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Influence of applied tension and nitric oxide on responses to endothelins in rat pulmonary resistance arteries: effect of chronic hypoxia

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1 The effect of basal tension (transmural tensions 235±29 mg wt (low tension: equivalent to ~16 mmHg) and 305±34 mg wt (high tension: equivalent to ~35 mmHg)) on rat pulmonary resistance artery responses to endothelin-1 (ET-1) and the selective ET_B-receptor agonist sarafotoxin S6c (S6c) were studied. The effects of nitric oxide synthase inhibition with N^{ω}-nitro-L-arginine methylester (L-NAME, 100 μM) on ET receptor-induced responses, as well as vasodilator responses to acetylcholine (ACh) and S6c, were also investigated. Changes with development of pulmonary hypertension, induced by two weeks of chronic hypoxia, were determined.

2 Control rat preparations showed greatest sensitivity for ET-1 when put under low tension (pEC_{50}: 8.1±0.1) compared with at the higher tension (pEC_{50}: 7.7±0.1) and there were significant increases in maximum contractile responses to S6c (~80%) and noradrenaline (~60%) when put under high tension.

3 In control pulmonary resistance arteries, both ET-1 and S6c produced potent vasoconstrictor responses. S6c was 12 fold more potent than ET-1 in vessels set at low tension (S6c pEC_{50}: 9.2±0.1) and 200 fold more potent than ET-1 when the vessels were set at high tension (S6c pEC_{50}: 9.0±0.1). Chronic hypoxia did not change the potencies of ET-1 and S6c but did significantly increase the maximum contractile response to ET-1 by 60% (at low tension) and 130% (at high tension).

4 In control rat vessels, L-NAME itself caused small increases in vascular tone (5–8 mg wt tension) in 33–56% of vessels. In the chronic hypoxic rats, in vessels set at high tension, L-NAME-induced tone was evident in 88% of vessels and had increased to 26.9±6.6 mg wt tension. Vasodilatation to sodium nitroprusside, in non-preconstricted vessels, was small in control rat vessels (2–6 mg wt tension) but increased significantly to 22.5±8.0 mg wt tension in chronic hypoxic vessels set at the higher tensions. Together, these results indicate an increase in endogenous tone in the vessels from the chronic hypoxic rats which is normally attenuated by nitric oxide production.

5 L-NAME increased the sensitivity to S6c 10 fold (low tension) and 6 fold (high tension) only in chronic hypoxic rat pulmonary resistance arteries. It had no effect on responses to ET-1 in any vessel studied.

6 Vasodilatation of pre-contracted vessels by ACh was markedly greater in the pulmonary resistance arteries from the chronic hypoxic rats (pIC_{50}: 7.12±0.19, maximum: 72.1±0.2.0%) compared to their age-matched controls (pIC_{50}: 5.77±0.15, maximum: 28.2±2.0%). There was also a 2.5 fold increase in maximum vasodilatation induced by ACh.

7 These results demonstrate that control rat preparations showed greatest sensitivity for ET-1 when set at the lower tension, equivalent to the pressure expected *in vivo* (~16 mmHg). Pulmonary hypertension due to chronic hypoxia potentiated the maximum response to ET-1. Pulmonary resistance arteries from control animals exhibited little endogenous tone, but exposure to chronic hypoxia increased endogenous inherent tone which is normally attenuated by nitric oxide. Endogenous nitric oxide production may increase in pulmonary resistance arteries from chronic hypoxic rats and attenuate contractile responses to ET_{B2} receptor stimulation. Relaxation to ACh was increased in pulmonary resistance arteries from chronic hypoxic rats.

Keywords: Pulmonary arteries; chronic hypoxia; basal tension; nitric oxide; endothelin

Introduction

The endothelins are a family of highly potent vasoconstrictor peptides (Inoue *et al*., 1989). At present two subtypes of endothelin (ET) receptor have been cloned and sequenced. The ET_A receptor shows selectivity for ET-1 over ET-3 and mediates vasoconstriction (Arai *et al*., 1990). The second receptor, ET_B, is non-isopeptide selective and mediates vasodilatation when present on the vascular endothelium (subsequently referred to as ET_{B1}) or contraction when present on vascular smooth muscle cells (ET_{B2}) (Sakurai *et al*., 1990; Masaki *et al*., 1991). Although at present there are no selective ET_A receptor agonists commercially available, several ET_B agonists have been identified including sarafotoxin S6c (S6c) (Williams *et al*., 1991). Due to their potent vasoconstrictor properties and their proliferative effect on vascular smooth muscle cells, the endothelins have been implicated in many pathophysiological conditions, including pulmonary hypertension (PHT). Elevated circulating ET-1 levels have been observed in patients with PHT, both primary and secondary

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forms (Stewart *et al*., 1991). It has been shown that pulmonary arterial responses to ET-1 are increased in the chronic hypoxic rat model of PHT, and that hypoxia enhances expression of the ET-1 gene in the rat lung (Elton *et al*., 1992; MacLean *et al*., 1995).

We have previously shown that the vasoconstrictor responses to endothelin in rat pulmonary resistance arteries are mediated via both ET_A and ET_{B2} receptors, whereas the responses to ET-1 in the large extrapulmonary arteries are mediated by the ET_A receptor alone (MacLean *et al*., 1994). In PHT, it is the pulmonary resistance arteries which contribute most to elevations in pulmonary vascular resistance and so, here, we investigated the vascular responses to ET-1 in isolated pulmonary resistance arteries from rats exposed to chronic hypoxia and their aged-matched controls.

As pulmonary resistance arteries from chronic hypoxic animals experience greater pressures *in vivo* due to their pulmonary hypertensive state, the role of initial resting tension on vascular responses to endothelin-receptor agonists was investigated. This has not previously been defined for pulmonary resistance arteries. In rat large pulmonary arteries, there is an increase in endogenous tone and this may be normally attenuated by NO production (MacLean *et al*., 1995; 1996). We have previously demonstrated that there is also an increase in endogenous vascular tone in pulmonary resistance arteries from pulmonary hypertensive rats (MacLean *et al*., 1996). We wished to investigate the influence of NO on this vascular tone and on responses to ET-1 and S6c in the pulmonary resistance artery. We did so by studying the effects of the NO synthase inhibitor N^{ω}-nitro-L-arginine methylester (L-NAME). There is some controversy as to whether endothelium-dependent vasodilatation of isolated perfused lungs are potentiated or blunted by chronic hypoxia (Adnot *et al*., 1991; Resta & Walker, 1996). Here, we examined acetylcholine (ACh)-induced vasodilatation in the isolated pulmonary resistance from the chronic hypoxic rat.

