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Chronic hypoxia differentially alters the responses of pulmonary arteries and veins to endothelin-1 and other agents

Harbans Lal, K. Ivor. Williams *, Brian Woodward

Department of Pharmacy and Pharmacology, University of Bath, Bath, BA2 7AY, UK

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Abstract

The effects of chronic hypoxia on the responses of rat large pulmonary arteries and veins to vasoactive agents have been examined. Endothelin-1-induced contractions of pulmonary arteries and pulmonary veins were reduced by chronic hypoxia. In contrast, chronic hypoxia augmented sarafotoxin 6c-induced contractile responses in pulmonary veins but not in pulmonary arteries. Chronic hypoxia augmented the constrictor effect of phenylephrine in pulmonary arteries, but not in pulmonary veins. The thromboxane receptor agonist, U46619 (9,11-dideoxy-9_{α},11_{α}-epoxy-methanoprostaglandin $f_{2\alpha}$) contracted pulmonary arteries and pulmonary veins, and although maximal responses were not altered in chronically hypoxic preparations, the EC_{50} value in pulmonary arteries was increased following chronic hypoxia. The relaxant effects of acetylcholine and isoprenaline on pulmonary arteries were potentiated by chronic hypoxia. In contrast, ionomycin-mediated relaxations of pulmonary arteries and pulmonary veins were reduced, while sodium nitroprusside-induced relaxation of pulmonary arteries and veins were not altered by chronic hypoxia. Previous studied have looked primarily at the effects of chronic hypoxia on pulmonary arteries. This data provides evidence that chronic hypoxia also causes selective changes in the reactivity of large pulmonary veins.

Keywords: Hypoxia, chronic; Pulmonary artery; Pulmonary vein; Vasoconstriction; Vasorelaxation; Endothelin-1

1. Introduction

Endothelin-1, the most potent vasoconstrictor yet described (Yangisawa et al., 1988), is known to be released by the lung (Goldie et al., 1996; Smith et al., 1997). In previous studies using the isolated perfused rat lung we have shown that endothelin-1, and the selective endothelin ET_B receptor agonist sarafotoxin 6c, act differentially to produce constriction of pulmonary arteries and veins, i.e., endothelin-1 causes a preferential venoconstriction via activation of endothelin ET_A receptors whereas sarafotoxin 6c produces arterial constriction via stimulation of endothelin ET_B receptors (Lal et al., 1995). Therefore the relative contributions of endothelin ET_A and ET_B receptor subtypes to endothelin-1-induced contractions differ considerably at different levels in the pulmonary vasculature. Other groups have also shown differential sensitivities of pulmonary blood vessels to the actions of the endothelins. For example, MacLean et al. (1994) showed that in large pulmonary arteries, constriction was primarily due to activation of endothelin ET_A receptors, while in smaller pulmonary arteries endothelin ET_B receptors were predominant. Also, pulmonary veins are more sensitive to the spasmogenic actions of endothelin-1 than are pulmonary arteries (Rodman et al., 1992; Toga et al., 1992). In addition to these differences, the mechanisms underlying endothelin-1-induced vasoconstriction in pulmonary arteries and veins can differ. For example, in sheep pulmonary arteries endothelin-1-induced contractions are thromboxane A_2-independent, whereas those of pulmonary veins are thromboxane A_2-dependent (Toga et al., 1992).

Chronic hypoxia leads to increased circulating levels of endothelin-1 (Elton et al., 1992; Bialecki et al., 1998) and endothelin receptor antagonists have been shown to prevent the development of pulmonary hypertension in this situation (Eddahibi et al., 1995; Oparil et al., 1995). In view of the fact that prolonged stimulation of endothelin receptors, particularly endothelin ET_B receptors, leads to tachyphylaxis (Le Monnier De Gouville et al., 1990;

* Corresponding author. Tel.: +44-1225-826842; Fax: +44-1225-826114

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Thompson et al., 1995) it might be expected that when circulating levels of endothelin-1 have been elevated by chronic hypoxia, the responsiveness of pulmonary arteries and veins to endothelins could be altered.

MacLean and McCulloch (1998) recently reported that in the rat, chronic hypoxia did not affect the contractile response to endothelin-1 in large and small pulmonary artery rings while responses to sarafotoxin 6c were enhanced in small pulmonary artery rings. In contrast Bialecki et al. (1998) showed that endothelin-1-induced contractions were reduced in pulmonary arteries from chronically hypoxic rats, while Adnot et al. (1991) reported an enhanced pressor response to endothelin-1. In an attempt to address this controversy, we have carried out studies to examine the actions of the endothelins in pulmonary arteries from rats which have been subjected to chronic hypoxia. In addition, we have also examined the effects of endothelin receptor agonists on pulmonary veins from chronically hypoxic animals as there are very few studies of chronic hypoxia on pulmonary veins.

It has been reported that there is an up-regulation of nitric oxide (NO) synthase in lungs from chronically hypoxic rats and an increased production of NO (Le Cras et al., 1996). This could also alter the vascular responsiveness of pulmonary blood vessels to the endothelins as, in the whole lung, they have pulmonary vasodilator actions which are due to activation of endothelin ET_B receptors on endothelial cells and the release of NO (Lal et al., 1996). As dilator responses to endothelins are reduced in the arterial bed of the whole lung of chronically hypoxic rats (Eddahibi et al., 1993) this suggests that a down-regulation of the endothelin ET_B receptors may be more important than the capacity for increased NO production. However, there are contradictory reports in the literature regarding the effects of chronic hypoxia on endothelial-dependent vasodilation in the pulmonary circulation of the rat. In contrast to the observations of Eddahibi et al. (1993), who showed a decreased endothelial-dependent relaxation, Russ and Walker (1993) showed that NO activity, as assessed by arginine vasopressin-induced vasodilation, was not affected by chronic hypoxia, while Oka et al. (1993) and Isaacson et al. (1994) described an enhanced NO production in chronically hypoxic rat lungs.

In all of the studies described above the effects of chronic hypoxia on the responsiveness of pulmonary veins to vasoactive agents were not examined. In view of the fact that changes in venous tone have implications for the development of hydrostatic oedema the present experiments were designed to compare the vascular responses of pulmonary arteries and pulmonary veins from chronically hypoxic and normoxic rats. The effects of several contractile agents (endothelin-1, sarafotoxin 6c, phenylephrine and, a thromboxane A_2 receptor agonist U46619 (Coleman et al., 1981), were studied, as were responses to endothelial-dependent (acetylcholine, ionomycin) and directly acting relaxants (sodium nitroprusside, isoprenaline). The involvement of nitric oxide (NO) generation in these effects was studied using the NO synthase inhibitor nitro-L-arginine (L-NOARG). Preliminary results from these studies have been presented to the British Pharmacological Society (Lal et al., 1998a,b).

