Chronic hypoxia augments endothelin-B receptor-mediated vasodilation in isolated perfused rat lungs

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Muramatsu, Masashi, Masahiko Oka, Yoshiteru Morio, Sanae Soma, Hideki Takahashi, and Yoshinosuke Fukuchi. Chronic hypoxia augments endothelin-B receptor-mediated vasodilation in isolated perfused lung. Am. J. Physiol. 276 (Lung Cell. Mol. Physiol. 20): L358–L364, 1999.—To investigate whether chronic hypoxia affects endothelin-B (ETB) receptor-mediated pulmonary vasodilation, we compared the vasodilator responses to IRL-1620, a selective ETB-receptor agonist, in isolated perfused lungs from normoxic and chronically hypoxic adult male rats. IRL-1620 caused a dose-dependent vasodilation that was greater in the hypertensive lungs than in the normotensive lungs. In normotensive lungs, a nitric oxide (NO) synthase inhibitor, N-nitro-L-arginine (L-NNA; 300 µM), and an ATP-sensitive potassium (KATP) channel inhibitor, glibenclamide (Glib; 10 µM), each reduced the vasodilator response to IRL-1620 (1 nM), but the combination of L-NNA and Glib inhibited it more effectively than either drug alone. In contrast, L-NNA alone, but not Glib alone, completely blocked IRL-1620-induced vasodilation in hypertensive lungs. The vasodilator response to a KATP-channel opener, NIP-121 (1 µM), but not the response to sodium nitroprusside (1 µM), was enhanced in hypertensive lungs. We also found increased expression of mRNA for the ETB receptor in lung tissue after hypoxic exposure. In addition, semiquantitative immunohistochemistry demonstrated higher expression levels of ETB receptors in the endothelium of distal segments of the pulmonary artery in hypoxic than in normoxic rats. These results suggest that ETB receptor-mediated pulmonary vasodilation is augmented after chronic hypoxic exposure and that release of NO may be the sole mechanism of this vasodilation in hypertensive lungs, whereas both release of NO and activation of KATP channels are involved in normotensive lungs. We speculate that the underlying mechanism responsible for this augmentation may partly be related to upregulation of ETB receptors in the endothelium of pulmonary resistance arteries in hypertensive lungs.

vasodilation; endothelin-B; pulmonary hypertension; nitric oxide; potassium channels

ENDOTHELIN (ET)-1, a 21-amino acid polypeptide produced by the vascular endothelium, is the most potent long-lasting vasoconstrictor ever described (32). ET-1 can also promote vasodilation in various vascular beds (5, 7, 9, 10, 15, 25). The functions of ET-1 are mediated through at least two distinct receptor subtypes (ETA and ETB) (1, 28). ETA receptors are present on smooth muscle cells, and their activation is believed to cause vasoconstriction. ETB receptors expressed on endothelial cells mediate vasodilation, whereas ETB receptors expressed on smooth muscle cells mediate vasoconstriction (13, 18). Several studies suggested that nitric oxide (NO) release (5, 25) and the activation of ATP-sensitive potassium (KATP) channels (7, 9, 15) mediate the vasodilator response to ET-1 by acting through ETB receptors in the pulmonary vascular endothelium, whereas most studies (5, 7, 9, 15) demonstrated that cyclooxygenase metabolites do not mediate this response. ETA and ETB receptors on smooth muscle cells mediate vasoconstriction by a direct receptor-dependent action, which results in an increase in intracellular calcium (13, 18).

Recently, it has been suggested that ET-1 may play a major role in the development of chronically hypoxic pulmonary hypertension. Li et al. (14) observed increased expression of both ET-1 and its receptors in hypoxic hypertensive rat lungs, and other studies (3, 4, 6) showed that the development of hypoxic pulmonary hypertension is prevented in rats treated with ET-1-receptor blockers. However, the vasodilator actions of ET-1 in the pulmonary circulation of animals exposed to chronic hypoxia have not been fully elucidated. For instance, whether the pulmonary vasodilator response to ET-1 is impaired (7) or augmented (25) after chronic hypoxia is controversial. Because pulmonary vasodilation induced by ET-1 activation of ETB receptors may modulate the development of pulmonary hypertension, it is important to reexamine this question to better understand the actions of ET-1 in the pathogenesis of hypoxic pulmonary hypertension. Therefore, the major purpose of the present study was to clarify whether the vasodilator response induced by ETB-receptor activation in the pulmonary vasculature is increased or decreased after exposure to chronic hypoxia.

