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Hypoxia augments conversion of big-endothelin-1 and endothelin ET_B receptor-mediated actions in rat lungs

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Abstract

We have examined the effect of endothelin-1, sarafotoxin-6C, big-endothelin-1 and other agents on perfused lungs from chronically hypoxic rats. Increases in pulmonary perfusion pressure induced by big-endothelin-1, endothelin-1, phenylephrine and potassium chloride were enhanced in hypoxic lungs, while the constrictor action of sarafotoxin-6C was not increased. When basal pulmonary perfusion pressure was raised, low doses of endothelin-1 and sarafotoxin-6C produced decreases in pulmonary perfusion pressure which were significantly greater in chronically hypoxic lungs, whereas responses to sodium nitroprusside were unchanged. Endothelin ET_B receptor-mediated bronchoconstrictor responses were also potentiated in hypoxic lungs, whereas responses to carbachol were not. In hypoxic lungs, conversion of big-endothelin-1 to endothelin-1 was significantly increased. These data provide evidence for a generalised increase in vasomotor activity in chronically hypoxic lungs, and a more selective increase in endothelin ET_B receptor-mediated vasodilator and bronchoconstrictor responses. Hypoxia also augments the conversion of big-endothelin-1 to endothelin-1.

Keywords: Chronic hypoxia; Lung; (Rat); Endothelin-1; Big-endothelin-1; Sarafotoxin-6C; Pulmonary vasoconstriction; Pulmonary vasodilation; Bronchoconstriction; Endothelin ET_B receptor

1. Introduction

Chronic hypoxia leads to the development of pulmonary hypertension which is associated with an increased endogenous production of endothelin-1 (Shirakami et al., 1991; Ferri et al., 1995). Investigations have shown a positive correlation between plasma endothelin-1 levels and pulmonary hypertension (Allen et al., 1993), and numerous studies have shown hypoxia-induced pulmonary hypertension in animals is prevented by endothelin receptor antagonists (DiCarlo et al., 1995; Eddahibi et al., 1995). In the lung, endothelin-1 acts on multiple endothelin receptor subtypes. Activation of endothelin ET_A and endothelin ET_B receptors on pulmonary vascular smooth muscle produces pulmonary vasoconstriction (Lal et al., 1995; Sato et al., 1995), while stimulation of endothelin ET_B receptors, located on the vascular endothelium, leads to pulmonary vasodilation which is mediated by nitric oxide and prostanoids (Lal et al., 1996).

We have previously shown that repeated injections of endothelin-1, or the selective endothelin ET_B receptor agonist sarafotoxin-6c, in perfused heart and lung preparations leads to desensitisation of endothelin ET_B receptor-mediated vasodilator responses (Thompson et al., 1995; Lal et al., 1996). In view of this, we speculated that the increased production of endothelin-1 in lungs from chronically hypoxic rats would attenuate its pulmonary vasodilator activity and augment pulmonary vasoconstriction. However, we have shown that in rings of large pulmonary arteries and veins from chronically hypoxic rats, endothelin-1-induced contractions were significantly reduced (Lal et al., 1999a). Similarly, Bialecki et al. (1998) have shown reduced endothelin-1 induced contractions in large pulmonary arteries from chronically hypoxic rats. However, other investigators, using receptor binding studies, have shown that endothelin ET_A receptors are increased in the lungs of chronically hypoxic rats (Li et al., 1994; Soma et

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al., 1999), but the functional consequences of this were not clear. Eddahibi et al. (1993) have reported that endothelin-1- and endothelin-3-induced vasodilator responses were attenuated in isolated lungs of chronically hypoxic rats perfused in a re-circulating mode. In contrast, others have shown increased endothelin-1-mediated pulmonary vasodilator responses in chronically hypoxic lungs (Muramatsu et al., 1999). Therefore, to clarify the controversy, we wanted to reinvestigate the dilator responses to endothelin-1 and the selective endothelin ET_B receptor agonist sarafotoxin-6C in chronically hypoxic rat lungs.

The earlier studies mentioned above have simply examined pulmonary vascular responses to endothelins in chronically hypoxic animals. However, the use of a perfused lung model (Lal et al., 1994), which allows bronchial tone to be assessed at the same time as vascular tone, gives us the opportunity to study pulmonary vascular and bronchial reactivity simultaneously. The bronchial actions of endothelins are also complex. Previous studies have shown that endothelin-1 may contract or relax isolated bronchial preparations (Battistini et al., 1994). The activation of endothelin ET_B receptors on bronchial smooth muscle mediates the bronchoconstrictor actions of endothelin-1 (Lal et al., 1995; Uhlig and Featherstone, 1997), whereas activation endothelin ET_A receptors causes both a contraction and an epithelium-dependent relaxation that is mediated by nitric oxide release (Battistini et al., 1994; Emanueli et al., 1998). Moller et al. (1999) have recently shown that in bronchial biopsies taken from patients with chronic airway obstruction, endothelin ET_B receptor mRNA is significantly increased. However, to our knowledge, the functional effects of endothelins on bronchial tone have not been studied in chronically hypoxic animals. In the present study, we report on the bronchial reactivity to endothelin-1 and sarafotoxin-6C in lungs from chronically hypoxic rats. Comparisons have been made with other known constrictor and relaxant agents.

The fact that circulating levels of endothelin-1 are reported to be higher in chronically hypoxic compared with normal animals and in patients with pulmonary hypertension (Shirakami et al., 1991), and that the lung is an important site for the production and metabolism of endothelins, prompted us to examine the effects of chronic hypoxia on the conversion of big-endothelin-1 to endothelin-1 in the lung.