2. Methods

2.1. Isolated pulmonary arteries and veins

Male Wistar rats with final weight 300–350 g were used throughout. Animals designated for chronic hypoxia were housed for 3 weeks in a recirculating normobaric environmental chamber maintained at 10% pO_2 similar to that described by Cryer and Bartley (1974). The air in the chamber was passed through a silica gel column to remove water vapour, while CO_2 was absorbed using a soda lime column. Oxygen tension was continuously monitored using a Servomex 1440C gas analyser.

Prior to removing tissues for experimental purposes animals were anaesthetised with pentobarbitone sodium (60 mg kg^{-1}, i.p.), heparin (500 IU) was injected (i.v.) via the tail vein and 5 min later the chest was opened and the heart and lungs were removed. Right ventricular wet weight and left ventricular wet weight were recorded. The main pulmonary artery (5–6 mm long) wet weight, and haematocrit values were also recorded. The large pulmonary arteries and veins were cleared of all visible connective tissue and cut into rings of 2–2.5 mm in length. All rings were mounted on stainless steel wire hooks in Krebs' solution at 37°C continuously bubbled with 20% O_2/5% CO_2/75% N_2. The Krebs' solution was of following composition (mM): KCl 4.7, KH_2PO_4 1.2, $CaCl_2$ 1.25, $MgSO_4$ 1.2, NaCl 118, $NaHCO_3$ 25, glucose 11.1. Isometric contractions were measured using force transducers (Biegestab K30) connected to a 4 channel MacLab/4S recorder. Each ring was equilibrated for 1 h at the mean optimal resting tension (7 mN for pulmonary veins and 10 mN for pulmonary arteries). Cumulative concentration–response curves for agonists were then constructed. In a separate set of experiments receptor antagonists or inhibitors were added after 30 min, and 30 min prior to agonist addition.

In a different protocol, basal tone in pulmonary arteries and pulmonary veins was raised using U46619 (300 nM) $\approx$ 80% of the maximal contraction in both control and chronically hypoxic vessels and vasorelaxant responses to acetylcholine, the calcium ionophore ionomycin, isoprenaline, and sodium nitroprusside were studied.

2.2. Drugs

BQ123 (cyclo[D-Asp-L-Pro-D-Val-L-Leu-D-Trp]) and BQ788 ([*N-cis*-2,6-dimethylpiperidinenocarbonyl-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 Peptide Institute (Japan), phenylephrine, U46619, isoprenaline, acetylcholine sodium nitroprusside from Sigma. Ionomycin was from Calbiochem. U46619 (9,11-dideoxy-9_{α},11_{α}-epoxy-methanoprostaglandin $f_{2\alpha}$) was dissolved in 95% ethanol and was further diluted in normal saline and stored at −80°C. BQ788 and ionomycin were dissolved in dimethylsulfoxide, the final concentration of dimethylsulfoxide was less than 0.1%. Stock solutions of BQ123, endothelin-1, sarafotoxin 6c and big endothelin-1 were prepared in normal saline and were stored at −20°C. Phenylephrine, acetylcholine, isoprenaline and sodium nitroprusside were dissolved in normal saline prepared on the same day and were kept in ice.

2.3. Statistical analysis

In pulmonary arteries and pulmonary veins, agonist-induced contractions were expressed as cumulative concentration–response curves and values expressed as absolute contractions in mN relative to basal tone. The vasodilator responses in pulmonary arteries and veins were expressed as percent relaxation relative to the contraction produced by U46619. Data are expressed as mean ± standard error of the mean (S.E.M.). An unpaired student t test was used to test the level of statistical significance between control and chronically hypoxic preparations. Probability values of $P < 0.05$ were considered significant.

3. Results

3.1. Right ventricular wet weight, pulmonary artery wet weight and haematocrit

Chronic hypoxia increased the ratio of right ventricular to total ventricular wet weight (0.34 ± 0.001 vs. 0.21 ± 0.001, $n = 12$, $P < 0.001$) as compared with control animals. In addition pulmonary artery wet weight as a percentage of body weight ratio was significantly increased in chronic hypoxia (0.0045 ± 0.0002, $n = 11$) vs. control (0.0016 ± 0.0002, $n = 5$, $P < 0.001$).

The haematocrit was also significantly increased in chronically hypoxic animals ($62 \pm 1\%$, $n = 11$) vs. control rats ($32 \pm 7\%$, $n = 5$, $P < 0.01$).

3.2. Vasoconstrictor responses

3.2.1. Endothelin-1

3.2.1.1. Pulmonary arteries. Fig. 1 shows that in pulmonary arteries from control rats endothelin-1 (0.01–100 nM) produced a concentration-dependent contraction. In chronically hypoxic rats endothelin-1-induced contractions

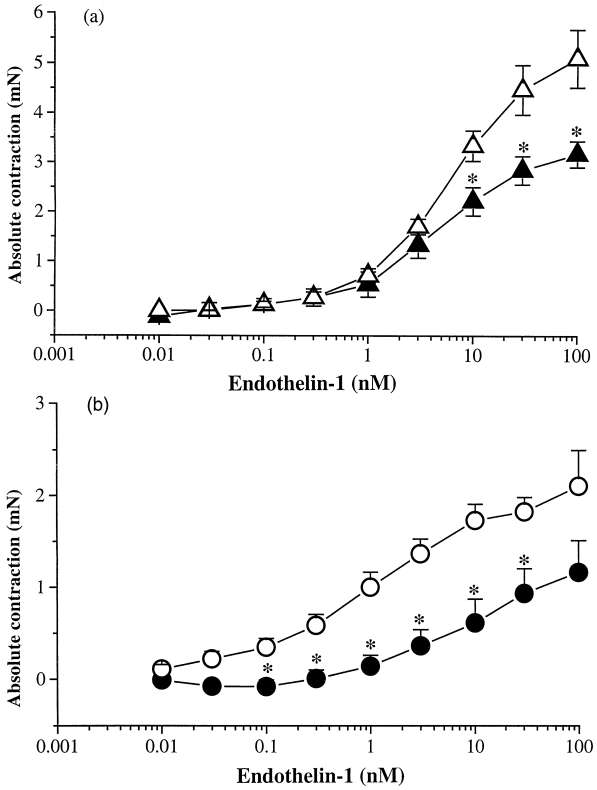

Fig. 1. Endothelin-1-induced contractions of: (a) pulmonary arteries, and (b) pulmonary veins from control (open symbols) and chronically hypoxic (filled symbols) animals, mean ± S.E.M., $n = 8–10$. *$P < 0.05$ significant difference c.w. control.

were significantly reduced. The absolute contraction caused by endothelin-1 (100 nM) in pulmonary arteries from chronically hypoxic animals was markedly reduced (3.1 ± 0.26 mN, $n = 10$) when compared with control preparations (5.3 ± 0.66 mN, $P < 0.05$, $n = 10$).

