Differences in acute hypoxic pulmonary vasoressponsiveness between rat strains: role of endothelium

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J. Appl. Physiol. 87(1): 356–362, 1999.—Intact Madison (M) rats have greater pulmonary pressor responses to acute hypoxia than Hilltop (H) rats. We tested the hypothesis that the difference in pressor response is intrinsic to pulmonary arteries and that endothelium contributes to the difference. Pulmonary arteries precontracted with phenylephrine (10–7 M) from M rats had greater constrictor responses [hypoxic pulmonary vasoconstriction (HPV)] to acute hypoxia (% O2) than those from H rats: 473 ± 30 vs. 394 ± 29 mg (P < 0.05). Removal of the endothelium or inhibition of nitric oxide (NO) synthase by N-nitro-L-arginine (L-NA, 10–3 M) significantly blunted HPV in both strains. Inhibition of cyclooxygenase by meclofenamate (10–3 M) or blockade of endothelin type A and B receptors by BQ-610 (10–3 M) + BQ-788 (10–5 M), respectively, had no effect on HPV. Constrictor responses to phenylephrine, endothelin-1, and prostaglandin F2α were similar in pulmonary arteries from both strains. The relaxation response to ACh, an NO synthase stimulator, was significantly greater in M than in H rats (80 ± 3 vs. 62 ± 4%, P < 0.01), but there was no difference in response to sodium nitroprusside, an NO donor. L-NA potentiated phenylephrine-induced contraction to a greater extent in pulmonary arteries from M than from H rats. These findings indicate that at least part of the strain-related difference in acute HPV is attributable to differences in endothelial function, possibly related to differences in NO production.

The intensity of hypoxic pulmonary vasoconstriction (HPV) differs between species as well as between individuals within species. Along these lines, the Madison (M) and Hilltop (H) strains of Sprague-Dawley rat consistently manifest differences in pulmonary vascular responses to hypoxia. Previous investigations on intact animals (19) and isolated lungs (9) have demonstrated that acute hypoxic pulmonary vasoressponsiveness is greater in the M than in the H strain. Mechanisms responsible for this difference are unknown.

Pulmonary arteries isolated from a variety of species including humans contract in response to acute hypoxia and have been used to study the mechanisms underlying HPV (5, 6, 23). These studies suggest that the sensor and effector of HPV are indigenously present in the vessel wall, that the intensity of HPV is endothelium dependent, and that hypoxic contraction is at least partially related to the inhibition of endogenous nitric oxide (NO) production by hypoxia (12, 22).

In the present investigation we hypothesized that the different pulmonary vascular responses of the M and H strains to acute hypoxia are related to properties intrinsic to the vessels and that we would be able to replicate the previously demonstrated vasoconstrictor differences in isolated pulmonary vessels. Furthermore, we hypothesized that the differences in vasoreactivity are attributable to functional differences of the endothelium as opposed to the medial and adventitial layers. To test these hypotheses, we isolated pulmonary arteries from normoxic M and H rats and tested responses to acute hypoxia, determined passive stretch curves, and assessed pharmacological responses to vasoconstrictors, vasodilators, and inhibitors of selective vasoactive mediators. Our findings demonstrate differences in acute hypoxic vasoreactivity between M and H rats in isolated pulmonary arteries. Furthermore, our findings suggest that the endothelial production of NO contributes to these differences.

METHODS

Animals and materials. Adult male Sprague-Dawley rats (4–6 wk old, 200–275 g) were obtained from Harlan Sprague Dawley Laboratories (Madison, WI; M rats) and Hilltop Laboratories (Scottsdale, PA; H rats). All reagents were purchased from Sigma Chemical (St. Louis, MO), except where otherwise stated. The following vasoactive agents were used: L-phenylephrine (PE), ACh chloride (ACh), sodium nitroprusside (SNP), prostaglandin F2α, N-nitro-L-arginine (L-NA), sodium mafenamate, BQ-610, BQ-788 (Peptides International, Louisville, KY), and endothelin-1 (ET-1; Genentech, South San Francisco, CA). All reagents were prepared in deionized water except for BQ compounds and ET-1. BQ compounds were first dissolved in DMSO; ET-1 was first dissolved in 0.1% acetic acid and then diluted with water.

