responses were calculated as a percent reversal of U-46619-induced vasoconstriction.

Statistical analysis. All data are expressed as means ± SE, and values of n refer to the number of animals in each group. A t-test, one-way ANOVA, or two-way ANOVA was used to make comparisons when appropriate. If differences were detected by ANOVA, individual groups were compared with the Student-Newman-Keuls test. A probability of P ≤ 0.05 was accepted as significant for all comparisons.

RESULTS

Arterial Blood Gases

Arterial blood gases were measured on the first day of H-IH and E-IH cycling. Before the first hypoxic stimulus, PO2 values were not different between H-IH and E-IH groups (Fig. 1A, Pre). Both H-IH and E-IH rats showed the anticipated decrease in PO2 during hypoxic exposure (Fig. 1A, Nadir), and both groups recovered to prestimulus PO2 values during the air flush (Fig. 1A, Post). In addition, PCO2 values were not different between groups before exposure to hypoxia and CO2 (Fig. 1B, Pre); however, H-IH animals were hypocapnic, whereas E-IH animals remained eucapnic during nadir O2 and peak CO2 exposures (Fig. 1B, Post). During the air flush, H-IH animals remained slightly hypocapnic (Fig. 1B, Post). As expected, pH measurements were inversely related to PCO2 (Fig. 1C).

Right Ventricular Hypertrophy

Greater right ventricle-to-total weight ratios were observed for CH rats compared with the normoxic group (Fig. 2), thus demonstrating right ventricular hypertrophy indicative of pulmonary hypertension. Consistent with our hypothesis, right ventricle-to-total weight ratios were significantly greater in H-IH animals compared with air controls, and this difference was not present following E-IH. There were no differences between groups in left ventricle plus septal weight normalized to body weight (data not shown).

Polycythemia

CH rats exhibited polycythemia as indicated by a significantly greater hematocrit compared with normoxic control rats (Fig. 3). H-IH also resulted in elevated hematocrit compared...
with sham-treated animals, and this increase was not statistically different from CH. E-IH elicited a modest increase in hematocrit that was significantly lower than both H-IH and CH groups.

**Pulmonary Arterial Remodeling**

Figure 4 illustrates percent wall thickness for pulmonary arteries with external diameters of 50–100 μm (Fig. 4A) and 50–100 μm (Fig. 4B) from each group of rats. Both CH and H-IH exposure increased wall thickness compared with control groups in each range of vessel diameters. Although wall thickness in H-IH tended to be less than with CH, this difference reached statistical significance only in arteries <50 μm in diameter (Fig. 4A). Consistent with the observed inhibitory effects of E-IH on the development of right ventricular hypertrophy (Fig. 2), prevention of hypocapnia with supplemental CO₂ prevented IH-induced arterial remodeling (Fig. 4, A and B).

**eNOS Protein Expression**

Both CH and H-IH resulted in greater eNOS expression in intrapulmonary arteries compared with their respective control groups. In contrast, intermittent CO₂ exposure prevented IH-induced upregulation of eNOS (Fig. 5B). Statistical comparisons were not made between Fig. 5, A and B, because these were derived from separate blots.

**Isolated Lung Experiments**

**Perfusate PO₂, PCO₂ and pH.** No differences in PO₂, PCO₂, or pH were observed in perfusate effluent samples between groups, as depicted in Table 1.

**Baseline vascular resistances.** Consistent with previous reports (32, 37), baseline vascular resistances were significantly greater in lungs from CH rats compared with normoxic conditions.
controls (Table 2). In contrast, no differences in basal resistance were detected between sham, H-IH, and E-IH groups.

**Ionomycin-induced pulmonary vasodilation.** Lungs were constricted with U-46619 to produce similar increases in vascular resistance between groups (Table 2) before assessment of ionomycin-induced vasodilation. Lungs isolated from CH rats exhibited greater responsiveness to ionomycin (100 nM) compared with those of normoxic control animals (Fig. 6) as previously demonstrated following 4 wk CH (32, 37). In contrast, vasodilatory responses to ionomycin were not different between lungs from H-IH, E-IH and sham groups, and ionomycin produced less vasodilation in H-IH and E-IH than in CH lungs.