We presented preliminary data from this study at the 4th International Conference on Endothelin (McCulloch & MacLean, 1995).

Methods

Chronic hypoxic rat model of pulmonary hypertension

Pulmonary hypertensive rats were prepared according to MacLean *et al*. (1995). Briefly, male Wistar rats (specific pathogen free, Harlan UK Ltd, 28–30 days old at the start of the experiment, ~65 g) were placed in a hypobaric chamber. This was depressurized to 550mbar (oxygen concentration reduced to 10%) over two days. The temperature in the chamber was maintained at 21–22°C and the chamber was ventilated with air at approximately 45 l min^{-1}. Rats were maintained in these hypoxic, hypobaric conditions for two weeks and studied immediately after removal from the chamber. Age-matched controls were maintained in room air. PHT was assessed by measuring the ratio of right ventricular (RV)/total ventricular (TV) weight. The right ventricle was carefully dissected free from the septum and left ventricle and both were blotted lightly and weighed. This is a reliable index of the degree of PHT in rats (Hunter *et al*., 1974; Leach *et al*., 1977).

Isolated pulmonary resistance arteries

Rats were killed by an overdose of sodium pentobarbitone (60 mg kg^{-1}, i.p.) and the lungs carefully dissected out and placed in cold Krebs. Using a microscope, second order-intralobal resistance arteries (150–200 μm) were dissected out and cleaned of surrounding parenchyma. The vessels were taken from the same area of lung in both controls and pulmonary hypertensive animals. Control and pulmonary hypertensive vessel pairs were then mounted as ring preparations (~2 mm long) in the same bath of a Mulvany Halpern small vessel myograph. This was achieved by passing two stainless steel wires (40 μm diameter) through the lumen of the vessel and attaching the ends of the wire to mounting jaws (Mulvany & Halpern, 1977). The vessels were bathed in Krebs solution at 37°C and bubbled with 16%O_2/5% CO_2 balance N_2. This gave a final bath oxygen tension of approximately 120 mmHg and CO_2 tensions of around 35–36 mmHg to give values equivalent to alveolar and pulmonary arterial oxygen tensions. Gas tensions were measured with an oxygen electrode (Strathkelvin Instruments, U.K.) and verified by a blood gas analyser (Corning 166).

Basal tension

By use of the Laplace relationship, normotensive and hypertensive rodents vessels can be tensioned to simulate a required resting transmural pressure (Mulvany & Halpern, 1977). Vessels from both groups of animals were set up under two tensions. Low tension — equivalent to 16.6 ± 0.2 mmHg ($n = 54$) and high tension — equivalent to 33.4 ± 0.4 mmHg ($n = 54$), which is approximately the *in vivo* pulmonary artery pressure of control and pulmonary hypertensive animals, respectively (Herget *et al*., 1978). For simplicity these two equivalent transmural pressures will be referred to subsequently as 'low tension' and 'high tension'.

Experimental protocols

After 1 h equilibration vessels were stimulated with 50 mM KCl and following washout and return to resting tension, the integrity of the vascular endothelium was assessed by the ability of 1 μM ACh to cause relaxation after preconstriction with 1 μM noradrenaline.

Cumulative concentration-response curves (CCRCs) to ET-1 and S6c (0.01 pM–300 nM) were constructed with or without 100 μM N^{ω}-nitro-L-arginine methylester (L-NAME, preincubated for 30 min).

Experiments were also carried out to investigate the possible vasodilator effects of S6c. Preparations were first preconstricted with 10 μM 5-hydroxytryptamine (5-HT) and following this 1 μM ACh was added to verify the presence of an intact vascular endothelium. 5-HT was used as this induced a greater maximum contractile response than noradrenaline and, in preliminary studies, S6c failed to exhibit a vasodilator response when tone was raised with noradrenaline. Following washout and a 30 min rest period, vessels were again preconstricted with 10 μM 5-HT, and CCRCs to S6c (0.01 pM to 0.3 nM) were conducted, with 1 min intervals allowed between the addition of each concentration of S6c. To ensure that any vasodilatation observed was not due to time-related changes in tone, some preparations were run as time controls i.e. no addition of S6c. These experiments were carried out with control rat pulmonary resistance arteries at low tension and chronic hypoxic pulmonary resistance arteries at high tension only. In another group of rats, this protocol was repeated except that 0.01 nM to 100 μM ACh was administered in place of S6c to examine further endothelium-dependent responses.

To assess the endogenous tone present in the vessels 1 μM sodium nitroprusside (SNP) was administered, before the

addition of KCl or any drugs, and following washout the response to 50 mM KCl was tested.

Drugs and solutions

The composition of the Krebs-bicarbonate buffer (pH 7.4) was as follows: (in mM): NaCl 118.4, Na HCO_3 25, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5 and glucose 11. The following drugs were used: (-)-noradrenaline bitartrate, 5-HT, aceylcholine chloride, N^{ω}-nitro-L-arginine methylester, sodium nitroprusside and S6c (Sigma, Poole, Dorset, U.K.); endothelin-1 (BioMac Glasgow, U.K.). All drugs were dissolved in distilled water.

Data analysis

pEC_{50} values were calculated by computer interpolation from individual CCRCs. Due to financial restraints, the maximum concentration of ET-1 used was 0.3 μM. Calculation of pEC_{50} values assumes that responses to ET-1 are maximal. Previous preliminary studies have demonstrated that 1 μM ET-1 does not induce significantly greater vasoconstriction than 0.3 μM and hence we justify using 0.3 μM as a maximum concentration for the calculation of pEC_{50} values. Vasodilatation induced by ACh was calculated and, for each concentration, shown as the percentage of the level of preconstriction to 5-HT remaining in each preparation. Data are presented as mean of n = number of rats.

Statistical comparisons of the means of groups of data were made by one-way analysis of variance (ANOVA) with a Tukey post test for multiple comparisons; $P<0.05$ was considered statistically significant.

Results

Assessment of pulmonary hypertension

Pulmonary hypertension was assessed by measuring the right ventricular to total ventricular ratio. We have previously published data on responses to ET-1 in large pulmonary arteries removed from the same cohort of control and chronic hypoxic rats used in the present study and described the heart and body weight changes in these rats in some detail (MacLean *et al.*, 1995). The right ventricular to total ventricular ratio was greater in the CH rats (0.392 ± 0.008 vs 0.246 ± 0.006, $n=12$; $P<0.001$) indicating a significant degree of pulmonary hypertension.

