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Evidence for a dilator function of 8-iso prostaglandin $F_{2\alpha}$ in rat pulmonary artery

Karen B. Jourdan, Timothy W. Evans, Nicholas P. Curzen & [1]Jane A. Mitchell

Unit of Critical Care Medicine, Royal Brompton Hospital, Sydney Street, London SW3 6NP

1 8-Iso prostaglandin $F_{2\alpha}$ (8-iso $PGF_{2\alpha}$) is one of a series of prostanoids formed independently of the cyclo-oxygenase pathway. It has been shown to be upregulated in many conditions of oxidant stress where its formation is induced by free radical-catalysed actions on arachidonic acid. As 8-iso $PGF_{2\alpha}$ is formed *in vivo* in diseases in which oxidant stress is high such as septic shock, we have assessed the relative potency and efficacy of this compound in pulmonary arteries from control and lipopolysaccharide (LPS)-treated rats.

2 Several studies have characterized the contractile actions of 8-iso $PGF_{2\alpha}$ on various smooth muscle preparations, but its potential dilator actions have not been addressed. Thus these studies examined both the contractile and dilator actions of 8-iso $PGF_{2\alpha}$ in rat pulmonary artery rings. The thromboxane mimetic U46619, PGE_2 sodium nitroprusside (SNP) and acetyl choline (ACh) were used for comparison. Each prostanoid had to be dissolved in ethanol to a maximum concentration of 1×10^{-2} M. At high concentrations, ethanol directly contracted pulmonary vessels. We were therefore limited by the actions of the vehicle such that we were unable to add prostanoids at concentrations higher than 1×10^{-4} M. In some cases this meant that maximum responses were not achieved and in these cases the E_{max} and pD_2 values are apparent estimates.

3 The following rank order of potency was obtained from contractile studies; U46619 > 8-iso $PGF_{2\alpha}$ > PGE_2, each prostanoid producing concentration-dependent contractions ($10^{-10}-3\times10^{-4}$ M, $10^{-9}-10^{-4}$ M, $10^{-8}-10^{-4}$ M, respectively). As has been shown previously for other smooth muscle preparations, the thromboxane receptor (TP) antagonist ICI 192605, (1×10^{-6}, 1×10^{-5} and 1×10^{-4} M), inhibited the contractions of 8-iso $PGF_{2\alpha}$ in a concentration-dependent fashion.

4 The nitric oxide synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME; 1×10^{-4} M), enhanced the contractile function of both 8-iso $PGF_{2\alpha}$ and PGE_2, but had no effect on that caused by U46619. Similarly, L-NAME inhibited the dilator function of all agents tested except the exogenous nitric oxide (NO) donor SNP, indicating that PGE_2 and 8-iso $PGF_{2\alpha}$ like ACh, act through the release of NO. The specificity of the effects of L-NAME were confirmed in studies with the inactive enantiomer D-NAME (1×10^{-4} M), which did not affect the contractile or the dilator actions of 8-iso $PGF_{2\alpha}$. Furthermore, ICI 192605 enhanced the dilator actions of 8-iso $PGF_{2\alpha}$, suggesting that the dilator component of 8-iso $PGF_{2\alpha}$ was achieved via activation of a non-TP receptor.

5 Isoprostanes may modulate vascular tone by a direct action on TP receptors to cause contraction and via a distinct receptor leading to the release of NO to cause dilatation.

Keywords: Sepsis; prostaglandins; thromboxane (TP) receptor; PGE_2; N^G-nitro-L-arginine methyl ester; 8-iso prostaglandin $F_{2\alpha}$; pulmonary artery; U46619; endotoxin; nitric oxide

Introduction

Isoprostanes are a newly-described group of prostaglandin-like compounds which can be produced independently of the cyclo-oxygenase pathway (Morrow & Roberts, 1996). Isoprostanes are formed under conditions of oxidant stress through a free radical action on arachidonic acid in cell membranes and are subsequently cleaved, presumably by the action of phospholipase enzymes. Free and esterfied F_2 isoprostanes are amongst the more abundant forms with *in vivo* levels ranging between 5 and 166 pg ml^{-1} in normal human plasma (Morrow *et al*., 1990a; Nourooz-Zadeh *et al*., 1995). Moreover, increased plasma levels of F_2 isoprostanes have been demonstrated in selenium-deficient rats subjected to free radical-catalysed lipid peroxidation induced by diquat, and in normal rats after carbon tetrachloride administration (Morrow *et al*., 1990b). In addition, in man plasma F_2 isoprostane levels increase with age and are elevated in smokers, possibly due to oxidants found in cigarette smoke (Morrow *et al*., 1995). Further, 8-iso $PGF_{2\alpha}$ specifically, is increased approximately three fold in individuals with non-insulin dependent diabetes mellitus (Gopaul *et al*., 1995), a disease state associated with endothelial dysfunction and oxidative damage.

