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Influences of the endothelium and hypoxia on α_1- and α_2-adrenoceptor-mediated responses in the rabbit isolated pulmonary artery

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1 The effects of the inhibitor of nitric oxide synthase, N^{ω}-nitro-L-arginine methylester (L-NAME, 10^{-4} M), mechanical disruption of the endothelium and hypoxia on contraction to noradrenaline (α_1- and α_2-adrenoceptor agonist), phenylephrine (α_1-adrenoceptor agonist) and UK 14304 (α_2-adrenoceptor agonist) were compared in the rabbit isolated pulmonary artery. The effects of the selective antagonists rauwolscine (10^{-6} M, α_2-adrenoceptors) and prazosin (10^{-7} M, α_1-adrenoceptors) on the contractions to noradrenaline before and after exposure to L-NAME were also assessed.

2 Noradrenaline, phenylephrine and UK 14304 all produced concentration-dependent increases in vascular tone. The responses to noradrenaline were sensitive to both rauwolscine and prazosin (effect of prazosin >> rauwolscine). L-NAME increased the potency of both noradrenaline and UK 14304, and also the maximum tension achieved. It had no effect on the responses to phenylephrine. After L-NAME, contractions to noradrenaline, although still sensitivie to both rauwolscine and prazosin, were now more sensitive to inhibition by rauwolscine.

3 Endothelium removal augmented the potency and maximum contractions to noradrenaline, phenylephrine and UK 14304.

4 Hypoxia decreased both the potency of phenylephrine and its maximum contractile response, but increased the maximum response to noradrenaline without effecting responses to UK 14304.

5 In conclusion, in the rabbit pulmonary artery, augmentation of contractile responses to noradrenaline by L-NAME involves a potentiation of α_2-adrenoceptor-mediated contraction probably through an effect on the synthesis of endothelium-derived nitric oxide. Experimental hypoxia had differential effects on all three agonists and did not mimic the effect of nitric oxide synthase inhibition.

Keywords: Pulmonary artery; nitric oxide; adrenoceptors; N^{ω}-nitro-L-arginine methylester; hypoxia

Introduction

A decrease in oxygen tension induces a vasodilatation in most systemic arteries but induces a vasoconstriction in the pulmonary arterial bed (Staub, 1985). Hypoxia-induced pulmonary vasoconstriction (HPV) aids in the regulation of ventilation-perfusion matching in normal subjects, in that it selectively re-distributes blood towards better ventilated alveoli (Dawson, 1984). However, this reflex also contributes to the pulmonary hypertension occurring in patients with hypoxia-related pulmonary abnormalities such as chronic obstructive pulmonary disease, cystic fibrosis, chronic bronchitus and emphysema (Rubin, 1984).

In 1984, Holden & McCall demonstrated, using porcine pulmonary arteries, that disruption of the vascular endothelium inhibited hypoxia-induced contractions. HPV is, therefore thought to be an endothelium-dependent phenomenon. It has been known for some time that removal of the vascular endothelium can potentiate responses to exogenously applied noradrenaline (Cocks & Angus, 1983) and other α_1- and α_2-adrenoceptor agonists (Egleme *et al.*, 1984; Lues & Schumann, 1984). Nitric oxide (or a closely related compound) has been identified as the major endothelium-derived relaxing factor (Ignarro *et al.*, 1987; Palmer *et al.*, 1988; Moncada *et al.*, 1988; 1989). We have recently demonstrated that inhibition of the synthesis of nitric oxide with the nitric oxide synthase inhibitor, N^{ω}-nitro-L-arginine methylester (L-NAME) potentiated neurogenic responses in the rabbit isolated pulmonary artery, and that this involved α_2-adrenoceptors (MacLean *et al.*, 1993). We have also demonstrated, in a preliminary study, that L-NAME can potentiate contraction to exogenously applied noradrenaline in the rat perfused lung and in rabbit isolated pulmonary arteries although the adrenoceptor subtype(s) involved in this effect have not been clearly identified (Shaw *et al.*, 1992).

Transient inhibition of nitric oxide synthesis is responsible for the transient vasoconstrictor response to acute hypoxia in rabbit pulmonary arteries (MacLean & McGrath, 1991). Endothelium derived relaxing factor is inhibited by hypoxia in bovine pulmonary artery endothelial cells and in rabbit pulmonary arteries (Warren *et al.*, 1989; Johns *et al.*, 1989). Recently it has been shown that moderate hypoxia can inhibit the activity of the isoform of nitric oxide synthase present in vascular endothelium, primarily through depletion of oxygen, one of the substrates for the enzyme (Rengasamy & Johns, 1991).

The aim of this study was to determine the α-adrenoceptor subtype involved in the potentiating effect of L-NAME on rabbit pulmonary arteries. The ability of endothelial cell removal to mimic the effect of L-NAME on response to α-adrenoceptor agonists was also examined. We also determined if the degree of hypoxia enforced in day-long *in vitro* experiments mimicked the effects of nitric oxide synthase inhibition, by studying the effects of experimental hypoxia on responses to α-adrenoceptor agonists.

Methods

NZW rabbits (2–3 kg) were killed by stunning followed by cervical dislocation. The lungs and heart were removed and

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the first left branch of the pulmonary artery (3–5 mm i.d.) dissected out and cleaned. The arteries were cut into rings which were suspended in 40 ml organ baths in Krebs saline under 2 g wt. tension. The Krebs solution was gassed with 5% O_2, 6% CO_2 balance nitrogen (to mimic the *in vivo* tensions in mixed arterio-venous pulmonary blood) and the tissues allowed to equilibrate for at least 1 h before starting the experiment. When hypoxia was induced the Krebs was gassed with 0% O_2, 6% CO_2 balance nitrogen (bath O_2 tension equivalent to 2.5%).