2. Material and methods

2.1. Chronic hypoxia

Male Wistar rats (250–270 g) were kept in a normobaric hypoxic chamber at 10% pO_2 for a period of 3 weeks prior to use. Humidity, pO_2 and temperature inside the chamber were monitored continuously as previously described (Lal et al., 1999b). In the earlier experiments, as an indicator of chronic hypoxia-induced cardiovascular changes, haematocrit values together with the ratios of right to total ventricular wet weight were monitored.

2.2. Isolated perfused lung preparation

Rats were anaesthetised (Sagatal 60 mg kg^{-1}, i.p.) and heparinised (500 i.u.) via a tail vein. Lungs were isolated and perfused as described previously (Lal et al., 1994). Briefly, the pulmonary artery was cannulated and the left atrium and main ventricular mass removed. The trachaea was also cannulated. Lungs were then removed and perfused via the pulmonary artery at 5 ml min^{-1} with modified Kreb's solution of the following composition (mM), sodium chloride (118), potassium chloride (4.7), potassium dihydrogen phosphate (1.2), calcium chloride (1.25), magnesium sulphate (1.2), sodium bicarbonate (25) and glucose (11.1), gassed with 20% O_2/5% CO_2/75% N_2). Lungs were suspended from an isometric transducer for recording changes in lung weight and ventilated via the trachea with room air (28 strokes min^{-1}, stroke volume 1 ml). Pulmonary inflation pressure and pulmonary perfusion pressure were monitored with pressure transducers attached to a MacLab data acquisition system. In previous studies, we have shown this model is stable for 2 h (Lal et al. 1994). After an initial 20 min stabilisation period bolus injections (10–100 μl) of big-endothelin-1, endothelin-1, sarafotoxin-6C, carbachol, phenylephrine and potassium chloride were given via the pulmonary artery cannula. Except for the phenylephrine and potassium chloride studies, where responses were rapidly reversible, only one agonist was used in each lung preparation. In the phenylephrine and potassium chloride study, the dose response to phenylephrine was completed before carrying out the potassium chloride dose response. In the carbachol, endothelin-1, sarafotoxin-6C and big-endothelin-1 studies, pulmonary inflation pressure responses did not return to base line values between each dose as previously reported (Lal et al., 1994, 1996). Therefore, the increases in pulmonary inflation pressure quoted in the results refer to the absolute increase in pressure caused by each dose of drug. In all cases, a minimum of four doses was given as indicated in Section 3.

In other experiments where we examined endothelin ET_B receptor-mediated vasodilator responses, vascular tone was raised by approximately 10 mm Hg by infusion of the thromboxane mimetic U46619 (20–80 nM). Vasodilator responses were expressed as percentage of the increase in pulmonary perfusion pressure produced by U46619.

2.3. Inhibitors and receptor antagonists

In some experiments, the effects of the endothelin ET_A receptor antagonist BQ123 (10 μM) and the endothelin ET_B receptor antagonist BQ788 (10 μM) were examined

on the actions of endothelin-1 and sarafotoxin-6C in chronically hypoxic lungs. These antagonists were perfused for 15 min prior to agonist addition.

The effects of sarafotoxin-6C in the presence of the nitric oxide synthase inhibitor nitro-L-arginine (100 μM) alone, or in combination with the cyclo-oxygenase inhibitor indomethacin (10 μM), were also studied in chronically hypoxic lungs. These inhibitors were added 30 min prior to agonist injection.

2.4. Determination of big-endothelin-1 conversion

In a different protocol, bolus injections of big-endothelin-1 were given into the pulmonary artery of control or chronically hypoxic lungs and the effluent collected for 10 min. Endothelin-1 was extracted from the perfusate and measured with a human endothelin-1 ELISA kit as described previously (Smith et al., 1997).

2.5. Statistical analysis

Increases in pulmonary perfusion pressure and pulmonary inflation pressure are taken as increases above the basal values prior to each dose of drug. Endothelin-1-, sarafotoxin-6C- and sodium nitroprusside-induced vasodilator effects are expressed as percentage decreases of the rise in pulmonary perfusion pressure induced by U46619. Data are expressed as mean ± S.E.M. Students' *t*-test and one-way ANOVA followed by Dunnett's tests were used to test the level of significance as appropriate. Probability values of $P < 0.05$ were considered significant.

2.6. Drugs

BQ123 (cyclo[D-Asp-L-Pro-D-Val-L-Leu-D-Trp]) and BQ788 (*N-cis*-2,6-dimethylperidinenocarbonyl-L-γMe-Leu-D-Trp (COOMe)-D-Nle-One]) were supplied by Rhone-Poulenc Rorer (Dagenham, England). Endothelin-1, sarafotoxin-6C and big-endothelin-1 were obtained from Peninsula U46619 (9,11-dideoxy-9_{α}, 11-epoxy-methano-prostaglandin $F_{2\alpha}$), sodium nitroprusside, potassium chloride, nitro-L-arginine and indomethacin were from Sigma. The human endothelin-1 ELISA kit was obtained from R&D Systems Europe (Oxford, UK).

U46619 was dissolved in 95% ethanol and further diluted in normal saline and stored at −80°C. BQ788 was dissolved in dimethylsulfoxide; the final concentration was less than 0.1%. Stock solutions of BQ123, big-endothelin-1, endothelin-1, sarafotoxin-6C, phenylephrine and carbachol were prepared in normal saline and stored at −20°C. Sodium nitroprusside and potassium chloride were prepared in normal saline before the experiment.

3. Results

3.1. Chronic hypoxia-induced changes in vascular and pulmonary parameters

Chronic hypoxia significantly increased the ratios of right ventricular to total ventricular weight when compared with control rats (0.34 ± 0.01 vs. 0.25 ± 0.01, $P < 0.01$, $n = 20$). Haematocrit values were also significantly higher in chronic hypoxic ($59 \pm 1\%$, $n = 20$) compared with control ($43 \pm 1\%$, $n = 20$, $P < 0.01$) rats.

