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Noradrenaline, β-adrenoceptor mediated vasorelaxation and nitric oxide in large and small pulmonary arteries of the rat

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1 Noradrenaline induces a meagre vasoconstriction in small muscular pulmonary arteries compared to large conduit pulmonary arteries. We have examined whether this may be partially related to differences in the β-adrenoceptor-mediated vasorelaxation component and, in particular, β-adrenoceptor-mediated NO release.

2 Noradrenaline induced a bell-shaped concentration-response in large (1202±27 μm) and small (334±12 μm) pulmonary arteries of the rat. In large arteries tension increased to 95.6±1.8% of 75 mM KCl (KPSS; $n=8$) at 2 μM, above which tension declined. The response in small arteries was meagre (12±1.5% KPSS, $n=9$), peaking at 0.2 μM. N^G-monomethyl-L-arginine (L-NMMA; 100 μM) abolished the decline in tension induced by higher concentrations of noradrenaline in large arteries, and increased maximum tension (117±3.5% KPSS, $n=5$, $P<0.05$). In small arteries peak tension doubled (22.0±3.4% KPSS, $n=6$, $P<0.01$), but still declined above 0.2 μM.

3 Propranolol (1 μM) abolished the decline in tension at higher concentrations of noradrenaline in both groups, but increased tension substantially more in small (37.4±3.7% KPSS, $n=5$, $P<0.001$) than in large arteries (112.2±3.7% KPSS, $n=9$, $P<0.05$). In the presence of L-NMMA, propranolol had no additional effect on large arteries, whereas in small arteries there was greater potentiation than for either agent alone (67.8±5.9% KPSS, $n=4$).

4 β-Adrenoceptor-mediated relaxation was examined in arteries constricted with prostaglandin $F_{2\alpha}$ (50 μM). In the presence of propranolol isoprenaline caused an unexpected vasoconstriction, which was abolished by phentolamine (10 μM). In the presence of phentolamine, isoprenaline caused a maximum relaxation of 43.3±2.1% ($n=6$) in large, and 49.0±4.5% ($n=6$) in small arteries. L-NMMA substantially reduced relaxation in large arteries (7.4±1.5%, $n=6$, $P<0.01$), but was less effective in small arteries (26.8±5.8, $n=5$, $P<0.05$).

5 Atenolol (β_1-antagonist, 5 μM) reduced relaxation to isoprenaline (large: 34.8±4.5%, $n=5$; small: 35.0±1.9%,$n=6$), but in combination with L-NMMA had no additional effect over L-NMMA alone. ICI 118551 (β_2-antagonist, 0.1 μM) reduced isoprenaline-induced relaxation more than atenolol (large: 18.0±4.6%, $n=6$, $P<0.05$; small: 25.6±10.7%, $n=6$, $P<0.05$). ICI 118551 in combination with L-NMMA substantially reduced relaxation (large: 4.8±2.6%, $n=9$; small: 6.5±3.6%, $n=5$).

6 Salbutamol-induced relaxation was reduced substantially by L-NMMA in large arteries (control: 34.7±6.4%, $n=6$; +L-NMMA: 8.3±1.3%, $n=5$, $P<0.01$), but to a lesser extent in small arteries (control: 50.9±7.5%, $n=6$; +L-NMMA: 23.0±0.7%, $n=5$, $P<0.05$). Relaxation to forskolin was also partially antagonized by L-NMMA.

7 These results suggest that the meagre vasoconstriction to noradrenaline in small pulmonary arteries is partially due to a greater β-adrenoceptor-mediated component than in large arteries. β-Mediated vasorelaxation in large arteries was largely NO-dependent, whereas in small arteries a significant proportion was NO-independent. Noradrenaline stimulation was also associated with NO release that was independent of β-adrenoceptors.

Keywords: Pulmonary artery; endothelium; nitric oxide; nitric oxide synthase; β-adrenoceptors; noradrenaline

Introduction

Sympathetic stimulation has been shown to cause changes in pulmonary vascular resistance (PVR) that are mediated via noradrenaline and α_1-, α_2-, and β_2-adrenoceptors (Hyman *et al.*, 1990). However, the functionally significant small muscular pulmonary arteries (100–500 μm internal diameter), which are primarily responsible for alterations in PVR, have a very poor vasoconstrictor response to noradrenaline compared to larger conduit arteries (Leach *et al.*, 1992). This diversity in response has not been adequately explained, and could involve differences in either the α-adrenoceptor-mediated vasoconstrictor component, the β-adrenoceptor-mediated vasodilator component, and/or nitric oxide (NO) production. There have been relatively few studies concerning the influence of adrenoceptor agonists on NO production in the pulmonary circulation, although significant variability has been obtained between other vascular beds (Ohgushi *et al.*, 1993).

It is known that removal of the endothelium or inhibition of NO synthesis with analogues of L-arginine increases noradrenaline-induced contraction of systemic vascular smooth muscle (Cederqvist *et al.*, 1991; Kaneko & Sunano, 1993), and the L-arginine analogue N^G-nitro-L-arginine methylester (L-NAME) has been shown to potentiate the response to both neurogenic stimulation and exogenous noradrenaline in isolated pulmonary artery of the rabbit (MacLean *et al.*, 1993a, b). There is therefore convincing evidence that noradrenaline-induced vasoconstriction is associated with an increase in NO production. There are several potential mechanisms that could contribute to this increase, including the rise in vascular tone *per se* (Graves & Poston, 1993; Amerini *et al.*, 1995), and a direct effect mediated by α-adrenoceptors (Kaneko & Sunano, 1993; Ohgushi *et al.*,

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1993; Tulloh *et al*., 1994). There is also evidence from the systemic circulation that β-agonists can stimulate release of NO from the endothelium.

Whereas the classical view is that β-adrenoceptor-induced vasodilatation is mediated entirely via activation of adenylate cyclase in the smooth muscle, there are now several findings suggesting that it may be, at least in part, endothelium dependent, as isoprenaline-induced relaxation in rat systemic arteries has been shown to be inhibited by removal of the endothelium or inhibition of NO synthesis (Gray & Marshall, 1992; Blankesteijn & Thien, 1993; Graves & Poston, 1993; Delpy *et al*., 1996), although the degree of inhibition obtained is variable. However, Moncada *et al*. (1991) have shown that isoprenaline-induced relaxations in rat aorta are not endothelium-dependent, and Eckly *et al*. (1994) showed no alteration of isoprenaline-induced changes in aortic smooth muscle adenosine 3′:5′-cyclic monophosphate (cyclic AMP) or guanosine 3′:5′-cyclic monophosphate (cyclic GMP) following removal of the endothelium. The role of NO in the response to β-agonists has not so far been examined in the pulmonary vasculature.

In this study we have focussed on the role of NO in β-adrenoceptor-mediated relaxation of the pulmonary vasculature and have examined whether this mechanism could partly underlie the differences in response to noradrenaline of large conduit (1–2 mm i.d.) and small muscular (200–450 μm i.d.) pulmonary arteries of the rat.

