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Potentiation of cyclic AMP-mediated vasorelaxation by phenylephrine in pulmonary arteries of the rat

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1 α_1-adrenoceptor agonists may potentiate relaxation to β-adrenoceptor agonists, although the mechanisms are unclear. We compared relaxations induced by β-adrenoceptor agonists and cyclic AMP-dependent vasodilators in rat pulmonary arteries constricted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or the α_1-adrenoceptor agonist phenylephrine (PE). In addition, we examined whether differences were related to cyclic AMP- or nitric oxide (NO) and cyclic GMP-dependent pathways.

2 Isoprenaline-induced relaxation was substantially potentiated in arteries constricted with PE compared with $PGF_{2\alpha}$. Methoxamine was similar to PE, whereas there was no difference between $PGF_{2\alpha}$ and 30 mM KCl. The potentiation was primarily due to a marked increase in the NO-independent component of relaxation, from $9.1 \pm 1.7\%$ for $PGF_{2\alpha}$ to $55.1 \pm 4.4\%$ for PE. NO-dependent relaxation was also enhanced, but to a lesser extent ($\sim 50\%$). Relaxation to salbutamol was almost entirely NO-dependent in both groups, and was potentiated $\sim 50\%$ by PE.

3 Relaxation to forskolin (activator of adenylate cyclase) was also enhanced in PE constricted arteries. Part of this relaxation was NO-dependent, but the major effect of PE was to increase the NO-independent component. Propranolol diminished but did not abolish the potentiation. There was no difference in response to CPT cyclic AMP (membrane permeant analogue) between PE and $PGF_{2\alpha}$, suggesting that mechanisms distal to the production of cyclic AMP were unchanged.

4 Relaxation to sodium nitroprusside (SNP) was the same for PE and $PGF_{2\alpha}$, although relaxation to acetylcholine (ACh) was slightly depressed. This implies that potentiation by PE does not involve the cyclic GMP pathway directly.

5 Mesenteric arteries constricted with PE did not show potentiation of isoprenaline-induced relaxation compared to those constricted with $PGF_{2\alpha}$, suggesting that this effect may be specific to the pulmonary circulation.

6 These results clearly show that PE potentiates both the NO-independent and -dependent components of cyclic AMP-mediated relaxation in pulmonary arteries of the rat, although the effect on the former is more profound. We suggest that potentiation of both components is largely due to direct activation of adenylate cyclase *via* α_1-adrenoceptors, within the smooth muscle and endothelial cells respectively.

Keywords: Pulmonary artery; phenylephrine; cyclic AMP; adenylate cyclase; α-adrenoceptors; β-adrenoceptors; nitric oxide

Abbreviations: ACh, acetylcholine; cyclic AMP, adenosine 3′,5′-cyclic monophosphate; cyclic GMP, guanosine 3′,5′-cyclic monophosphate; CPT cyclic AMP, 8-(4-chlorophenylthio)-adenosine 3′,5′-cyclic monophosphate; KPSS, physiological salt solution containing 75 mM KCl, equimolar substitution for NaCl; L-NMMA, L-N^G-monomethylarginine; ME, methoxamine; NO, nitric oxide; PDE, phosphodiesterase; PE, phenylephrine; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; PSS, physiological salt solution; SNP, sodium nitroprusside

Introduction

The action of vasodilators may be dependent on the type of vasoconstrictor agent, (e.g. Plane & Garland, 1996), and it has been suggested that stimulation of α_1-adrenoceptors may potentiate relaxation to β-adrenoceptor agonists in pulmonary arteries (Peng *et al*., 1996; Priest *et al*., 1997). This could have functional significance, as sympathetic stimulation causes changes in pulmonary vascular resistance that are mediated *via* noradrenaline and α- and β-adrenoceptors (Hyman *et al*., 1990). We have previously shown that relaxation of $PGF_{2\alpha}$ constricted rat pulmonary arteries by β-adrenoceptor agonists is largely endothelium dependent and mediated *via* nitric oxide (NO) (Priest *et al*., 1997), and there is evidence that noradrenaline-induced vasoconstriction of pulmonary arteries may be depressed due to stimulation of NO synthesis *via* both α- and β-adrenoceptors (MacLean *et al*., 1993; Tulloh *et al*., 1994; Priest *et al*., 1997). Constriction of porcine pulmonary arteries with the α_1-adrenoceptor agonist phenylephrine (PE) has also been associated with an endothelium-dependent rise in cyclic GMP (Pepke-Zaba *et al*., 1993). This is consistent with studies on systemic arteries that have proposed that α_1-adrenoceptor agonists directly stimulate NO synthesis (Kaneko & Sunano, 1993).

It is not clear however whether the putative PE-induced potentiation of relaxation to β-adrenoceptor agonists involves NO. There is known to be significant cross-talk between cyclic AMP and cyclic GMP pathways (Lincoln & Cornwell, 1993; Vigne *et al*., 1994), and activators of guanylate cyclase have been shown to potentiate isoprenaline stimulated cyclic AMP accumulation and relaxation in vascular smooth muscle (Maurice & Haslam, 1990; Delpy *et al*., 1996). However PE has also been shown to stimulate or potentiate accumulation of cyclic AMP directly in a variety of other cell types (Pedarzani

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& Storm, 1996; Traish *et al*., 1997; Ruan *et al*., 1998), although there are few reports on vascular smooth muscle (Izumi *et al*., 1996).

In order to determine the mechanisms by which PE might potentiate cyclic AMP-induced relaxation, we compared the effects of constriction with either $PGF_{2\alpha}$, PE or 30 mM KCl on the vasorelaxant properties of β-adrenoceptor agonists and cyclic AMP-dependent vasodilators in pulmonary arteries of the rat, and examined whether any enhancement of response was related to cyclic AMP- or NO/cyclic GMP-dependent pathways.

Methods

Tissue preparations

Adult, male Wistar rats (250–350 g) were killed by anaesthetic overdose (intraperitoneal injection of pentarbitone, 50 mg kg^{-1}), as approved by the Home Office Inspector. The heart and lungs and a section of large intestine 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. Pulmonary (1141 ± 22 μm i.d., $n = 142$) and mesenteric arteries (344 ± 69 μm, $n = 20$) were dissected free of connective tissue and mounted in a small vessel myograph as previously described (Leach *et al*., 1992; Priest *et al*., 1997), and equilibrated with 5% CO_2 in O_2, (pH 7.35–7.40, 37°C). In some experiments 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; Priest *et al*., 1997). Arteries producing less than 1 mN mm^{-1} were discarded. After washing with PSS the arteries returned to baseline tone.