**U-46619-induced pulmonary vasoconstriction.** To determine the effect of H-IH and E-IH on pulmonary vasoconstrictor reactivity, vasoconstrictor responses to the thromboxane analog U-46619 were assessed in isolated lungs from each group of rats. Responses to U-46619 were greater in lungs from CH rats compared with normoxic controls (Fig. 7A). Increases in vasoreactivity to U-46619 were also observed in lungs from H-IH and E-IH vs. sham animals (Fig. 7B), although this increase reached statistical significance only at the 200 nM concentration. Furthermore, responses to U-46619 were less in H-IH and E-IH compared with CH lungs.

**DISCUSSION**

The major findings of this study are 1) similar to CH, H-IH induces right ventricular hypertrophy, polycythemia, pulmonary arterial remodeling and upregulation of arterial eNOS, and these responses to IH are inhibited by coincident maintenance of eucapnia; 2) although both CH and H-IH increase arterial eNOS expression, augmented ionomycin-dependent vasodilation was observed only in lungs from CH rats; and 3) both H-IH and E-IH increase pulmonary vasoconstrictor reactivity to the thromboxane analog U-46619 but to a lesser extent than with CH. These findings support a novel effect of hypocapnia to exacerbate IH-induced pulmonary hypertension.
and associated upregulation of arterial eNOS expression and reveal important differences between continuous and intermittent hypoxia with respect to the development of pulmonary hypertension and alterations in vasoreactivity.

Pulmonary hypertension during wakefulness has been reported to occur in 12–43% of patients with SA (2, 46) and may be as severe as 70 Torr (29). Furthermore, SA coincident with chronic obstructive lung disease (COPD) results in a 75% occurrence of pulmonary hypertension, suggesting that SA exacerbates pulmonary hypertension resulting from COPD (5, 13, 22). Despite growing recognition of the magnitude of this disorder, the cardiovascular sequelae leading to the progression of SA-induced pulmonary hypertension in humans remains poorly understood. Therefore, we investigated effects of IH on indexes of pulmonary hypertension and vasoreactivity using a rat model of SA that has previously been documented to induce systemic hypertension (1, 20), a common condition in patients with SA.

As expected, in vivo blood gas measurements indicate that the hypoxic stimulus employed by our model resulted in arterial hypoxemia in both H-IH and E-IH animals during intermittent hypoxia, whereas both groups returned to normal arterial Po2 levels during the air flush. IH animals demonstrated a fall in arterial PCO2 during hypoxia, indicative of hyperventilation in response to hypoxia. Furthermore, supplemental CO2 in the E-IH group prevented the development of hypocapnia during hypoxic exposure. Consistent with these changes in PCO2, arterial pH remained unchanged in E-IH animals during hypoxia, whereas H-IH animals became alkaloic during IH and pH remained elevated compared with E-IH during normoxic re-exposure. Although we did not measure blood gases in 2-wk CH rats, our laboratory has previously shown that 4-wk CH rats have a greater P2O2 and reduced pH and PCO2 under both normoxic and hypoxic conditions compared with control animals, reflecting hyperventilation and acid-base adjustments to CH (36). Whether similar acclimation occurs with long-term IH exposure remains to be determined.

Consistent with previous studies of episodic hypoxia in rodent models (9, 41), our present results have demonstrated an effect of H-IH to induce RV hypertrophy, pulmonary arterial remodeling, and polycythemia indicative of pulmonary hypertension. However, increases in right ventricular weight are not consistently observed in animal models employing frequent cycling of hypoxia plus CO2 supplementation (11, 12), suggesting a possible protective influence of intermittent hypocapnia or eucapnia to attenuate the pulmonary hypertensive response to IH. Indeed, continuous CO2 supplementation interferes with the development of pulmonary hypertension resulting from CH in rats (21, 31). In agreement with these previous observations, we found that CO2 supplementation prevented the development of right ventricular hypertrophy and pulmonary arterial remodeling and blunted the polycythemic response to H-IH. Although E-IH would be expected to produce cyclical increases in pulmonary arterial pressure as a result of intermittent hypoxic vasoconstriction, the lack of right ventricular hypertrophy in these animals argues against the possibility that sustained pulmonary hypertension develops in these animals during noncycling conditions.