Concentration-response curves to noradrenaline, phenylephrine and UK 14304 were constructed before and after administration of L-NAME (10^{-4} M). In further experiments, concentration-response curves to noradrenaline were constructed before, then 45 min after, administration of rauwolscine (10^{-6} M). Prazosin (10^{-7} M) was then added, still in the presence of rauwolscine, and further concentration-response curves constructed 45 min later. This procedure with noradrenaline, rauwolscine and prazosin was also performed in the presence of 10^{-4} M L-NAME. In other experiments, the vascular endothelium was removed by gently rubbing the intimal surface with a pair of forceps on a Krebs-soaked tissue. Concentration-response curves to all three agonists were then established in these rings as well as intact preparations from the same animals. At the end of these experiments, tone was raised with 10^{-7} mM noradrenaline and substance P (10^{-8} M) used as a stimulant for nitric oxide production to show that only preparations with an intact endothelium relaxed. This ensured that endothelium removal had been successful. Fluorescent dyes were also used to examine histologically the structure of rubbed endothelial cells in at least ten random preparations (Daly *et al.*, 1992). To study the effect of hypoxia on agonist responses, the vessels were equilibrated in the normoxic conditions, concentration-response curves performed and the vessels were then re-equilibrated in the hypoxic conditions before constructing further concentration-response curves.

Drugs and solutions

The composition of the Krebs-bicarbonate saline was as follows (in mM): NaCl 118.4, $NaHCO_3$ 25, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, glucose 11, Na_2EDTA 0.023. The following drugs were used: prazosin (Pfizer), rauwolscine HCl (Roth), UK 14304 (5-bromo-6[2-imazolin-2-yl-amino] quinoxaline) bitartrate (Pfizer), N^{ω}-nitro-L-arginine methylester (Sigma). All drugs were dissolved in distilled water.

The gas tensions and pH were determined regularly by use of both an oxygen electrode inserted into one of the organ baths and a blood gas analyser (Instrumentation Laboratory system 1302).

Analysis of results

As the tension transducer was calibrated with a 2 g weight, contractions were expressed in equivalent weight units, i.e. mg wt. tension (see Miller, 1988). To standardize maximum responses for statistical comparison they are expressed, in the tables, as a percentage of the response obtained to 100 mM KCl in each preparation. For concentration-response curves, however, control contractions are expressed as a percentage of their maximum response to the relevant agonist. Subsequent contractions achieved after drug treatment or exposure to hypoxia are expressed as a percentage of their control maximum response. EC_{50}s for agonists were calculated for each preparation and meaned within groups. The EC_{50} is expressed as the $-\log$ concentration (M) and was calculated using a computer extrapolation. Statistical analysis of these results was performed by a paired Student's *t* test and confirmed by one-way analysis of variance where appropriate.

In the experiments comparing agonist responses in endothelium rubbed and unrubbed preparations, a slightly different analysis was required. In Table 3, to standardize maximum responses, for statistical comparison, contractions are expressed as a percentage of the maximum response obtained to 100 mM KCl in each preparation. For the concentration-response curves, however, in order to illustrate alterations in agonist potency, contractions achieved in endothelium-rubbed preparations are expressed as a percentage of their own maximum response to the relevant agonist. Statistical analysis of the difference between unrubbed preparations and rubbed ones was performed by use of unpaired Student's *t* test.

Results

In normoxic conditions, the bath gas tensions and pH were as follows: $P_{O_2} = 45.4 \pm 0.5$ mmHg, $P_{CO_2} = 35.6 \pm 0.3$ mmHg and pH $= 7.39 \pm 0.03$, $n = 6$.

The effect of 10^{-4} M L-NAME on concentration-response curves to noradrenaline, phenylephrine and UK 14304

L-NAME itself had variable effects on vascular tone. In some preparations (approximately 60%) it caused small increases in vascular tone (approximately 5% of the maximum contractile response to 100 mM KCl) whilst in others it had no effect on vascular tone. For example, in 8 preparations in which it did increase tone, it increased vascular tone by 206 ± 47 mg wt. tension. The maximum response to 100 mM KCl in these preparations was 4338 ± 535 mg wt. tension.

Figure 1 shows the effect of L-NAME on the concentration-response curves to noradrenaline, phenylephrine and UK 14304 in the rabbit isolated pulmonary artery by expressing contractile responses in the presence of L-NAME as a percentage of the maximum response achieved prior to its administration. Table 1 gives the corresponding EC_{50} values and maximum responses to each agonist expressed as a percentage of the maximum response obtained with 100 mM KCl.

Figure 1a and Table 1 show that L-NAME increased the potency of noradrenaline as well as increasing the maximum tension it was able to achieve in these vessels (by approximately 60% of control absolute maximum tensions). L-NAME augmented noradrenaline-evoked contractions regardless of whether or not it had any effect on vascular tone itself.

Figure 1b shows that L-NAME had no effect on the concentration-response curves to phenylephrine and, correspondingly, Table 1 shows no effect of L-NAME on the EC_{50} or on maximum responses to phenylephrine.

L-NAME increased the potency of UK 14304 as well as increasing the maximum tension it was able to achieve in these vessels by approximately 125% of control absolute maximum tensions (Figure 1c, Table 1).