ET-1 and S6c responses and the effect of basal tension

Control pulmonary resistance arteries KCl 50 mM-induced contractions were of the same magnitude at low and high tensions in control rats, being 235 ± 29 mg wt ($n=16$) and 305 ± 34 mg wt ($n=16$), respectively. All pEC_{50} values are summarized in Table 1. S6c was significantly more potent than ET-1 and an increase in the resting tension in control vessels caused a significant decrease in the tissue sensitivity to ET-1 (Table 1, Figure 1). The maximum contraction achieved to ET-1 was unaffected by changes in resting tension (Figure 1). S6c produced maximum contractions of around 60% of the maximum to ET-1 in control rat vessels set at the low tension ($P<0.05$, S6c vs ET-1). The potency of S6c was not significantly altered by changes in tension but there was a significant increase ($\sim100\%$, $P<0.05$) in maximum contraction to S6c in vessels set at high tension (Table 1, Figure 1).

Chronic hypoxic pulmonary resistance arteries KCl 50 mM-induced contractions were of the same magnitude at low and high tensions in chronic hypoxic rats, being 246 ± 30 mg wt

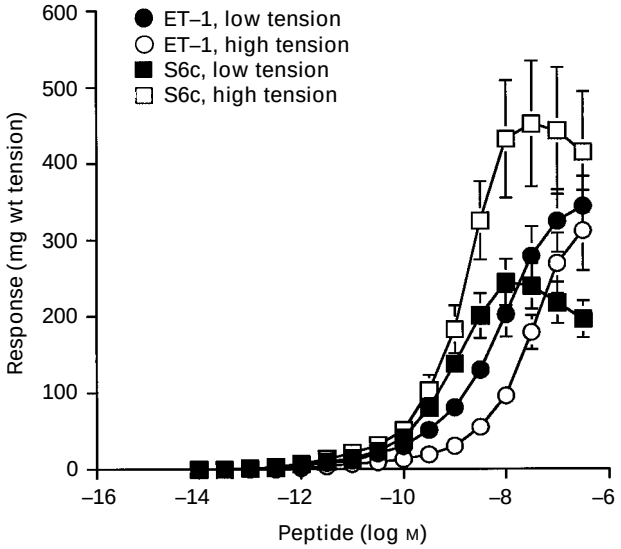

Figure 1 Endothelin-1 (ET-1) and Sarafotoxin S6c (S6c) - induced vasoconstriction in control rat pulmonary resistance arteries. Cumulative concentration-response curves for ET-1 in control rate pulmonary resistance arteries at: low tension ($n=8$), high tension ($n=6$). Cumulative concentration-response curves for S6c in control animals at: low tension ($n=7$), and high tension ($n=5$). Data are expressed as absolute response to ET-1 in mg wt tension. Each point represents the mean and vertical lines show s.e.mean.

Table 1 pEC_{50} values for endothelin-1 and sarafotoxin S6c in control and pulmonary hypertensive rat pulmonary resistance arteries: effect of pretreatment with 100 μM L-NAME

	Low tension (~16 mmHg)				*High tension* (~35 mmHg)			
	Control		*Chronic hypoxic*		*Control*		*Chronic hypoxic*	
Group	pEC_{50}	n	pEC_{50}	n	pEC_{50}	n	pEC_{50}	n
ET-1 control	8.1 ± 0.1	8	8.1 ± 0.1	6	7.7 ± 0.1^{a}	6	8.1 ± 0.2	6
S6c	9.2 ± 0.1***	7	8.9 ± 0.1*	6	9.0 ± 0.1***	5	9.3 ± 0.1***	7
ET-1 + L-NAME	8.1 ± 0.1	7	8.3 ± 0.1	7	8.1 ± 0.1^{b}	6	8.6 ± 0.1	7
S6c + L-NAME	9.1 ± 0.2	6	10.1 ± 0.3^{c}	6	9.1 ± 0.1	6	9.9 ± 0.2^{d}	6

ET-1, endothelin-1; S6c, sarafotoxin S6c; L-NAME, N^{ω}-nitro-L-arginine methylester. *n*, number of animals. Statistical comparisons were made by one way analysis of variance (ANOVA). Values are mean $\pm$ s.e.mean. *$P<0.05$; ***$P<0.001$ S6c vs ET-1; [a]$P<0.05$, ET-1 control low vs control high; [b]$P<0.05$ ET-1 control high vs control high + L-NAME; [c]$P<0.001$ S6c chronic hypoxic low vs chronic hypoxic low + L-NAME; [d]$P<0.05$ S6c chronic hypoxic high vs chronic hypoxic high + L-NAME.

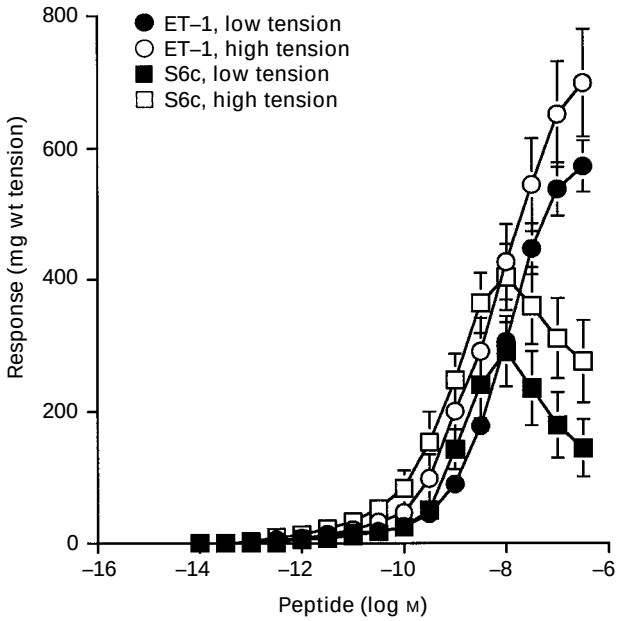

Figure 2 Endothelin-1 (ET-1) and sarafotoxin S6c (S6c) - induced vasoconstriction in chronic hypoxic rat pulmonary resistance arteries. Cumulative concentration-response curves for ET-1 in control rat pulmonary resistance arteries at: low tension ($n=6$), high tension ($n=6$). Cumulative concentration-response curves for S6c in control animals at: low tension ($n=6$), and high tension ($n=7$). Data are expressed as absolute response to ET-1 in mg wt tension. Each point represents the mean and vertical lines show s.e.mean.