Methods

Tissue preparations

Adult, male Wistar rats (250–350 g) were anaesthetized with ether and killed by cervical dislocation, as approved by the local Home Office Inspector. The heart and lungs were excised and placed in a physiological salt solution (PSS) containing (in mM): NaCl 118, $NaHCO_3$ 24, $MgSO_4$ 1, NaH_2PO_4 0.435, glucose 5.56, Na-pyruvate 5, $CaCl_2$ 1.8 and KCl 4. Large pulmonary arteries (1202 ± 27 μm i.d., $n=117$) and small intrapulmonary arteries (5th–6th order branch, 334 ± 12 μm, $n=83$) were then dissected free of connective tissue and mounted in a small vessel myograph as previously described in detail (Leach *et al*., 1992; 1994), and equilibrated with 5% CO_2 in O_2 (pH 7.35–7.40, 37°C). In some experiments on large arteries only the endothelium was disrupted *in situ* by gently rubbing the luminal surface of the artery with a 40 μm wire or human hair. The presence of a functioning endothelium was determined by application of acetylcholine (ACh; 10 μM) following agonist-induced contraction. After 60 min equilibration the arteries were subjected to a standard run up procedure of three 4 min exposures to PSS containing high K^+ (KPSS 75 mM [K^+], equimolar substitution for NaCl) (Leach *et al*., 1992; 1994). Arteries producing less than 1 mN mm^{-1} were discarded. After washing with PSS the arteries returned to baseline tone.

Experimental protocols

Noradrenaline Cumulative concentration-response relationships were determined for noradrenaline (1 nM–10 μM) in large and small pulmonary arteries. Noradrenaline was added directly to the bath, and tension was allowed to plateau before the next addition (4 min). Tension is expressed in terms of the immediately preceding response to KPSS. The involvement of β-adrenoceptors in the response to noradrenaline was examined by following the same protocol in the presence of the non-selective antagonist propranolol (1 μM). The role of NO was similarly examined by pre-incubation for 20 min with the NO synthase (NOS) inhibitor N^G-monomethyl-L-arginine (L-NMMA, 100 μM). Experiments were also performed in the presence of both propranolol and L-NMMA. The noradrenaline concentration-response relationship was also determined following removal of the endothelium in large arteries alone.

β-Adrenoceptor agonists Cumulative concentration-response relationships were constructed for the vasorelaxant actions of the non-selective β-adrenoceptor agonist isoprenaline and the β_2-adrenoceptor agonist salbutamol in large and small pulmonary arteries, following stable pre-contraction with 50 μM prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, EC_{80}). Tension was allowed to stabilize following every addition and is expressed in terms of the initial $PGF_{2\alpha}$-induced tension. The role of NO was investigated following pre-incubation for 20 min with L-NMMA (100 μM). Initial experiments with the β_1-agonist dobutamine caused substantial variations in tension, with transient relaxations followed by contraction on each addition, and the response was very variable between individual arteries. It was therefore not used in these experiments. The relative components of isoprenaline-induced vasorelaxation due to β_1- and β_2-adrenoceptors were therefore studied in the presence of the nominally selective antagonists atenolol (5 μM) and ICI 118551 (0.1 μM), respectively. However, it should be recognized that neither antagonist has absolute selectivity and ICI 118551 may cause some inhibition of β_1-adrenoceptors at this concentration (Bilski *et al*., 1983).

At concentrations above 100 nM isoprenaline caused a vasoconstriction in large pulmonary arteries, which was substantially enhanced in the presence of 1 μM propranolol and abolished by the α-adrenoceptor antagonist phentolamine (10 μM, see Figure 2). All further experiments involving isoprenaline were therefore performed in the presence of phentolamine, for both sizes of artery.

Forskolin β-Adrenoceptor agonists stimulate adenylate cyclase and increase cyclic AMP, and it has been suggested that β-adrenoceptor-induced relaxation mediated by NO may be related to a rise in cyclic AMP. The vasorelaxant actions of the adenylate cyclase activator forskolin (10 nM–3 μM) were therefore examined by use of the same protocol as for isoprenaline, and following preincubation with L-NMMA (100 μM).

Chemicals and solutions

All drugs were obtained from Sigma (U.K.) with the exception of $PGF_{2\alpha}$ (Upjohn Pharmaceuticals Ltd., Crawley, U.K.), propranolol (ICI, Macclesfield, U.K.), ICI 118551 (erythro-1-(7-methylindan-4-yloxy-3-isopropylamino-butan-2-ol) Tocris Cookson, Brisol, UK) and L-NMMA (Novabiocem, Notts, U.K.) and were racemic mixtures unless otherwise designated. Other chemicals were of Analar quality (BDH, Southampton, U.K.). All drugs were prepared as stock solutions in deionized water with the exception of forskolin, which was dissolved in dimethylsulphoxide (DMSO; final bath concentration $<0.05\%$). PSS was made up for each experiment in water freshly drawn from a reverse osmosis-deionization plant with u.v. irradiation (Elgastat, Elga Ltd, U.K.).

Data and statistical analysis

Initial developed tensions are given as mN mm^{-1} artery length (Leach *et al*., 1992); tensions thereafter are expressed as percentage of the initial tension or of the response to KPSS as appropriate. The EC_{50} and maximum response were estimated for individual concentration-response curves by use of non-linear least-squares regression (SigmaStat, Jandel Scientific, U.S.A.) where appropriate. EC_{50} values were converted to negative logarithmic values for all statistical analysis, although for ease of comprehension EC_{50} values ($\pm$95% confidence limits) are given in the text. All other values are given as mean$\pm$s.e.mean. Data were compared by use of an unpaired Student's *t* test or one-way analysis of variance as appropriate (SigmaStat, Jandel Scientific, U.S.A.). Differences were considered significant at $P<0.05$.

Results

Noradrenaline-induced contraction and effect of propranolol

Noradrenaline induced a bell-shaped concentration-response curve in both large and small pulmonary arteries (Figure 1). In large arteries tension increased up to a maximum of 95.6±1.8% KPSS at 2 μM noradrenaline, thereafter tension fell ($n=8$). In small arteries noradrenaline induced only a meagre constriction which was substantially smaller than that in large arteries, with a maximum contraction of 12±1.5% KPSS ($n=9$, $P<0.001$ compared to large) at 0.2 μM noradrenaline, and the decline in tension at higher concentrations was more pronounced, such that at 10 μM tension was only 4.3±1.5% KPSS (Figure 1). Propranolol (1 μM) potentiated contraction to noradrenaline and reduced or abolished the decline in tension observed at higher concentrations in both large and small arteries (Figure 1). The effect of propranolol was substantially greater in small arteries, such that tension was effectively doubled at 0.2 μM (21±3.3% KPSS, $n=5$, $P<0.01$) and tension at 10 μM increased to 37.4±3.7% KPSS (Figure 1). In large arteries maximum tension only increased to 112.2±3.7% KPSS ($n=9$, $P<0.05$).