In order to see if the endothelin-1-induced contractions of pulmonary arteries were reduced in chronic hypoxia because of an increased NO production, the NO synthase inhibitor L-NOARG was used. Inclusion of L-NOARG (100 μM) 30 min prior to endothelin-1 in pulmonary artery rings from chronically hypoxic rats increased the basal tone (1.3 ± 0.27 mN, $n = 10$) which was greater than that seen in control preparations (0.41 ± 0.1 mN, $n = 6$, $P < 0.05$). However, in the presence of L-NOARG the absolute contraction of pulmonary arteries from chronically hypoxic animals caused by the highest concentration of endothelin-1 used (100 nM), 2 ± 0.51 mN, was not significantly different when compared with chronically hypoxic preparations in the absence of L-NOARG (3.1 ± 0.26 mN).

BQ123 (3 μM) inhibited endothelin-1-induced contractions in control pulmonary artery rings. Endothelin-1 100 nM in control preparations produced 5.3 ± 0.66 mN, $n = 10$ contraction, this response was significantly reduced to 1.5 ± 0.74 mN, $P < 0.01$, $n = 4$ in the presence of BQ123 (3 μM).

3.2.1.2. Pulmonary veins. Fig. 1 shows that endothelin-1 also produced a concentration-dependent contraction of pulmonary veins. As with pulmonary arteries, the contraction of pulmonary veins in response to endothelin-1 in vessels from chronically hypoxic rats was significantly reduced when compared with control pulmonary veins ($P < 0.05$, $n = 8$–10). The addition of L-NOARG (100 μM) to pulmonary veins produced small increases in basal tone in veins from chronically hypoxic (0.06 ± 0.03 mN, $n = 6$) and control animals (0.08 ± 0.05 mN, $n = 8$). These increases were not significantly different from each other. L-NOARG also had no significant effect on endothelin-1-induced contractions in pulmonary veins from chronically hypoxic animals. For example, in the presence of L-NOARG, endothelin-1 (30 nM) produced an increase in tension of 1.0 ± 0.16 mN ($n = 5$), while in the absence of L-NOARG the contraction was 1.0 ± 0.34 mN ($n = 6$).

BQ123 (3 μM) inhibited endothelin-1 responses in control pulmonary veins. Endothelin-1 (30 nM) produced a contraction of pulmonary venous rings from control rats (1.8 ± 0.15 mN, $n = 8$), this response was significantly reduced in the presence of BQ123 (0.52 ± 0.18 mN, $n = 4$, $P < 0.05$).

3.2.2. Sarafotoxin 6c

3.2.2.1. Pulmonary arteries. Fig. 2 shows that the endothelin ET_B receptor agonist sarafotoxin 6c (0.01–30 nM) produced small contractions of pulmonary arteries ($EC_{50} = 2.45 \pm 0.68$ nM, $n = 6$) from control animals, the maximal absolute contraction caused by sarafotoxin 6c (30 nM) in pulmonary arteries was significantly lower 0.29 ± 0.08 mN, $n = 6$, $P < 0.001$, than that caused by endothelin-1 (30 nM) (4.5 ± 0.5 mN, $n = 10$, $P < 0.001$). In chronically hypoxic rats, sarafotoxin 6c-induced contractions of pulmonary arteries were not significantly different to control preparations, the maximal contractions being 0.29 ± 0.08 mN, ($n = 6$) vs. 0.29 ± 0.16 mN ($n = 5$) respectively. In the presence of L-NOARG (100 μM) these sarafotoxin 6c (0.01 nM–30 nM) responses in pulmonary artery of control and chronically hypoxic rats were not altered (data not shown).

3.2.2.2. Pulmonary veins. Fig. 2 shows that sarafotoxin 6c contracted pulmonary veins from control rats ($EC_{50} = 0.61 \pm 0.11$ nM, $n = 5$). The maximal contraction caused by sarafotoxin 6c in pulmonary veins (1.04 ± 0.18 mN) was significantly greater than in pulmonary arteries (0.29 ± 0.08 mN, $n = 8$, $P < 0.05$).

In contrast to pulmonary arteries, the response to sarafotoxin 6c in pulmonary veins from chronically hypoxic rats tended to be augmented at the lower concentrations used (10–300 pM) when compared with control pulmonary veins (Fig. 2), but this only achieved statistical significance at the 100 pM concentration (chronically hypoxic 0.8 ± 0.2 mN, $n = 4$ compared to the control response of 0.03 ± 0.02 mN, $n = 5$ $P < 0.05$). The maximal constrictor response to sarafotoxin 6c in pulmonary veins was not affected by chronic hypoxia.

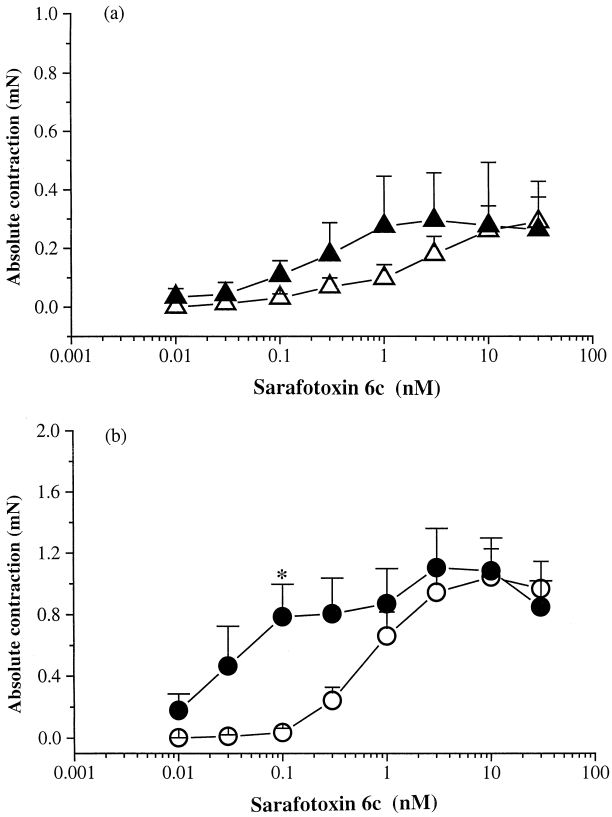

Fig. 2. Sarafotoxin 6c-induced contractions of: (a) pulmonary arteries, and (b) pulmonary veins from control (open symbols) and chronically hypoxic (filled symbols) animals, mean ± S.E.M., $n = 4$–6. $^*P < 0.05$ significant difference c.w. control.