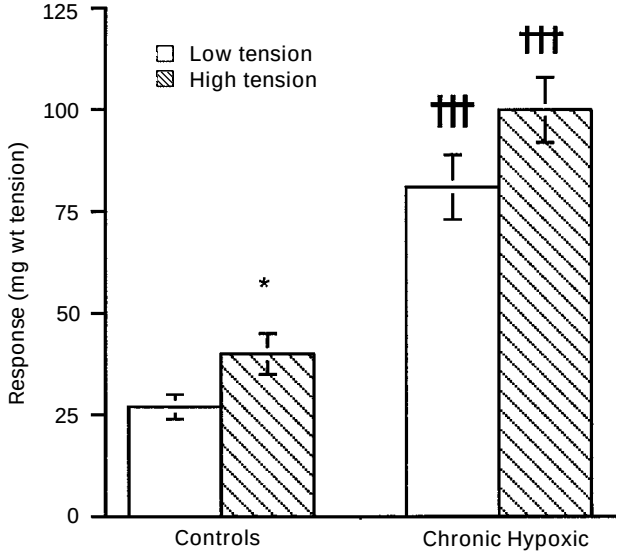

Figure 3 Maximum contraction to 1 μM noradrenaline in control and chronic hypoxic rat pulmonary resistance arteries. Data from rat pulmonary resistance arteries at low tension (control, $n=15$; chronic hypoxic, $n=15$) and high tension (control, $n=15$; chronic hypoxic, $n=15$) are shown. Data are expressed as the absolute contractile response to noradrenaline in mg wt tension. Each column represents the mean $\pm$ s.e.mean. Statistical comparisons were made by one way analysis of variance (ANOVA). High vs low tension, $^*P<0.05$.; control vs chronic hypoxic $\dagger\dagger\dagger P<0.001$.

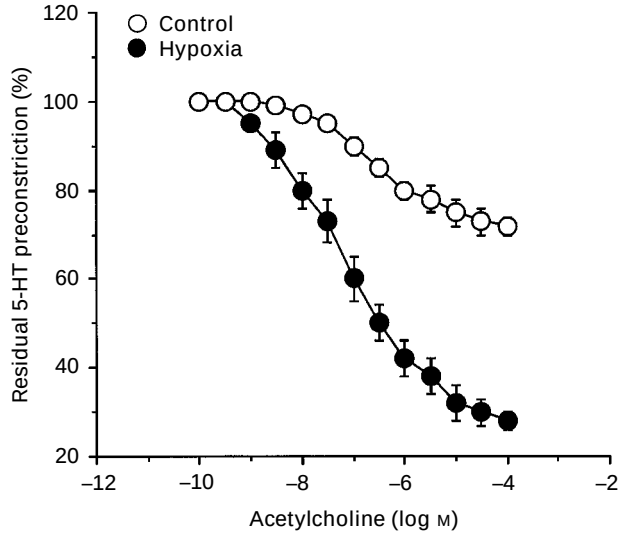

Figure 4 Vasodilatation induced by acetylcholine in pulmonary resistance arteries preconstricted with 10 μM 5-HT in control rats ($n=10$), and chronic hypoxic rats ($n=10$). Data are expressed as a percentage of the contraction to 10 μM 5-HT remaining after acetylcholine administration. Each point represents the mean and vertical lines show s.e.mean.

($n=16$) and 264 ± 33 mg wt ($n=16$), respectively; and did not differ from control rat responses to 50 mM KCl. Figure 2 shows CCRCs for ET-1 and S6c in chronic hypoxic pulmonary resistance arteries. pEC_{50} values are summarized in Table 1. S6c was more potent than ET-1 and an increase in the resting tension had no effect on the potency of ET-1 (Figure 2, Table 1). The potency of ET-1 was not significantly altered in the chronic hypoxic pulmonary resistance arteries compared with controls (Table 1). Maximum ET-1-induced contractions were significantly increased in chronic hypoxic vessels at both low and high tensions when compared with vessels from control age-matched controls (low tension: 343 ± 39.3 mg wt tension (control) vs 572.5 ± 58.8 (chronic hypoxic, $P<0.05$); high tension: 312 ± 52.5 mg wt tension (controls) vs 692 ± 81.5 (chronic hypoxic, $P<0.01$), Figures 1 and 2). S6c produced maximum contractions of around 50–60% of the maximum to ET-1 at both high and low tensions ($P<0.05$, S6c vs ET-1, Figure 2). S6c was equipotent in control and chronic hypoxic pulmonary resistance arteries at low and high tensions (Table 1). Maximum contractions to S6c in chronic hypoxic vessels were not significantly different from those seen in control vessels at either high or low tensions (low tension: 244.4 ± 30.6 mg wt tension (control) vs 291 ± 53.5 (chronic hypoxic); high tension: 452 ± 82.6 mg wt tension (controls) vs 403.7 ± 50.3 (chronic hypoxic), Figures 1 and 2).

Integrity of the vascular endothelium

Control and chronic hypoxic pulmonary resistance arteries preconstricted with 1 μM noradrenaline relaxed to 1 μM acetylcholine (ACh) at both low and high tensions. The maximum response to noradrenaline was significantly increased by chronic hypoxia (Figure 3). However, noradrenaline (1 μM) evoked small and poorly maintained contractions, especially in the control rat vessels. Further analysis of ACh-induced vasodilatation was examined in vessels preconstricted with 10 μM 5-HT which produced a well maintained contraction. Responses to ACh were increased by chronic hypoxia (Figure 4). The pIC_{50} for ACh in the control rat vessels was 5.77 ± 0.15 ($n=10$) and in the chronic hypoxic rat vessels it was 7.12 ± 0.19 ($n=10$, $P<0.001$ vs controls). The overall maximum relaxation was also greater in chronic hypoxic rat (Figure 4, $P<0.001$). However, 5-HT-induced tone was greater in the chronic hypoxic preparations being