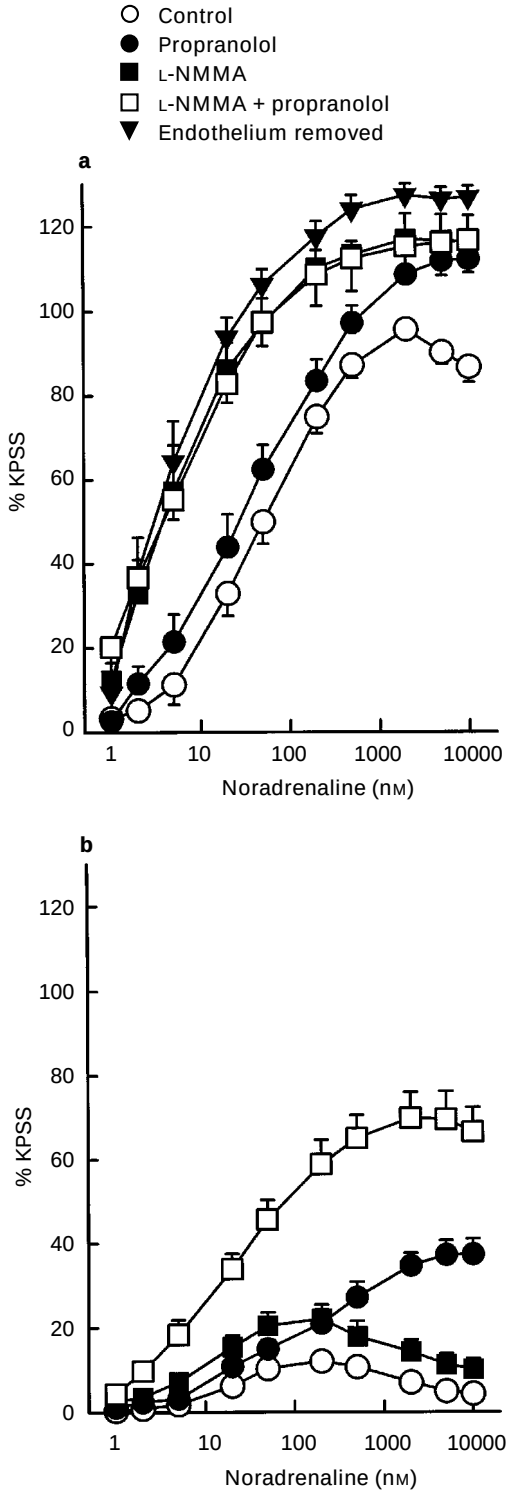

Figure 1 Noradrenaline concentration-response curves in (a) large and (b) small pulmonary arteries. The responses are expressed in terms of tension developed to 75 mM KCl PSS, iso-osmolar substitution for NaCl (KPSS). Each point is the mean of 4–10 experiments and vertical lines show s.e.mean. Where no error bar is shown, the error is smaller than the symbol.

Effect of L-NMMA on noradrenaline-induced contraction

Noradrenaline-induced contraction was potentiated by L-NMMA (100 μM) in both large and small arteries (Figure 1). In large arteries the reduction in tension above 2 μM was abolished, with an increase in maximum tension to 117±3.5% KPSS ($n=5$; $P<0.001$). There was also a significant shift to the left of the concentration response curve (EC_{50}: control: 45.0 (−21.3, +40.3); L-NMMA: 7.79 (−3.91, +7.87) nM, $P<0.05$). Although the peak tension of small arteries was nearly doubled, the bell-shape of the response curve was unchanged, and maximum tension (22.0±3.4% KPSS; $n=6$; $P<0.01$) occurred as before at 0.2 μM noradrenaline. In large arteries in which the endothelium had been removed the response to noradrenaline was not significantly different from that in the presence of L-NMMA (100 μM) (Figure 1).

Effect of propranolol and L-NMMA combined on noradrenaline-induced contraction

In large pulmonary arteries 100 μM L-NMMA and 1 μM propranolol in combination potentiated noradrenaline-induced contraction (maximum tension: 116.6±7.6% KPSS, $n=10$, $P<0.05$), abolished the decline in tension at higher concentrations, and shifted the curve to the left (EC_{50}: 4.59 (−2.09, +3.85) nM), but this effect was not significantly different from that of L-NMMA alone, indicating that propranolol had no additional effect (Figure 1). However, in marked contrast, in small pulmonary arteries the combination of L-NMMA and propranolol caused a significantly greater potentiation than either agent alone (maximal contraction 67.8±5.9% KPSS $n=4$, $P<0.001$), and a shift to the left of the response curve (EC_{50}: 19.41 (−5.94, +8.97) nM). In the absence of agonist-induced tension L-NMMA caused only a small increase in baseline resting tension in either small (1.48±0.56% KPSS, $n=5$) or large (5.5±2.4% KPSS, $n=6$) pulmonary arteries.

Effects on $PGF_{2\alpha}$-induced contraction

$PGF_{2\alpha}$ at 50 μM caused a sustained contraction of similar size in large and small arteries (large: 2.49±0.14 mN mm^{-1}, $n=47$; small: 2.20±0.19 mN mm^{-1}, $n=26$), equivalent to ~80% of the maximum response to $PGF_{2\alpha}$ (EC_{80}) in both size of artery. L-NMMA (100 μM) caused a small and consistent increase in $PGF_{2\alpha}$-induced tone, but this did not reach significance between groups (+L-NMMA: large: 3.13±0.25 mN mm^{-1}, $n=23$; small: 2.42±0.15 mN mm^{-1}, $n=18$). In large arteries following removal of the endothelium there was no significant change in $PGF_{2\alpha}$-induced tension (2.58±0.46 mN mm^{-1}, $n=11$). Phentolamine (10 μM), propranolol (1 μM), atenolol (5 μM) and ICI 118551 (0.1 μM) had no effect on either basal or $PGF_{2\alpha}$-induced tension.

Effects of isoprenaline

Isoprenaline caused a concentration-dependent relaxation of $PGF_{2\alpha}$-induced tension in small pulmonary arteries, with a maximum relaxation of 61.9±2.9% of initial tone at 300 nM ($n=6$, Figure 2), with a slight increase in tension at higher concentrations. In the presence of 100 μM L-NMMA the relaxation was significantly reduced (35.5±5.2%, $n=4$). In large pulmonary arteries, however, isoprenaline caused a relatively small relaxation that reached a maximum at 100 nM (18.8±1.5%, $n=7$), followed by an increase in tension as the concentration increased, so that at 3 μM there was a net contraction to 118.0±5.6% of initial tone (Figure 2). In the presence of 100 μM L-NMMA the relaxation component was completely abolished, leaving an apparent concentration-dependent contraction (Figure 2). The contractile activity of isoprenaline in large arteries was considerably enhanced following β-adrenoceptor blockade with propranolol (1 μM) (152±14% initial tone, $n=7$), and under these conditions a significant contraction was also apparent in small arteries (110±8% initial tone, $n=4$). The constrictor activity of isoprenaline that was uncovered by propranolol was abolished in the presence of the non-selective α-adrenoceptor antagonist phentolamine (10 μM) (Figure 2), suggesting that in this tissue isoprenaline has some cross-selectivity to α-adrenoceptors. It should be noted that dobutamine exhibited some characteristics similar to those of isoprenaline, but that tension was unstable even in the presence of phentolamine. In the absence of any agonist-induced tension, and in the presence of propranolol, isoprenaline also caused a concentration-dependent contraction from baseline tension in both large and small arteries, which was similarly abolished by phentolamine (data not shown). All further experiments with isoprenaline were therefore performed in the presence of 10 μM phentolamine.