We have, therefore, compared the vasodilator responses to IRL-1620, a highly selective ETB-receptor agonist (29), in normotensive and hypertensive rat lungs. To learn more about the mechanisms of this vasodilator response, we examined the effects of N-nitro-L-arginine (L-NNA), an inhibitor of NO synthase (NOS), and glibenclamide (Glib), an inhibitor of KATP channels, on IRL-1620-induced vasodilation in normotensive and hypertensive lungs. Furthermore, additional experiments were performed to test whether chronic hypoxia alters the vasodilator responses to the NO donor sodium nitroprusside (SNP) and to the KATP-channel opener NIP-121 (17). Alteration of ETB-receptor expression after chronic hypoxic exposure may affect the vasodilator response to IRL-1620, so we also investigated the changes in ETB-receptor mRNA tran...
script levels and immunoreactivity in the pulmonary vasculature after chronic hypoxia.

METHODS

All protocols and surgical procedures were approved by the Institutional Animal Use Committee of Juntendo University School of Medicine (Tokyo, Japan) in accordance with National Institutes of Health and American Physiological Society guidelines.

Animals. Experiments were performed with two groups of adult male Sprague-Dawley rats (300–400 g). The pulmonary normotensive rats (the control group) were housed at the ambient barometric pressure (760 mmHg), and the chronically hypoxic pulmonary hypertensive rats (the experimental group) were housed in a hypobaric chamber, with the barometric pressure maintained at 380 mmHg for 3–4 wk. The chamber was flushed continuously with room air to prevent the accumulation of CO₂, NH₃, and H₂O. The hypobaric pressure was maintained 24 h/day except when the chamber was opened for 10–15 min once or twice a day to remove rats or to clean the cages and replenish the food and water. All rats were exposed to a 12:12-h light-dark cycle and allowed free access to standard rat food and water.

Isolated perfused lungs. Isolated lungs were prepared as previously described (21), with minor modifications. The lungs were isolated from normoxic and chronically hypoxic rats after intraperitoneal administration of 30 mg of pentobarbital sodium and intracardiac injection of 100 U of heparin. The lungs of the pulmonary hypertensive rats were isolated within 1 h of their removal from the hypobaric chamber. Isolated lungs were ventilated with a humid mixture of 21% O₂–7% CO₂–74% N₂ at 60 breaths/min, with an inspiratory pressure of 9 cmH₂O and an end-expiratory pressure of 2.5 cmH₂O. They were perfused through a main pulmonary arterial cannula with a peristaltic pump at a constant flow of 0.04 ml·g body wt⁻¹·min⁻¹. The perfusate was a physiological salt solution containing (in mM) 116.3 NaCl, 5.4 KCl, 0.83 MgSO₄, 19.0 NaHCO₃, 1.04 NaH₂PO₄, 1.8 CaCl₂·2H₂O, and 5.5 µ-g glucose (Earle’s balanced salt solution; Sigma, St. Louis, MO). Ficoll (4 g/100 ml, type 70; Pharmacia, Uppsala, Sweden) was included as a colloid. After the blood was flushed out of the lungs with 20 ml of the physiological salt solution, the lungs were perfused with a recirculated volume of 30 ml. Effluent perfusate drained from a left ventricular cannula into a perfusate reservoir. Lung and perfusate temperatures were maintained at 38°C, and the pH of the perfusate was kept between 7.3 and 7.4. The pulmonary perfusion pressure was measured continuously with a transducer and a pen recorder, and the lungs were equilibrated for 20 min before vascular responses were elicited.