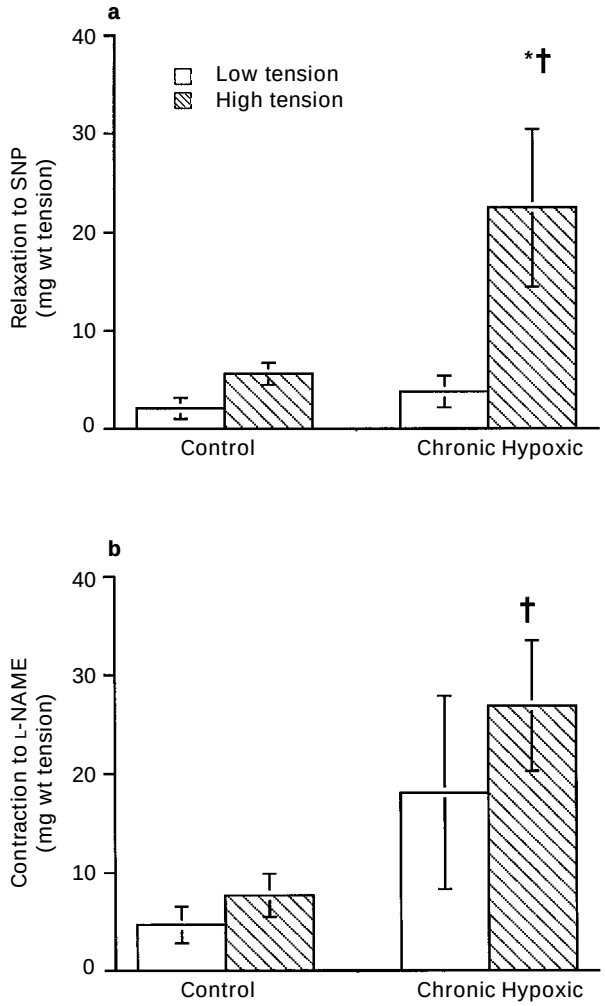

Figure 5 (a) Vasodilatation to 1 μM sodium nitroprusside (SNP) in control and chronic hypoxic rat pulmonary resistance arteries. Data from rat pulmonary resistance arteries at low tension (control $n=8$; chronic hypoxic, $n=6$) and high tension (control, $n=6$; chronic hypoxic, $n=8$) are shown. Data are expressed as the absolute vasodilatation to sodium nitroprusside in mg wt tension. (b) Effect of 100 μM L-NAME on vascular tone. Data from rat pulmonary resistance arteries at low tension (control, $n=8$; chronic hypoxic, $n=8$) and high tension (control, $n=8$; chronic hypoxic, $n=8$) are shown. Data are expressed as the absolute contraction to L-NAME in mg wt tension. Each column represents the mean $\pm$ s.e.mean. Statistical comparisons were made using one way analysis of variance (ANOVA) high vs low tension $^*P<0.05$; control vs chronic hypoxic $\dagger P<0.05$.

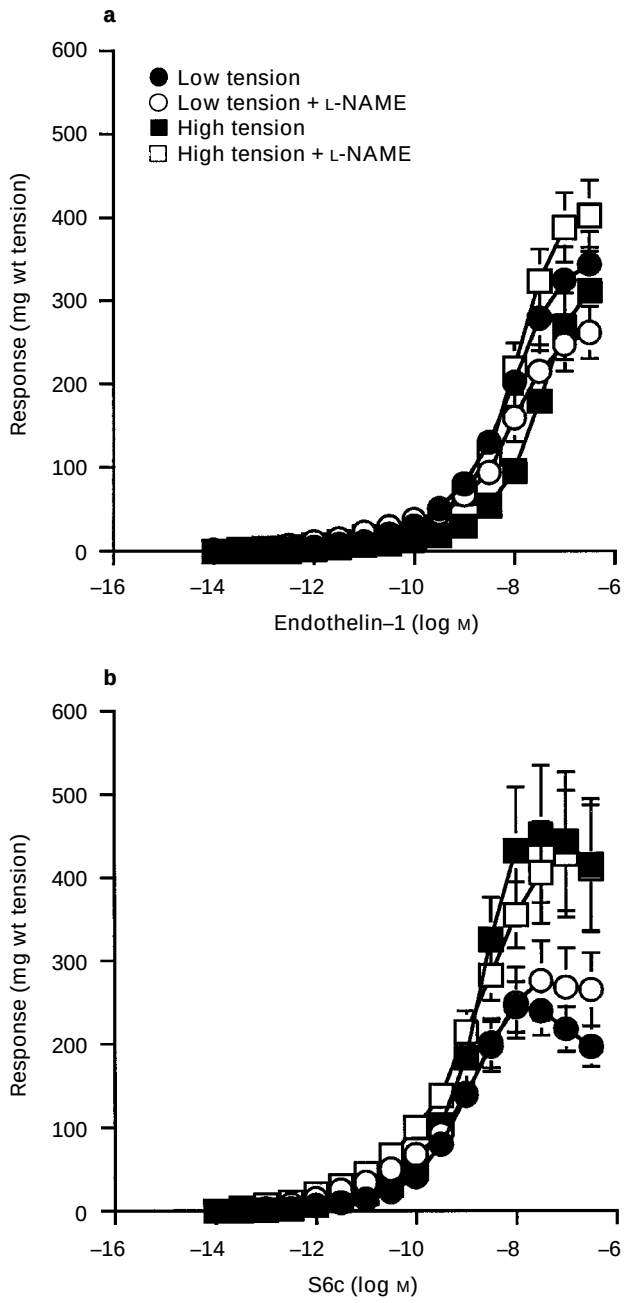

Figure 6 Endothelin-1 (ET-1)- and sarafotoxin S6c (S6c)-induced vasoconstriction in control rat pulmonary resistance arteries: effect of 100 μM L-NAME. (a) Cumulative concentration-response curves to ET-1 in control rat pulmonary resistance arteries at low tensions ($n=8$), control at low tension + L-NAME ($n=7$), control rat pulmonary resistance arteries at high tension ($n=6$) and control at high tension + L-NAME ($n=6$). Data are expressed as the absolute response to ET-1 in mg wt tension. (b) Cumulative concentration-response curves to S6c in control rat pulmonary resistance arteries at low tension ($n=7$), control at low tension + L-NAME ($n=6$), control rat pulmonary resistance arteries at high tension ($n=5$) and control rat pulmonary resistance arteries at high tension + L-NAME ($n=6$). Data are expressed as the absolute response to S6c in mg wt tension. Each point represents the mean and vertical lines show s.e.mean.