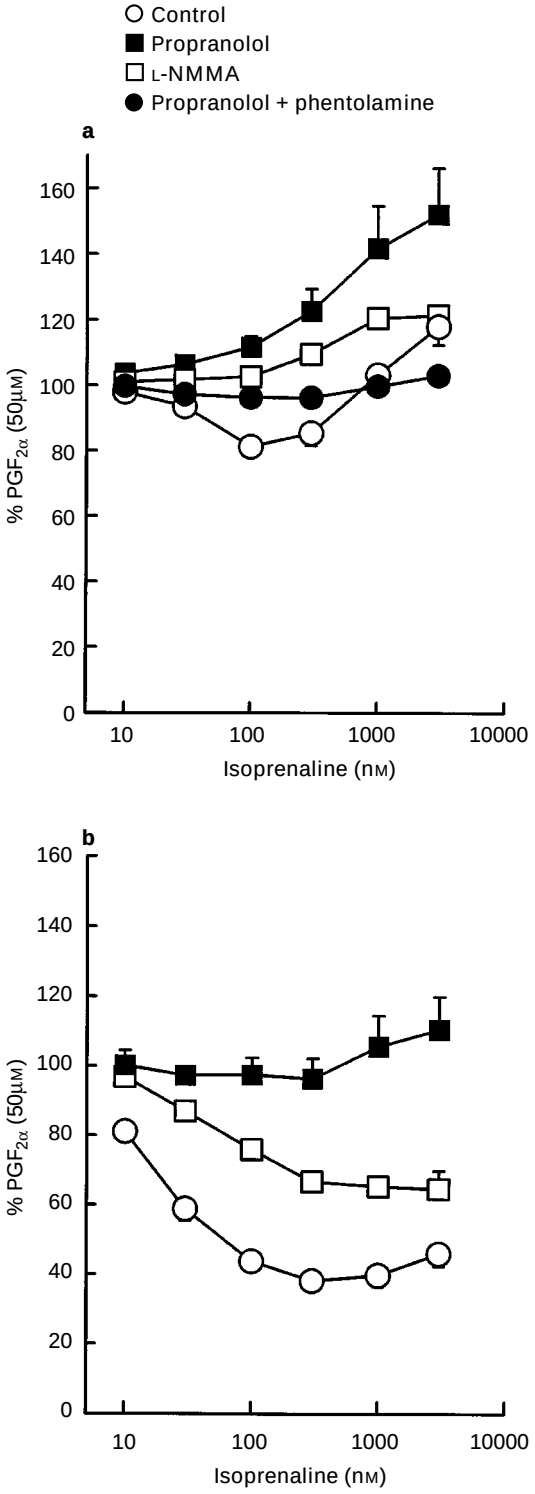

Figure 2 Isoprenaline concentration-response curves in large (a) and small (b) arteries constricted with 50 μM $PGF_{2\alpha}$. Each point is the mean of 4–7 experiments and vertical lines show s.e.mean. Where no error bar is shown, the error is smaller than the symbol.

Effects of L-NMMA, atenolol and ICI 118551 on isoprenaline-induced vasorelaxation

In the presence of phentolamine, relaxation to isoprenaline was greater in large arteries than in its absence (Figure 3), (maximum relaxation: 43.3±2.1%, $P<0.01$; EC_{50}: 309 (−102, +154) nM, $n=6$). In contrast, in small arteries the maximum relaxation was significantly smaller than in the absence of phentolamine (49.0±4.5%, $P<0.05$; EC_{50}: 151 (−34, +43) nM, $n=6$). L-NMMA (100 μM) reduced isoprenaline-induced relaxation by more than 80% in large arteries, but by less than 50% in small arteries (large: 7.4±1.5%, $n=6$, $P<0.01$; small: 26.8±5.8, $n=5$, $P<0.05$). There was no significant change in the EC_{50} (large: 291 (−106, +166) nM; small: 366 (−113, +163) nM). The response to isoprenaline was also substantially reduced in large arteries denuded of endothelium, with a maximum relaxation of 12.3±3.5% ($n=4$), and this was not significantly different from that in the presence of L-NMMA (Figure 3). This suggests that in large pulmonary arteries constricted with $PGF_{2\alpha}$ the large majority of β-adrenoceptor-mediated relaxation is related to NO, whereas in small arteries only about half is related to this mechanism.

The experiments were repeated in the presence of atenolol (5 μM, β_1-adrenoceptor antagonist) and ICI 118551 (0.1 μM, β_2-adrenoceptor antagonist). Experiments were again performed in the presence of phentolamine. ICI 118551 0.1 μM effectively abolished relaxation to 3 μM salbutamol, and 0.1 μM ICI 118551 and 5 μM atenolol in combination abolished relaxation to 3 μM isoprenaline. In the presence of atenolol, maximum relaxation to isoprenaline was reduced from control values in both small and large arteries (Figure 4), but this only reached significance in small arteries (large: 34.8±4.5%, $n=5$, NS; small: 35.0±1.9%, $n=6$, $P<0.05$). There was no significant change in the EC_{50} (large: 300 (−108, +170) nM, $n=5$; small: 303 (−126, +217) nM, $n=6$). In large arteries, L-NMMA in combination with atenolol caused a further substantial reduction in isoprenaline-induced relaxation to 8.5±2.3% ($n=4$, $P<0.01$), whereas in small arteries the reduction was smaller (to 24.0±2.1%, $n=5$, $P<0.05$). In both large and small arteries the maximum relaxation in the presence of atenolol and L-NMMA was not significantly different from the effect of L-NMMA on isoprenaline alone. There was no significant change in EC_{50} (large: 604 (−218, +341) nM; small: 171 (−96, +219) nM).

In the presence of the β_2-adrenoceptor antagonist ICI 118551 there was considerable variability between individual

arteries, similar but less extensive than that seen for the β_1-adrenoceptor agonist dobutamine. ICI 118551 approximately halved the relaxation to isoprenaline in both large and small pulmonary arteries (large: 18.0±4.6%, $P<0.05$; EC_{50}: 211 (−127, +322) nM; $n=6$; small: 25.6±10.7%, $P<0.05$; EC_{50}: 224 (−160, +565) nM; $n=6$) (Figure 5). In the presence of both L-NMMA and ICI 118551 isoprenaline-induced relaxation was very small, and at 3 μM isoprenaline relaxation was not significantly different from zero (large: 4.8±2.6%, $n=9$; small: 6.5±3.6%, $n=5$). It was not possible to fit the data.

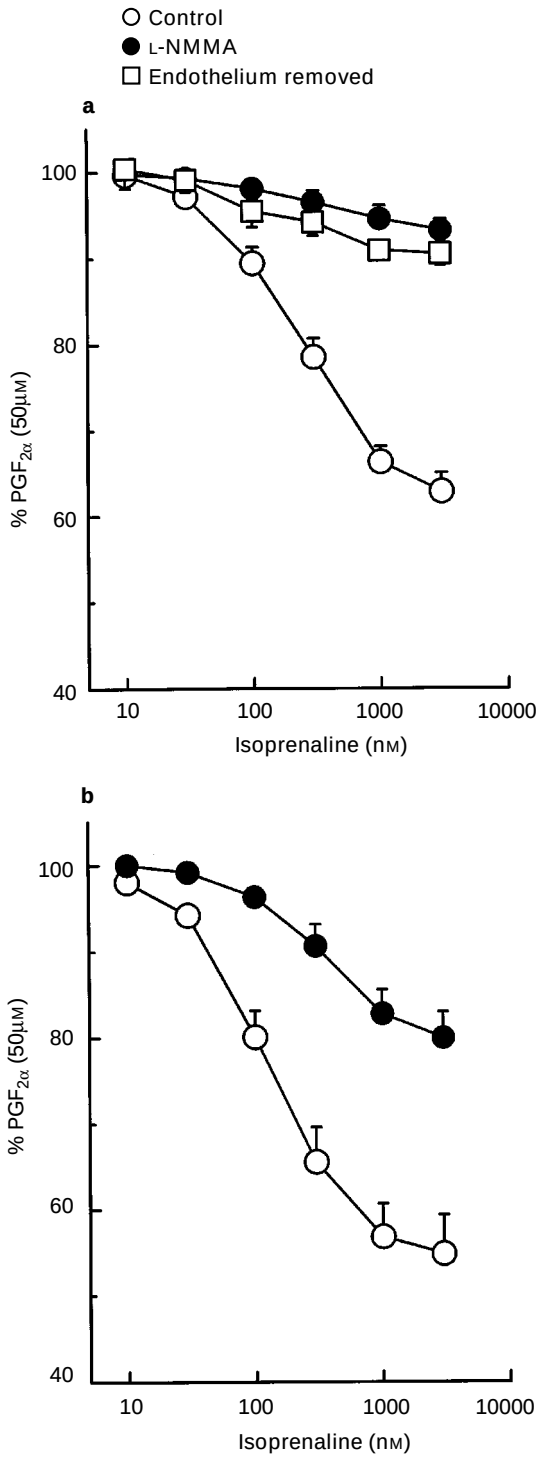

Figure 3 Isoprenaline concentration-response curves in large (a) and small (b) arteries constricted with 50 μM $PGF_{2\alpha}$, in the presence of phentolamine (10 μM). Each point is the mean of 4–6 experiments and vertical lines show s.e.mean. Where no error bar is shown, the error is smaller than the symbol.