Experimental protocols

Cumulative concentration-response relationships were constructed for PE and $PGF_{2\alpha}$, and following pre-incubation for 20 min with L-N^G-monomethylarginine (L-NMMA; 100 μM). Experiments with PE were also performed in the presence of 10 μM propranolol, and propranolol and L-NMMA combined. Experiments were time matched in separate arteries.

Influence of vasoconstrictor type on subsequent relaxation to 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 pulmonary arteries, following stable precontraction with either $PGF_{2\alpha}$ (50 μM), the α_1-adrenergic agonists PE (10 μM) or methoxamine (ME, 10 μM), or PSS containing 30 mM KCl (equimolar substitution for NaCl). These concentrations were chosen so as to elicit the same degree of tension (~80% KPSS), and therefore match any stimulation of NO induced by stretch alone. Similar experiments were performed on mesenteric arteries for isoprenaline, following preconstriction with $PGF_{2\alpha}$ or PE only. We have previously demonstrated that isoprenaline concentrations above 100 nM cause a small but significant α-adrenoceptor-mediated vasoconstriction in pulmonary arteries of the rat (Priest *et al*., 1997). With the exception of experiments using PE or ME as the vasoconstrictor, all other studies involving isoprenaline were therefore performed in the presence of 10 μM phentolamine; we have previously shown that phentolamine has no effect on salbutamol-induced relaxation (Priest *et al*., 1997). Tension was allowed to stabilize following every addition, and is expressed in terms of the initial induced tension. The role of nitric oxide (NO) and NO synthase was investigated in some experiments following pre-incubation for 20 min with L-NMMA (100 μM).

Influence of vasoconstrictor type on cyclic AMP-mediated relaxation

β-adrenoceptors agonists act *via* adenylate cyclase to increase cyclic AMP. We therefore also investigated whether vasoconstriction with $PGF_{2\alpha}$ or PE affected vasorelaxation induced by forskolin, a direct activator of adenylate cyclase; CTP cyclic AMP (8-(4-chlorophenylthio)-adenosine 3′,5′-cyclic monophosphate), a membrane permeant analogue of cyclic AMP; and papaverine, a phosphodiesterase (PDE) inhibitor that primarily causes relaxation by reducing the breakdown of cyclic AMP (Holzmann *et al*., 1977). Experiments were performed as described above. As forskolin has an NO-dependent component in this preparation (Priest *et al*., 1997), the effect of L-NMMA on forskolin-induced vasorelaxation was also examined. PE has been reported to enhance forskolin-induced relaxation *via* β-adrenoceptors in corpus cavernosum smooth muscle (Traish *et al*., 1997), and some experiments were therefore also performed in the presence of 10 μM propranolol.

Influence of vasoconstrictor type on NO- and cyclic GMP-mediated relaxation

We have previously shown that β-adrenoceptor-mediated vasorelaxation in pulmonary arteries is partly related to NO. In order to determine whether the type of vasoconstrictor influenced other vasorelaxants mediated by cyclic GMP, cumulative concentration response relationships were constructed for acetylcholine (ACh) and sodium nitroprusside (SNP) following preconstriction with $PGF_{2\alpha}$ (50 μM) or PE (10 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.), L-NMMA and CTP cyclic AMP (Novabiocem, Notts., U.K.). Other chemicals were of Analar quality (BDH, Southampton, U.K.). Drugs were prepared as stock solutions using PSS, with the exception of forskolin which was dissolved in DMSO (final bath concentration $<0.05\%$). PSS was made up for each experiment using 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. Concentration-response curves to the vasoconstrictors are expressed as a percentage of the response to 75 mM KCl (iso-osmolar substitution for NaCl). Relaxation is expressed as a percentage of the initial tension. The EC_{50} and extrapolated maximum response were estimated for individual concentra-

tion-response curves using non-linear least-squares regression (SigmaStat, Jandel Scientific, U.S.A.) where appropriate. EC_{50} values were converted to negative logarithmic values (PD_2) for all statistical analysis, although for each of comprehension EC_{50} values [±95% confidence limits] are given in the text. All other values are given as means±s.e.mean. Data were compared using 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

As we found that propranolol significantly increased constriction to 10 μM PE in pulmonary arteries (see below), we examined the effect of propranolol on the concentration-response relationship for PE, and in the presence of L-NMMA (Figure 1). Propranolol (10 μM) increased the maximum constriction to PE in pulmonary arteries (Control: 65.2±5.2% response to 75 mM KCl, $n=11$; propranolol: 85.7±5.6%, $n=5$; $P<0.05$), and caused a shift to the left of the response curve, although this did not reach significance (EC_{50}: Control: 91 [−23, +31] nM; propranolol: 70 [−43, +117] nM). In the presence of L-NMMA alone maximum constriction was also increased, and the EC_{50} significantly reduced (104±7%, $n=6$, $P<0.001$; EC_{50}. 31 [−8, +12] nM, $n=6$, $P<0.001$; both compared to control). There was no difference between the response to PE in the presence of L-NMMA alone or L-NMMA and propranolol combined (Combined: 95.3±5.8%; EC_{50}: 21.5 [−12.1, +27.8] nM, $n=4$). The response to $PGF_{2\alpha}$ in the presence of L-NMMA was also examined to determine whether the L-NMMA-sensitive component of PE constriction was specific, or related to developed tension (Figure 1). Although L-NMMA also significantly decreased the EC_{50} for $PGF_{2\alpha}$ (Control: 8.5 [−4.2, +8.6] μM, $n=6$; L-NMMA: 2.0 [−1.0, +1.9], $n=4$; $P<0.05$), there was no change in maximum constriction (Control: 105±4%, $n=6$; L-NMMA: 100±5%, $n=4$).