34.5 $\pm$ 3.2% of 50 mM KCl contraction in controls and 87.6 $\pm$ 5.2% in the chronic hypoxic rat vessels ($P<0.01$).

Assessment of endogenous tone in pulmonary resistance arteries

The percentage of vessels that relaxed to SNP (1 μM) in each group were: control vessels/low tension: 29%; control vessels/high tension: 88%; chronic hypoxic vessels/low tension: 75%; chronic hypoxic vessels/high tension: 93%. Results shown in Figure 5a are from those preparations which did relax to SNP. There was a significant increase in SNP-mediated relaxation in the vessels from the chronic hypoxic animals, set up at the higher tension (Figure 5a). As these vessels were not preconstricted, this indicates an increase in endogenous tone.

The effect of L-NAME (100 μM)

The percentage of vessels contracting in each group were: control vessels/low tension: 33%; control vessels/high tension: 53%; chronic hypoxic vessels/low tension: 56%; chronic hypoxic vessels/high tension: 88%. Results shown in Figure

5b are from those preparations which did respond to L-NAME. There was a significant increase in L-NAME-mediated vasoconstriction in the vessels from the chronic hypoxic animals, set up at the higher tension (Figure 5b). This indicates that the increased endogenous tone in these vessels is normally opposed by NO release. L-NAME had no significant effect on tissue sensitivity or the maximum contraction to ET-1 in control vessels set at low or high tension (Figure 6a, Table 1). Likewise, L-NAME had no significant effect on tissue sensitivity or maximum contraction to S6c in control vessels at low or high tension (Figure 6b, Table 1).

In vessels removed from chronic hypoxic rats, tissue sensitivity and maximum contraction to ET-1 at low or high tension was not significantly affected by L-NAME (Figure 7a, Table 1). However, L-NAME did significantly increase the sensitivity of chronic hypoxic pulmonary resistance vessels to S6c at both low and high tensions (Table 1). This increase in sensitivity occurred without an alteration in the observed maximum contraction to S6c in these preparations (Figure 7b).

Vasodilator role of sarafotoxin S6c

No vasodilatation to S6c was observed in preconstricted vessels from control rats, but significant vasoconstrictor responses were observed from 0.1 pM onwards, compared with time controls (Figure 8a). Likewise, in preconstricted vessels from chronic hypoxic rats only vasoconstrictor responses were observed from 10 pM onwards. All vessels studied were shown to have intact vascular endothelium in that ACh-induced contractions were evident.

Discussion

The results from this study confirm the results of earlier studies, with older rats, that ET_{B2}-like receptors mediate vasoconstriction in rat pulmonary resistance arteries in that sensitivity to S6c is greater than that to ET-1 (MacLean *et al*., 1994). This makes the rat an appropriate model for study as we have shown that ET_{B2}-like receptors partially mediate responses to ET-1 in human pulmonary resistance arteries (McCulloch *et al*., 1996). This contrasts with rat and human large pulmonary arteries, where the ET_A receptor predominates (MacLean *et al*., 1994; Fukuroda *et al*., 1994). We have recently demonstrated that ET-1-mediated contraction in rat pulmonary resistance arteries is not competitively inhibited by the selective ET_A-receptor antagonists FR139317 and BMS 182874, neither is it inhibited by the selective ET_B-receptor antagonist BQ788 or a combination of BMS 182874 and BQ788. It is inhibited by the mixed ET_A-/ET_B-receptor antagonist SB209670, as are responses to ET-1 in human pulmonary resistance arteries (McCulloch *et al*., 1996; 1998). A similar profile has been described in rabbit and guinea-pig bronchus (Hay *et al*., 1996; Hay & Luttmann, 1997). These studies suggest the presence of an atypical ET_{B2}-like receptor in pulmonary arteries and bronchi.

We consistently observed a biphasic CCRC to S6c, with responses decreasing in magnitude after reaching a maximum around 0.1 nM. This was not due to NO production as this component of the curve persisted in the presence of L-NAME. We can only speculate that the decrease in the response to S6c was due to desensitization. Indeed, precontracted vessels do not relax to single-dose administration of S6c at concentrations greater than 0.1 nM (unpublished observations).

In the present study, a major aim was to assess the influence of applied basal tension on responses to ET-1 and S6c in both control and pulmonary hypertensive, chronic hypoxic rats. We chose tensions equivalent to ~16 mmHg and ~35 mmHg, as these are the pressures expected *in vivo* in control and chronic hypoxic rats, respectively (Herget *et al*., 1978). In control rat pulmonary resistance arteries, ET-1 was significantly less potent when vessels were placed under the higher resting