Salbutamol-induced vasorelaxation

Phentolamine had no effect on the response to salbutamol and was therefore not used in these experiments. Salbutamol-induced relaxation was abolished by 1 μM propranolol ($n=4$)

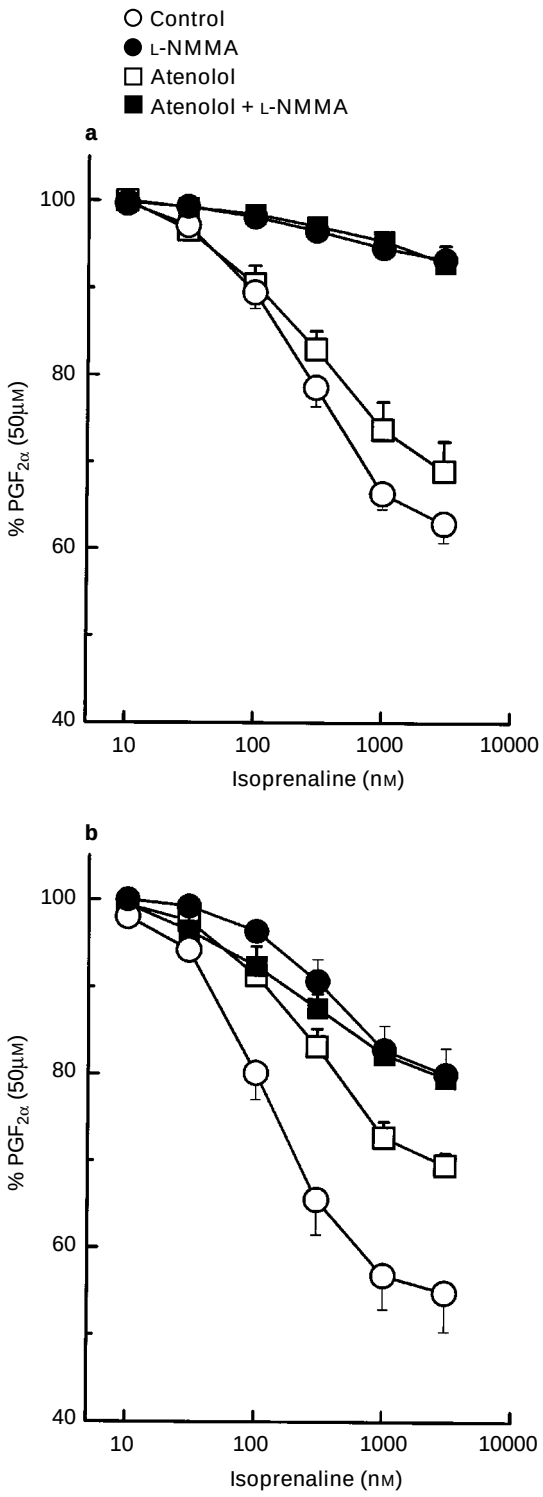

Figure 4 Isoprenaline concentration-response curves in large (a) and small (b) arteries constricted with 50 μM $PGF_{2\alpha}$ in the presence of phentolamine (10 μM) and atenolol (5 μM). The control and L-NMMA data from Figure 3 are also shown to aid comparison. Each point is the mean of 4–6 experiments and vertical lines show s.e.mean. Where no error bar is shown the error is smaller than the symbol.

(Figure 6). Salbutamol caused a concentration-dependent relaxation in both large and small pulmonary arteries constricted with $PGF_{2\alpha}$; the maximum relaxation in small arteries was significantly greater than that in large arteries (large: $34.7 \pm 6.4\%$, EC_{50}: 135 $(-50, +79)$ nM, $n=6$; small: $50.9 \pm 7.5\%$, EC_{50}: 116 $(-42, +67)$ nM, $n=6$; $P<0.05$). In the presence of L-NMMA salbutamol-induced relaxation was reduced by about 55% in small arteries, but by about 80% in large arteries (large: $8.3 \pm 1.3\%$, $n=5$, $P<0.01$; small: $23.0 \pm 0.7\%$, $n=5$, $P<0.05$), and there was a shift to the right in concentration response curves, although this only just reached significance in the small arteries (large: EC_{50}: 987 $(-497, +832)$ nM, $P<0.01$; small: EC_{50}: $216(-30, +49)$ nM, $P<0.05$) (Figure 6).

Forskolin-induced vasorelaxation

Forskolin caused a concentration-dependent vasorelaxation which was significantly greater in large arteries than in small

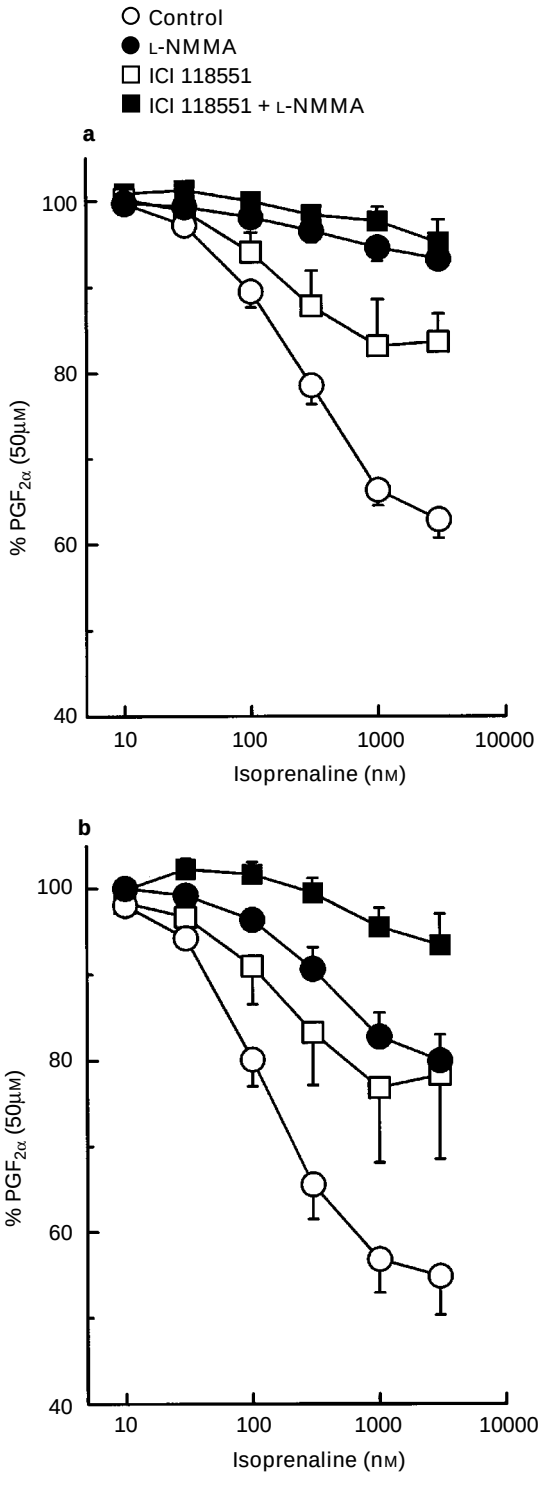

Figure 5 Isoprenaline concentration-response curves in large (a) and small (b) arteries constricted with 50 μM $PGF_{2\alpha}$ in the presence of phentolamine (10 μM) and ICI 118551 (0.1 μM). The control and L-NMMA data from Figure 3 are also shown to aid comparison. Each point is the mean of 4–6 experiments and vertical lines show s.e.mean. Where no error bar is shown, the error is smaller than the symbol.