Isolated artery preparation. Rats were anesthetized with pentobarbital sodium (100 mg/kg ip) and exsanguinated by cutting the abdominal aorta. Heart and lungs were removed en bloc and placed in Earle's balanced salt solution (EBSS) containing (in mM) 116.3 NaCl, 5.4 KCl, 0.83 MgSO4, 19.0 NaHCO3, 1.04 NaH2PO4, 1.8 CaCl2, 2H2O, 5.5 d-glucose, and 0.03 phenol red as a pH indicator. The distal extrapulmonary and proximal intrapulmonary arteries from left lung (−1.5−2.0 mm ID) and thoracic aorta (−2.0−2.5 mm ID) were isolated, cut into rings (2−3 mm long), and mounted in 20-ml organ chambers filled with EBSS and bubbled with 95% O2-5% CO2, as described previously (8). In some experiments the endothelium was removed by gently rubbing the lumen

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with a roughened stainless steel wire. Force was measured in milligrams by using isometric force transducers (Grass FT03) and recorded on a polygraph (model 790, Grass).

Hypoxic response. The response to acute hypoxia was obtained as described previously (23). Because the response to acute hypoxia was found to be more robust and reproducible in PE-precontracted rings (23), the artery preparations were precontracted with a submaximal concentration of PE (10^{-7} M) and subjected to hypoxia by bubbling the organ chambers with a hypoxic gas mixture containing 5% PO_2 (85 Torr), 3% (PO_2 = 45 Torr), and 0% O_2 (PO_2 = 10 Torr) in a graded fashion. PO_2 in organ baths was monitored with an O_2 electrode (model 125/05, Instech Laboratories, Horsham, PA). Each 10-min exposure was followed by 10 min of normoxia for reequilibration during hypoxic exposure. All gas mixtures contained 5% CO_2-95% N_2. Other investigators have described monophasic (23, 29) or biphasic (1, 17) responses to hypoxia in isolated pulmonary arteries, the latter characterized by an initial (phase 1) contraction followed by relaxation and then a more sustained (phase 2) contraction. In this study we examined the monophasic response, as this has been described as a suitable in vitro model for evaluation of acute hypoxic vasoconstriction (23). The hypoxic contractile response was determined as the difference between baseline tone (after PE-induced precontraction) and the peak force developed during the hypoxic challenge. In some experiments, responses to acute hypoxia were obtained in pulmonary artery rings that were denuded of the endothelium or pretreated with L-NA (10^{-6} M), medoflenamate (10^{-5} M), or BQ-610 + BQ-788 (10^{-5} M).

Passive length-tension relationship. The vessel passive length-tension relationship was determined by the method of Coflesky et al. (3). Briefly, to determine the relationship between resting tension and vessel internal circumference (C_v), a mounted segment was stretched circumferentially on the wires until a force was detected by the transducer. By applying the law of Laplace, the effective circumference (C_eff) at which the measured tension would be equivalent to that produced by an intraluminal pressure of 10 Torr was derived from C_v. Passive resting tensions were then measured (in mg) at increments of C_v from 0.7 to 1.2 C_v and expressed as percentage of C_eff.

Pharmacological responses. The pulmonary artery and aortic rings were gradually stretched to a predetermined optimal passive resting tension of 0.5 and 1.0 g, respectively (8), and allowed to stabilize for 60 min. After stabilization, viability of the preparations and functionality of the endothelium were demonstrated by vessel contraction with PE (10^{-10}-10^{-8} M) and subsequent relaxation with ACh (10^{-2} M). The endothelium was considered denuded when there was no response to ACh. Viability of denuded ring preparations was demonstrated by contractions with PE and relaxation with SNP. The preparations were washed with fresh EBSS and allowed to return to baseline tension. To investigate whether the strain differences are unique to hypoxia or generalized, we obtained cumulative concentration-response curves to the contractile agonist ET-1 (10^{-10}-10^{-7} M) and prostaglandin F_2a (10^{-6}-10^{-5} M). In some preparations, relaxation responses were assessed in vessels precontracted with PE (10^{-7} M) by obtaining cumulative concentration-response curves to the endothelium-dependent vasodilator ACh (10^{-7}-10^{-2} M) and the endothelium-independent vasodilator SNP (10^{-9}-10^{-6} M). To determine whether contractile behavior of the vessels would differ in response to NO synthase (NOS) inhibition, L-NA (10^{-5}-10^{-3} M) was administered to PE (10^{-7} M)-precontracted vessels.