Response to vasorelaxants: initial tensions

There was no difference between the tension developed in pulmonary arteries in response to 50 μM $PGF_{2\alpha}$ (2.65 ±0.15 mN mm^{-1}, $n=42$), 10 μM PE (2.69±0.13 mN mm^{-1}, $n=41$), 10 μM ME (2.45±0.53 mN mm^{-1}, $n=6$), or 30 mM [K^+] (2.53±0.25 mN mm^{-1}, $n=10$). Preincubation with L-NMMA (100 μM) did not significantly increase $PGF_{2\alpha}$-induced tension (3.00±0.34 mN mm^{-1}, $n=15$), but did increase PE-induced tension (3.75±0.08 mN mm^{-1}, $n=17$; $P<0.001$), consistent with the data obtained from the cumulative concentration-response experiments above. In the presence of 10 μM propranolol PE-induced tension was also significantly enhanced (3.57±0.36 mN mm^{-1}, $n=10$; $P<0.01$), but in the presence of both propranolol and L-NMMA there was no further significant increase in tension (4.11±0.23 mN mm^{-1}, $n=4$). Propranolol had no effect on either basal or $PGF_{2\alpha}$-induced tension ($n=5$). In mesenteric arteries, there was no significant difference between tension developed to 50 μM $PGF_{2\alpha}$ (3.00±0.27 mN mm^{-1}, $n=5$) and 10 μM PE (3.40±0.53 mN mm^{-1}, $n=4$).

Isoprenaline and salbutamol-induced vasorelaxation

Pulmonary arteries constricted with PE showed a much larger maximum relaxation to isoprenaline (102.0±1.6% initial tone; $n=6$; $P<0.001$) and a smaller EC_{50} (65 [−15, +20] nM; $n=6$; $P<0.001$) compared to those constricted with $PGF_{2\alpha}$ (38.5±4.0%; EC_{50}: 238 [−89, +142] nM; $n=6$) (Figure 2). L-NMMA (100 μM) caused a significant reduction in the maximum relaxation to isoprenaline in both PE (55.1±4.4%; $n=6$; $P<0.001$) and $PGF_{2\alpha}$ constricted arteries (9.1±1.7%; $n=8$; $P<0.001$), although the maximum relaxation in arteries constricted with PE was still substantially greater than in those constricted with $PGF_{2\alpha}$ ($P<0.001$). L-NMMA increased the EC_{50} for isoprenaline in PE constricted arteries (217 [−101, +187] nM; $n=6$; $P<0.01$), but did not alter the EC_{50} in $PGF_{2\alpha}$ constricted arteries (272 [−75, +105] nM; $n=8$; NS). In the presence of L-NMMA there was no longer any significant

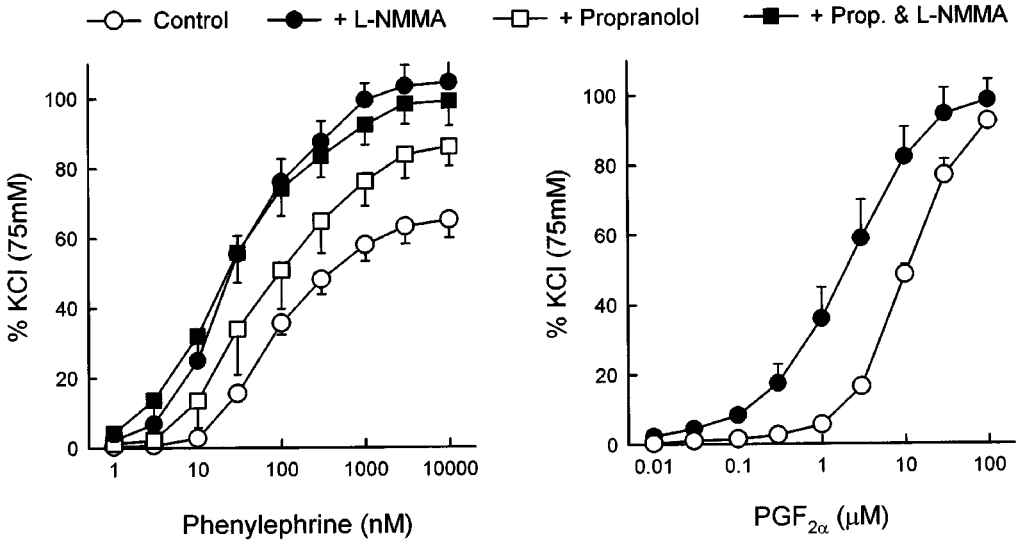

Figure 1 PE (left hand panel) and $PGF_{2\alpha}$ (right hand panel) concentration-response curves in pulmonary arteries, and for PE in the presence of 100 μM L-NMMA, 10 μM propranolol, and L-NMMA and propranolol combined. The response is expressed in terms of tension developed to 75 mM KCl PSS (KPSS). Each point is the mean of 4–11 experiments, and symbols are mean±s.e.mean; where no error bar is shown, the error is smaller than the symbol.

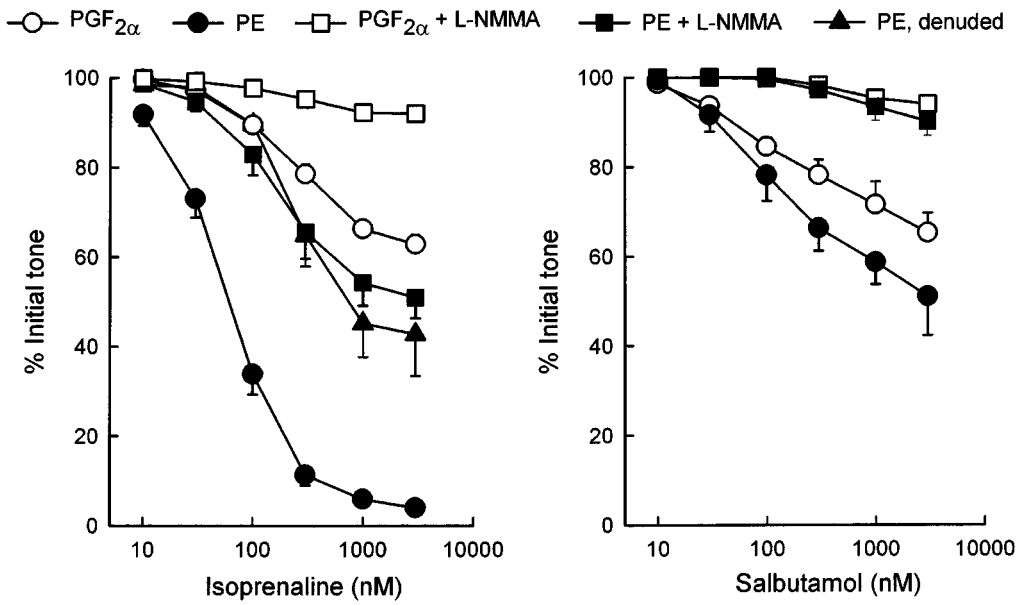

Figure 2 Isoprenaline (left hand panel) and salbutamol (right hand panel) concentration-response curves in pulmonary arteries constricted with 50 μM $PGF_{2\alpha}$ or 10 μM PE, and in the presence of 100 μM L-NMMA, or removal of the endothelium. Each point is the mean of 4–8 experiments, and symbols are mean ± s.e.mean; where no error bar is shown, the error is smaller than the symbol.

difference in EC_{50} between PE and $PGF_{2\alpha}$ constricted arteries. Removal of the endothelium had a similar effect to application of L-NMMA in PE constricted arteries ($n=4$; Figure 2).