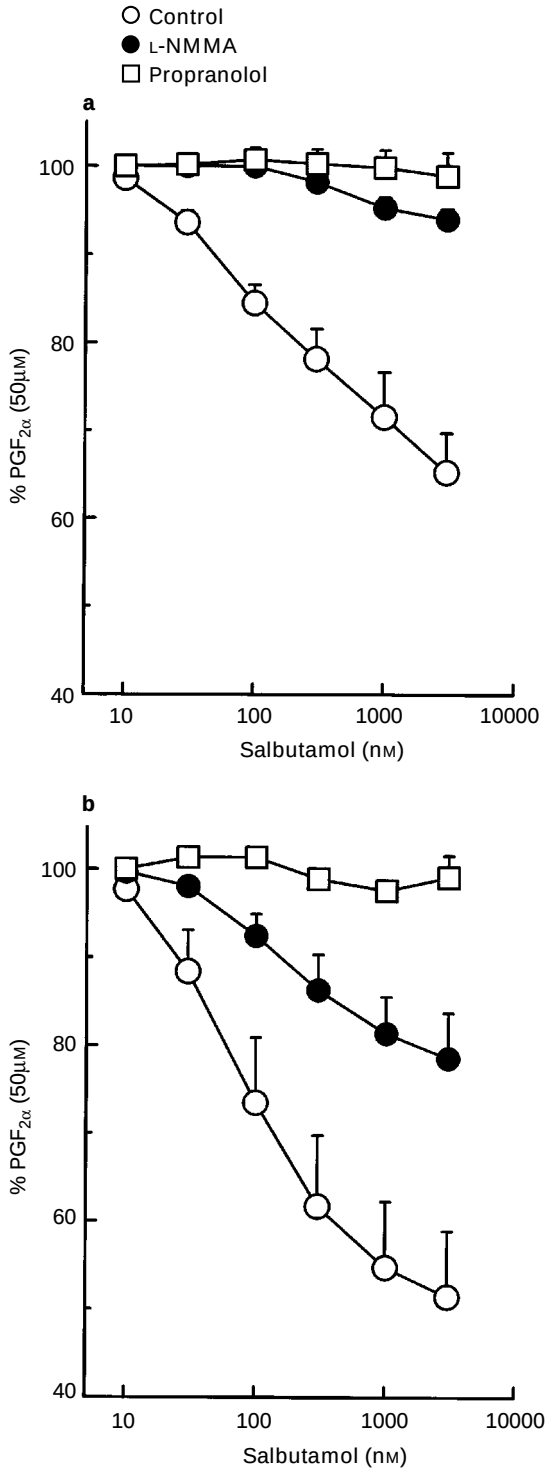

Figure 6 Salbutamol concentration-response curves in large (a) and small (b) arteries constricted with 50 μM $PGF_{2\alpha}$. Each point is the mean of 4–6 experiments and vertical lines show s.e.mean. Where no error bar is shown, the error is smaller than the symbol.

arteries (large: 92.9±4.8%, EC_{50}: 300 (−71, +93) nM, $n=7$; small: 56.2±5.2%, $P<0.01$, EC_{50}: 436 (−175, +293) nM, $n=5$) (Figure 7). L-NMMA (100 μM) caused a significant rightward shift of the response curves (large: EC_{50}: 1536 (−666, +1177) nM, $P<0.01$, $n=6$; small: EC_{50}: 1140 (−470, +799) nM, $P<0.05$, $n=6$), although there was no significant change in the fitted maximum relaxation (large: 65.7±3.4%, $n=6$; small: 47.0±5.3%, $n=6$). These results suggest that at concentrations below 1 μM a large proportion of forskolin-induced relaxation, particularly in large pulmonary arteries, is mediated by an L-NMMA-dependent mechanism (Figure 7).

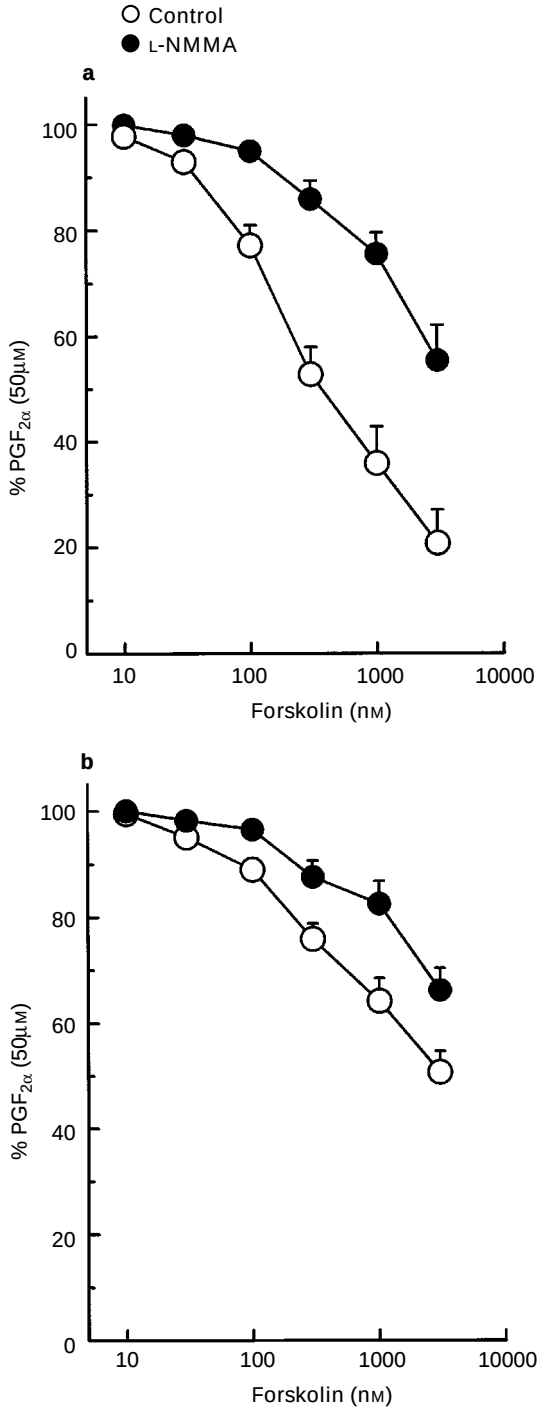

Figure 7 Forskolin concentration-response curves in large (a) and small (b) arteries constricted with 50 μM $PGF_{2\alpha}$. Each point is the mean of 5–7 experiments and vertical lines show s.e.mean. Where no error bar is shown, the error is smaller than the symbol.