Figure 2a shows the effect of 10^{-6} M rauwolscine and 10^{-7} M prazosin on the concentration-response curves to noradrenaline in the rabbit isolated pulmonary artery in control preparations. Figure 2b shows the effect of these antagonists on the concentration-response curves to noradrenaline in preparations pretreated with L-NAME. The EC_{50}s for noradrenaline in all conditions are summarized in Table 2. The results show that rauwolscine shifts the concentration-response curve to noradrenaline significantly to the right in both treated and untreated groups but it has a significantly greater effect on those preparations treated with L-NAME (see Table 2). To verify the statistical analysis of this effect of L-NAME, a one-way analysis of variance (ANOVA) was also performed on these data. This showed that the EC_{50} for noradrenaline in the presence of rauwolscine was significantly different from control values and also significantly different from the EC_{50} for noradrenaline obtained in the present of L-NAME as well as rauwolscine. Rauwolscine also decreased the maximum response to nora-

drenaline in the treated group (by approximately 30% of control absolute maximum tensions). Prazosin together with rauwolscine had a more profound effect on the noradrenaline concentration-response curves than rauwolscine alone, shifting them further to the right. Table 2 shows that prazosin plus rauwolscine had a greater effect than rauwolscine alone on the EC_{50}s to noradrenaline but this combination was less effective in the present of L-NAME. To verify the statistical analysis of this effect of L-NAME, a one-way analysis of variance (ANOVA) was also performed on these data. This showed that the EC_{50} for noradrenaline in the presence of both rauwolscine and prazosin was significantly different from control values and also significantly diferent from the EC_{50} for noradrenaline obtained in the presence of L-NAME, rauwolscine and prazosin.

The effect of endothelium-removal on concentration-response curves to noradrenaline, phenylephrine and UK 14304

In control preparations precontracted with noradrenaline, substance P induced a relaxation. For example, if tone was raised to 710 ± 30 mg wt. tension, 3×10^{-8} M substance P

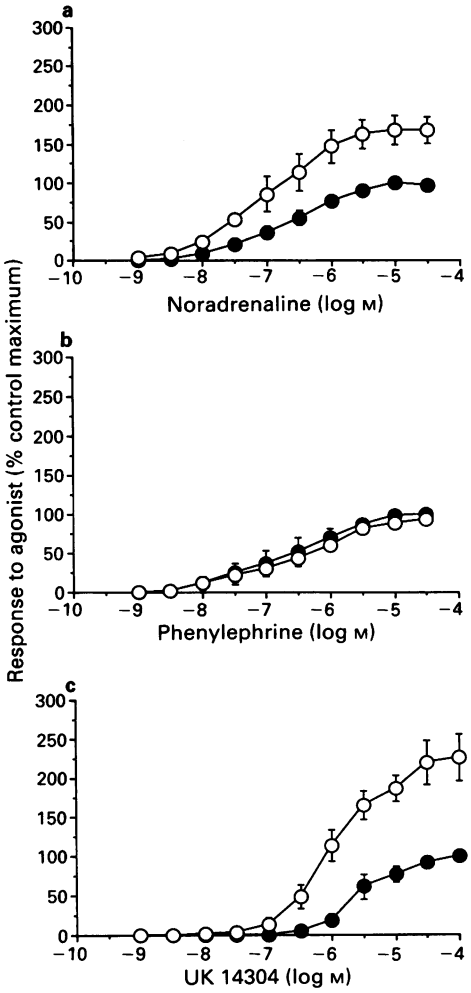

Figure 1 Vasoconstriction of rabbit isolated pulmonary arteries by noradrenaline (a), phenylephrine (b) and UK 14304 (c) in the absence (controls, ●) and presence of 10^{-4} M N^{ω}-nitro-L-arginine methylester (L-NAME, ○). Data are expressed as a percentage of the maximum response, to each agonist, achieved in the controls. Each point represents the mean response of 12 preparations from 6 animals with vertical bars representing ± s.e.mean.

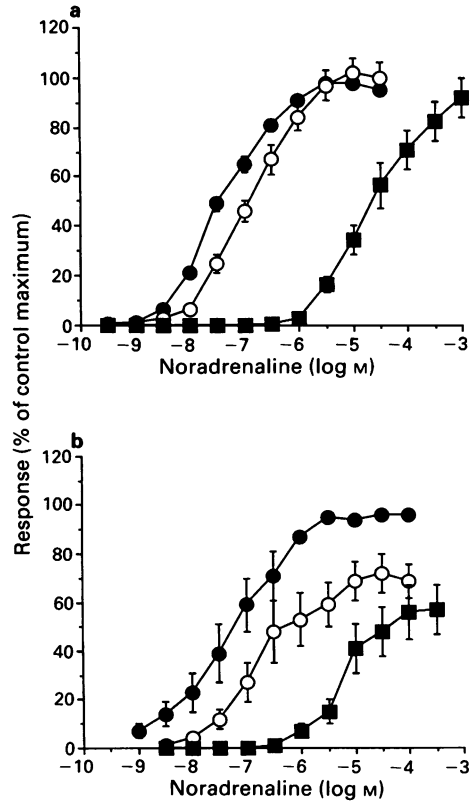

Figure 2 Vasoconstriction of rabbit isolated pulmonary arteries by noradrenaline (a) and after administration of 10^{-4} M N^{ω}-nitro-L-arginine methylester (L-NAME, b) and in the absence (●) and presence of 10^{-6} M rauwolscine (□) and 10^{-6} M rauwolscine plus 10^{-7} M prazosin (■). Data are expressed as a percentage of the maximum response achieved in the absence of either antagonist. Each point represents the mean responses of 10 preparations from 5 animals with vertical bars representing ± s.e.mean.