In contrast to isoprenaline, relaxation mediated by the β_2-adrenoceptor agonist salbutamol differed less dramatically between PE and $PGF_{2\alpha}$-constricted pulmonary arteries (Figure 2). Maximum relaxation to salbutamol in PE constricted pulmonary arteries was 49.7 ± 1.9% ($n=5$), which was significantly greater than that in $PGF_{2\alpha}$ constricted arteries (34.7 ± 6.4%; $n=6$; $P<0.05$). There was no significant difference in EC_{50} (PE: 143 [−48, +72] nM; $PGF_{2\alpha}$: 135 [−50, +79] nM). In the presence of L-NMMA, salbutamol induced only a very small relaxation in either PE or $PGF_{2\alpha}$ constricted arteries (Figure 2). It was not possible to adequately fit the data from these latter experiments; however the relaxation induced by 3000 nM salbutamol was not significantly different between PE (9.8 ± 3.2%; $n=4$) and $PGF_{2\alpha}$ (6.0 ± 1.3%; $n=5$) constricted arteries (Figure 2).

In order to determine whether the differences in isoprenaline-induced relaxation in pulmonary arteries were specific to either PE or $PGF_{2\alpha}$, experiments were also performed with methoxamine, another α_1-adrenoceptor agonist, and 30 mM KCl, which causes constriction by depolarization. Isoprenaline-induced relaxation was no different in arteries constricted with methoxamine compared to PE (ME: max relation: 105 ± 3.4%; EC_{50}: 74.7 [−30.2, +50.6] nM; $n=6$) (Figure 3). There was also no difference between arteries constricted with 30 mM KCl and those constricted with $PGF_{2\alpha}$ (KCl: max relaxation: 42.9 ± 7.0%; EC_{50}: 184 [−98, +208] nM; $n=6$). As $PGF_{2\alpha}$ and 30 mM KCl induce constriction by entirely different mechanisms, this would imply that there is a specific α_1-adrenoceptor-mediated potentiation of isoprenaline-induced relaxation.

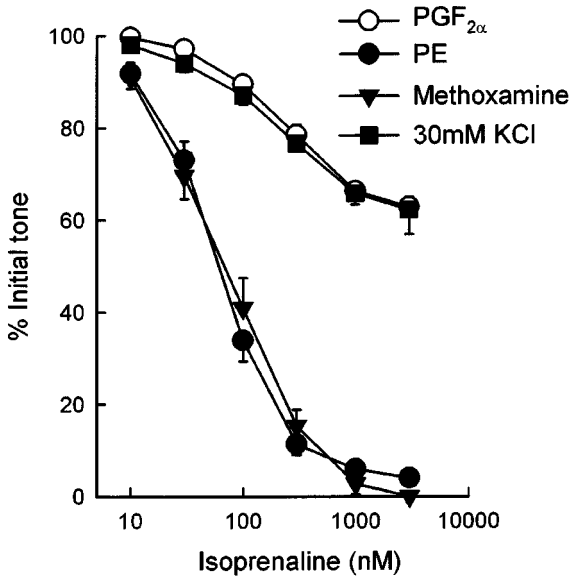

Figure 3 Isoprenaline concentration-response curves in pulmonary arteries constricted with 50 μM $PGF_{2\alpha}$, 10 μM PE, 10 μM methoxamine, or 30 mM KCl. Each point is the mean of six experiments, and symbols are mean ± s.e.mean; where no error bar is shown, the error is smaller than the symbol.

cyclic AMP-mediated relaxation

Isoprenaline binds to β-adrenoceptors, stimulates adenylate cyclase and so increases cyclic AMP. In order to determine whether the potentiation of isoprenaline-induced relaxation was due to an action at or distal to the receptor, we compared the effects of PE and $PGF_{2\alpha}$ constriction on relaxation to forskolin (direct activator of adenylate cyclase), papaverine (phosphodiesterase inhibitor), and CPT cyclic AMP (membrane permeant analogue of cyclic AMP) in pulmonary arteries. The response to forskolin is shown in Figure 4. Forskolin caused complete relaxation of developed tension in both PE (99.4 ± 3.7%; $n=8$) and $PGF_{2\alpha}$ constricted arteries

(97.6±1.1%; $n=6$). However the EC_{50} for forskolin was significantly less in PE (43.2 [−10.8, +14.4] nM; $P<0.001$) compared with $PGF_{2\alpha}$ constricted arteries (281 [−68, +81] nM). L-NMMA shifted the forskolin response curves to the right for both PE and $PGF_{2\alpha}$, although the extent of the shift was greater for $PGF_{2\alpha}$ (EC_{50}: PE: 150 [−37, +50] nM; $n=7$; $P<0.001$; $PGF_{2\alpha}$: 1824 [−627, +956] nM; $n=4$; $P<0.005$), and estimated maximum relaxation was substantially reduced in $PGF_{2\alpha}$, but not PE constricted arteries (PE: 107.7±1.9%; $n=8$; $PGF_{2\alpha}$: 59.7±5.5%; $n=4$; $P<0.01$). This is consistent with a NO-dependent component of forskolin-induced relaxation, as previously described (Priest *et al.*, 1997).