Discussion

Our results are consistent with previous studies in that the vasoconstrictor response to noradrenaline was considerably less in small compared to large pulmonary arteries (Altiere *et al*., 1983; Leach *et al*., 1992), with peak tension in small arteries only ~15% of that in large (Figure 1). The noradrenaline concentration-response curve was also bell-shaped, with a reduction in tone at higher concentrations. This was more significant in small arteries where tension started to decline at concentrations about 0.2 μM, and by 10 μM had fallen back almost to the baseline. There was no decline in tension in large arteries until the concentration of noradrenaline was above 2 μM. A similar profile has been observed in pulmonary arteries, but not aorta, of the rabbit (Altiere *et al*., 1983). The meagre response to noradrenaline in small arteries could be related to either a reduced efficacy of α-adrenoceptor agonists for inducing contraction, or an increased component of vasorelaxant mechanisms. As noradrenaline also has β-adrenoceptor agonist activity, it is reasonable to suggest that at higher concentrations the reduction in tension may be due to activation of β-adrenoceptors with a lower affinity for noradrenaline than α-adrenoceptors. Indeed, in the presence of propranolol to block β-adrenoceptors, the reduction in tension at higher concentrations was abolished, although the increase in maximum tension was more substantial in small arteries (Figure 1). The results suggest that in contrast to large conduit pulmonary arteries, where the propranolol-sensitive component of tension was only ~20% at 10 μM noradrenaline, in small arteries the β-mediated response starts to predominate at concentrations of noradrenaline above 0.2 μM.

It has been proposed that both α- and β-adrenoceptor activation can lead to release of NO (Gray & Marshall, 1992; Graves & Poston, 1993; Kaneko & Sunano, 1993; Ohgushi *et al*., 1993), and it has been shown that removal of the endothelium or inhibition of NO synthase potentiates noradrenaline induced-vasoconstriction in pulmonary arteries (Cederqvist *et al*., 1991; Leach *et al*., 1992; Shinozuka *et al*., 1992). In the present experiments the L-arginine analogue L-NMMA significantly increased tension developed to noradrenaline in both large and small pulmonary arteries (Figure 1), but, the response to L-NMMA differed substantially between large and small arteries. In large arteries the decline in tension at concentrations of noradrenaline above 2 μM was abolished and there was both a 20% increase in peak tension and a 10 fold reduction in the EC_{50}. In small arteries peak tension was approximately doubled at 0.2 μM, yet the shape of the concentration-response curve remained unchanged (Figure 1). The response curves in the presence of L-NMMA were significantly different from those in the presence of propranolol in both groups, indicating that mechanisms other than those involving β-adrenoceptor activation were contributing to the stimulation of NO. However, in the presence of both L-NMMA and propranolol the response curve for large arteries did not differ from that in the presence of L-NMMA alone, nor from the response in the absence of an endothelium. This suggests that in large pulmonary arteries the effects of β-adrenoceptor activation under these conditions are entirely mediated via NO and the endothelium. In small arteries the effects of L-NMMA and propranolol in combination were radically different from those in large arteries, and showed a large increase in tension at all concentrations of noradrenaline that was substantially greater than that induced by either agent alone. This suggests that in small pulmonary arteries a significant component of the β-adrenoceptor-mediated response is independent of NO. These potentially important differences were investigated further by use of β-agonists and selective antagonists, and are discussed below.

Our results indicate that in large arteries at least, noradrenaline-induced vasoconstriction is associated with an NO-dependent component that is largely independent of β-adrenoceptors. It has been suggested that an increase in tension or wall stretch alone can increase NO production (Amerini

et al., 1995), and L-NAME has been shown to increase substantially the sensitivity and maximum response of rat mesenteric arteries to K^+ depolarization (Graves & Poston, 1993). However, in these experiments L-NMMA caused a significant increase in tension in large arteries even at 2 nM noradrenaline and below, when in the absence of L-NMMA tension was extremely low at this concentration (Figure 1), suggesting that other mechanisms may be involved. Studies on both systemic arteries (Kaneko & Sunano, 1993; Ohgushi *et al*., 1993) and pulmonary artery of the pig (Tulloh *et al*., 1994), have suggested that α-adrenoceptor stimulation may directly stimulate NO production, although the mechanism remains unclear.

Isoprenaline is a classical non-selective β-adrenoceptor agonist and has been used extensively in the study of β-adrenoceptor mechanisms over many years, and as far as we are aware has not been previously shown to have any vasoconstrictor activity at reasonable (<50 μM) concentrations. The vasoconstriction to isoprenaline was potentiated by propranolol, obviating any β-adrenoceptor-mediated mechanism in either the smooth muscle, endothelium or neural endings. However, it was abolished by phentolamine (10 μM, Figure 2), and substantially reduced by the α_1-antagonist prazosin (1 μM, data not shown), suggesting activity at α-adrenoceptors. It was also not dependent on the presence of $PGF_{2\alpha}$, as in the presence of propranolol vasoconstriction could be induced from the baseline (data not shown). This phenomenon has not been previously described as far as we are aware, and apparently does not occur in systemic arteries of the rat (e.g. mesenteric, Graves & Poston, 1993; Hucks and Ward, unpublished observations), although isoprenaline has been shown to increase Ca^{2+} in rat parotid acinar cells via an α-adrenoceptor-mediated mechanism (Tanimura *et al*., 1990). It should be noted that the efficacy of isoprenaline as an α-agonist in our preparation was relatively low, although in large pulmonary arteries significant vasoconstriction was apparent at 100 nM in the presence of propranolol, and that it was significantly less effective in small arteries (Figure 2). As expected, the β_2-agonist salbutamol had no vasoconstrictor activity even in the presence of propranolol. These unusual findings have important implications both in terms of the interpretation of other studies, and α-adrenoceptor heterogeneity and selectivity between different vascular beds and species. They clearly require further investigation, although this falls outside the remit of the present study. All subsequent experiments involving isoprenaline were as a result performed in the presence of 10 μM phentolamine throughout.

In the presence of phentolamine, isoprenaline induced a concentration-dependent relaxation against $PGF_{2\alpha}$-induced contraction that was not significantly different between large and small pulmonary arteries, with a maximum relaxation and EC_{50} of around 45% and 225 nM, respectively. The maximum relaxation was somewhat less than that previously shown for rat small mesenteric arteries contracted with KPSS (66%), whereas the EC_{50} was around 10 fold higher (Graves & Poston, 1993). Inhibition of NO synthesis with L-NMMA reduced isoprenaline-induced relaxation in both groups, but to a much greater extent in large arteries, where more than 80% of the relaxation was abolished by L-NMMA, compared to less than 50% in small arteries. A similar effect was observed in large arteries denuded of endothelium (Figure 3). These results are consistent with those from the noradrenaline studies and imply that in large pulmonary arteries the large majority of β-agonist-induced relaxation is mediated by NO, whereas in small pulmonary arteries approximately half the relaxation is related to other mechanisms, presumably the classically described increase in cyclic AMP. The proportion of NO-independent relaxation induced by isoprenaline in small pulmonary arteries was similar to that previously described in rat small mesenteric arteries (Graves & Poston, 1993). Our results are in clear agreement with previous studies on systemic arteries, that have suggested that isoprenaline-induced relaxation is at least partly dependent on NO and the endothelium (Gray & Marshall, 1992; Blankesteijn & Thien, 1993; Graves & Poston, 1993; Delpy *et al*., 1996).