In control preparations the endothelin ET_B receptor antagonist BQ788 (3 μM) completely blocked the responses to sarafotoxin 6c (0.01–30 nM, $n = 4$).

3.2.3. Phenylephrine

3.2.3.1. Pulmonary arteries and pulmonary veins. Fig. 3 shows that phenylephrine (1 pM–1 μM) produced contractions of pulmonary artery in control and chronically hypoxic rats. Responses to the lower concentrations of phenylephrine were potentiated in pulmonary arteries from chronically hypoxic animals ($EC_{50} = 2.8 \pm 1.2$ nM, $n = 8$) compared with control ($EC_{50} = 13 \pm 3.1$ nM, $n = 10$, $P < 0.01$). Interestingly, at concentrations up to 1 μM, phenylephrine did not contract pulmonary veins from control or chronically hypoxic rats (Fig. 3).

3.2.4. Thromboxane A_2 receptor agonist U46619

3.2.4.1. Pulmonary arteries and pulmonary veins. Fig. 4 shows the responses of U46619 in control and chronically

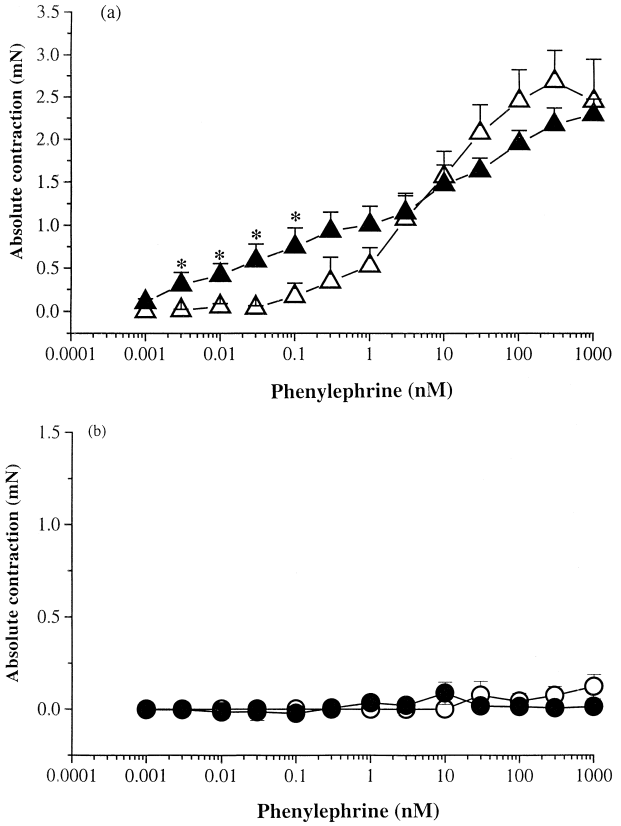

Fig. 3. Phenylephrine-induced contractions of: (a) pulmonary arteries, and (b) pulmonary veins from control (open symbols) and chronically hypoxic (filled symbols) animals, mean ± S.E.M., $n = 8–10$, $^*P < 0.05$ significant difference c.w. control.

hypoxic pulmonary vessels. In control vessels U46619 (0.3 nM–1 μM) produced a greater absolute maximal response in pulmonary arteries (3.3 ± 0.38 mN, $n = 7$) than in pulmonary veins (1.48 ± 0.09 mN, $n = 6$). In chronically hypoxic preparations, responses to U46619 in pulmonary veins were not altered, the EC_{50} values in chronic hypoxia vs. control were 31 ± 7 nM ($n = 8$), vs. 30 ± 7 nM ($n = 6$) respectively ($P > 0.05$). In pulmonary arteries from chronically hypoxic rats there was decreased ($P < 0.05$) sensitivity to U46619 which was reflected in an increased EC_{50} value (25 ± 3 nM, $n = 5$), compared to the control EC_{50} (9.3 ± 2.4 nM, $n = 7$). Despite this change in the EC_{50} value there was no significant change in the maximal contractile response to U46619 in pulmonary arteries from chronically hypoxic rats (Fig. 4).

3.3. Vasodilator responses

In order to determine whether chronic hypoxia affected vasorelaxant responses in pulmonary arteries and veins, basal tone was raised by pre-incubating rings with U46619 (300 nM). This concentration was used because its effects on chronically hypoxic and control pulmonary arteries and veins were not significantly different (Fig. 4). The relaxant responses to acetylcholine, sodium nitroprusside, ionomycin, and isoprenaline were then studied.

3.3.1. Acetylcholine

3.3.1.1. Pulmonary arteries. The responses to acetylcholine in control and chronically hypoxic preparations are shown in Fig. 5. In control pulmonary arteries, acetylcholine produced a concentration-dependent relaxation. In pulmonary arteries from chronically hypoxic animals the acetylcholine-induced relaxations were augmented as indicated by a reduction in the EC_{50} from 370 ± 50 nM, ($n = 7$ control) to 150 ± 42 nM, ($n = 8$ chronic hypoxia, $P < 0.05$). Although the maximum relaxation of pulmonary arteries caused by acetylcholine in chronically hypoxic preparations tended to be greater ($89 \pm 10\%$) than in the control pulmonary arteries ($60 \pm 5.5\%$) it was not significantly different ($P > 0.05$).