In isolated lungs basal pulmonary perfusion pressure was significantly higher in chronically hypoxic lungs (12 ± 0.52 mm Hg, $n = 47$) compared with control lungs (7.6 ± 0.48 mm Hg, $n = 47$, $P < 0.01$).

Basal pulmonary inflation pressure in chronically hypoxic lungs (5 ± 0.3 mm Hg, $n = 47$) was significantly lower than in control lungs (6.6 ± 0.4 mm Hg, $n = 47$).

3.2. Effects of chronic hypoxia on pulmonary vasoconstrictor responses

3.2.1. Big-endothelin-1

Fig. 1 shows the effects of big-endothelin-1 (50–1600 pmol) and endothelin-1 (50–800 pmol) on pulmonary perfusion pressure in control and chronically hypoxic lungs. Responses to low doses of big-endothelin-1 (50–800 pmol) were not altered in chronically hypoxic lungs. However, at the highest dose used, big-endothelin-1 (1600 pmol) produced a significantly larger increase in pulmonary perfusion pressure in chronically hypoxic lungs (21 ± 2.2 mm Hg, $n = 4$) than in control lungs (6.6 ± 1.3 mm Hg, $n = 3$, $P < 0.01$).

3.2.2. Endothelin-1

From Fig. 1 it can be seen that at all doses used, endothelin-1-induced increases in pulmonary perfusion pressure were augmented in lungs from chronically hypoxic compared to control lungs. These changes were statistically significant at doses above 200 pmol. For example, the 400 pmol dose of endothelin-1 caused an increase in pulmonary perfusion pressure of 19 ± 2 mm Hg ($n = 6$) in hypoxic lungs and 8 ± 1 mm Hg ($n = 5$) in control lungs ($P < 0.01$).

The effects of endothelin receptor antagonists on endothelin-1-induced increases in pulmonary perfusion pressure in hypoxic lungs are shown in Fig. 2. The endothelin ET_A receptor antagonist, BQ123 (10 μM), significantly blocked endothelin-1 (50–800 pmol)-induced increases in pulmonary perfusion pressure, while the endothelin ET_B antagonist, BQ788 (10 μM), was much less effective.

3.2.3. Sarafotoxin-6C

Fig. 3 shows that unlike endothelin-1 and big-endothelin-1, the increase in pulmonary perfusion pressure

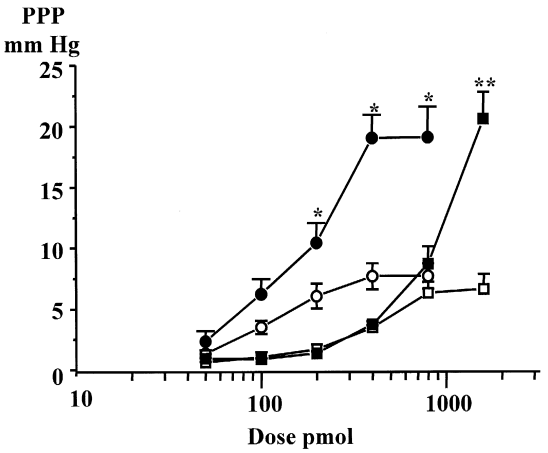

Fig. 1. Endothelin-1 (○, ●) and big endothelin-1 (□, ■)-induced increases in pulmonary perfusion pressure (PPP mm Hg) in control (open symbols) and chronically hypoxic lungs (filled symbols). Each point represents mean ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$, significantly different from appropriate controls $n = 4–6$.

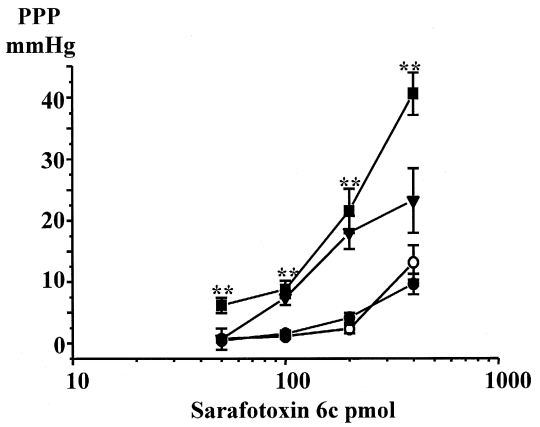

Fig. 3. Sarafotoxin-6C-induced increases in pulmonary perfusion pressure (PPP) in control lungs (○, $n = 4$), chronically hypoxic lungs (●, $n = 4$), chronically hypoxic lungs plus L-NOARG (100 μM) (▼, $n = 3$), and chronically hypoxic lungs plus L-NOARG (100 μM) and indomethacin (10 μM) (■, $n = 5$). Each point represents mean ± S.E.M. $^{**}P < 0.01$, significantly different from sarafotoxin-6C responses in control or chronically hypoxic lungs.

induced by sarafotoxin-6C (50–400 pmol) was not significantly affected by chronic hypoxia. As sarafotoxin-6C stimulates endothelin ET_B receptors located on the endothelial cells to release nitric oxide, we were interested to see if enhanced production of nitric oxide could be attenuating the constrictor effects of sarafotoxin-6C in hypoxic lungs. The effects of the nitric oxide synthase inhibitor nitro-L-arginine alone (100 μM), or in combination with indomethacin (10 μM), on sarafotoxin-6C-induced changes in pulmonary perfusion pressure were then examined.

The presence of nitro-L-arginine alone increased pulmonary perfusion pressure by 2.66 ± 1 mm Hg ($n = 3$), while nitro-L-arginine in combination with indomethacin increased pulmonary perfusion pressure by 2.9 ± 0.6 mm Hg ($n = 5$).