In the presence of propranolol, the response curve in PE constricted arteries was also shifted to the right (134.2 [−41.0, +58.9] nM; $n=6$; $P<0.01$), but the EC_{50} was still significantly less than that for $PGF_{2\alpha}$ ($P<0.01$) (Figure 4). Propranolol and

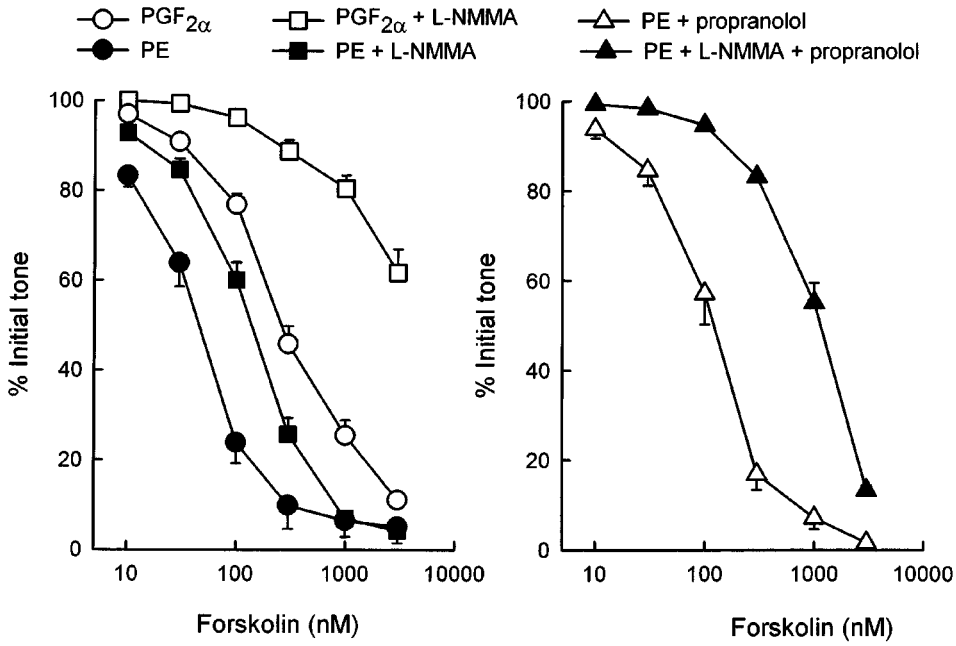

Figure 4 Forskolin concentration-response curves in pulmonary arteries constricted with 50 μM $PGF_{2\alpha}$ or 10 μM PE, and in the presence of 100 μM L-NMMA. The right hand panel shows the response to forskolin in PE constricted arteries in the presence of 10 μM propranolol, and L-NMMA plus propranolol. Each point is the mean of 4–8 experiments, and symbols are mean±s.e.mean; where no error bar is shown, the error is smaller than the symbol.

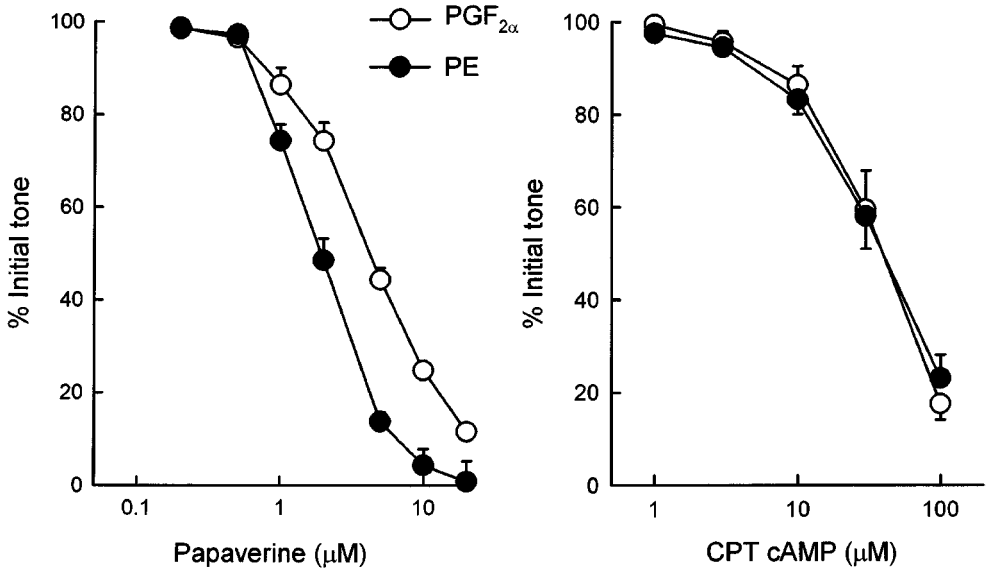

Figure 5 Papaverine (left hand panel) and CPT cyclic AMP (right hand panel) concentration-response curves in pulmonary arteries constricted with 50 μM $PGF_{2\alpha}$, or 10 μM PE. Each point is the mean of 4–6 experiments, and symbols are mean±s.e.mean; where no error bar is shown, the error is smaller than the symbol.

L-NMMA in combination caused a further increase in EC_{50} in PE constricted arteries (1126 [−261, +339] nM; $n=4$; $P<0.001$), such that there was no longer any difference in EC_{50} compared to $PGF_{2\alpha}$ in the presence of L-NMMA. However, maximum relaxation was unaltered in the presence of propranolol and L-NMMA, and was still substantially greater in PE constricted arteries (105.6±4.4%; $n=4$; $P<0.001$) (Figure 4).

The response to papaverine and CPT cyclic AMP is shown in Figure 5. Papaverine caused relaxation below the baseline for both PE (106±4%; $n=4$) and $PGF_{2\alpha}$ (123±8%; $n=4$) constricted arteries. The EC_{50} for papaverine was less in PE (2.10 [−0.40, +0.49] μM; $n=4$; $P<0.05$) compared to $PGF_{2\alpha}$ constricted arteries (6.66 [−2.27, +3.44] μM; $n=4$). Constriction with 30 mM K^+ showed a similar response to $PGF_{2\alpha}$ (30 mM K^+: 5.20 [−1.53, +2.17] μM; $n=4$), and propranolol had no effect on the response to papaverine in PE constricted arteries (105.5±1.4%; $n=4$; EC_{50}: 2.38 [−1.11, +2.08] μM; $n=4$) (data not shown in Figure 5). There was no difference in the response to CPT cyclic AMP between arteries constricted with PE and $PGF_{2\alpha}$ (PE: 35.6 [−11.1, +16.3] μM; $n=6$; $PGF_{2\alpha}$: 32.7 [−11.8, +18.4] μM; $n=6$) (Figure 5).