Table 1 The effect of N^{ω}-nitro-L-arginine methylester (L-NAME) on responses to α-adrenoceptor agonists in the rabbit isolated pulmonary artery

	Controls		*+ L-NAME* (10^{-4} M)		
Agonist	EC_{50}	*Maximum response to agonist*	EC_{50}	*Maximum response to agonist*	n
Noradrenaline	6.5 ± 0.2	44 ± 7	7.1 ± 0.4*	72 ± 12*	12
Phenylephrine	6.2 ± 0.4	51 ± 4	6.6 ± 0.3	45 ± 5	12
UK 14304	5.6 ± 0.1	17 ± 4	6.0 ± 0.2*	43 ± 4***	12

EC_{50} is expressed as the − log concentration (M) and the maximum response of each agonist as the percentage of the maximum response obtained with 100 mM KCl in each ring preparation. Data are expressed as mean ± s.e.mean. n = the number of preparations from six animals. Statistical comparisons with controls were carried out using Student's paired t test. *$P<0.05$; ***$P<0.001$

Table 2 The effect of rauwolscine and prazosin on the EC_{50} of noradrenaline in the rabbit isolated pulmonary artery in untreated preparations and preparations treated with 10^{-4} M N^{ω}-nitro-L-arginine methylester (L-NAME)

Antagonists	EC_{50} [-log concentration (M)] Untreated	+ L-NAME
Control	7.5 ± 0.1	7.3 ± 0.3
Rauwolscine (10^{-6} M)	6.9 ± 0.1*	6.4 ± 0.1*††
Rauwolscine (10^{-6} M) plus prazosin (10^{-7} M)	4.6 ± 0.4***	5.6 ± 0.1***††

Statistical comparisons with controls were carried out using a Student's paired t test. *$P<0.05$; ***$P<0.001$. Data are expressed as the mean (10 preparations from 5 animals) ± s.e.mean. Statistical comparisons between rauwolscine-treated preparations (untreated controls vs + L-NAME group) were carried out using a Student's unpaired t test. ††$P<0.01$.

induced a vasodilatation of 97 ± 11 mg wt. tension. Higher doses of substance P relaxed preconstricted rings by up to 50–60%, dependent on the level of preconstriction induced. Removal of the vascular endothelium totally inhibited the ability of substance P (10^{-7} M–10^{-6} M) to relax preconstricted vessels. In addition, in those preparations examined histologically, all endothelial cells appeared damaged by this rubbing procedure. We are confident, therefore, that rubbing produced maximal disruption of the vascular endothelium.

Figure 3 compares the potencies of noradrenaline, phenylephrine and UK 14304 in endothelium-intact and endothelium-rubbed preparations. Table 3 gives the calculated values for the EC_{50} and also gives the calculated maximum contraction to each agonist expressed as a percentage of the maximum response to 100 mM KCl.

Figure 3a shows that endothelial removal shifted the concentration-response curve for noradrenaline to the left, i.e. increased its potency. Table 3 shows the resultant effect of endothelium removal on EC_{50} values and also shows that there was an increase in the maximum tension induced by noradrenaline in endothelium rubbed vessels.

In a similar fashion, Figures 3b and 3c and Table 3 show that endothelial cell removal also increased both the potency of, and maximum contractile responses to, phenylephrine and UK 14304.

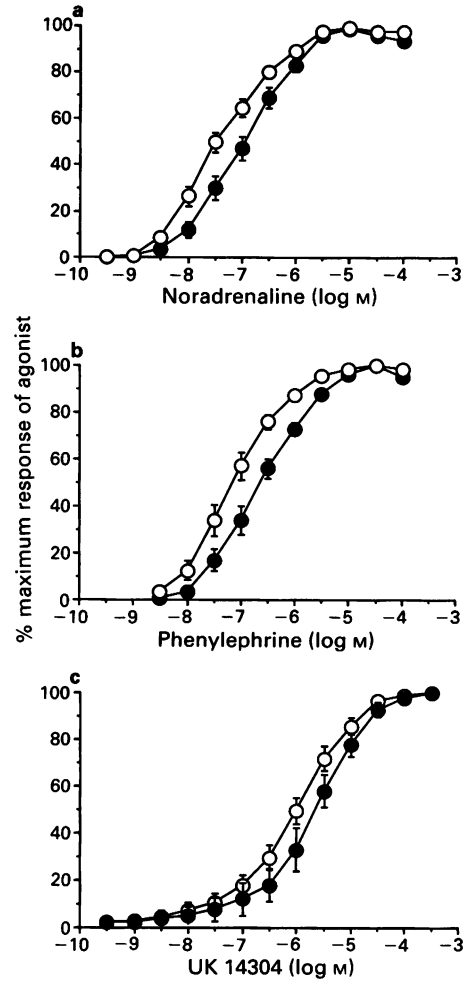

Figure 3 Vasoconstriction of rabbit isolated pulmonary arteries by noradrenaline (a), phenylephrine (b) and UK 14304 (c) in intact preparations (●) and preparations with a disrupted endothelium (○). Data are expressed as a percentage of the maximum response achieved by each agonist in each preparation. Each point represents the mean responses of 12 preparations (a,b) or 14 preparations (c) from 6 animals with vertical bars representing ± s.e.mean.

The effect of hypoxia on concentration-response curves to noradrenaline, phenylephrine and UK 14304

In hypoxic conditions, the bath gas tensions and pH were as follows: $P_{O_2} = 5.7 \pm 0.6$ mmHg, $P_{CO_2} = 35.8 \pm 0.5$ mmHg, pH = 7.37 ± 0.01, $n = 6$.