Microscopy. After completion of the functional studies, the vessels were flushed with fresh EBSS and allowed to return to the baseline tension of 0.5 g. Then EBSS in the organ baths was replaced with 10% neutral buffered formal saline. The vessels were fixed overnight at this setting and were embedded in epoxy resin. Microscopic (1-µm) sections were cut and stained with toluidine blue (2% in 1% borax). The following measurements were made using a modification of the method of Coflesky et al. (3). Total vessel luminal area and medial thickness (MT, from the external edge of the internal elastic lamina to the internal edge of the external elastic lamina) were determined by using image analysis software from Scion Image. From these parameters, percent wall thickness was determined using the following equation: (2MT/d) * 100, where d is diameter.

Statistical analysis. The response to vasoconstrictors was expressed as force (mg) and to vasodilators as percentage of PE-induced contraction. Values are means ± SE; n is number of rats. ANOVA or Student’s t-test was used where appropriate to determine statistically significant differences. When ANOVA revealed significant F ratios, the significance of differences between pairs of means was tested using Student’s t-test. Differences were considered statistically significant at P < 0.05.

RESULTS

Effects of hypoxia on artery preparations. Under normoxic conditions, pulmonary arteries from M and H rats were precontracted with PE (10^{-7} M) so that initial tensions were 333 ± 19 and 325 ± 20 mg (n = 19–20, P > 0.05), respectively. Decreasing PO_2 to 85 Torr by bubbling the organ baths with 5% O_2 had no contractile effect on the pulmonary arteries. Further decreasing the PO_2 increased the force in the precontracted pulmonary arteries from the M and H rats (Fig. 1A). The peak response to 3% O_2 in the pulmonary artery of M rats (391 ± 32 mg, n = 19) was not significantly different from that in H rats (334 ± 23 mg, n = 20). However, the contraction induced by 0% O_2 was significantly greater in M rats (473 ± 30 mg, n = 23, P < 0.05) than in H rats (394 ± 29 mg, n = 22). Removal of the endothelium completely abolished hypoxic contraction in both strains (Fig. 1B). In contrast to the pulmonary arteries, PE-precontracted aortic rings relaxed in response to acute hypoxia. Increasing the severity of hypoxia increased the relaxation response to a similar magnitude in both strains (Fig. 1C).

Pulmonary artery microscopy and passive length-tension relationship. To elucidate whether pulmonary arteries from M and H rats manifested structural or passive mechanical differences, we determined MT, total area, and percent wall thickness of excised vessel rings and passive length-tension relationships, respectively. The MT, total area, and percent wall thickness (Table 1) and passive length-tension curves (Fig. 2) were similar in vessels isolated from M and H rats.

Responses to constrictor agents. To determine whether M rats had greater constrictor responses than H rats to agents other than hypoxia, we measured isometric force generated by pulmonary artery rings exposed to PE, ET-1, and PGF_2alpha (Fig. 3). All three vasoconstrictors produced similar concentration-dependent increases in force in pulmonary artery rings of both strains (Fig. 3).
Responses to vasorelaxant agents. ACh, an endothelium-dependent vasodilator, induced concentration-dependent relaxation of PE (10\(^{-7}\) M)-precontracted pulmonary artery rings in the two strains (Fig. 4A). Relaxation was significantly greater in pulmonary arteries of M than of H rats at 10\(^{-3}\) M ACh (71 ± 5 vs. 46 ± 7% of PE-induced contraction, \(n = 20–25\), \(P < 0.01\)) and 10\(^{-2}\) M ACh (80 ± 3 vs. 62 ± 4% of PE-induced contraction, \(n = 20–25\), \(P < 0.01\)). However, SNP, an endothelium-independent vasodilator, induced similar relaxation responses in pulmonary arteries from both rat strains (Fig. 4B).

Effects of inhibitors of endothelium-derived vasoactive mediators. L-NA (10\(^{-3}\) M), an NOS inhibitor, increased baseline force by 198 ± 48 and 153 ± 34 mg (\(n = 8–14\), \(P > 0.05\)) in vessels from M and H rats, respectively. L-NA also amplified PE (10\(^{-7}\) M)-induced contractions in a concentration-dependent manner in pulmonary artery rings isolated from both strains (Fig. 5). The amplification was significantly greater in vessels from M than from H rats. Pretreatment with L-NA (10\(^{-3}\) M) significantly reduced hypoxic contraction in PE-precontracted arteries from M rats and eliminated it in those from H rats (Fig. 6). In contrast, pretreatment with the cyclooxygenase inhibitor meclofenamate (10\(^{-5}\) M) or combined endothelin type A and B receptor blockade with BQ-610 + BQ-788 (10\(^{-5}\) M each) did not significantly affect HPV in PE-precontracted pulmonary artery rings from either strain (Fig. 6).