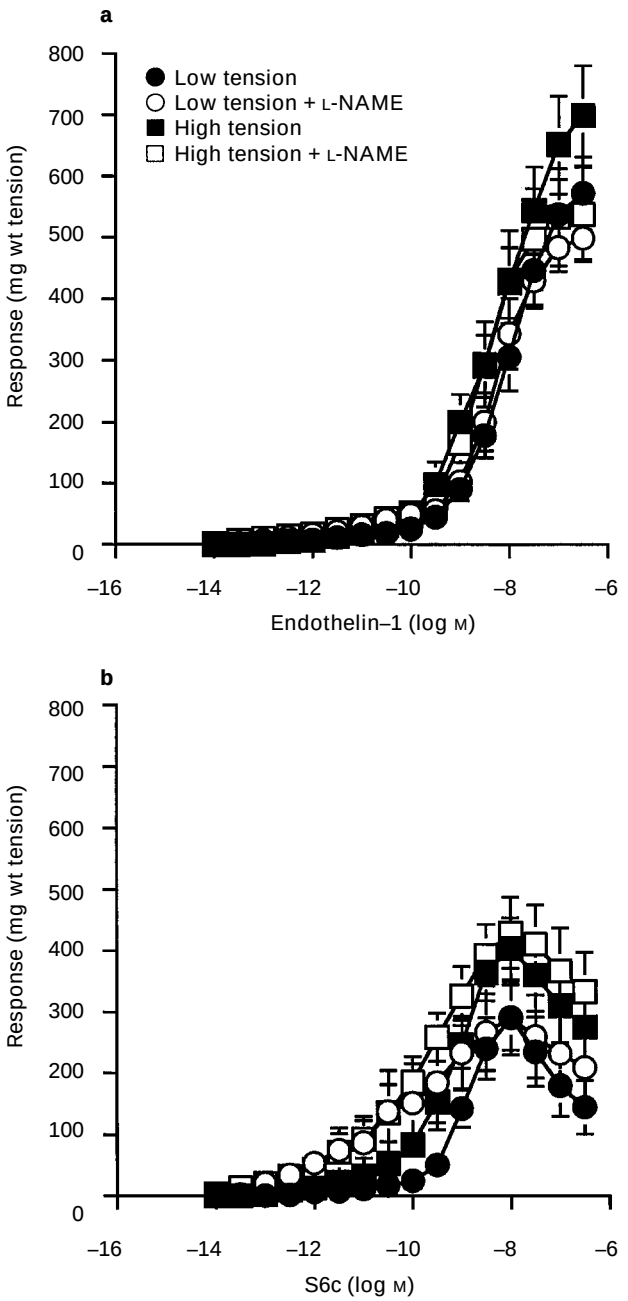

Figure 7 Endothelin-1 (ET-1) and sarafotoxin S6c (S6c) induced vasoconstriction in chronic hypoxic rat pulmonary resistance arteries: effect of 100 μM L-NAME. (a) Cumulative concentration-response curves to ET-1 in chronic hypoxic rat pulmonary resistance arteries at low tension ($n=6$), and chronic hypoxic at low tension + L-NAME ($n=7$), chronic hypoxic pulmonary resistance arteries at high tension ($n=6$) and chronic hypoxic at high tension + L-NAME ($n=7$). Data are expressed as the absolute response to ET-1 in mg wt tension. (b) Cumulative concentration-response curves to S6c in chronic hypoxic rat pulmonary resistance arteries at low tension ($n=6$) and chronic hypoxic at low tension + L-NAME ($n=6$), chronic hypoxic pulmonary resistance arteries at high tension ($n=7$) and chronic hypoxic at high tension + L-NAME ($n=6$). Data are expressed as the absolute response to S6c in mg wt tension. Each point represents the mean and vertical lines show s.e.mean.

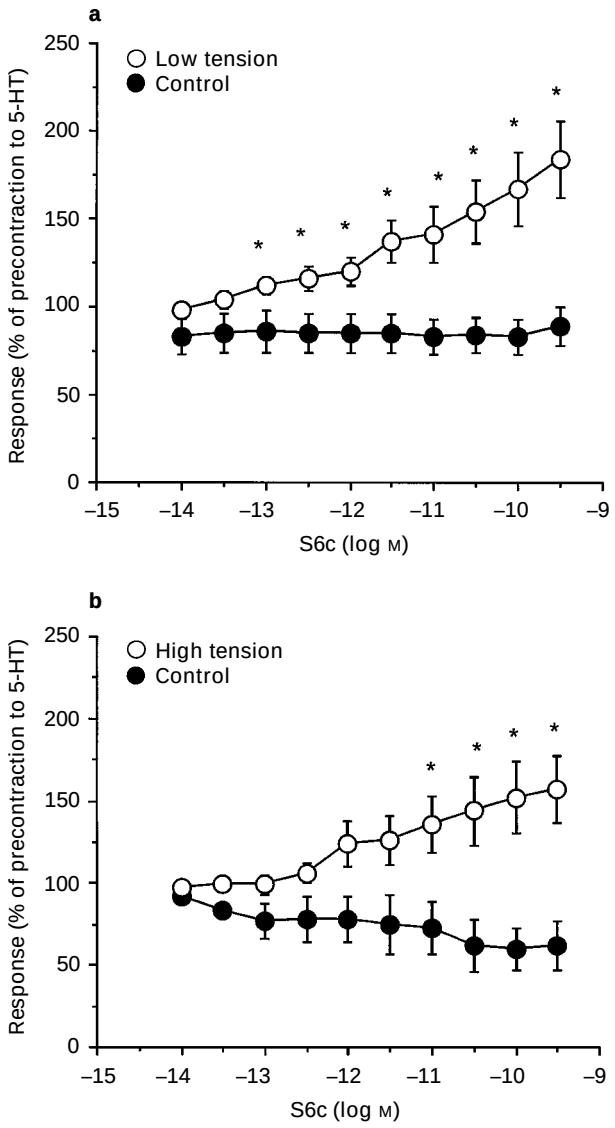

Figure 8 Responses to sarafotoxin S6c (S6c) in presence of raised tone induced by 5-hydroxy-tryptamine (5-HT). (a) Time controls for control rat pulmonary resistance arteries at low tension ($n=3$) and cumulative concentration-response curves to S6c in control pulmonary resistance arteries ($n=6$). (b). Time controls for chronic hypoxic rat pulmonary resistance arteries at high tension ($n=3$) and cumulative concentration-response curves to S6c in chronic hypoxic pulmonary resistance arteries ($n=5$). Data are expressed as a percentage of the contraction induced by 10 μM 5-HT. Each point represents the mean and vertical lines show s.e.mean. Statistical comparisons were made by one way analysis of variance (ANOVA). Time control vs S6c, $^*P<0.05$.

tension. This may have been due to over-stretching of the smooth muscle layers causing damage. However, this seems unlikely as responses to S6c were not significantly decreased in sensitivity under the same conditions and maximum responses to ET-1, noradrenaline and KCl were not decreased. The decreased sensitivity to ET-1 at high tension was not observed in vessels pretreated with L-NAME. This suggests that NO release, in response to the increased vessel stretch at high tensions, may be responsible for the decrease in sensitivity to ET-1. Indeed, shear stress and stretch have been shown to stimulate NO production from endothelial cells (Tesfamariam & Cohen, 1988; Hynes *et al*., 1995). Sensitivity to S6c in control vessels was unaffected by setting up at the higher tension, whilst the maximum contraction achieved to S6c was significantly increased. This may be a length-tension phenomenon, as it has been previously demonstrated that the maximum developed tension to 75 mM potassium in adult rat pulmonary resistance arteries was achieved when vessels were stretched to an equivalent transmural pressure of 30 mmHg (Leach *et al*., 1992). However as this pressure is approaching the values seen in pulmonary hypertensive states, it is clearly an inappropriate pressure at which to be setting control vessels. Rogers *et al*. (1992) have also shown that responses to different contractile agonists appear to show different length-tension relationships when set up at equivalent transmural pressures of 17 mmHg and 35 mmHg.