Experimental protocols in isolated perfused lungs. Initial experiments compared the vasodilator responses of normotensive and hypertensive lungs to IRL-1620 during increased vascular tone induced by infusion of 9,11-dideoxy-11α,9α-epoxymethanoprostaglandin F₂α (U-46619; a thromboxane mimetic; Sigma). Previous studies (5, 7, 9, 15) showed that prostacyclin generation is not involved in the mechanism of ET-1-induced pulmonary vasodilation, and our preliminary experiments showed that medofenamate, an inhibitor of synthesis of prostacyclin (31), does not alter the vasodilator response to IRL-1620, so medofenamate (3.1 µM) was added to the perfusate in all of the experiments. After a 20-min equilibration, infusion of U-46619 was started via the pulmonary arterial line to achieve a pressor response of 5 mmHg in 15 min with an infusion pump (model STC-521, Terumo). The dose of U-46619 was adjusted to achieve the same rate of increase in pulmonary perfusion pressure in each lung. Infusion of U-46619 caused a gradual increase in the perfusion pressure, and the pressure kept increasing unless the infusion was terminated (see Fig. 1). After infusion of U-46619 for 15 min, IRL-1620 (0.1–5.0 nM; Ciba-Geigy, Takarazuka, J apan) (29) or its vehicle (DMSO) was added to the perfusate reservoir at 2-min intervals.

The next experiment tested whether pretreatment with either an inhibitor of NOS, L-NNA (Aldrich, Milwaukee, WI), an inhibitor of K ATP channels, Glib (Sigma), or both inhibited the vasodilator response to IRL-1620 in normotensive and hypertensive lungs. Fifteen minutes after the addition of either L-NNA (300 µM), Glib (10 µM), L-NNA plus Glib, or vehicle (DMSO), the U-46619 infusion was started. IRL-1620 (1 nM) was added to the perfusate reservoir after 15 min of U-46619 infusion, and the vasodilator response was measured. The concentrations of L-NNA and Glib used in this experiment were based on the previous results reported in isolated rat lungs (9, 25). The vasodilator response is expressed as (immediate depressor response to the vasodilator/ respective pressor response to U-46619) × 100 (in percent).

To test whether chronic hypoxia affects vasoreactivity to an NO donor or a K ATP-channel opener, we compared the vasodilator responses to SNP (Sigma) or NIP-121 (Nissan Chemical, Tokyo, Japan) (17) in normotensive and hypertensive lungs. SNP (1 µM) or NIP-121 (1 µM) was added to the perfusate reservoir after 15 min of U-46619 infusion. We also tested whether Glib (10 µM) blocks the vasodilator response to NIP-121. NIP-121 (1 µM) was added to the perfusate reservoir after 15 min of U-46619 infusion with and without Glib pretreatment in normotensive lungs. The vasodilator response was calculated as described above.

Measurement of lung ET B-receptor mRNA levels by Northern blot analysis. Northern blot analysis was carried out as previously described (23). Briefly, 10 µg of denatured total RNA isolated from normotensive and hypertensive rat lungs were electrophoresed on 1.0% agarose-formaldehyde gel and transferred to a nylon membrane (Hybond-N+, Amersham Japan, Tokyo, Japan). After prehybridization, the membrane was hybridized for 48 h at 42°C with a random-primed 32P-labeled ET B-receptor cDNA probe. The primers used for PCR were 5’–CTGCTGTTGCGAAGCATTGTGAG-3’ (sense) and 5’–CATCGGTCTTCTAGGTGTA-3’ (antisense). After hybridization, the membrane was washed at a final stringency in 0.1× sodium-saline citrate (0.15 M NaCl and 0.015 M sodium citrate, pH 7.4)–0.1% SDS at 65°C. Autoradiography was performed, and the bands were quantified by densitometry with a computer-assisted image analyzer (BAS 2000, Scananalytics, Circle Billerica, MA). After the membrane was probed with ET B-receptor cDNA, it was stripped and re-probed with a 32P-labeled 28S rRNA probe as a positive control. The amount of ET B-receptor mRNA is expressed as the ratio of ET B-receptor mRNA to 28S rRNA.