**DISCUSSION**

We found that pulmonary arteries isolated from M rats have greater constrictor responses to acute hypoxia than those from H rats. This observation is consistent with the results of our previous studies showing greater acute HPV responses in M than in H rats, in intact animals (19) as well as in isolated, blood-perfused lung preparations (9). The present finding demonstrates that the increased acute vasoconstrictor response to hypoxia in M rats is at least partly an intrinsic property of pulmonary arteries independent of the effects of circulating vasoactive mediators or other extravascular signaling mechanisms. However, our study does not exclude the possibility that these latter extrinsic factors may contribute to differences in vasoreactivity in intact lungs or animals. Aortic ring preparations from both strains relaxed to the same extent in Table 1. Comparison of medial thickness, total area, and percent wall thickness of Hilltop and Madison rat pulmonary arteries

<table>
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<th>Hilltop ((n = 13))</th>
<th>Madison ((n = 10))</th>
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<tr>
<td>Medial thickness, µm</td>
<td>42.0 ± 3.4</td>
<td>44.2 ± 1.8</td>
</tr>
<tr>
<td>Total luminal area, mm(^2)</td>
<td>0.18 ± 0.02</td>
<td>0.16 ± 0.007</td>
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<tr>
<td>%Wall thickness</td>
<td>6.5 ± 0.5</td>
<td>7.7 ± 0.5</td>
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Values are means ± SE; \(n\), number of animals.
response to hypoxia, suggesting that the strain differences are unique to the pulmonary circulation.

Removal of the endothelium from the isolated pulmonary arteries markedly blunted hypoxia-induced contractions and eliminated differences between vessels isolated from M or H rats. These findings suggest that a mediator derived from the endothelium or some interaction between endothelium and smooth muscle cells is responsible for the greater contractile responses of pulmonary arteries in the M rats. Previous studies have found that endothelial stripping markedly inhibits contractile responses to hypoxia in isolated pulmonary arteries (10, 12, 23). However, our observation is the first to suggest that strain differences in hypoxic pulmonary vasoresponsiveness may be related to properties of the endothelium.

Other potential mechanisms by which isolated pulmonary arteries may manifest different contractile responses to hypoxia include differences in vessel structure or passive mechanical properties. Regardless of the role of the endothelium, thicker or more muscular arteries could conceivably generate more force in response to constrictor agents. However, morphometric studies of pulmonary arteries fixed at the same tension revealed no differences in MT or vascular smooth muscle luminal area. Consistent with this observation, previous histological studies on tissue sections from intact lungs from M and H rats showed no morphological differences under normoxic conditions (4, 14). In addition, passive stretch-tension curves were virtually identical between pulmonary arteries isolated from the different strains. Accordingly, structural or passive mechanical differences do not explain the different contractile responses.

In response to various vasoconstrictor agents including PE, ET-1, and prostaglandin F\textsubscript{2\alpha}, isolated pulmonary arteries from M and H rats responded similarly. These results are in accordance with those observed in isolated blood-perfused lungs, where the response to acute hypoxia was different between the M and H rats but the response to ANG II was the same (9). Taken together, these results demonstrate that the enhanced contractile responses of the arteries were relatively

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**Fig. 3.** Contractile force developed in response to increasing concentrations of phenylephrine (A, n = 29–32), endothelin-1 (B, n = 6–7), and prostaglandin F\textsubscript{2\alpha} (C, n = 4–10) by pulmonary arteries isolated from Madison and Hilltop rats. Values are means ± SE.

**Fig. 4.** Relaxation of phenylephrine (10\textsuperscript{-7} M)-precontracted pulmonary arteries isolated from Madison and Hilltop rats in response to increasing concentrations of Ach (A, n = 20–25) and sodium nitroprusside (B, n = 20–25). Values are means ± SE. **P < 0.001 compared with Hilltop.
specific for hypoxia and that a generalized hyperresponsiveness to contractile agents cannot explain the differences between the strains. Likewise, in response to the endothelium-independent vasodilator SNP, relaxation responses were similar in isolated pulmonary arteries precontracted with PE. However, the percent relaxation was significantly greater among M than among H rats in response to the endothelium-dependent vasodilator ACh. This enhanced vasorelaxation of isolated pulmonary arteries from M rats suggests greater release of NO from the endothelium in response to ACh.

We did not measure NO levels or investigate the mechanisms involved in the presumed greater NO release in M rats, but possibilities include increased muscarinic receptor density or activity, enhanced intracellular signaling pathways, enhanced endothelial NOS activity, or increased availability of L-arginine, the substrate for NOS. Alternatively, ACh could stimulate increased release of endothelium-derived hyperpolarizing factor(s) from M rats (2).