In a different set of experiments L-NOARG (100 μM) inhibited acetylcholine (1 nM–30 μM)-induced relaxations in control and chronically hypoxic pulmonary artery rings. Acetylcholine-induced maximal relaxant responses in the absence of L-NOARG ($75 \pm 3\%$) were converted to small contractions by L-NOARG in control pulmonary arteries

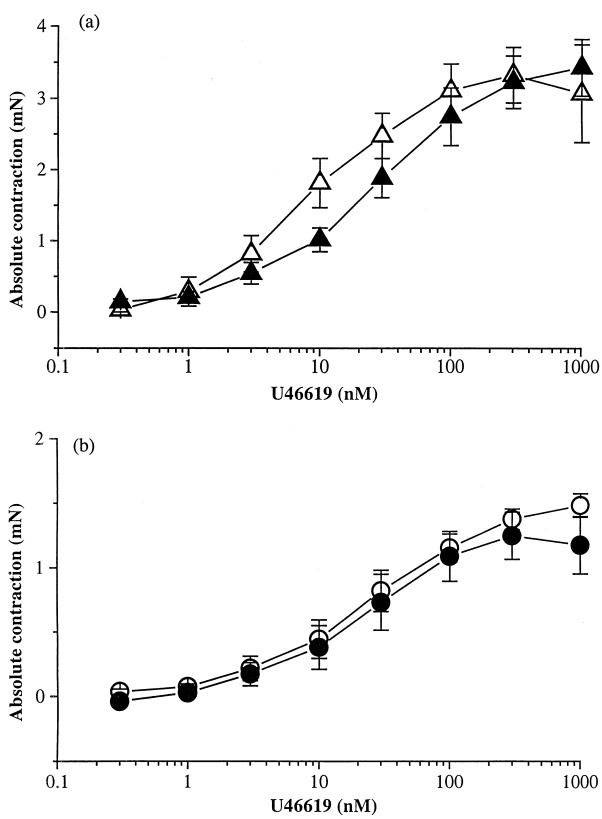

Fig. 4. U46619-induced contractions of: (a) pulmonary arteries, and (b) pulmonary veins from control (open symbols) and chronically hypoxic (filled symbols) animals, mean ± S.E.M., $n = 5–10$, $^*P < 0.05$ significant difference c.w. control.

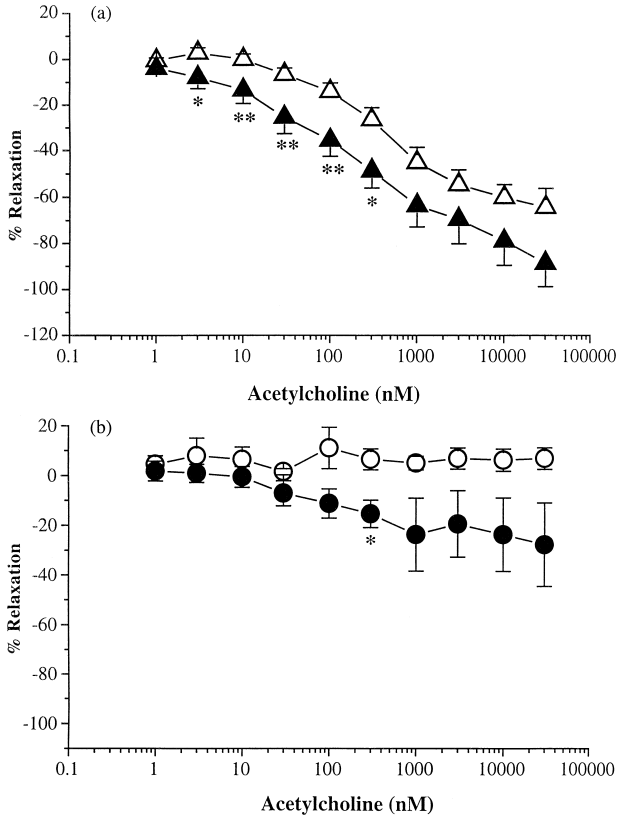

Fig. 5. Acetylcholine-induced relaxation of U46619 precontracted pulmonary arteries (a), and pulmonary veins (b), from control (open symbols) and chronically hypoxic (filled symbols) animals, mean ± S.E.M., $n = 5–8$, $^{*}P < 0.05$, $^{**}P < 0.01$ significant difference c.w. control.

($4 \pm 5\%$, $n = 4$, $P < 0.001$). Similarly, in pulmonary arteries from chronically hypoxic rats, acetylcholine-induced maximal responses ($60 \pm 10\%$) were significantly reduced ($90.5 \pm 2.5\%$, $n = 4$, $P < 0.01$) in the presence of L-NOARG.

3.3.1.2. Pulmonary veins. Unlike pulmonary arteries, control pulmonary veins did not relax in response to acetylcholine (1 nM–30 μM, $n = 6$) (Fig. 5). Acetylcholine did cause a small relaxation of pulmonary veins from chronically hypoxic animals, however, this was only statistically significant when compared with the control at the 300 nM concentration ($15 \pm 5.4\%$, $n = 5$, $P < 0.05$). L-NOARG (100 μM) abolished acetylcholine-induced relaxations.

3.3.2. Ionomycin

3.3.2.1. Pulmonary arteries. Fig. 6 shows the vasorelaxant responses to ionomycin in control vs. chronically hypoxic vessels. In control pulmonary artery rings, ionomycin (1 nM–3000 nM) produced a concentration-dependent relaxation ($EC_{50} = 41 \pm 7$ nM, $n = 4$) which was significantly reduced in preparations from chronically hypoxic animals ($EC_{50} = 220 \pm 55$ nM, $n = 6$, $P < 0.05$). The maximal relaxation caused by ionomycin in pulmonary arteries from chronically hypoxic rats was also significantly decreased ($24 \pm 7\%$, $P < 0.01$) when compared with control pulmonary artery rings ($67 \pm 11.5\%$, $n = 4$).

3.3.2.2. Pulmonary veins. In control pulmonary venous rings ionomycin produced a similar degree of relaxation to that seen in control pulmonary artery rings. In pulmonary venous rings from chronically hypoxic rats, responses to ionomycin ($EC_{50} = 233 \pm 90$ nM, $n = 6$) tended to be reduced when compared with control preparations ($EC_{50} = 80 \pm 30$ nM) but this did not achieve statistical significance ($P > 0.05$). The maximal relaxation produced by ionomycin in pulmonary venous rings from control ($53 \pm 12\%$, $n = 7$) vs. chronically hypoxic rats ($43 \pm 5\%$, $n = 6$) were not significantly different ($P > 0.05$, Fig. 6).