In the presence of nitro-L-arginine, sarafotoxin-6C-induced increases in pulmonary perfusion pressure were enhanced in hypoxic lungs. For example, the increase in pulmonary perfusion pressure caused by sarafotoxin-6C (400 pmol) in the presence of nitro-L-arginine (23 ± 5.2 mm Hg, $n = 3$) was significantly higher than that caused by 400 pmol of sarafotoxin-6C in control hypoxic lungs (9.6 ± 1.6 mm Hg, $n = 4$, $P < 0.05$). Moreover, in the presence of nitro-L-arginine (100 μM) and indomethacin (10 μM), the response to sarafotoxin-6C (400 pmol) was even greater (40 ± 3.4 mm Hg, $n = 5$, $P < 0.001$).

In order to see if enhanced pulmonary constrictor responsiveness of hypoxic lungs to endothelin-1 was selective, vasoconstrictor responses to phenylephrine and potassium chloride were also examined in hypoxic lungs.

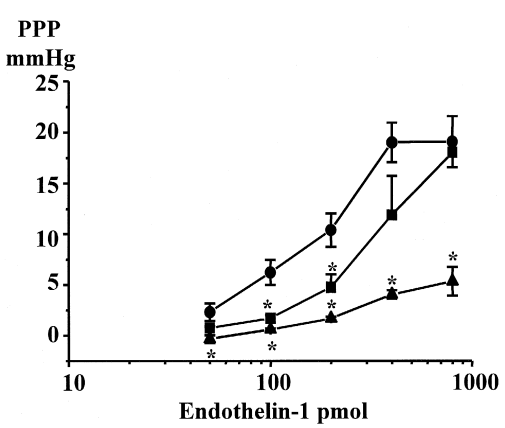

Fig. 2. Action of endothelin-1 on pulmonary perfusion pressure (PPP mm Hg) in chronically hypoxic lungs (●) and chronically hypoxic lungs in the presence of BQ788 (10 μM) (■) or BQ123 (10 μM) (▲). Each point represents mean ± S.E.M. $^{*}P < 0.05$, significantly different from endothelin-1 alone lungs $n = 5$.

3.2.4. Phenylephrine

As with endothelin-1, the phenylephrine-induced increases in pulmonary perfusion pressure were significantly potentiated in hypoxic lungs compared to control lungs at all doses used (Fig. 4).

3.2.5. Potassium chloride

Potassium chloride (25–400 μmol)-induced increases in pulmonary perfusion pressure were also potentiated in lungs from chronically hypoxic rats compared to control lungs (Fig. 5). In chronically hypoxic lungs, 400 μmol of potassium chloride increased pulmonary perfusion pressure by 31 ± 4 mm Hg ($n = 5$). This was significantly higher than the response seen in control lungs (12.5 ± 1.3 mm Hg, $n = 4$, $P < 0.05$).

3.3. Effects of chronic hypoxia on pulmonary vasodilator responses

In order to examine vasodilator responses, basal pulmonary perfusion pressure was increased in control and

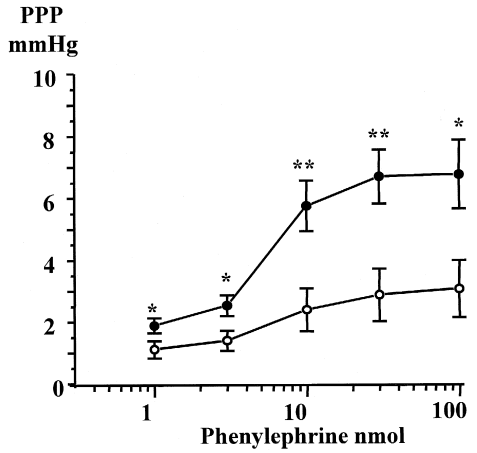

Fig. 4. Phenylephrine-induced increases in pulmonary perfusion pressure (PPP) in control (○) and chronically hypoxic (●) lungs. Each point represents mean ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$, significantly different from control $n = 10$.

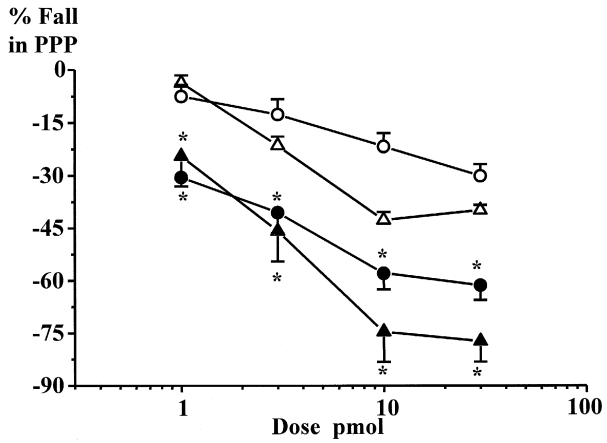

Fig. 6. Graph showing the percentage fall in pulmonary perfusion pressure (% fall PPP) in response to endothelin-1 (○, ●) and sarafotoxin-6C (△, ▲) in control (open symbols) and chronically hypoxic (filled symbols) lungs pre-contracted (≏10 mm Hg) with U46619. Each point represents mean ± S.E.M. $^{*}P < 0.05$ significantly different compared with appropriate control n = 3–5.

chronically hypoxic lungs using U46619. U46619 was infused at a concentration sufficient to increase pulmonary perfusion pressure by ≈ 10 mm Hg. In control lungs, this response was produced by 80 nM U46619, while in chronically hypoxic lungs, a lower concentration was required (20 nM).