In chronic hypoxic animals, resting tension had no effect on the potency of ET-1. A change in resting tension did not have a significant effect on the potency, or maximum contraction, to S6c. Our results suggest that the major effect of an increase in tension was to cause a small, 2.5 fold, increase in the potency of ET-1. Chronic hypoxia itself increased the maximum response to ET-1, although it did not affect ET-1 potency. Chronic hypoxia is known to cause pulmonary vascular remodelling, in which small pulmonary resistance arteries become muscularised (Hunter *et al*., 1974). If it were simply the case that all vasoconstrictor responses were increased due to pulmonary vascular remodelling, then we would have seen an increase in the maximum contraction to all vasoconstrictors tested. It could be argued that we do not see a significant increase in the S6c response due to receptor desensitization. However, we have looked at the absolute contractions to S6c at each concentration step before desensitization, and there was no significant difference between control and chronic hypoxic pulmonary resistance arteries at any of these points. In addition, maximal responses to KCl were not increased. The increase in the maximum response to ET-1 could be due to an increase in ET_A-receptor activation. Indeed, we have shown that this increase is inhibited by the ET_A antagonist FR139317 (McCulloch *et al*., 1998). We have previously examined the effect of chronic hypoxia on contractile responses to ET-1 and S6c in the main pulmonary artery and pulmonary artery branches of the rat. We demonstrated an increased sensitivity to ET-1 in the pulmonary artery branch, and an uncovering of ET_{B2}-mediated responses in both the main pulmonary artery and the pulmonary artery branches (MacLean *et al*., 1995). Hence, not only do ET-receptor subtypes vary with the size or location of the pulmonary artery, but the effect of pulmonary hypertension, due to chronic hypoxic exposure, also varies with the vessel type studied.

We investigated the effect of ACh-induced vasodilatation in 5-HT preconstricted vessels. ACh was more potent in the vessels from the chronic hypoxic rats and produced a considerably greater maximum vasodilatation. Endothelium-dependent vasodilatation to arginine vasopressin has also been shown to increase in the isolated-perfused lung from chronic hypoxic rats (Eichinger & Walker, 1994; Resta & Walker, 1996). This was not associated with an increase in vasodilatation induced by NO donors and was mediated by NO and not endothelium-derived hyperpolarizing factor (EDHF) (Resta & Walker, 1996; Resta *et al*., 1997). Collectively, these results are compatible with studies that indicate *de novo* eNOS production in the small pulmonary arteries from chronic hypoxic rats (Xue *et al*., 1994; Le Cras *et al*., 1996). Gambone *et al*. (1997) have identified a component of the vasodilator response to ACh in canine pulmonary arteries which may be mediated EDHF. However, ACh-induced vasodilatation of human and rodent pulmonary arteries is thought to be mediated by NO (Norel *et al*., 1996; Kysela & Torok, 1996). However, the role of EDHF

may well be increased in pulmonary hypertensive states (Garland *et al*., 1995).

Administration of L-NAME produced increases in vascular tone in some, but not all, vascular preparations. A higher percentage of vessels from controls and chronic hypoxic rats contracted to L-NAME if set at the higher tension. The percentage of preparations which contracted was greatest in the chronic hypoxic rat vessels set at high tension and the magnitude of the response was also significantly increased in these vessels. Above, we suggested that stretching the control vessels to higher tension may cause increased release of NO. The results suggest that either artificially applied tension or inherent tone induced by PHT may increase basal NO production. However, the observation that L-NAME itself caused greater contraction in chronic hypoxic rat pulmonary resistance arteries set at the high tension suggests that there may be a greater degree of inherent tone in pulmonary hypertensive vessels, which is normally attenuated by NO. We investigated this possibility by examining the ability of SNP to reduce the tone of vessels which were not preconstricted and had not previously been treated with any drug. Vasodilatation to SNP was infrequent in control preparations at low and high tension, increasing in frequency in chronic hypoxic vessels, and was significantly greater in chronic hypoxic vessels at high tension. Collectively, the results suggest that, at the transmural pressure pulmonary resistance arteries experience *in vivo*, chronic hypoxia causes an increase in inherent tone which is normally opposed by NO. This conclusion is supported by the work of Barer *et al*. (1993), who demonstrated that L-NAME induced an increased pulmonary pressure in isolated perfused lungs from chronic hypoxic but not control rats. However, control preparations would show an increase in perfusion pressure if the pulmonary circulation was in a pre-contracted state. This suggests that tonic vasoconstriction could be the stimulus for increased release of basal NO. There is also an increase in endogenous tone in large calibre pulmonary arteries which is normally attenuated by NO (MacLean *et al*., 1995; Wanstall *et al*., 1995).

L-NAME had no effect on responses to ET-1 in control preparations at either tension. L-NAME had no significant effect on ET-1 responses at either tension in chronic hypoxic preparations. However, there was a significant increase in the potency of S6c. This indicates that NO may normally suppress responses to S6c, and therefore ET_{B2}-like receptors, in chronic hypoxic pulmonary resistance arteries. This provides further support for the suggestion that there is increased NO production in the chronic hypoxic vessels. An alternative possibility is that there may be endothelial ET_{B1}-receptors releasing NO, and that pretreatment with L-NAME removes this inhibition and subsequently increases the sensitivity to S6c. However, we were unable to demonstrate any vasodilatation to S6c in preconstricted pulmonary resistance arteries from control or chronic hypoxic rat lungs, although it may be that the endothelial ET_{B1} receptors comprise only a small percentage of the total ET_B-receptor population and that contractile ET_{B2} receptors overwhelm their vasodilator activity.

The differential effect of L-NAME on the potencies of S6c and ET-1 has also been observed in rabbit pulmonary resistance arteries (Docherty & MacLean, 1996; 1998). This contributes to the growing evidence that there are subtypes of ET_{B2} receptors which are not only differentially influenced by receptor antagonists but also by NO (Hay *et al*., 1996; McCulloch *et al*., 1996; Hay & Luttman, 1997).

In conclusion, the potency of ET-1 in pulmonary resistance arteries from control rats is greatest when vessels are set at their equivalent transmural pressure *in vivo*. Maximum contractile responses to ET-1 are increased by chronic hypoxia which also increases the role of endogenous NO on vascular reactivity. Endogenous NO may suppress responses to ET_{B2}-like receptors stimulated by S6c in pulmonary resistance arteries, whilst having no effect on responses to ET-1. Isolated pulmonary resistance arteries from chronic hypoxic rats exhibit inherent tone and this is normally attenuated by NO production. ACh-induced vasodilatation is increased in pulmonary resistance arteries from chronic hypoxic rats.

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