Biochemical studies suggest that O2, one of the substrates for NOS, becomes a rate-limiting factor in the production of NO by NOS during hypoxia (20, 21, 27). Several studies in isolated pulmonary arteries and endothelial cells in culture suggest that hypoxic contraction is a consequence of the inhibition of NOS and subsequent reduction in the relaxing effect of endogenous NO on pulmonary vascular tone (11, 12, 22, 26). Consistent with this possibility, the acute hypoxic contractile response and the relaxation response to the NO-dependent vasodilator carbachol are blunted in pulmonary arteries from chronically hypoxic rats compared with vessels from normoxic rats (12). This study demonstrates a parallel between the vessel’s ability to release NO and the magnitude of hypoxic contraction; in vessels with more NO-releasing capability at baseline, inhibition of NO release during hypoxia is greater, which permits greater contraction. The similarity between these observations and the responses in the M vs. the H rats suggests that the enhanced HPV in the M rats might be a consequence of greater NO-releasing capability at baseline and greater inhibition of NOS in response to hypoxia.
This possibility is further supported by our finding that precontracted pulmonary arteries from the M rats manifested greater contractile response to NOS inhibition with L-NA than did H rats. Not only did L-NA potentiate submaximal contractions to PE, but it also inhibited any further contractile response to hypoxia in PE-precontracted vessels from H rats, consistent with previous observations of other investigators using NOS inhibitors (12, 22, 29). These findings are consistent with the hypothesis that once NOS is inhibited by L-NA, no more inhibition occurs in response to hypoxia, and the hypoxic contractile response is prevented. In M rat pulmonary arteries, on the other hand, there was a small residual response to hypoxia in the presence of L-NA, raising the possibility that other mediators in addition to NO may be involved.

Potential endothelium-derived vasoactive mediators that could contribute to the enhanced hypoxic contractile responses include not only NO but also prostaglandins and ET-1. Accordingly, we performed inhibitor studies in isolated pulmonary arteries with intact endothelium to obtain evidence for the potential involvement of these pathways. We found no evidence that increased release of ET-1 is involved on the basis of the complete absence of any effect of combined endothelin type A and B receptor blockade on hypoxic contractile responses, consistent with the results of other studies in isolated pulmonary arteries (7, 15) and perfused lungs (24). Nevertheless, ET-1 may serve a role in the mediation of HPV in other experimental models (25), including intact rats (18).

Consistent with previous studies in isolated rat pulmonary arteries (1, 23), the cyclooxygenase inhibitor meclofenamate had no effect on hypoxic responsiveness in isolated pulmonary arteries. This observation renders unlikely the possible involvement of cyclooxygenase metabolites, including prostacyclin, in the contractile differences between the M and H rat strains. However, prostaglandins could still play a role in the differences among other animal species, because pretreatment with flurbiprofen, the cyclooxygenase inhibitor, diminished HPV in isolated pulmonary arteries from sheep (5) and humans (6).

A number of limitations should be considered in interpreting our results. We used only distal extrapulmonary or proximal intrapulmonary arteries in our study, which may respond differently from smaller resistance arteries (13, 17). However, the fact that hypoxic contractile responses in these vessels parallel the hypoxic pressor responses observed in intact animals (19) and isolated lungs (9) suggests that they and resistance vessels respond similarly. The behavior of pulmonary arteries in isolated preparations may not correspond directly to in situ behavior. In addition, observations on endothelium-denuded vessels must be interpreted with great caution. The process of denudation may injure the remaining medial layer, causing injury-related changes in contractile behavior, rather than changes due to the endothelial stripping per se. To minimize this possibility, we removed the endothelial surface with great care and established that PE-induced contractions and SNP-induced relaxations were present before studying denuded vessels. Nevertheless, our results do not exclude the possible role of potassium channels or other vascular smooth muscle-related mechanisms in mediating the strain-related differences in response to acute hypoxia.

Our findings implicate the endothelium as an important modulator of the intensity of HPV contributing to differences in vasoresponsiveness between strains and, presumably, between species as well. In addition, our evidence points to the NOS system as the most likely endothelium-derived vasoactive mediator system that contributes to the strain-related differences. Additional studies that examine gene expression of NOS and release of NO from pulmonary arteries under basal and hypoxia-stimulated conditions are needed to establish the mechanisms implicated in this study.

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