Figure 4 shows the effect of hypoxia on the concentration-response curves to noradrenaline, phenylephrine and UK 14304 in the rabbit isolated pulmonary artery by expressing contractile responses in hypoxic conditions as a percentage of the maximum response achieved in normoxic conditions. Table 4 gives the corresponding values for the EC_{50} and maximum responses to each agonist expressed as a percentage of the maximum response obtained with 100 mM KCl. Figure 4a and Table 4 show that hypoxia had no effect on the potency of noradrenaline but increased the maximum tension it was able to achieve in these vessels (by approximately 20% of control absolute maximum tensions).

Figure 4b and Table 4 show that hypoxia shifted the concentration-response curve to phenylephrine to the right

Table 3 The effect of mechanical disruption of the vascular endothelium on responses to α-adrenoceptor agonists in the rabbit isolated pulmonary artery

	Endothelium intact		*Endothelium disrupted*		
Agonist	EC_{50}	*Maximum response to agonist*	EC_{50}	*Maximum response to agonist*	n
Noradrenaline	7.0 ± 0.1	113 ± 6	7.4 ± 0.1*	142 ± 6**	12
Phenylephrine	6.7 ± 0.1	101 ± 5	7.2 ± 0.1**	124 ± 9*	12
UK 14304	5.6 ± 0.1	16 ± 5	6.0 ± 0.1*	49 ± 14**	14

EC_{50} is expressed as the − log concentration (M) and the maximum response of each agonist as the percentage of the maximum response obtained with 100 mM KCl in each ring preparation. Data are expressed as mean ± s.e.mean. n = the number of preparations from six animals. Statistical comparisons with controls were carried out using a Student's unpaired t test. *$P<0.05$; **$P<0.01$.

and hence decreased its potency. These results also show that the maximum response achieved by phenylephrine was likewise reduced by hypoxia (by approximately 20% of control absolute maximum tensions).

Figure 4c and Table 4 show the effect of hypoxia on the concentration-response curves to UK 14304. The results show that hypoxia had no significant effect on responses to UK 14304 in these vessels.

At the end of some experiments, arterial rings (six in total from three animals) that had been exposed to hypoxia were examined histologically by use of fluorescent dyes. There was no structural damage to the endothelial cells. In addition, pre-constricted arterial rings exposed to hypoxia for the duration of these experiments were still able to relax to substance P to the same extent as control preparations.

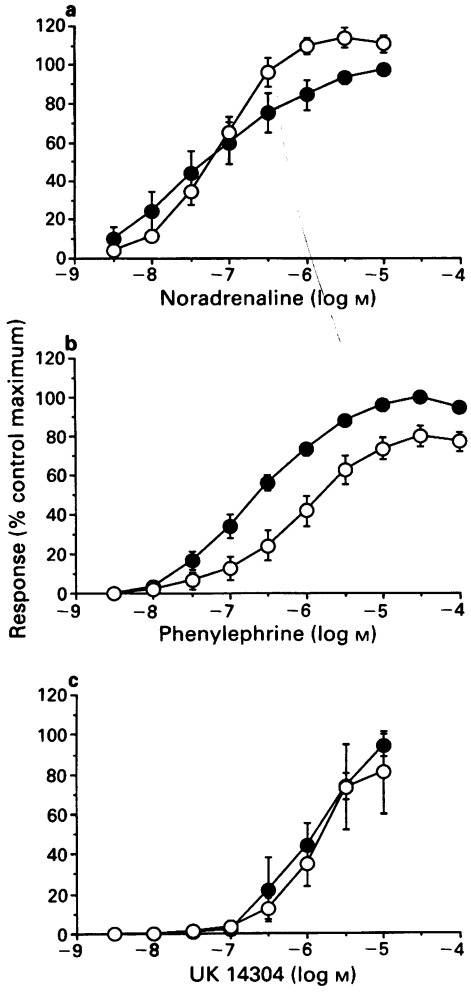

Figure 4 Vasoconstriction of rabbit isolated pulmonary arteries by noradrenaline (a), phenylephrine (b) and UK 14304 (c) in normoxia (Po_2 45 mmHg, ●) or hypoxia (Po_2 6 mmHg, ○). Data are expressed as a percentage of the maximum response achieved by each agonist in each normoxia. Each point represents the mean responses of 12 preparations (a,b) or 9 preparations (c) from 6 animals with vertical bars representing ± s.e.mean.

Discussion

The results show that inhibition of nitric oxide synthase with L-NAME potentiates responses to noradrenaline in rabbit pulmonary arteries. In a limited number of preliminary experiments we have also been able to demonstrate this phenomenon in human pulmonary artery rings (unpublished observations). This may have far reaching implications for our understanding of the mechanisms underlying the development of pulmonary hypertension. Endothelial cell disruption has been observed in experimental pulmonary hypertension, and in patients with hypoxia-related pulmonary hypertension (Rosenberg & Rabinovitch, 1986; Rabinovitch *et al.*, 1986). It has also been demonstrated that endothelium-dependent relaxation induced by acetylcholine is impaired in isolated perfused lungs from patients with chronic lung disease (Cremona *et al.*, 1992) and this is believed to be due to lack of oxygen leading to reduced nitric oxide release (Dinh-Xuan, 1992). If reduced nitric oxide production leads to the increased sensitivity of pulmonary arterial smooth muscle to circulating catecholamines, this would contribute to the persistent pulmonary vasoconstriction typical in hypoxia-induced pulmonary hypertension.

We have also shown that L-NAME can also potentiate the responses of rabbit pulmonary artery rings to nerve stimulation (MacLean *et al.*, 1993). The combined effect of the increased sensitivity to nerve stimulation and circulating catecholamines would further aggravate the pulmonary hypertension. Indeed, recent studies have shown that inhalation of nitric oxide in patients with chronic lung disease and pulmonary hypertension relieves the hypertension and exerts a selective effect on the pulmonary circulation (Adnot *et al.*, 1992). This is somewhat of a breakthrough in the clinical approach to treatment, as the biggest problem facing clinicians is the lack of selectivity of most vasodilators used in the treatment of pulmonary hypertension. Due to pulmonary vascular remodelling, diseased lungs are relatively resistant to vascular therapy and standard vasodilators exert greater effects on the systemic circulation leading to unwanted side-effects.