3.3.3. Isoprenaline

3.3.3.1. Pulmonary arteries. Fig. 7 shows that isoprenaline produced a greater maximal relaxation in pulmonary arteries of chronically hypoxic animals compared to controls. However the EC_{50} value in pulmonary artery rings of

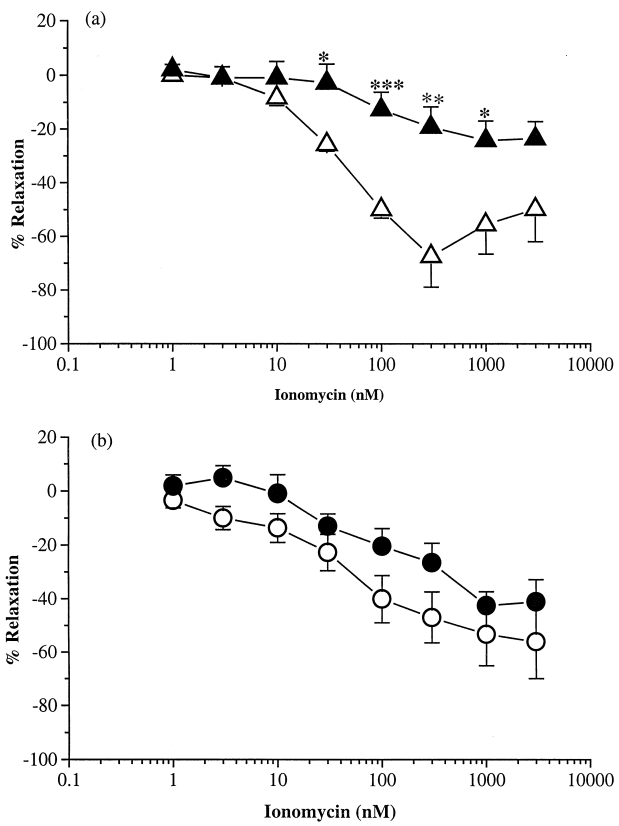

Fig. 6. Ionomycin-induced relaxation of U46619 precontracted pulmonary arteries (a), and pulmonary veins (b), from control (open symbols) and chronically hypoxic (filled symbols) animals, mean ± S.E.M., $n = 4–8$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ significant difference compared to controls.

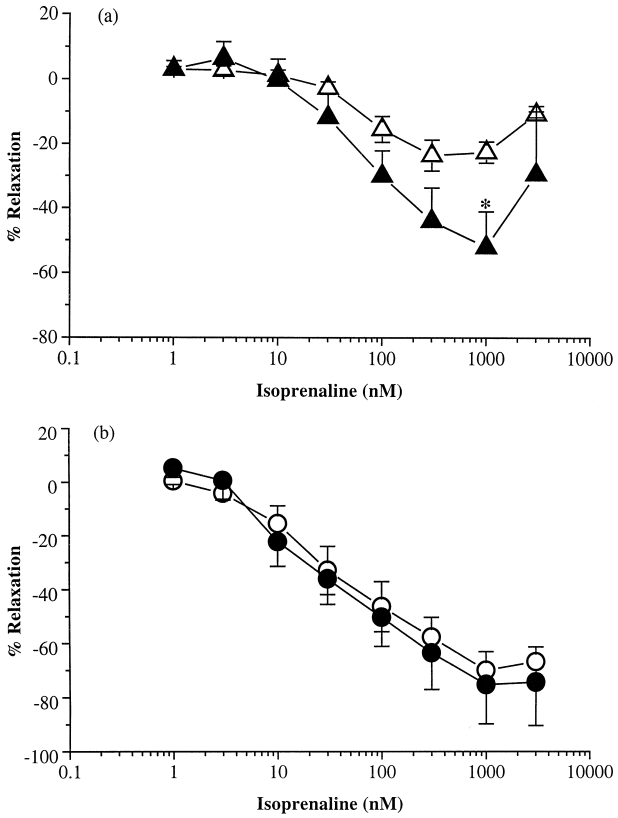

Fig. 7. Isoprenaline-induced relaxation of U46619 precontracted pulmonary arteries (a), and pulmonary veins (b), from control (open symbols) and chronically hypoxic (filled symbols) animals, mean ± S.E.M., $n = 5–8$. $^*P < 0.05$, significant difference compared to controls.

control animals was not different to that from chronically hypoxic animals, the EC_{50} values being 104 ± 28 nM, ($n = 5$) and 124 ± 45 nM, ($n = 8$), respectively. At the highest concentration used, the pulmonary artery relaxant action of isoprenaline in control and chronically hypoxic preparations showed some evidence of tachyphylaxis.

3.3.3.2. Pulmonary veins. Fig. 7 shows that in control preparations isoprenaline produced a greater relaxation in pulmonary veins ($70 \pm 7\%$, $n = 5$) than in pulmonary arteries ($23 \pm 3\%$, $n = 5$, $P < 0.05$). In chronically hypoxic animals the isoprenaline-induced relaxation of pulmonary veins were not significantly different to that of control preparations.

3.3.4. Sodium nitroprusside

3.3.4.1. Pulmonary arteries and veins. The EC_{50} values for sodium nitroprusside (1–1000 nM)-induced relaxation of control pulmonary arteries (35 ± 14 nM, $n = 3$) and pulmonary veins (50 ± 18 nM, $n = 3$), and the maximal effect produced $100 \pm 2\%$ and $87 \pm 8\%$, respectively were not statistically different. These relaxant effects were not significantly affected in preparations from chronically hypoxic rats.

4. Discussion

While there have been a number of studies examining the effects of vasoactive agents on pulmonary arteries from chronically hypoxic animals there has been very little work carried out on pulmonary veins. Chronic changes in the reactivity of pulmonary veins clearly have important implications for pulmonary capillary pressure and the development of pulmonary oedema. For this reason we have examined, and shown, that there are selective changes in the responses of pulmonary arteries and veins to different vasoconstrictor and vasodilator agents.

After 3 weeks of normobaric hypoxia there was a significant increase in the ratio of right ventricular to total ventricular weight. Haematocrit values and pulmonary artery wet weight were also increased in chronically hypoxic rats. These changes are all indicative of pulmonary hypertension, right ventricular hypertrophy and pulmonary hyperplasia which are well characterised in this model (Emery et al., 1981; Barer et al., 1993).

In preparations from normoxic animals endothelin-1 was found to be a more potent constrictor of pulmonary veins compared with pulmonary arteries. This finding is in agreement with the observations of Rodman et al. (1992) and Toga et al. (1992). The constrictor responses to ET-1 in pulmonary arteries and veins of normoxic animals were significantly reduced in the presence of the selective endothelin ET_A receptor antagonist BQ123 (Ihara et al., 1992), suggesting the presence of endothelin ET_A receptors in these vessels.