Our present finding that arterial eNOS expression is increased in both 2-wk CH and H-IH hypertensive rats is consistent with earlier studies of lungs from 4-wk hypoxic rats (32, 33). The relative contributions of hypoxia per se and altered vascular mechanical forces associated with pulmonary hypertension in mediating eNOS upregulation with CH, however, are controversial. Le Cras and colleagues (23) found that even after banding the left pulmonary artery to minimize CH-induced increases in pulmonary hypertension and vascular shear forces, lung eNOS mRNA and protein levels were increased in response to hypoxic exposure, suggesting that eNOS gene expression is induced by hypoxia. In contrast, several lines of evidence from our laboratory support a role for mechanical forces associated with pulmonary hypertension in this response (32, 33). For example, eNOS is upregulated selectively within the pulmonary arterial circulation, which correlates with the site of pulmonary hypertension and where

Table 1. Saline perfusate Po2, PCO2, and pH in lungs isolated from normoxic, chronically hypoxic, sham-treated, hypocapnic intermittently hypoxic, and eucapnic intermittently hypoxic rats

<table>
<thead>
<tr>
<th></th>
<th>Po2, Torr</th>
<th>PCO2, Torr</th>
<th>pH</th>
<th>n</th>
</tr>
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<tbody>
<tr>
<td>Normoxic</td>
<td>135 ± 1</td>
<td>32 ± 2</td>
<td>7.40 ± 0.01</td>
<td>9</td>
</tr>
<tr>
<td>CH</td>
<td>138 ± 1</td>
<td>31 ± 1</td>
<td>7.41 ± 0.01</td>
<td>10</td>
</tr>
<tr>
<td>Sham</td>
<td>139 ± 2</td>
<td>33 ± 1</td>
<td>7.41 ± 0.01</td>
<td>9</td>
</tr>
<tr>
<td>H-IH</td>
<td>139 ± 2</td>
<td>33 ± 1</td>
<td>7.40 ± 0.01</td>
<td>9</td>
</tr>
<tr>
<td>E-IH</td>
<td>140 ± 2</td>
<td>33 ± 1</td>
<td>7.42 ± 0.01</td>
<td>10</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. CH, chronically hypoxic; H-IH, hypocapnic intermittently hypoxic; E-IH, eucapnic intermittently hypoxic. There are no significantly differences.

Table 2. Baseline vascular resistances and changes in resistance to U-46619 in isolated lungs

<table>
<thead>
<tr>
<th></th>
<th>Baseline Resistance</th>
<th>n</th>
<th>Δ Resistance</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxic</td>
<td>0.100 ± 0.006</td>
<td>10</td>
<td>0.305 ± 0.017</td>
<td>4</td>
</tr>
<tr>
<td>CH</td>
<td>0.155 ± 0.012*</td>
<td>10</td>
<td>0.255 ± 0.040</td>
<td>4</td>
</tr>
<tr>
<td>Sham</td>
<td>0.079 ± 0.005</td>
<td>12</td>
<td>0.323 ± 0.039</td>
<td>4</td>
</tr>
<tr>
<td>H-IH</td>
<td>0.078 ± 0.005†</td>
<td>11</td>
<td>0.290 ± 0.006</td>
<td>4</td>
</tr>
<tr>
<td>E-IH</td>
<td>0.091 ± 0.004‡</td>
<td>11</td>
<td>0.264 ± 0.019</td>
<td>4</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. Resistances are in Torr·ml⁻¹·min⁻¹·kg⁻¹ body wt. *P < 0.05 vs. normoxic. †P < 0.05 vs. CH.
shear forces are predicted to be highest as a result of arterial constriction, remodeling and polycythemia. Evidence that arterial eNOS levels are similarly elevated in nonhypoxic models of pulmonary hypertension (33, 42) provides additional support for mechanical stimuli rather than hypoxia per se in mediating this response to CH. In further agreement with a role for mechanical stimuli rather than hypoxia per se in the mechanism of vasoconstriction involving enhanced Rho kinase sensitization, a response associated with enhanced arterial RhoA and Rho kinase activity and increased Rho kinase phosphorylation or decreased caveolar localization, which limits eNOS activity despite increased protein levels (27). Indeed, a recent study reported sequestration of eNOS away from its functional caveolar localization in pulmonary artery endothelial cells from pulmonary hypertensive rats (26). Given that ionomycin-dependent dilation is only partially inhibited by N\textsuperscript{G}-nitro-L-arginine (37), it is additionally possible that enhanced reactivity to ionomycin in CH lungs is mediated in part by an endothelium-derived hyperpolarizing factor-type response, which may not be similarly upregulated in response to H-IH or E-IH. Future studies are necessary to evaluate the contribution of ROS or alternative vasodilatory mechanisms to the dissociation between eNOS levels and ionomycin-induced vasodilation following H-IH exposure.