As with many other noradrenergic responses, this study shows that the response to noradrenaline in the rabbit pulmonary artery is mediated primarily by α_1-adrenoceptors. There was, however a significant effect of rauwolscine on these responses and some agonist activity of the selective α_2-adrenoceptor agonist, UK 14304. Together this suggests that there is a functional population of α_2-adrenoceptors in the rabbit pulmonary artery.

A parallel, as yet unpublished, study has demonstrated that, in the rabbit isolated pulmonary artery, 10^{-6} M rau-

Table 4 The effect of hypoxia on responses to α-adrenoceptor agonists in the rabbit isolated pulmonary artery

	Normoxia (Po_2 45 mmHg)		*Hypoxia* (Po_2 6 mmHg)		
Agonist	EC_{50}	*Maximum response to agonist*	EC_{50}	*Maximum response to agonist*	n
Noradrenaline	7.3 ± 0.3	78 ± 11	7.0 ± 0.2	97 ± 14**	12
Phenylephrine	6.6 ± 0.1	101 ± 5	6.1 ± 0.2**	83 ± 6 **	12
UK 14304	6.0 ± 0.2	24 ± 6	5.0 ± 1.0	22 ± 7	9

EC_{50} is expressed as the − log concentration (M) and the maximum response of each agonist as the percentage of the maximum response obtained with 100 mM KCl in each ring preparation. Data are expressed as mean ± s.e.mean. n = the number of preparations from six animals. Statistical comparisons with controls were carried out using a Student's unpaired t test. $^{**}P<0.01$.

wolscine caused a ten fold rightward shift in UK 14304 concentration-response curves demonstrating a rauwolscine-sensitive, α_2-adrenoceptor-selective effect of UK 14304. There was, however, a component of the response of UK 14304 which was sensitive to 10^{-7} M prazosin. This study demonstrated that the rabbit pulmonary artery behaves in a similar fashion to the rabbit isolated distal saphenous artery in that the α_2-adrenoceptor response is dependent, to some extent, upon a positive influence from the α_1-adrenoceptor (Dunn *et al.*, 1991b).

In the present study, the effect of UK 14304 and rauwolscine were significantly enhanced in the presence of L-NAME. Indeed, rauwolscine also halved the effect of L-NAME on the maximum contractile response to noradrenaline. As L-NAME virtually doubled the maximum response to noradrenaline, this suggests recruitment of α_2-adrenoceptors contributed to this increase in maximum response.

This is consistent with previous studies in which we have shown that in order to observe responses mediated by postjunctional α_2-adrenoceptors in arterial preparations, the vascular conditions must be modified. For example, in the rat tail artery, when vascular tone is raised with vasopressin, endothelin-1 or 5-hydroxytryptamine, a previously quiescent α_2-adrenoceptor population is 'uncovered' (Templeton *et al.*, 1989; MacLean & McGrath, 1990). The presence of angiotensin II selectively facilitates responses mediated via postjunctional α_2-adrenoceptors in the saphenous artery and ear vein of the rabbit (Dunn *et al.*, 1991a). In addition, inhibition of neuronal uptake will reveal α_2-adrenoceptor-mediated nerve responses in the rat tail artery (Papanicolaou & Medgett, 1986). Hence, the results described here are compatible with the view that α_2-adrenoceptors require specific vascular conditions before their effect is revealed. In the case of pulmonary arteries, one way to reveal a contribution by α_2-adrenoceptors is to inhibit nitric oxide synthesis. This is the case regardless of whether or not L-NAME itself increases vascular tone. We have previously described the variable effect of L-NAME on vascular tone which seems to be related to vessel diameter, the smaller pulmonary arteries being influenced more (MacLean & McGrath, 1991). In this study, any tone increase was very small and unrelated to the effect of L-NAME on contractile responses. For example, L-NAME always augmented responses to α-adrenoceptor agonists regardless of whether or not it caused an increase in vascular tone. In addition, any increase in tone was negligible compared to the absolute increase in α-adrenoceptor-induced contractions.

As L-NAME had no effects on responses to phenylephrine and actually decreased the effectiveness of prazosin it may be that the effect of L-NAME is selective to α_2-adrenoceptors. This may, however, be species-dependent as we have shown potentiation of phenylephrine-induced responses by L-NAME in the rat perfused lung (Shaw *et al.*, 1992) and Liu *et al.* (1991) demonstrated that nitric oxide synthase inhibition augmented contractile responses to phenylephrine in guinea-pig isolated pulmonary arteries. Accordingly, we are currently investigating the receptors involved in noradrenergic responses in other mammalin species (human, bovine, ovine). Contractile responses to α_2-adrenoceptor activation are typically well-maintained and could contribute to persistent pulmonary hypertension. If selective α_2-adrenoceptor facilitation does exist in human pulmonary arteries, this would be a pharmacological advantage for selective therapeutic intervention. α_2-Adrenoceptor antagonists could be directed at the pulmonary circulation in pulmonary hypertensives in which there is endothelial cell damage and an associated reduction in nitric oxide synthase activity.