In vessels from chronically hypoxic rats endothelin-1-induced contractions of pulmonary arteries were reduced when compared with preparations from normoxic animals. Our observation on pulmonary artery is in agreement with the recent study by Bialecki et al. (1998). However, this contrasts with a very recent paper by MacLean and McCulloch (1998) who showed that following two weeks of chronic hypoxia the constrictor action of endothelin-1 in pulmonary artery was enhanced. The reason for this discrepancy is not clear. It is unlikely to be due to the duration of the hypoxic treatment period, or the strain of rat used, because Bialecki et al. (1998) used a two week treatment period and Sprague–Dawley rats, while our study, and that of MacLean and McCulloch (1998) used Wistar rats.

Bialecki et al. (1998) and MacLean and McCulloch (1998) did not examine the effects of chronic hypoxia on pulmonary veins. In our study we have also shown that the sensitivity of large pulmonary veins to endothelin-1 is decreased following chronic hypoxia. The precise mechanism(s) underlying the attenuated responses to endothelin-1 in pulmonary arteries and veins from chronically hypoxic animals is not clear. It might be expected that following chronic hypoxia, when circulating levels of endothelin-1 are increased (Bialecki et al., 1998) there could be a down-regulation of endothelin receptors in blood vessels.

In support of this, it has been shown that in lungs of chronically hypoxic pigs, endothelin ET_A receptors in the pulmonary arteries were reduced (Gosselin et al., 1997; Noguchi et al., 1997). In contrast in a study in chronically hypoxic rats, increased levels of mRNA for endothelin ET_A receptors have been reported, however receptors protein levels were not determined (Li et al., 1994).

Alternatively, it is possible that increased levels of vasodilator mediators are being released from the pulmonary arteries and veins of chronically hypoxic rats, either under basal conditions or as a result of endothelin-1 application and that these are causing a physiological antagonism of the constrictor responses to endothelin-1. Recently we have shown that in chronically hypoxic lungs an inhibitor of cyclooxygenase, indomethacin, produced pulmonary vasoconstriction which suggests increased basal production of vasodilator prostanoids (Lal et al., 1999). Although in the present study the effects of indomethacin on endothelin-1 induced contractions were not studied, we have shown previously that indomethacin had no significant effect on endothelin-1 induced vasoconstriction in lungs from normal rats (Lal et al., 1995). Another possible mediator would be NO, the production of which has been reported to be increased in chronically hypoxic rats (Barer et al., 1993). When the NO synthase inhibitor L-NOARG was added to pulmonary arteries from chronically hypoxic animals it did cause a greater increase in tone than that seen in control pulmonary arteries, which is indicative of an increased basal level of NO in these chronically hypoxic vessels. This effect of NO synthase inhibition in pulmonary arteries is in agreement with the studies of Le Cras et al. (1996) and Resta et al. (1997). However, even in the presence of L-NOARG the maximal pulmonary artery response to endothelin-1 was still depressed when compared to the control preparations. Eddahibi et al. (1993) also reported a depressed endothelin-1 mediated, endothelial-dependent vasodilation in lungs from chronically hypoxic rats. This indicates that although the basal production of NO was increased in pulmonary arteries from chronically hypoxic animals it was not responsible for the depressed endothelin-1 constrictor response. The fact that constrictor responses to U46619 and phenylephrine were not reduced in pulmonary arteries of chronically hypoxic rats also argues against a role for an enhanced basal production of some endogenous vasodilator as being the mechanism underlying the reduced constrictor response to endothelin-1 in pulmonary arteries. When L-NOARG was added to pulmonary veins it produced a very small increase in tone which did not differ in control and chronically hypoxic vessels. This difference in the effect of L-NOARG on pulmonary arteries and veins indicates that there was a selective up-regulation of NO production in pulmonary arteries of chronically hypoxic rats compared with pulmonary veins. Despite these differences, the constrictor responses of endothelin-1 were depressed in both pulmonary arteries and veins of chronically hypoxic animals. This again argues against a generalised increase in some basal physiological antagonism which could account for the reduced endothelin-1-induced vasoconstrictor responses, and favours down-regulation of the endothelin ET_A receptors which are responsible for endothelin-1 induced contractions in large pulmonary arteries (MacLean et al., 1994) and pulmonary veins (Toga et al., 1992).

The selective endothelin ET_B receptor agonist sarafotoxin 6c (Williams et al., 1991) was also studied in pulmonary artery and pulmonary venous rings from control and chronically hypoxic animals. Unlike endothelin-1, sarafotoxin 6c caused a greater contraction of control pulmonary veins than pulmonary arteries. The selective endothelin ET_B receptor antagonist BQ788 (Ishikawa et al., 1994), completely blocked pulmonary venous responses to sarafotoxin 6c, suggesting the presence of endothelin ET_B receptors in these vessels. The poor response of large pulmonary artery rings to sarafotoxin 6c is in accord with the observations of Bialecki et al. (1998), but it contrasts with our studies in the perfused whole lung model where sarafotoxin 6c produced arterial vasoconstriction (Lal et al., 1995). However, this whole lung preparation also includes microvascular resistance arterioles. MacLean et al. (1994) have also reported that smaller pulmonary artery rings are more responsive to sarafotoxin 6c than large pulmonary artery rings. This comparison with our earlier study in the whole lung provides further evidence that endothelin receptors are differentially located along the pulmonary vascular bed.

In order to investigate if NO production, in control tissues, contributed to the smaller responses to sarafotoxin 6c in control pulmonary artery compared to pulmonary veins, sarafotoxin 6c-induced contractions were studied in the presence of L-NOARG. The results showed that L-NOARG had no effect on the responses to sarafotoxin 6c in pulmonary arteries and veins. This shows that the poor contractile response of pulmonary artery to sarafotoxin 6c is not due to physiological antagonism caused by NO production. Therefore, the most likely explanation for poor responsiveness is a lack of endothelin ET_B receptors on the large pulmonary artery smooth muscle cells.

In pulmonary arteries of chronically hypoxic rats the small sarafotoxin 6c-induced contractile responses were similar to those of control preparations. In contrast, in pulmonary veins from chronically hypoxic rats, the sarafotoxin 6c-induced contractions were augmented suggesting that endothelin ET_B smooth muscle receptors were upregulated in pulmonary veins from chronically hypoxic animals.