It was noticeable that in small arteries the maximum relaxation to isoprenaline was significantly less in the presence of phentolamine, and, although not commented upon in the paper, relaxation to isoprenaline has been shown to be greater in mesenteric arteries constricted with noradrenaline than with K^+ (Graves & Poston, 1993).We have also found that endothelium-dependent relaxation is greater following constriction with phenylephrine as compared to $PGF_{2\alpha}$ or KPSS (Peng *et al*., 1996). These results imply that α-adrenoceptor stimulation might potentiate NO-mediated relaxation, although the mechanism remains to be elucidated.

Further experiments were performed in order to determine the β-adrenoceptor subtypes involved in the NO-dependent and -independent components of the response to isoprenaline. Graves and Poston (1993) have previously shown that the vasorelaxant response to the nominal β_1-agonist dobutamine could be abolished by L-NAME in the rat mesenteric arteries. We were unable to elicit repeatable responses to this agent in our preparation, which has also been shown to promote significant tachyphylaxis (Graves & Poston, 1993). However, in the presence of the β_2-antagonist ICI 118551, L-NMMA substantially reduced relaxation to isoprenaline in both large and small pulmonary arteries (Figure 5), suggesting that in this preparation β_1-adrenoceptor-mediated relaxation was also largely dependent on NO release. In contrast to the results of Graves and Poston (1993), who showed that salbutamol-induced relaxation in small mesenteric arteries was unaffected by L-NAME, we found that both salbutamol-induced relaxation (Figure 6) and isoprenaline-induced relaxation in the presence of the β_1-antagonist atenolol (Figure 4) were reduced by L-NMMA, suggesting that β_2-adrenoceptor-mediated relaxation also involves NO in the pulmonary vasculature. Gray & Marshall (1992) have previously demonstrated that removal of the endothelium abolished salbutamol-induced relaxation in rat thoracic aorta and, in large pulmonary arteries, we also found that the majority of the β_2-mediated relaxation was inhibited by L-NMMA, leaving a very small NO-independent component. In small arteries this NO-independent component was larger, but in the case of salbutamol-induced relaxation was still less than 50% of the total. The NO-dependent relaxation induced by isoprenaline in the presence of atenolol was smaller than that induced by salbutamol, although in the presence of L-NMMA the maximum relaxations to isoprenaline, isoprenaline plus atenolol, and salbutamol were all not significantly different from each other for small or large arteries. The relatively small difference between the NO-dependent component of salbutamol and isoprenaline plus atenolol could be explained by either some cross-reactivity of salbutamol and/or atenolol to β_1- and β_2-adrenoceptors respectively, or a smaller efficacy of isoprenaline for β_2-adrenoceptors in this preparation. The role of NO in the response to salbutamol may depend on the specific vascular bed, as although Graves & Poston (1993) showed no effect of L-NAME in rat small mesenteric arteries, Wang *et al*. (1993) found that vasorelaxation to salbutamol was inhibited by L-NAME in rat aorta, and in the *in vivo* study of Gardiner, *et al*. (1991) vasodilatation to salbutamol was attenuated by L-NAME in rat hind limb.

These results suggest that the large majority of β_1-adrenoceptor-mediated relaxation in large and small pulmonary arteries involves NO, whereas β_2-adrenoceptor-mediated relaxation has a significant proportion that is NO-independent, but only in small arteries. If the NO-dependent component relates to a direct action on the endothelium, as proposed by Gray & Marshall (1992), this may reflect differences in either β-adrenoceptor density or efficacy in the smooth muscle. It is worth noting that the total β_2-adrenoceptor-mediated relaxation in these pulmonary arteries was greater than that mediated by β_1-adrenoceptors, whereas in mesenteric arteries salbutamol-induced relaxation has been found to be relatively modest (Graves & Poston, 1993). This is consistent with the findings of O'Donnell & Wanstall (1981) that although there is

a mixed population of β_1- and β_2-adrenoceptors in main pulmonary artery of the rat, β_2-adrenoceptors predominate. β_2-Adrenoceptors are also reported to be of greater importance in the control of pulmonary vascular resistance (Hyman *et al*., 1990).

The mechanisms by which β-adrenoceptor stimulation can lead to NO-dependent relaxation are unclear. β-Adrenoceptors are linked to adenylate cyclase via a G-protein, and vasorelaxation was previously thought to be mediated entirely via activation of adenylate cyclase and a subsequent increase in cyclic AMP. However, Gray & Marshall (1992) showed that removal of the endothelium of rat aorta preparations or addition of L-NOARG, another inhibitor of NO synthase, abolished the vasorelaxation to isoprenaline and forskolin, a direct activator of adenylate cyclase, and that both isoprenaline and forskolin caused an increase in smooth muscle cyclic GMP. They postulated that an increase in cyclic AMP in the endothelial cell could cause activation of NO synthase (Gray & Marshall, 1992), and more recently Rebich *et al*. (1995) have shown an association between the elevation of cyclic AMP and release of NO in pig pial arteries. Graier *et al*. (1992) have proposed a possible mechanism for this process, whereby forskolin or elevation of cyclic AMP causes hyperpolarization of the endothelial cell, thereby increasing the electrochemical inward driving force for Ca^{2+}, and thus Ca^{2+} entry, and hence stimulating NO synthase (Graier *et al*., 1992). However Graves & Poston (1993) showed a slight enhancement of forskolin-induced relaxation in the presence of L-NAME in rat mesenteric arteries, and Kuhn *et al*. (1991) were unable to demonstrate any increase in NO release from bovine cultured endothelial cells following experimental increases in cyclic AMP. Our results in pulmonary arteries are more similar to those of Delpy *et al*. (1996) than Gray & Marshall (1992), in that the forskolin concentration-response curve was shifted to the right by L-NMMA, with no change in maximum relaxation. In direct contrast to the β-agonists examined in our preparation, forskolin was a more effective vasodilator in large as opposed to small pulmonary arteries.

Although our results would be consistent with both β-adrenoceptor-mediated NO release and, possibly, stimulation of NO synthase in the endothelial cell by cyclic AMP, they are by no means conclusive. 8-Bromo-cyclic GMP and activators of guanylate cyclase have been shown to potentiate the isoprenaline-induced rise in cyclic AMP and subsequent relaxation, and it has been suggested that the effects of inhibition of NO synthesis on β-adrenoceptor mediated relaxation could be explained in terms of a reduction in basal NO, and hence loss of this potentiation (Maurice & Haslam, 1990; Lugnier & Komas, 1993; Delpy *et al*., 1996). In contrast Gray & Marshall (1992) have shown that L-NOARG abolishes the rise in smooth muscle cyclic GMP induced by isoprenaline without decreasing cyclic AMP. Moreover, if the L-NMMA-sensitive and -insensitive components of both forskolin- and β-agonist-induced relaxations are all mediated by changes in smooth muscle cell cyclic AMP, it is difficult to explain why in our experiments β-agonists were in general more effective in small pulmonary arteries, whereas forskolin was more effective in large arteries.

Whatever the mechanisms involved, our results show conclusively that β-agonist-induced vasorelaxation in pulmonary arteries is at least partially dependent on NO, and that in large arteries this pathway forms by far the largest component. As inhibition of NO synthesis also affected vasorelaxation to forskolin, there is clear evidence that in pulmonary arteries there is substantial interaction between cyclic GMP- and cyclic AMP-mediated mechanisms, either at the level of the NO synthase in the endothelium or in the smooth muscle cell itself. Although the results also suggest that the meagre vasoconstriction to noradrenaline in small pulmonary arteries is partially due to a greater β-adrenoceptor-mediated component than in large arteries, noradrenaline stimulation was associated with NO release that was independent of β-adrenoceptors. It is unclear whether this is directly related to α-adrenoceptor-stimulation and we are currently investigating the mechanisms involved.

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