Immunohistochemistry for lung ET B receptor. The rats that had been exposed to air or hypoxia were anesthetized with an intraperitoneal injection of 60 mg of pentobarbital sodium. After intracardiac injection of 100 U of heparin, the lungs were isolated from the rats. After cannulas had been inserted into the pulmonary artery and left atrium, the lungs were perfused at 36 cmH₂O through the pulmonary arterial cannula with phosphate-buffered saline. The left lung was perfused with 4% paraformaldehyde (PFA), then was inflated by infusion of 4% PFA through the cannulas inserted in the trachea to a pressure of 24 cmH₂O and fixed in 4% PFA overnight at 4°C. After the PFA was removed, the lung tissue was filled with and embedded in optimum cutting temperature compound and stored at −80°C until used for immunohis-
tochemical studies. Frozen sections (4 µm) were incubated with 10% normal goat serum to reduce nonspecific binding of secondary antibodies. The serum was drained from the sections before they were incubated with rabbit anti-ET<sub>B</sub>-receptor polyclonal antibody (IBL, Gunma, Japan) at a concentration of 5 mg/mL for 12 h at 4°C. The primary antibody used in this study was raised against a synthetic polypeptide corresponding to the COOH-terminal amino acid sequence of the human ET<sub>B</sub> receptor and has a specificity for human, rat, and bovine ET<sub>B</sub> receptors. We confirmed that this antibody exhibits no cross-reactivity with the ET<sub>A</sub>-receptor peptide sticking to the plastic plate. Next, the sections were incubated with a biotinylated goat anti-rabbit IgG antibody (diluted 1:100) for 30 min, followed by the avidin-biotin-peroxidase complex (diluted 1:100; Vector Laboratories, Burlingame, CA) for 30 min. Subsequently, the immunoperoxidase color reaction was developed by incubation for 15 min in Tris-HCl containing 0.05% 3,3′-diaminobenzidine tetrahydrochloride and 0.01% hydrogen peroxide. Negative controls were prepared with rabbit nonimmune serum instead of primary antibody or by omitting steps in the avidin-biotin peroxidase procedure. The sections were examined by light microscopy without knowledge of the treatment groups, and the intensity of immunostaining was graded semiquantitatively from 0 to 3: grade 0, no staining; grade 1, focal staining or weak staining; grade 2, diffuse moderate staining; and grade 3, strong staining. To assess the changes in ET<sub>B</sub>-receptor expression after exposure to hypoxia, the immunostaining grade of the pulmonary arteries was estimated in lung sections from each animal and was calculated in each group. Serial sections were stained with elastic Van Gieson’s method to distinguish arteries and veins by the presence of internal elastic lamina (24). For each rat, the pulmonary arteries were grouped according to the external diameter: over 400, 201–400, 121–200, and 61–120 µm. The number of animals in each group was four.

Right ventricular hypertrophy. To assess the severity of pulmonary hypertension in the chronically hypoxic rats, the hearts were dissected, and an index of right ventricular hypertrophy was calculated as the ratio of the wet weight of the free wall of the right ventricle to the wet weight of the left ventricular wall plus septum.

Statistics. Data are expressed as means ± SE. Statistical analysis was done by either Student’s t-test, analysis of variance (ANOVA) with Fisher’s post hoc test for multiple comparisons, or repeated-measures ANOVA followed by Dunn-type multiple comparison. Differences were considered significant at <i>P</i> < 0.05.

RESULTS

Right ventricular hypertrophy. The severity of pulmonary hypertension in chronically hypoxic rats was reflected by an increased ratio of right ventricular weight to left ventricular plus septal weight, which averaged 0.57 ± 0.01 (n = 34) vs. 0.31 ± 0.01 (n = 47) in the normoxic control rats (<i>P</i> < 0.05).

Vasodilator responses to IRL-1620. The baseline perfusion pressures were 5.4 ± 0.1 (n = 43) and 8.3 ± 0.2 (n = 30) mmHg in normotensive and hypertensive lungs, respectively (<i>P</i> < 0.05). During conditions of increased pulmonary vascular tone induced by infusion of U-46619, IRL-1620 caused a concentration-dependent vasodilation that was greater in the hypertensive lungs than in the normotensive lungs (Fig. 1). Vehicle (DMSO) alone did not cause any dilation, and the perfusion pressure kept increasing gradually at almost the same rate in both normotensive and hypertensive lungs (Fig. 1). The U-46619 dose required to constrict the lungs from hypoxic rats (63 ± 6 pmol/min) was significantly less than that required to constrict the lungs from normoxic rats (133 ± 10 pmol/min).