Mechanical disruption of the vascular endothelium mimicked the effect of L-NAME on contractile responses to noradrenaline and UK 14304 consistent with L-NAME inhibiting endothelium-derived nitric oxide synthase. In addition, it also augmented responses to phenylephrine. The vascular endothelium produces and releases many other factors such as the relaxant prostacyclin and the constrictor endothelin-1. As the response to phenylephrine was augmented by endothelium disruption, this implies that such endothelial cell derived vasoactive factors also exert effects on α_1-adrenoceptor activation. Hence, where disruption of endothelial cell structure is associated with pulmonary hypertension (Rabinovitch *et al.*, 1986), the pulmonary vascular responses to circulating catecholamines are likely to be enhanced through augmentation of both α_1- and α_2-adrenoceptor-mediated contractile responses. This would further aggravate the pulmonary hypertension.

Acute experimental hypoxia increased the maximum contractile response to noradrenaline. This was evidently not through an effect on α_1-adrenoceptor activation alone as phenylephrine-induced responses were significantly depressed by hypoxia. Neither does it appear to act through α_2-adrenoceptor activation alone, as it had no significant effect on the responses to UK 14304. Marriott & Marshall (1990) also reported that equivalent experimental hypoxia potentiated noradrenaline-evoked contractions in rabbit pulmonary arteries. They concluded, from their studies, that either hypoxia was facilitating noradrenaline-induced influx of calcium through receptor-operated mechanisms or that combined depolarization by hypoxia and noradrenaline may facilitate calcium influx through voltage-operated calcium channels. Our study indicates that whichever mechanism is responsible, to observe the effect of hypoxia seems to require the simultaneous stimulation of both α_1- and α_2-adrenoceptors. It has previously been suggested that, in certain vascular beds, simultaneous activation of α_1- and α_2-adrenoceptors is required before the responses to α_2-adrenoceptor stimulation are observed, e.g. in the rabbit saphenous artery (Dunn *et al.*, 1991b) and rat tail artery (Xiao & Rand, 1989). It is likely, therefore, that such interactions may involve complex facilitatory interactions between α_1- and α_2-adrenoceptor-induced calcium ion channel activation and membrane potential changes.

As discussed, chronic pulmonary hypoxia associated with hypoxia-related lung disease results in inhibition of endothelium-dependent relaxation and this is likely to be due to a reduction in nitric oxide synthesis. These results certainly do not prove that experimental hypoxia can inhibit nitric oxide synthesis, but they do not disprove this theory either. What they do show is an inability to mimic the effect of chronic hypoxia exposure using the experimental hypoxia prevalent in *in vitro* experiments. Hence, caution must be taken when comparing *in vivo* hypoxic conditions with *in vitro* ones. Experiments are planned to overcome these experimental problems by use of an appropriate chronic hypoxic animal model.

We have previously reported that, in rabbit pulmonary arteries, a sudden hypoxic challenge may transiently inhibit nitric oxide synthase (MacLean & McGrath, 1991). It is evident, however, that the degree of hypoxia produced in the organ bath, for the length of time described here, is not sufficient to inhibit nitric oxide synthase persistently.

The results suggest that in conditions in which the pulmonary vascular endothelium is disrupted, or where the nitric oxide system is inhibited (conditions prevalent in pulmonary hypertension), pulmonary vascular responses to circulating catecholamines may be potentiated and this may involve α_2-adrenoceptor-activation.

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References

ADNOT, S., KOUYOUMDJIAN, C., FRATACCI, M.D., DEFOUILLOY, C., ANDRIVET, P., SEDIAME, S. & HERIGAUT, R. (1992). Response to infusion of acetylcholine and inhalation of nitric oxide in patients with chronic lung disease and pulmonary hypertension. *J. Vasc. Res.*, **29**, 76, 3P.

COCKS, T.M. & ANGUS, J.A. (1983). Endothelium-dependent relaxation of coronary arteries by noradrenaline and serotinin. *Nature*, **305**, 627–630.

CREMONA, G., HIGENBOTTAM, T.W., WOOD, A.M., PEPKE-ZABA, J., BUTT, A.Y. & DINH-XUAN, A.T. (1992). Endothelium-dependent relaxation induced by acetylcholine is impaired in isolated perfused lungs from patients with chronic lung disease. *J. Vasc. Res.*, **29**, 98, 66P.

DALY, C.J., GORDON, J.F. & MCGRATH, J.C. (1992). The use of fluorescent nuclear dyes for the study of blood vessel structure and function: novel applications of existing techniques. *J. Vasc. Res.*, **29**, 41–48.

DAWSON, C.A. (1984). Role of pulmonary vasomotion in the physiology of the lung. *Physiol. Rev.*, **64**, 544–616.

DINH-XUAN, A.T. (1992). Endothelial dysfunction in chronic hypoxic pulmonary hypertension. *J. Vasc. Res.*, **29**, 103, 82P.

DUNN, W.R., DALY, C.J., MCGRATH, J.C. & WILSON, V.G. (1991a). A comparison of the effects of angiotensin II and Bay K 8544 on responses to noradrenaline mediated via postjunctional α_1- and α_2-adrenoceptors in rabbit isolated blood vessels. *Br. J. Pharmacol.*, **103**, 1475–1483.

DUNN, W.R., MCGRATH, J.C. & WILSON, V.G. (1991b). Postjunctional α-adrenoceptors in the rabbit distal saphenous artery: indirect sensitivity to prazosin of responses to noradrenaline mediated via postjunctional α_2-adrenoceptors. *Br. J. Pharmacol.*, **103**, 1484–1492.

EGLEME, C., GODFRAIND, T. & MILLER, R.C. (1984). Enhanced responsiveness of rat isolated aorta to clonidine after removal of the endothelial cells. *Br. J. Pharmacol.*, **81**, 16–18.