3.3.1. Endothelin-1

Endothelin-1 (1–30 pmol)-induced decreases in pulmonary perfusion pressure were significantly larger in chronically hypoxic lungs compared with control lungs (Fig. 6). The ED_{50} value for endothelin-1 in control lungs (5.2 ± 0.62 pmol, $n = 3$) being reduced to 1.77 ± 0.42 pmol ($n = 4$) in hypoxic lungs ($P < 0.05$). In addition, the maximum decrease in pulmonary perfusion pressure caused by endothelin-1 (30 pmol) in control lungs ($30 \pm 3\%$, $n = 3$) was significantly augmented in hypoxic lungs ($62 \pm 6\%$, $n = 3$, $P < 0.05$). Doses of endothelin-1 higher than 30 pmol produced increases in pulmonary perfusion pressure in both sets of lungs (data not shown).

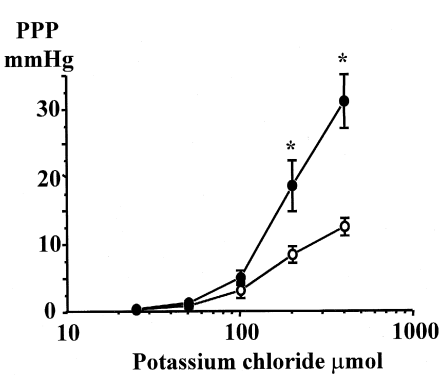

Fig. 5. Potassium chloride-induced increases in pulmonary perfusion pressure (PPP) in control (○) and chronically hypoxic (●) lungs. Each point represents mean ± S.E.M. $^{*}P < 0.05$, significantly different from control $n = 5$.

3.3.2. Sarafotoxin-6C

Sarafotoxin-6C (1–30 pmol)-induced decreases in pulmonary perfusion pressure were also significantly potentiated in hypoxic lungs (Fig. 6). The ED_{50} value in control lungs (3.3 ± 0.35 pmol, $n = 4$) was reduced in hypoxic lungs (1.8 ± 0.44 pmol, $n = 4$, $P < 0.05$). In addition, the maximum decrease in pulmonary perfusion pressure caused by 30 pmol of sarafotoxin-6C in control lungs ($40 \pm 1.5\%$) was significantly augmented in hypoxic lungs ($77 \pm 5.8\%$, $n = 5$, $P < 0.01$).

3.3.3. Sodium nitroprusside

In contrast to the enhanced dilator responses to endothelin-1 and sarafotoxin-6C, sodium nitroprusside (10–3000 pmol)-mediated pulmonary vasodilator responses in chronically hypoxic and control lungs were not significantly different throughout the dose response curve. The decreases in pulmonary perfusion pressure induced by 300 and 1000 pmol of sodium nitroprusside in control and hypoxic lungs were $62.5 \pm 4\%$ and $59 \pm 10\%$, and $83 \pm 16\%$, ($n = 4$) and $88 \pm 16\%$, respectively ($P > 0.05$, $n = 5$).

3.4. Effects of chronic hypoxia on bronchoconstrictor responses

3.4.1. Big-endothelin-1

Low doses of big-endothelin-1 (50–800 pmol) caused similar increases in pulmonary inflation pressure in hypoxic and control lungs. However, at the highest dose of big-endothelin-1 (1600 pmol), the increase in pulmonary inflation pressure in hypoxic lungs (5.9 ± 0.74 mm Hg, $n = 4$) was significantly higher than in control lungs (3.4 ± 0.36 mm Hg, $n = 3$, $P < 0.05$).

3.4.2. Endothelin-1

As with big-endothelin-1, the endothelin-1 (50–400 pmol)-induced increases in pulmonary inflation pressure in hypoxic lungs were also potentiated when compared to control responses (Fig. 7).

The increased effect of endothelin-1 on pulmonary inflation pressure in hypoxic lungs was not attenuated by the endothelin ET_A receptor antagonist BQ 123 (10 μM). For example, in the presence of BQ123, a 400-pmol dose of endothelin-1 increased pulmonary inflation pressure by 5.68 ± 1.0 mm Hg, ($n = 6$) while in the absence of BQ 123, the response was 4.5 ± 0.57 mm Hg ($n = 4$). In contrast, the endothelin ET_B receptor antagonist BQ788 (10 μM) did inhibit endothelin-1-induced increases in pulmonary inflation pressure in hypoxic lungs. In the presence of BQ788, a 400-pmol dose of endothelin-1 only increased pulmonary inflation pressure by 2.4 mm Hg ($n = 5$) compared with the control hypoxic response of 4.5 ± 0.57mm Hg ($n = 5$, $P < 0.05$).

3.4.3. Sarafotoxin-6C

Fig. 7 shows that sarafotoxin-6C (50–400 pmol)-induced increases in pulmonary inflation pressure were enhanced in hypoxic lungs. In chronically hypoxic lungs, the ED_{50} was 95 ± 2.6 pmol ($n = 4$), while in control lungs, the ED_{50} was 127 ± 14.8 pmol ($n = 4$). The pulmonary inflation pressure response caused by 200 pmol of sarafotoxin-6C was almost doubled in hypoxic lungs (9.8 ± 0.3 mm Hg, $n = 4$) compared with a control response of 5.8 ± 1.1 mm Hg ($n = 5$, $P < 0.05$).

In hypoxic lungs, the presence of nitro-L-arginine (100 μM) alone, or in combination with indomethacin (10 μM), did not affect sarafotoxin-6C-induced increases in pulmonary inflation pressure (data not shown).

In order to see if the increased bronchial reactivity of hypoxic lungs is selective for endothelins, responses to a muscarinic agonist, carbachol, were examined.

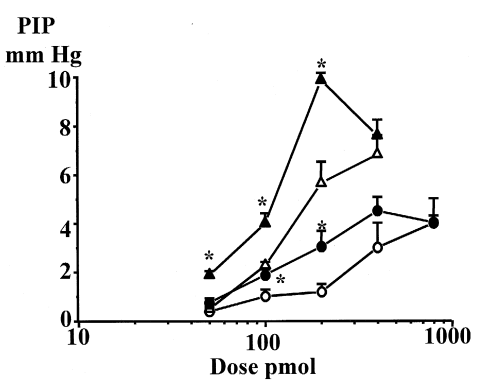

Fig. 7. Endothelin-1 (○, ●) and sarafotoxin-6C (△, ▲)-induced increases in pulmonary inflation pressure (PIP) in control (open symbols) and chronically hypoxic (filled symbols) lungs. Each point represents mean ± S.E.M. * $P < 0.05$, significantly different from appropriate control $n = 4$–6.