ACh- and cyclic GMP-mediated relaxation

In contrast to the potentiating effect of PE constriction on isoprenaline- and forskolin-induced relaxation in pulmonary arteries, the ACh-response curve was shifted to the right in PE

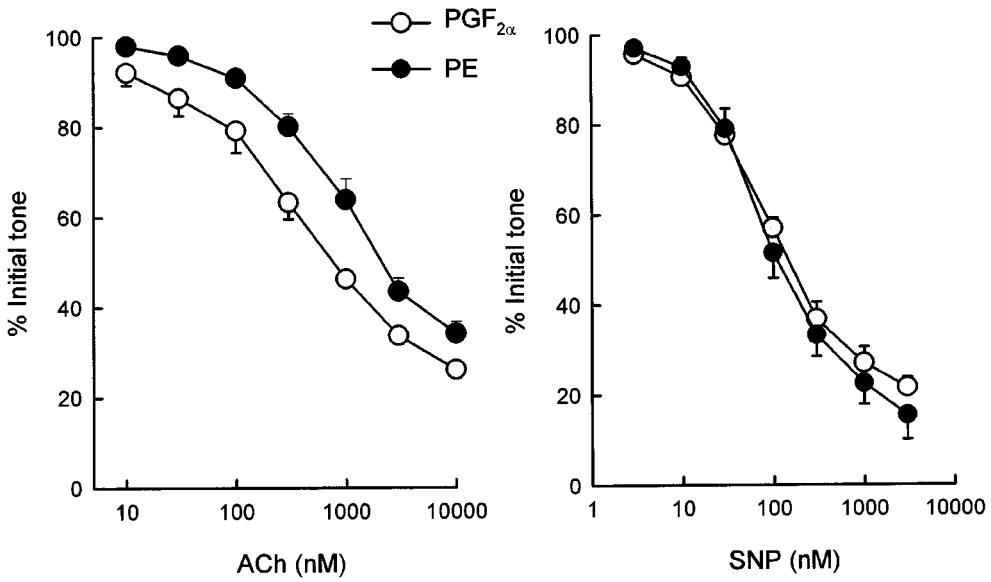

Figure 6 ACh (left hand panel) and SNP (right hand panel) concentration-response curves in pulmonary arteries constricted with 50 μM $PGF_{2\alpha}$, or 10 μM PE. Each point is the mean of 6–8 experiments, and symbols are mean±s.e.mean; where no error bar is shown, the error is smaller than the symbol.

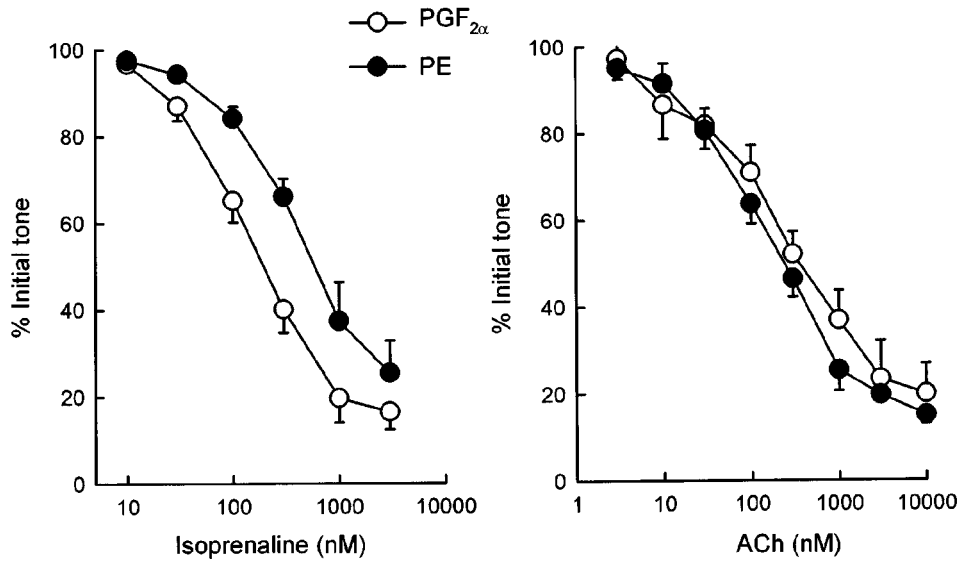

Figure 7 Isoprenaline (left hand panel) and ACh (right hand panel) concentration-response curves in mesenteric arteries constricted with 50 μM $PGF_{2\alpha}$, or 10 μM PE. Each point is the mean of 4–6 experiments, and symbols are mean±s.e.mean; where no error bar is shown, the error is smaller than the symbol.

compared to $PGF_{2\alpha}$ constricted arteries (EC_{50}: PE: 899 [−309, +471] nM; $n=7$; $PGF_{2\alpha}$: 251 [−124, +247] nM; $n=6$; $P<0.01$). Maximum relaxation was unaltered (PE: 73.0±2.1%; $PGF_{2\alpha}$: 74.4±3.1%) (Figure 6). The response to SNP was the same for both PE and $PGF_{2\alpha}$ (PE: 87.4±5.5%; 75.1 [−25.9, +39.6] nM; $n=7$; $PGF_{2\alpha}$: 81.3±2.7%; 74.7 [−9.9, +11.4] nM; $n=8$). In this preparation vasorelaxation to 10 μM ACh is abolished by 100 μM L-NMMA.

Mesenteric arteries

Figure 7 shows the response of mesenteric arteries to isoprenaline and ACh. Although there was no difference in the maximum relaxation to isoprenaline between arteries constricted with PE (86±9%; $n=4$) and $PGF_{2\alpha}$ (91±5%; $n=6$), mesenteric arteries constricted with PE showed a small significant increase in EC_{50} compared to those constricted with $PGF_{2\alpha}$ (PE: 426 [−86, +108] nM; $n=4$; $PGF_{2\alpha}$: 162 [−47, +66] nM; $n=6$; $P<0.01$). There was however no difference between the ACh response curves (Figure 7).

Discussion

Our results show a clear potentiation of isoprenaline and forskolin induced vasorelaxation in pulmonary arteries constricted with PE or methoxamine, relative to that with $PGF_{2\alpha}$ or 30 mM KCl. Both NO-dependent and independent components of relaxation to these agents were enhanced, though to different extents, which may indicate a common underlying mechanism. However the response to PE in this preparation appears to be quite complex, with additional direct effects on NO synthesis and possibly β-adrenoceptors.