In order to see if chronic hypoxia affected the responsiveness of pulmonary arteries and pulmonary veins to other vasoconstrictor agents, the α_1-adrenoceptor agonist phenylephrine and the thromboxane A_2 receptor agonist U46619 were used. In control preparations phenylephrine produced contractions of pulmonary arteries but not pulmonary veins, indicating that functional α_1-adrenoceptors

are not present in pulmonary veins. This is consistent with our observations in the perfused rat lung, which also contains microvascular resistance arterioles, where phenylephrine only constricted the arterial side of the pulmonary circulation (Lal et al., 1994). At low concentrations, phenylephrine-induced responses in pulmonary arteries were significantly augmented following chronic hypoxia. This shows that unlike endothelin-1, pulmonary arterial responsiveness to α_1-adrenoceptor agonists is enhanced following chronic hypoxia. McMurtry et al. (1978) and MacLean and McCulloch (1998) have reported an enhanced pulmonary vasoconstrictor response to the α-adrenoceptor agonist norepinephrine in lungs from chronically hypoxic rats which supports our observations with phenylephrine.

In contrast to phenylephrine, the thromboxane receptor agonist U46619 contracted pulmonary arteries and veins from control animals. The maximal contraction produced in pulmonary arteries was greater than in pulmonary veins. In vessels from chronically hypoxic rats these maximal contractions to U46619 were not altered. This provides further evidence that chronic hypoxia can differentially alter the responsiveness of pulmonary vessels to vasoconstrictor agonists.

In order to see if vascular relaxation was altered in pulmonary arteries and pulmonary veins of chronically hypoxic animals, we examined the actions of vasorelaxant drugs with differing mechanisms of action. These tissues were pre-constricted with U46619 as its maximal constrictor action in pulmonary arteries and pulmonary veins was not affected by chronic hypoxia.

In pre-contracted preparations acetylcholine relaxed control pulmonary arteries, but not pulmonary veins. This suggests that the endothelial muscarinic receptors which are activated by acetylcholine are located primarily on the arterial side of the pulmonary circulation. This is consistent with a previous study in guinea pig pulmonary vessels where acetylcholine relaxed pulmonary arteries but not pulmonary veins (Shi et al., 1997). In pulmonary arteries from chronically hypoxic animals we observed that the acetylcholine-induced relaxation was enhanced. An enhanced vasodilator effect of acetylcholine in large pulmonary arteries from chronically hypoxic rats has also been reported by MacLean and McCulloch (1998), while a study by Brown et al. (1998), using a monocrotaline model of pulmonary hypertension, also showed an enhanced acetylcholine-induced relaxation of pulmonary arteries. These observations are in contrast to those seen in patients with hypoxic lung disease where the relaxant response to acetylcholine in pulmonary arteries was attenuated (Dinh-Xuan et al., 1991). However, it should be noted that Adnot et al. (1991) reported a reduced vasodilator response to acetylcholine when using a perfused whole lung preparation from chronically hypoxic rats.

The increased vasodilator response to acetylcholine seen in our study could be due to an increase in the number of muscarinic receptors, and this may be part of a more generalised response to chronic hypoxia, as muscarinic receptors are up-regulated in the heart following chronic hypoxia (Kacimi et al., 1993). An alternative explanation could be that in pulmonary arteries of chronically hypoxic rats, there is a greater capacity for the production of NO following muscarinic receptor activation. The fact that L-NOARG produced a greater increase in basal tone in pulmonary arteries from chronically hypoxic rats compared with control preparations and, the observations of Barer et al. (1993), who studied the effects of NO synthase inhibitors in chronically hypoxic rats using an in situ blood perfused lung preparation, gives support to this suggestion. In the present study L-NOARG significantly blocked acetylcholine-induced relaxations in pulmonary vessels from control and chronically hypoxic rats which again suggests that enhanced acetylcholine-induced relaxations in pulmonary vessels are due to an increased NO production. Russ and Walker (1993) reported that the relaxations of pulmonary arteries and pulmonary veins to endothelium-dependent or directly acting NO donors were similar in normoxic and hypoxic lungs, this shows that any change in response to acetylcholine must have been upstream of guanyl cyclase as this is the enzyme which is activated by NO. In view of these observations our suggestion of an increased muscarinic receptor number would seem to be the most likely explanation for the enhanced response to acetylcholine.

The calcium ionophore, ionomycin, relaxed pulmonary arteries and veins. However, unlike the relaxant effect of acetylcholine, the responses to ionomycin were reduced in pulmonary arteries and veins from chronically hypoxic rats. Using a perfused whole lung preparation, Adnot et al. (1991) also reported that vasodilator responses to the calcium ionophore, A23187, were attenuated in chronically hypoxic lungs. Ionomycin increases influx of calcium into the endothelium by a non-receptor mediated process, and in rabbit femoral artery it has been shown to induce relaxation primarily by an NO-independent pathway involving endothelium derived hyperpolarising factor (EDHF) (Plane et al., 1995). If ionomycin has the same mechanism of action in rat pulmonary arteries this could indicate that chronic hypoxia is in some way attenuating the production, or action, of EDHF. The fact that sodium nitroprusside was capable of causing a maximal relaxation of the pulmonary arteries in control and chronically hypoxic vessels shows that the reduced response to ionomycin was not due to a defect in smooth muscle relaxation.

In control vessels isoprenaline relaxed pulmonary veins more than pulmonary arteries. This suggests that, unlike α-adrenoceptors, which are present primarily on pulmonary arteries (see above), functional β-adrenoceptors are more prevalent on the venous side of the pulmonary vascular bed. In hypoxic preparations pulmonary artery relaxation to isoprenaline was enhanced, whereas in pul-

monary veins it was not. The enhanced vasorelaxation in pulmonary arteries seen in the present study is consistent with a previous study (Emery et al., 1981) and is probably due to an up-regulation of β-adrenergic receptors (Birnkrant et al., 1993).

In summary, the major novel finding of these experiments concerns the effect of chronic hypoxia on pulmonary veins. Control pulmonary veins relaxed in response to ionomycin, isoprenaline and sodium nitroprusside but they did not relax in response to acetylcholine. In pulmonary veins from chronically hypoxic animals the relaxant effect of acetylcholine was enhanced while the effects of ionomycin were attenuated. This indicates that chronic hypoxia may compromise EDHF production in pulmonary veins. We have also provided evidence that functional β-adrenoceptors are present on pulmonary veins while α-adrenoceptors are absent. The constrictor effects of endothelin-1 on pulmonary arteries and pulmonary veins were attenuated in vessels from chronically hypoxic rats while the constrictor effect of sarafotoxin 6c was enhanced in pulmonary veins from these animals. This shows that chronic hypoxia causes regional and agonist-selective changes in vascular reactivity in large pulmonary vessels.

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