Effects of inhibitors of NO and K<sub>ATP</sub> channels on IRL-1620-induced vasodilation. The effects of L-NNA and Glib on IRL-1620-induced vasodilation in normotensive and hypertensive lungs are shown in Figs. 2 and 3, respectively. In normotensive lungs, either L-NNA or Glib alone attenuated the vasodilator response to IRL-1620, but together they had a stronger effect than either one alone (Fig. 2). By contrast, L-NNA alone, but not Glib alone, completely blocked the vasodilator response of hypertensive lungs to IRL-1620 (Fig. 3). L-NNA pretreatment significantly decreased the concentration of U-46619 required to constrict the lungs from normoxic (87 ± 13 pmol/min) and hypoxic (15 ± 7 pmol/min).
pmol/min) rats compared with untreated lungs. By contrast, Glib pretreatment significantly increased the concentration of U-46619 required to constrict the lungs from normoxic (646 ± 89 pmol/min) and hypoxic (698 ± 6 pmol/min) rats compared with untreated lungs.

Effects of an NO donor or a K<sub>ATP</sub>-channel opener. The vasodilator response to SNP was not enhanced in hypertensive lungs compared with normotensive lungs (Fig. 4A). The vasodilator response induced by NIP-121 in hypertensive lungs was greater than that in normotensive lungs (Fig. 4B). In an additional experiment, we observed that the vasodilator response induced by NIP-121 (45.6 ± 1.4%) was completely blocked by Glib pretreatment in normotensive lungs (n = 4 in each group; P < 0.05).

ET<sub>B</sub>-receptor mRNA in normotensive and hypertensive lungs. ET<sub>B</sub>-receptor mRNA transcripts, detected as a 5.0-kb band by Northern blot analysis, were expressed in normotensive control rat lungs. The ratio of ET<sub>B</sub>-receptor mRNA to 28S rRNA was about twofold greater in the lungs from rats exposed to hypoxia (Fig. 5) compared with that in normotensive rat lungs.

Fig. 4. Effects of sodium nitroprusside (SNP; 1 µM; A) and NIP-121 (1 µM; B) on elevated perfusion pressure induced by continuous infusion of U-46619 in NL and HL. Values are means ± SE; n = 4 lungs/group. *P < 0.05 vs. NL by t-test.

Fig. 5. Effects of exposure to hypoxia on endothelin-B (ET<sub>B</sub>) receptor mRNA levels in rat lungs. A: representative Northern blots of ET<sub>B</sub> receptor in lung tissue from rats exposed to air [control (C)] or hypoxia for 21 days (21d). B: quantification of ET<sub>B</sub>-receptor mRNA by densitometry. mRNA from each animal was quantified individually, then mean (±SE) for each group was determined; n = 4 rats/group. *P < 0.05 vs. control animals by t-test.
Immunohistochemistry for ET<sub>B</sub> receptor in lungs from normotensive and hypertensive rats. ET<sub>B</sub>-receptor immunoreactivity was faint in the intima of the arteries corresponding to the terminal and respiratory bronchioles and alveolar ducts in the normotensive control lungs. In the lungs from rats exposed to hypobaric hypoxia for 21 days, expression of ET<sub>B</sub> receptors in the intima of the distal segments of pulmonary muscular arteries was greater than that in normotensive lungs; semiquantitative analysis showed that the immunoreactivity for ET<sub>B</sub> receptors in the intima was 1.6-fold greater in arteries of 121–200 and 61–120 µm in diameter (Fig. 6).