Maximum tension induced by PE in pulmonary arteries was significantly enhanced by inhibition of NO synthase with L-NMMA, whereas that induced by $PGF_{2\alpha}$ was not. It therefore seems unlikely that stimulation of NO synthesis during constriction to PE can be attributed solely to an increase in tension *per se*, as suggested for noradrenaline (Graves & Poston, 1993; Amerini *et al*., 1995), although this may be responsible for leftward shift of the response curves seen for both agonists. This is consistent with previous reports of a direct action of α_1-adrenoceptor agonists on NO synthesis (Pepke-Zaba *et al*., 1993; Kaneko & Sunano, 1993; Priest *et al*., 1997). Surprisingly, propranolol also increased PE-induced tension, suggesting that in this tissue PE may have some cross-reactivity to β-adrenoceptors, similar to that described in human corpus cavernosum smooth muscle (Traish *et al*., 1997). Propranolol in combination with L-NMMA had the same effect as L-NMMA alone, implying that the depression of PE-induced tension uncovered by propranolol is largely mediated by NO. This suggests that stimulation of NO by PE in this preparation is partly mediated *via* β-, rather than α_1-adrenoceptors. It is possible that PE does not bind directly to β-adrenoceptors, but instead increases the efficacy of endogenous β-adrenoceptor agonists in a fashion similar to that described for isoprenaline below, or by an indirect action on the β-adrenoceptors themselves. However, as propranolol had no effect on $PGF_{2\alpha}$-induced tension, this implies that there is little endogenous β-adrenoceptor agonist activity in this preparation. Further experiments, including ligand-binding studies, are required to settle this question.

Pulmonary arteries constricted with PE showed a substantially enhanced relaxation to isoprenaline compared with those constricted with $PGF_{2\alpha}$ (Figure 2). This was primarily due to a dramatic increase in the L-NMMA-insensitive and endothelium independent component of relaxation, which in $PGF_{2\alpha}$ constricted arteries was very small. The L-NMMA sensitive, and thus presumably NO dependent component was increased by approximately 50%. The potentiation appears to be specific to α_1-adrenoceptor agonist stimulation, as similar results to those with PE were obtained for methoxamine, whereas the response in arteries constricted by a depolarizing solution containing 30 mM K^+ was identical to that in arteries constricted with $PGF_{2\alpha}$ (Figure 3). These results also suggest that the potentiation by PE and methoxamine is not related to any depolarizing influence that these agents may have, and is therefore unrelated to the mechanisms described by Plane & Garland (1996) to account for differences in ACh-induced relaxation in arteries constricted by noradrenaline or the thromboxane mimetic U46619, or to any possible hyperpolarizing action of β-adrenoceptor agonists (Randall & McCulloch, 1995).

The potentiation of salbutamol (β_2-adrenoceptor agonist)-induced relaxation by PE was less marked than that for isoprenaline (non-selective β-adrenoceptor agonist). This was entirely due to the lack of any significant potentiation of the NO-independent component of relaxation, as the NO-dependent component was enhanced to a similar extent for salbutamol as it was for isoprenaline (~50%, Figure 2). The lack of any significant NO-independent β_2-mediated relaxation in large pulmonary arteries, as reported here and previously (Priest *et al*., 1997), suggests that β_2-adrenoceptors are primarily located on the endothelium rather than the smooth muscle. In comparison, Ashikaga *et al*. (1996) have reported that whereas bovine aortic endothelial cells were more sensitive to catecholamines than smooth muscle cells, the latter were almost selective for β_1-adrenoceptor agonists.

β-adrenoceptor agonists stimulate production of cyclic AMP by adenylate cyclase, and we examined whether the potentiation of isoprenaline-induced relaxation by PE was due to mechanisms distal to the β-adrenoceptor. Although forskolin, a direct activator of adenylate cyclase, caused complete relaxation of pulmonary arteries constricted with both PE and $PGF_{2\alpha}$, the EC_{50} was significantly smaller in arteries constricted with PE, suggesting potentiation of adenylate cyclase activity. As previously described (Priest *et al*., 1997), forskolin relaxation was partially dependent on NO, but the effect of L-NMMA was more pronounced in $PGF_{2\alpha}$ compared with PE constricted arteries (Figure 4), and maximum relaxation was decreased.

However, although PE has also been reported to enhance forskolin-induced cyclic AMP accumulation in human corpus cavernosum smooth muscle, Traish *et al*. (1997) attributed this to activity of PE at β-adrenoceptors. As we found that propranolol increased PE-induced tension in pulmonary artery (see above), we also examined the response to forskolin in PE constricted arteries in the presence of propranolol. Propranolol shifted the forskolin concentration response curve to the right, although the EC_{50} was still significantly smaller than that for $PGF_{2\alpha}$ constricted arteries. However the shift induced by L-NMMA was greater in the presence of propranolol, such that there was no longer any apparent difference in EC_{50} between PE and $PGF_{2\alpha}$ constricted arteries, although there was still a large difference in maximum relaxation (Figure 4). These results suggest that part, but by no means all of the PE-induced potentiation of forskolin-induced relaxation is due to a β-adrenoceptor mediated pathway, and possible mechanisms for both components are discussed below. Even taking into account the reduced potentiation in the presence of propranolol, the effects of PE on forskolin-induced relaxation are consistent with those on isoprenaline-induced relaxation, in

that both are potentiated by PE relative to, and both show a greater NO-independent component than in $PGF_{2\alpha}$-constricted arteries.

The apparent effects on adenylate cyclase might also be related to changes in the rate of breakdown of cyclic AMP by PDEs, or by alterations in the sensitivity to cyclic AMP of end point mechanisms such as protein kinase A. The latter can be discounted, as there was no difference in the response to CPT cyclic AMP between PE and $PGF_{2\alpha}$ constricted arteries. In contrast, the papaverine concentration response curve was shifted to the left in the presence of PE, relative to those with $PGF_{2\alpha}$ or 30 mM K^+ (Figure 5). However, inhibition of PDE activity by papaverine would also be more effective if the rate of cyclic AMP production was increased, and any decrease in PDE activity might be expected to show some potentiation of the response to CPT cyclic AMP. As this did not occur, it seems reasonable to suggest that PE increases cyclic AMP production rather than inhibits cyclic AMP breakdown.