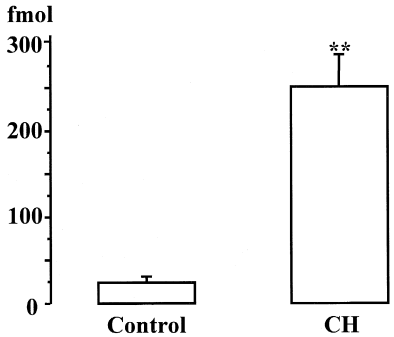

Fig. 8. Graph showing levels of endothelin-1 (fmol) in the lung perfusate after a bolus injection of big-endothelin-1 (1600 pmol) in control and chronically hypoxic lungs. Each point represents mean ± S.E.M. ** $P < 0.01$, significantly different from control $n = 4$.

3.4.4. Carbachol

In contrast to sarafotoxin-6C, neither the ED_{50} nor the maximal increase in pulmonary inflation pressure seen in response to carbachol (0.3–100 nmol) were significantly altered by chronic hypoxia. ED_{50} values in control and chronically hypoxic lungs were 4.3 ± 0.96 and 9.3 ± 1.3 nmol, respectively, ($n = 6$, $P > 0.05$). Maximal increases in pulmonary inflation pressure, seen at a carbachol dose of 30 nmol in chronically hypoxic and control lungs, were 5.9 ± 1.3 and 7.4 ± 1.5 mm Hg ($n = 6$), respectively ($P > 0.05$).

3.5. Effects of chronic hypoxia on conversion of big-endothelin-1

Basal endothelin-1 output in the effluent from chronically hypoxic lungs over the 10 min collection period tended to be greater than that of the control lungs, but this did not achieve a statistical significance (control, 0.51 ± 0.26 fmol vs. chronic hypoxia, 1.14 ± 45 fmol, $n = 4$). However, injection of big-endothelin-1 (1600 pmol), followed by collection of the perfusate for 10 min, resulted in a much higher level of endothelin-1 in the perfusate of the chronically hypoxic lungs (251 ± 37 fmol) compared with control lungs (24 ± 7 fmol, $n = 4$, $P < 0.01$, Fig. 8).

4. Discussion

In the present study, we have examined the conversion of big-endothelin-1 to endothelin-1 in perfused lungs from chronically hypoxic and control rats, and compared the broncho-pulmonary actions of big-endothelin-1, endothelin-1, sarafotoxin-6C, carbachol and phenylephrine in these lungs. The major observations provide evidence that there is an up-regulation of endothelin ET_B receptor-mediated responses in chronically hypoxic lungs, and that the pulmonary activity of endothelin converting enzyme is increased following chronic hypoxia.

Chronic hypoxia significantly increased the ratio of the right ventricular weight to total ventricular weight, and haematocrit, when compared to control animals. These changes, together with the increased basal pulmonary perfusion pressure in chronically hypoxic lungs, are indicative of hypoxia-induced pulmonary hypertension and right ventricular hypertrophy (Hunter et al., 1974; Emery et al., 1981; Barer et al., 1993).

In view of the fact that chronic hypoxia-induced pulmonary hypertension can be attenuated with endothelin receptor antagonists (DiCarlo et al., 1995; Eddahibi et al., 1995), we thought that basal effluent levels of endothelin-1 coming from the lung might be higher in the chronically hypoxic preparations. Although endothelin-1 levels in the effluent from the hypoxic lungs tended to be higher than in control lungs, they did not achieve a statistical significance probably because of the low number of experiments. However, the main aim of this series of experiments was to see if the conversion of big-endothelin-1 to endothelin-1 was enhanced in chronically hypoxic lungs. The results showed that following big-endothelin-1 administration, the level of endothelin-1 in the effluent coming from hypoxic lungs was about 10-fold higher than that of control lungs. This indicates that conversion of big-endothelin-1 is enhanced in the chronically hypoxic lung. Alternatively, there could be a decreased degradation of endothelin-1 in the hypoxic lungs. We feel the latter possibility is less likely because when functional responses to big-endothelin-1 in the form of increases in pulmonary perfusion pressure were studied, only the response to the highest dose was potentiated (Fig. 1). In addition, when authentic endothelin-1 was being studied, responses to the two lowest doses were not potentiated. If there were a decreased degradation of endothelin-1, it would be expected that these responses would have been enhanced in hypoxic lungs. Therefore, when looking at the effects of big-endothelin-1, these studies indicate that at the lower doses of big-endothelin-1 in both control and hypoxic lungs, endothelin converting enzyme activity was not a rate-limiting factor. However, at the higher doses of big-endothelin-1, an increased endothelin converting enzyme activity in the hypoxic lungs allowed for a more complete coversion to endothelin-1. If there had been decreased catabolism of endothelin-1, it would have been expected that responses to low doses of big-endothelin-1 would also have been enhanced. Therefore, on balance, we believe our data is consistent with an up-regulation of endothelin converting enzyme activity in hypoxic lungs.