**DISCUSSION**

We used IRL-1620, a selective ET<sub>B</sub>-receptor agonist (29), to clarify whether the vasodilation induced by ET<sub>B</sub>-receptor activation is increased or decreased in the pulmonary vasculature of chronically hypoxic rats that developed pulmonary hypertension. The results demonstrate that IRL-1620 caused a dose-dependent vasodilation that was greater in the lungs from chronically hypoxic rats compared with that in normoxic lungs. Furthermore, we found that two mechanisms were responsible for IRL-1620-induced vasodilation in normotensive lungs, release of NO and activation of K<sub>ATP</sub> channels, whereas in the hypertensive lungs, IRL-1620-induced vasodilation resulted from the release of NO only. We observed augmented ET<sub>B</sub> receptor-mediated vasodilation in hypertensive lungs that appeared to be associated with an increase in the number of ET<sub>B</sub> receptors in the endothelium of the pulmonary resistance arteries.

Chronic hypoxic exposure of rats causes an increase in pulmonary arterial resistance as a result of vasoconstriction, increased blood viscosity resulting from polycythemia, and pulmonary vascular remodeling (27). Although the mechanism of chronic hypoxia-induced pulmonary hypertension is not fully understood, recent evidence suggests that ET-1 is adduced as a possible mediator of the development of hypoxic pulmonary hypertension (2, 18). Indeed, upregulation of mRNA for ET-1, ET<sub>A</sub>, and ET<sub>B</sub> receptors and the overexpression of ET-1 in the whole lung have been observed in the rat model of chronically hypoxic pulmonary hypertension (14). Moreover, a number of groups have reported attenuation in the development of hypoxic pulmonary hypertension in rats treated with either an ET<sub>A</sub>-receptor blocker (3, 6) or both ET<sub>A</sub>- and ET<sub>B</sub>-receptor blockers (4). However, the precise role of ET-1 and its
receptors in the pathogenesis of hypoxic pulmonary hypertension is not fully understood because of the potential bidirectional effect of ET-1 on pulmonary vascular tone through ET\(_A\)- and ET\(_B\)-receptor subtypes (20). In particular, it remains unclear how the vasodilator action of ET-1 in the pulmonary vasculature is altered by chronic hypoxic exposure. Previously, Eddahibi et al. (7) demonstrated that the pulmonary vasodilator response to ET-1 was impaired in the lungs from chronically hypoxic rats, whereas a recent study (25) has shown that chronic hypoxia augmented the pulmonary vasodilator response to ET-1, probably due to increased NO activity. The reason for this discrepancy is unclear. In the present study, therefore, we used a selective ET\(_B\)-receptor agonist, IRL-1620, to address this discrepancy and found that IRL-1620 caused a dose-dependent vasodilation that was greater in the hypertensive lungs than in the normotensive rat lungs.

We then investigated the mechanism of IRL-1620-induced vasodilation. In normotensive lungs, we found that L-NNA and Glib each attenuated the pulmonary vasodilation induced by IRL-1620. These results are similar to a recent report that found that both L-NNA and Glib attenuate ET\(_B\) receptor-mediated vasodilation in rat conduit pulmonary arteries (10). In that study, a combination of L-NNA and Glib had no further inhibitory effect than either alone, suggesting that NO may hyperpolarize vascular smooth muscle by activating K\(_{ATP}\) channels (19, 22). In the present study, however, the combination of L-NNA and Glib inhibited the IRL-1620-induced vasodilation more effectively than either alone, indicating that the release of NO and activation of K\(_{ATP}\) channels may be independent mechanisms in IRL-1620-induced vasodilation. Thus the vasodilator response to IRL-1620 in normotensive rat lungs appears to be mediated partly by NO release and partly by activation of K\(_{ATP}\) channels. Although it is unclear why our results are different from those of Higashi et al. (10), the difference may depend on the region of the pulmonary circulation examined, i.e., the whole pulmonary circulation vs. the conduit pulmonary artery.