Classical endothelium-dependent vasodilators such as ACh activate NO synthase *via* Ca^{2+}-calmodulin and dissociation from caveolin; cyclic AMP is not involved in this pathway (Michel & Feron, 1997). However, there is a growing body of evidence that Ca^{2+}-independent processes can also activate NO synthase (Fleming *et al*., 1997). It has been suggested that the NO-dependent component of β-adrenoceptor agonist and forskolin induced relaxation is due to either direct activation of the NO synthase by cyclic AMP (Gray & Marshall, 1992; Rebich *et al*., 1995; Priest *et al*., 1997), or to cross-talk at the level of the cyclic AMP and cyclic GMP dependent PDEs (Lugnier & Komas, 1993; Vigne *et al*., 1994; Delpy *et al*., 1996). Potentiation of the NO-dependent component of β-adrenoceptor agonist induced relaxation by PE might therefore be mediated indirectly *via* the same mechanisms responsible for potentiation of NO-independent relaxation, i.e. those involving cyclic AMP, or by other mechanisms including a rise in endothelial cell Ca^{2+} or actions on the guanylate cyclase pathway itself. However relaxation to SNP or ACh was not enhanced by PE constriction relative to $PGF_{2\alpha}$ and indeed ACh was surprisingly slightly less effective (Figure 6). This implies that the potentiation of the NO-dependent component of β-adrenoceptor agonist induced relaxation by PE is *via* a mechanism that does not involve the cyclic GMP pathway directly, and is independent of mechanisms involved in ACh-stimulation of NO synthase. It could however involve cyclic AMP, as PE has previously been shown to increase endothelial cell cyclic AMP *via* an α_1-adrenoceptor mediated mechanism (Bacic *et al*., 1992). The reasons behind the small inhibition of ACh-relaxation by PE are unclear, but it is possible that there may be competition between the cyclic AMP and Ca^{2+}-calmodulin stimulatory pathways for NO synthase. This remains to be investigated.

The lack of potentiation of either ACh- or SNP-induced relaxation by PE also suggests that the potentiation of β-adrenoceptor agonist induced NO-dependent relaxation is not due to cross-talk between cyclic AMP and cyclic GMP pathways, which has previously been proposed as the mechanism underlying the endothelium-dependent component of β-adrenoceptor agonist and forskolin induced relaxation (Lugnier & Komas, 1993; Delpy *et al*., 1996). However it would be consistent with the alternative hypothesis of a direct action of cyclic AMP on the NO synthase (Gray & Marshall, 1992; Rebich *et al*., 1995; Priest *et al*., 1997).

There was no potentiation of isoprenaline-induced relaxation by PE in mesenteric arteries, and no change in ACh-induced relaxation. The lack of effect in mesenteric arteries could reflect either the absence of a mechanism linking α_1-adrenoceptors to the adenylate cyclase (see below), different adenylate cyclase isoforms, or different populations of α_1-adrenoceptor sub-types. Further experiments are required before this can be clarified, and indeed whether the lack of response in mesenteric arteries is common to other systemic artery types. It is notable that relaxation to another agent that increases cyclic AMP, the putative cyclic AMP specific PDE inhibitor tetramethylpyrazine (Lin *et al*., 1993), is also partially selective in the rat to pulmonary relative to mesenteric arteries (Peng *et al*., 1996).

Our results would be most consistent with the hypothesis that α_1-adrenoceptor agonists potentiate activity of adenylate cyclase, causing increased accumulation of cyclic AMP. PE has been shown to cause or potentiate cyclic AMP accumulation in other tissues, including neurons (Pedarzani & Storm, 1996), corpus cavernosum smooth muscle (Traish *et al*., 1997), Chinese hamster ovary (CHO) and Rat-1 fibroblast cells transfected with α_1-adrenoceptors (Horie *et al*., 1995; Ruan *et al*., 1998), and in particular aorta (Izumi *et al*., 1996) and endothelial cells (Bacic *et al*., 1992). As cyclic AMP has been reported to stimulate NO synthesis (Gray & Marshall, 1992; Rebich *et al*., 1995; Priest *et al*., 1997), we can speculate that the proposed α_1-adrenoceptor mediated stimulation of NO synthesis might also be related to a rise in endothelial cell cyclic AMP.

Several mechanisms have been suggested for stimulation of cyclic AMP accumulation by PE. PE increases smooth muscle intracellular Ca^{2+}, and Ca^{2+} is known to stimulate adenylate cyclase activity (Ho *et al*., 1995). However both $PGF_{2\alpha}$ and K^+ depolarization also increase Ca^{2+}, and Traish *et al*. (1997) ruled out a rise in Ca^{2+} as the mechanism underlying potentiation of forskolin-induced relaxation (Traish *et al*., 1997). In the latter study the response was attributed to cross-reactivity of PE on β-adrenoceptors (Traish *et al*., 1997), but although we have demonstrated that PE does have some effect mediated by β-adrenoceptors in rat pulmonary arteries, there was still significant potentiation of the relaxation to forskolin and papaverine in the presence of propranolol. It is also difficult to see how stimulation of β-adrenoceptors alone by PE could increase the maximum response to isoprenaline, as shown here, although a shift in the EC_{50} might be expected. Alternatively, PE and other α_1-adrenoceptor agonists could cause direct activation of G_S-adenylate cyclase *via* α_1-adrenoceptors. Horie *et al*. (1995) have shown just such an action in CHO cells transfected with α_{1B}-adrenoceptors, and Ruan *et al*. (1998) have shown that PE causes accumulation of cyclic AMP in Rat-1 fibroblasts transfected with α_{1A}-adrenoceptors. Although these two studies are convincing, further work is required before the precise mechanisms underlying the PE-induced potentiation of cyclic AMP-mediated relaxation appertaining to this tissue can be determined.

In conclusion, this study clearly shows that PE can potentiate both NO dependent and independent components of cyclic AMP-mediated relaxation in pulmonary arteries of the rat, and we suggest that this may be largely due to direct activation or synergistic potentiation of adenylate cyclase activity, mediated *via* α_1-adrenoceptors. The study also reiterates the point made by Plane & Garland (1996) that the action of vasodilators may depend on the type of vasoconstrictor, although the mechanisms in this case are clearly entirely different from those described in their paper.

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