We have also shown that endothelin-1-induced increases in pulmonary perfusion pressure were significantly potentiated in hypoxic compared to control lungs. This is in contrast to our previous study (Lal et al., 1999a) using rings of large pulmonary arteries and veins, where endothelin-1-induced contractions were attenuated in chronically hypoxic vessels. This suggests that chronic hypoxia differentially alters the responsiveness of fine resistance vessels and large conductance pulmonary vessels to endothelin-1. Endothelin receptors are known to be differentially located along the pulmonary vasculature, i.e. endothelin ET_A receptors are present primarily on larger pulmonary conductance arteries, whereas endothelin ET_B receptors predominate in the smaller pulmonary resistance vessels (MacLean et al., 1994). The fact that vascular effects of vasoactive drugs in perfused organs primarily reflects their action at the level of the resistance vessels, suggests that enhanced responses to endothelin-1 in the chronically hypoxic lungs should be via an increased endothelin ET_B-mediated constriction of fine resistance vessels. However, the fact that the selective endothelin ET_A receptor antagonist, BQ123, was more effective than BQ788 in blocking the endothelin-1-induced rise in pulmonary perfusion pressure in chronically hypoxic lungs, provides evidence that the enhanced response is primarily due to stimulation of endothelin ET_A receptors, with a smaller component coming from smooth muscle endothelin ET_B receptors. These findings are consistent with a recent study by Soma et al. (1999) who showed that endothelin ET_A receptors were significantly up-regulated in rat pulmonary resistance arteries following chronic hypoxia.

An alternative explanation for the enhanced endothelin-1-mediated increase in pulmonary perfusion pressure would be if endothelin ET_B receptor-mediated, endothelial cell-dependent, vasodilation was impaired in hypoxic lungs, as this would allow vasoconstrictor responses to endothelin-1 to be more pronounced. However, when vasodilator responses to sarafotoxin-6C and endothelin-1, which are mediated by endothelin ET_B receptors, were examined, they were actually enhanced in chronically hypoxic lungs. This indicates that the enhanced pressor response to endothelin-1 is due to a direct action on the pulmonary vascular smooth muscle, and it is not due to a diminished endothelin ET_B receptor-mediated vasodilator response.

The enhanced endothelin-1-mediated increase in pulmonary perfusion pressure seen in hypoxic lungs raises a question about the selectivity of this response. Is it selective for endothelin-1 or is it simply a physiological effect secondary to the pulmonary hyperplasia, which takes place following chronic hypoxia (Hunter et al., 1974)?

When the effects of the alpha-adrenoceptor agonist phenylephrine were examined on pulmonary perfusion pressure changes in chronically hypoxic lungs, it was shown that these were also enhanced when compared with control lungs. This is consistent with our previous findings in large isolated pulmonary artery rings where phenylephrine induced contractions were significantly potentiated (Lal et al., 1999a). Enhanced pulmonary vasoconstrictor responses to the α-adrenoceptor agonist norepinephrine (McMurty et al., 1978) and angiotensin-II (Emery et al., 1981) have also been described in chronically hypoxic lungs. This indicates that the enhanced vasoconstriction in response to endothelin-1 is not receptor specific effect, and that it represents a more generalised effect on vascular

smooth muscle. This is not surprising, as chronic hypoxia does lead to the development of pulmonary hypertension and vascular hyperplasia (Hunter et al., 1974), and we have shown that pulmonary artery weights are significantly higher in chronically hypoxic rats (Lal et al., 1999a), indicating an increased vascular smooth muscle mass. The fact that potassium chloride-mediated pulmonary vasoconstriction was also potentiated in hypoxic lungs provides further support for a non-specific effect on vascular smooth muscle, as potassium induced contractions are not receptor-mediated. Therefore, the most likely explanation of the enhanced vasoconstrictor response to endothelin-1 is vascular hyperplasia. However, it is possible that an increase in myofilament calcium sensitivity could also have a role to play (Evans et al., 1999). A further possibility is that the enhanced vasoconstrictor actions of endothelin-1, phenylephrine and potassium chloride in chronically hypoxic lungs are simply due to the fact that basal perfusion pressure was greater in these preparations. However, the fact that sarafotoxin-6C-induced constriction was not enhanced argues against this.

In contrast to endothelin-1, sarafotoxin-6C-induced increases in pulmonary perfusion pressure were not significantly altered in chronically hypoxic lungs. We believe the reason for this is that sarafotoxin-6C-induced vasodilation, which is endothelin ET_B receptor-mediated, and endothelial-dependent, is opposing the direct effect of sarafotoxin-6C on smooth muscle. The fact that nitro-L-arginine significantly potentiated sarafotoxin-6C-induced increases in pulmonary perfusion pressure in hypoxic lungs is in agreement with our previous studies in normal lungs (Lal et al., 1996) and provides evidence that sarafotoxin-6C-induced nitric oxide production is opposing its direct constrictor action. It is unlikely that an enhanced basal release of nitric oxide is responsible for this effect. If this was the explanation, an increased basal nitric oxide production would have been expected to attenuate the constrictor actions of endothelin-1, phenylephrine and potassium chloride; but this was not seen. However, a recent paper has reported that aminoguanidine, a relatively selective inhibitor of the inducible form of nitric oxide synthase, when added to pulmonary artery rings from animals subjected to acute *Pseudomonas aeruginosa* infection, did not enhance the effects of the constrictor agents phenylephrine, potassium chloride and prostaglandin $F_{2\alpha}$ to the same extent (Yaghi and McCormack, 1999). However, it is important to stress that this was a different model using a relatively large vessel preparation from an animal subjected to bacterial infection, while our perfused lung model contains very fine resistance vessels, which may behave differently.

When nitro-L-arginine was added in our study, it only increased basal pulmonary perfusion pressure by 2.66 ± 1 mm Hg, however, it potentiated the pressor action of the higher doses of sarafotoxin-6C by about 15 mm Hg (Fig. 3). This provides further evidence that an enhanced sarafotoxin-6C, endothelin ET_B-mediated, nitric oxide production is opposing the direct pressor action of sarafotoxin-6C. The finding that indomethacin, when added along with nitro-L-arginine, produced a further potentiation of the pulmonary perfusion pressure response to higher doses of sarafotoxin-6C, compared to its response in the presence of nitro-L-arginine alone, suggests that vasodilator cyclooxygenase products are also being released at the higher doses of sarafotoxin-6C. These products would also offset endothelin ET_B receptor-mediated vasoconstriction in chronically hypoxic lungs.