In contrast to the results obtained in the normotensive lungs, L-NNA alone, but not Glib alone, completely blocked the vasodilation induced by IRL-1620 in the hypertensive lungs, suggesting that the release of NO exclusively mediates IRL-1620-induced vasodilation under these conditions. The reason why Glib had no inhibitory effect on the IRL-1620-induced vasodilation is not clear. However, a reduction in the number or sensitivity of K\(_{ATP}\) channels on vascular smooth muscle seems unlikely because we found that the vasodilator response to a K\(_{ATP}\)-channel opener, NIP-121 (17), was preserved, and indeed augmented, in the lungs from hypoxic rats. Although the mechanism by which ET activates K\(_{ATP}\) channels has not been determined, one possibility is that ET\(_B\)-receptor activation stimulates the release of an endothelium-derived hyperpolarizing factor (EDHF), which causes hyperpolarization and relaxation of vascular smooth muscle through activation of potassium channels (8, 15). If this were the case, then our results could be interpreted as evidence that IRL-1620-induced vasodilation is mediated in part by the release of both NO and EDHF in normotensive lungs and that the release of EDHF may be impaired in hypertensive lungs.

There are several possible explanations for the augmented vasodilator response to IRL-1620 in the lungs from rats exposed to chronic hypoxia. Although we did not assess the endothelial NOS (eNOS) activity in the pulmonary vasculature in the present study, a number of studies have recently shown that NO-mediated vasodilation (11, 21, 25), NO production (11, 21), eNOS mRNA and protein levels (12, 30), and eNOS immunoreactivity in the pulmonary artery (24) are increased in the lungs from chronically hypoxic rats compared with the lungs from normoxic rats, suggesting strongly that chronic hypoxia increases eNOS activity within the pulmonary vasculature. Therefore, because IRL-1620, acting through ET\(_B\) receptors in the pulmonary vascular endothelium, causes the release of NO, we speculate that the greater IRL-1620-induced vasodilation observed after chronic hypoxia could at least partly be due to increased NO activity. Another possible explanation for the augmented vasodilator response is an increase in the number of ET\(_B\) receptors in the pulmonary vascular endothelium after exposure to chronic hypoxia. This possibility is supported by our present study and another study (14) that showed that ET\(_B\)-receptor mRNA is overexpressed in the whole lung after chronic hypoxia. Further strong support is provided by our immunohistochemical study results, which demonstrate that the lungs from chronically hypoxic rats have higher levels of ET\(_B\) receptors in the endothelium of the distal segments of the pulmonary arteries, which correspond to the terminal and respiratory bronchioles and alveolar ducts, which are thought to be the principal site of resistance in the pulmonary circulation, than in the normoxic lungs. Therefore, an increase in the number of ET\(_B\) receptors in the endothelium of pulmonary resistance arteries is likely to be partly responsible for the augmented IRL-1620-induced vasodilation observed after chronic hypoxia. Increased vascular smooth muscle sensitivity to NO or increased guanylate cyclase activity may be the third possible explanation for this augmentation. However, this possibility is unlikely because we have shown that the vasodilator response to the NO donor SNP was not enhanced, and indeed was reduced, in the lungs from rats exposed to chronic hypoxia compared with control lungs. This result is consistent with previous studies (16, 26) that showed blunted responses to SNP in hypertensive pulmonary arteries. Similarly, another laboratory (25) demonstrated that vasodilator responses to other NO donors were similar in lungs from normoxic and chronically hypoxic rats.

In summary, in isolated perfused rat lungs under increased vascular tone induced by an infusion of U-46619, the pulmonary vasodilator response to IRL-1620 was enhanced after chronic hypoxic exposure. The mechanism of the IRL-1620-induced vasodilation was not the same in normotensive and hypertensive lungs; only the release of NO appeared to be involved in the
mechanism of vasodilation in the hypertensive lungs, whereas both the release of NO and the activation of KATP channels were partly involved in the normotensive lungs. The enhanced vasodilator action of IRL-1620 after chronic hypoxia does not appear to be due to enhanced vascular smooth muscle sensitivity to NO; instead, the underlying mechanism of this augmentation may be partly related to the upregulation of ETB receptors in the endothelium of the pulmonary resistance arteries of hypoxic rats. Such a change in ETB receptor expression may have an important pathophysiological role in the modulation of the development of chronically hypoxic pulmonary hypertension.

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Received 21 July 1998; accepted in final form 28 October 1998.

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