Similar to our present observations of greater U-46619-induced vasoconstriction in lungs from CH, H-IH and E-IH rats, Thomas and Wanstall (41) found that IH (continuous 10% \textsubscript{0}2 for 8 h/day) enhanced vasoconstriction to various agonists, including U-46619, serotonin, and endothelin-1 in rat main pulmonary artery rings. CH additionally augmented pulmonary vasoconstritor responsiveness to several other agonists, including UTP, sphingosylphosphorylcholine, prostaglandin F\textsubscript{2\alpha}, angiotensin II, and norepinephrine (18, 25, 39). It is possible that greater Ca\textsuperscript{2+} entry through either store-operated or receptor-operated cation channels in pulmonary vascular smooth muscle from CH rats (24) mediates increased vasoreactivity to receptor-mediated agonists. However, a recent study by Nagaoka and colleagues (28) suggests that an additional mechanism of vasoconstriction involving enhanced Rho kinase activity represents a major component of the pulmonary hypertensive response to CH in rats. These findings are further consistent with recent observations by our laboratory that CH augments RhoA/Rho kinase-induced pulmonary vascular smooth muscle Ca\textsuperscript{2+} sensitization, but not PKC-dependent Ca\textsuperscript{2+} sensitization, a response associated with enhanced arterial RhoA and Rho kinase activity and increased Rho kinase expression (18). Whether similar mechanisms contribute to enhanced vasoconstrctor responsiveness following IH remains to be established.

The mechanism by which CO\textsubscript{2} reduces IH-mediated pulmonary hypertension remains to be established; however, it is possible that CO\textsubscript{2}-induced decreases in ROS are involved. CH elicits pulmonary hypertension in newborn rats, which is as-

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**Fig. 7.** Resistance changes to U-46619 (Torr \cdot ml\textsuperscript{-1} \cdot min\textsuperscript{-1} \cdot kg body wt) in lungs from normoxic and CH rats (A) and from sham-treated, H-IH, and E-IH rats (B). Values are means ± SE of n = 6–7 rats/group. *P < 0.05 vs. corresponding control group. #P < 0.05 vs. CH. *P < 0.05 vs. 100 nM U-46619.
associated with an increase in oxidant stress assessed by 8-isoprostane content in the lung (21). Interestingly, exposure of these rats to 10% CO2 during hypoxia reduced oxidant stress and attenuated CH-induced pulmonary hypertension. ROS have additionally been implicated in contributing to pulmonary hypertension based on evidence that there is increased oxidant stress in lungs from idiopathic pulmonary hypertension patients (4, 7). The administration of antioxidants blunts pulmonary hypertension (6, 16), and this response may be mediated by decreased oxidant-produced pulmonary vasoconstrictors, such as 8-isoprostane, peroxynitrite, and endothelin-1 (17). The effect of CO2 supplementation to prevent hypoxia-induced increases in lung 8-isoprostane is associated with decreased lung expression of the potent endothelin-derived vasoconstrictor peptide, endothelin-1 (21), suggesting that multiple mechanisms contribute to the protective effects of CO2 in the hypertensive pulmonary vasculature. Based on our present findings that CO2 attenuated both the pulmonary hypertensive and erythropoietin-dependent polycythemic responses to H-IH, it is possible that CO2 mediates these responses through a common mechanism of decreased hypoxia-inducible gene expression.

In summary, whereas CH, H-IH, and E-IH augmented pulmonary vasoconstriction to U-46619, only CH and H-IH were associated with increased expression of arterial eNOS, pulmonary arterial remodeling, and right ventricular hypertrophy indicative of pulmonary hypertension. Furthermore, augmented ionomycin-induced vasodilation was observed only in lungs from CH rats. Future studies are necessary to determine the contribution of ROS and myofilament Ca2+ sensitization to H-IH-induced pulmonary hypertension and associated changes in vasoreactivity, as well as mechanism by which supplemental CO2 attenuates the development of IH-induced pulmonary hypertension.

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