HOLDEN, W.E. & MCCALL, E. (1984). Hypoxia-induced contractions of porcine pulmonary artery strips depend on intact endothelium. *Exp. Lung Res.*, **7**, 101–102.

IGNARRO, L.J., BYRNS, R., BUGA, G.M. & WOOD, K.S. (1987). Endothelium-derived relaxing factor from pulmonary artery and vein possess pharmacological and chemical properties identical to those of nitric oxide radical. *Circ. Res.*, **61**, 866–879.

JOHNS, R.A., LINDEN, J.M. & PEACH, M.J. (1989). Endothelium-dependent relaxation and cyclic GMP accumulation in rabbit pulmonary artery are selectively impaired by moderate hypoxia. *Circ. Res.*, **65**, 1508–1515.

LIU, S.F., CRAWLEY, D.E., EVANS, T.W. & BARNES, P.J. (1991). Endogenous nitric oxide modulates adrenergic neural vasoconstriction in guinea-pig pulmonary artery. *Br. J. Pharmacol.*, **104**, 565–569.

LUES, I. & SCHUMANN, H.H. (1984). Effect of removing the endothelial cells on the reactivity of rat aortic segments to different α-adrenoceptor agonists. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **328**, 160–163.

MACLEAN, M.R. & MCGRATH, J.C. (1990). Effects of pre-contraction with endothelin-1 on α_2-adrenoceptor- and (endothelium-dependent) neuropeptide Y-mediated contractions in the isolated vascular rat tail. *Br. J. Pharmacol.*, **101**, 205–211.

MACLEAN, M.R. & MCGRATH, J.C. (1991). The effect of extracellular [Ca^{2+}] reduction nifedipine, ryanodine and L-NAME on *in vitro* hypoxic pulmonary vasoconstriction in the rabbit. *Br. J. Pharmacol.*, **104**, 119P.

MACLEAN, M.R., MCCULLOCH, K.M., MACMILLAN, J.B. & MCGRATH, J.M. (1993). Endothelial and hypoxia influences on neurogenic transmission in the rabbit isolated pulmonary artery. *Br. J. Pharmacol.*, **108**, 150–154.

MARRIOTT, J.F. & MARSHALL, J.M. (1990). Effects of hypoxia upon contractions evoked in isolated rabbit pulmonary artery by potassium and noradrenaline. *J. Physiol.*, **422**, 15–28.

MILLER, D.J. (1988). May the force be with you. *Trends Pharmacol Sci.*, **9**, 124–125.

MONCADA, S., PALMER, R.M.J. & HIGGS, E.A. (1988). The discovery of nitric oxide as the endogenous nitrovasodilator. *Hypertension*, **12**, 365–372.

MONCADA, S., PALMER, R.M.J. & HIGGS, E.A. (1989). Biosynthesis of nitric oxide from L-arginine, pathway for the regulation of cell function and communication. *Biochem. Pharmacol.*, **38**, 1709–1715.

PALMER, R.M.J., FERRIGE, A.G. & MONCADA, S. (1988). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*, **327**, 524–526.

PAPANICOLAOU, M. & MEDGETT, I.C. (1986). Effects of blockade of alpha 1- and alpha 2-adrenoceptors on vasoconstrictor responses to single and twin pulse stimulation in rat tail artery. *Eur. J. Pharmacol.*, **131**, 211–218.

RENGASAMY, A. & JOHNS, R.A. (1991). Characterization of endothelium-derived relaxing factor/nitric oxide synthase from bovine cerebellum and mechanisms of modulation by high and low oxygen tensions. *J. Pharmacol. Exp. Ther.*, **259**, 310–316.

RABINOVITCH, M., BOTHWELL, T., HAYAKAWA, B.N., WILLIAMS, W.G., TRUSLER, G.A., ROWE, R.D. & OLLEY, P.M. & CUTZ, E. (1986). Pulmonary artery endothelial abnormalities in patients with congenital heart defects and pulmonary hypertension. *Lab. Invest.*, **55**, 623–653.

ROSENBERG, H.C. & RABINOVITCH, M. (1986). Endothelial injury and vascular reactivity in monocrotaline pulmonary hypertension. *Am. J. Physiol.*, **255**, H1484–91.

RUBIN, L.J. (1984). Pulmonary hypertension secondary to lung disease. In *Pulmonary Hypertension*. ed. Weir, E.K. & Reeves, J.T. pp. 291–320. New York: Futura.

SHAW, A., MACLEAN, M.R., POLLOCK, D. & MCGRATH, J.C. (1992). Influence of L-NAME on α_1- and α_2-adrenoceptor and responses in the isolated rabbit pulmonary artery and the rat Krebs-perfused lung. *J. Vasc. Res.*, **29**, 197, 355P.

STAUB, N.C. (1985). Site of hypoxic pulmonary vasoconstriction. *Chest*, **88** (Suppl.), 240s–245s.

TEMPLETON, A.G.B., MACMILLAN, J., MCGRATH, J.C., STOREY, N.D. & WILSON, V.G. (1989). Evidence for prazosin-resistant, rauwolscine-sensitive α-adrenoceptors mediating contractions in the isolated vascular bed of the rat tail. *Br. J. Pharmacol.*, **97**, 563–571.

WARRNE, J.B., MALTBY, N.H., MACCORMACK, D. & BARNES, P.J. (1989). Pulmonary endothelium derived relaxing factor is impaired in hypoxia. *Clin. Sci.*, **77**, 671–676.

XIAO, X.-H. & RAND, M.J. (1989). α_2-Adrenoceptor agonist enhances responses to certain other vasoconstrictor agonists in the rat tail. *Br. J. Pharmacol.*, **96**, 539–547.

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