Further support for an increased sarafotoxin-6C-induced nitric oxide release in chronically hypoxic lungs came from experiments where we examined the vasodilator actions of sarafotoxin-6C and endothelin-1 in U46619 preconstricted preparations. In these studies, dilator responses to endothelin-1 and sarafotoxin-6C were enhanced in hypoxic lungs compared to control preparations. As the dilator responses to sarafotoxin-6C and endothelin-1 are the result of endothelin ET_B-induced nitric oxide release, this provides evidence for an increased endothelial endothelin ET_B responsiveness in hypoxic lungs. However, due to the design of the experiments, U46619 was used at different concentrations to increase basal perfusion pressure by 10 mm Hg in control and chronically hypoxic lungs. As the basal perfusion pressure was already higher in the hypoxic lungs, this meant that prior to the addition of endothelin-1, or sarafotoxin-6C, the starting pressures after U46619 addition were also higher in the hypoxic lungs. This clearly gives scope for a greater vasodilator response in the chronically hypoxic preparations, and it could be argued that this accounts for the enhanced endothelin-1 and sarafotoxin-6C dilator responses seen in hypoxic lungs. However, when vasodilator responses to sodium nitroprusside were examined, it was found that at all doses used, there was no significant difference between control and hypoxic lungs. Therefore, this provides good evidence that there is enhanced endothelin ET_B-mediated vasodilatation in hypoxic lungs which is not an artefact of the experimental design.

The enhanced vasodilator response to endothelin-1 and sarafotoxin-6C in hypoxic lungs seen in our experiments is in contrast to the observations of (Eddahibi et al., 1993) who showed a diminished vasodilator response to endothelin-1 and endothelin-3 in perfused lungs of chronically hypoxic rats. The reason for this discrepancy is not clear, although, a major difference is that these workers used a re-circulating mode of perfusion. The very recent paper by Muramatsu et al. (1999), which agrees with our data, has reported that vasodilator responses to the endothelin ET_B receptor agonist IRL-1620 were enhanced in lungs from chronically hypoxic rats. Muramatsu et al. (1999) also reported that endothelin ET_B receptors located on the pulmonary vascular endothelium are significantly increased in lungs from chronically hypoxic rats. Our observation that nitro-L-arginine and indomethacin, which inhibit the endothelial production of NO and prostanoids,

respectively, enhanced sarafotoxin-6C-mediated increases in pulmonary perfusion pressure in chronically hypoxic lungs provides further evidence that endothelial-dependent vasodilator responses are not inhibited in hypoxic lungs. Therefore, we are unable to explain the discrepancy between our data and that of Eddahibi et al. (1993).

In addition to their effects on the pulmonary vasculature, we have now shown that endothelin-1- and sarafotoxin-6C-induced bronchoconstriction are also enhanced in chronically hypoxic lungs; these effects were more pronounced with the lower doses used. The constrictor actions of these agents on bronchial smooth muscle are, like endothelin-induced vasodilation, mediated by endothelin ET_B receptors, as shown by their inhibition with the selective endothelin ET_B receptor antagonist BQ788. The fact that carbachol-induced bronchconstriction, which is mediated via muscarinic receptors, was not potentiated in hypoxic lungs shows that this effect was selective for endothelin ET_B receptor stimulation. To our knowledge, this is the first time that alterations in the endothelin receptor-mediated bronchial responses have been reported in lungs from chronically hypoxic animals. Interestingly, Moller et al. (1999) reported that in bronchial biopsies taken from patients with chronic airway, obstruction endothelin ET_B receptor mRNA is significantly increased. Furthermore, Chalmers et al. (1997) have reported that asthmatics exhibits bronchial hyper-reactivity to endothelin-1.

The fact that nitro-L-arginine alone, or in combination with indomethacin, had no effect on the basal bronchial tone suggests that nitric oxide and prostaglandins do not contribute to the low basal bronchial tone in chronically hypoxic lungs. Also, these inhibitors had no effect on the bronchoconstrictor responses to sarafotoxin-6C in chronically hypoxic lungs. This is in contrast to our previous studies in normal rat lungs, where nitro-L-argine significantly potentiated bronchial responses to sarafotoxin-6C (Lal et al., 1996). Others have also shown that endothelin-1-induced relaxation of guinea-pig trachaea involves nitric oxide release (Emanueli et al., 1998). In the present study, the lack of effect of nitro-L-arginine on sarafotoxin-6C-induced bronchoconstriction may indicate a reduced nitric oxide production in the airways of chronically hypoxic rats.

In summary, results from this study have shown that in chronically hypoxic lungs, the conversion of big-endothelin-1 to endothelin-1 is significantly increased, suggesting an increased endothelin converting enzyme activity. The findings that pulmonary perfusion pressure responses to big-endothelin-1, endothelin-1, phenylephrine and potassium chloride were augmented suggest that this is due to a non-specific increase in vascular reactivity. The fact that sarafotoxin-6C-induced vasoconstriction in chronically hypoxic lungs was significantly potentiated in the presence of nitro-L-arginine and indomethacin, together with enhanced endothelin-1 and sarafotoxin-6C vasodilator responses suggests an enhanced endothelin ET_B receptor-mediated effect in the vasculature of chronically hypoxic lungs. This, together with a selective enhancement of endothelin ET_B-mediated bronchoconstriction indicates that there is a generalised up-regulation of endothelin ET_B receptors in hypoxic lungs. These findings could have important implications for the treatment of hypoxia-induced pulmonary dysfunction.

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