The biological significance of isoprostane formation is not completely understood. Nevertheless, evidence increasingly suggests that isoprostanes have important contractile effects on smooth muscle function. Indeed, 8-iso $PGF_{2\alpha}$ has been shown to cause contraction of a variety of smooth muscle preparations including human myometrium (Crankshaw, 1995), guinea-pig and human airways (Kawikova *et al*., 1996), rat renal (Morrow *et al*., 1990b; Takabashi *et al*., 1992) and pulmonary vessels (Kang *et al*., 1993) and rabbit pulmonary vessels (Banerjee *et al*., 1992). Where tested, the contractile actions of 8-iso $PGF_{2\alpha}$ are blocked by thromboxane receptor (TP) antagonists (Morrow *et al*., 1990b; Takabashi *et al*., 1992; Banerjee *et al*., 1992; Kang *et al*., 1993; Crankshaw, 1995; Kawikova *et al*., 1996). 8-Iso $PGF_{2\alpha}$ also causes platelet aggregation (Morrow *et al*., 1992), possibly via TP receptor activation, although evidence suggests that specific isoprostane receptors may be present in some preparations (Fukunaga *et al*., 1993). In contrast to the contractile actions of 8-iso $PGF_{2\alpha}$, the putative dilator properties of 8-iso $PGF_{2\alpha}$ have not been

[1] Author for correspondence.

investigated. Thus, we have characterized the vascular (contractile and dilator) actions of 8-iso $PGF_{2\alpha}$ on rat pulmonary arteries.

The pulmonary vasculature is sensitive to changes in vasoactive mediators during conditions such as septic shock where a fall in systemic vascular resistance is often accompanied by pulmonary hypertension. Thus, the production of vasoconstrictor agents in the pulmonary circulation during sepsis can lead to raised pulmonary vascular resistance and secondary right heart failure which has a poor prognosis (Curzen *et al*., 1994). During sepsis the recruitment and activation of neutrophils in the lung contribute to high oxidant stress, conducive to the formation of isoprostanes. In addition, the release of cytokines during sepsis may influence the expression of prostanoid receptors or the associated signal transduction pathways. Thus, we have compared the potency and efficacy of 8-iso $PGF_{2\alpha}$ on pulmonary arteries from untreated (control) animals, and those rendered endotoxaemic by the administration of bacterial lipopolysaccharide (LPS). Specifically, we investigated the contribution of nitric oxide (NO) and cyclo-oxygenase products by employing the enzyme inhibitors N^G-nitro-L-arginine methyl ester (L-NAME) and indomethacin, respectively. To characterize the involvement of TP receptors we compared to potency of 8-iso $PGF_{2\alpha}$ with the thromboxane mimetic U46619 (9,11-dideoxy-9α,11α-epoxymethanoprostaglandin $F_{2\alpha}$), and utilized ICI 192605 (4(z)-6-(2,4,5cis)[2-(chlorophenyl)-4-(2-hydroxyphenyl)1,3-dioxan-5-yl]hexenoic acid), a TP receptor antagonist. The dilator actions of 8-iso $PGF_{2\alpha}$ were compared with those of acetylcholine (ACh), a known agonist for endothelial NO, and PGE_2, an eicosanoid with an established dilator function. Some of these results have been published in abstract form (Jourdan *et al*., 1996).

Methods

Organ bath experiments

Male Wistar rats were treated with either *Salmonella enteritidis* endotoxin (20 mg kg^{-1}, i.p.) or left untreated for 4 h before being killed by cervical dislocation. Pulmonary arteries were dissected, cut into rings 2×10^{-3} m long, mounted onto a pair of rigid, parallel wires and suspended in 2×10^{-3} l organ baths containing oxygenated (95% O_2-5% CO_2) Krebs-Henseleit (KH) solution at 37°C. The composition of the KH solution was as follows (mM): NaCl 118, KCl 5.9, $MgSO_4$ 1.2, $CaCl_2$ 2.5, NaH_2PO_4 1.2, $NaHCO_3$ 25.5 and glucose 5.6. One of the wires was fixed, and one attached to a force transducer (FT.03 Grass Instruments, Quincy, MA). Changes in isometric force were recorded on a polygraph multichannel recorder (Grass model 7). After 15 min of equilibration time, the rings were contracted with KCl (4×10^{-2} M) and relaxed to a uniform baseline tension of 4.9 mN by repeated washing with KH solution. Rings were left to equilibrate in the bath for a total of 30 min and washed every 15 min.

Protocol design for contractile responses

From each rat, four pulmonary artery rings were obtained, one of which was used as a control, the others being incubated with either L-NAME (1×10^{-4} M dissolved in KH solution) or indomethacin (3×10^{-5} M dissolved in 5% $NaCO_3$), or L-NAME together with indomethacin for 30 min. L-NAME caused a direct increase in pulmonary artery tone in both control (mean ± s.e.mean, 0.447 ± 0.176 mN; $n=13$) and LPS-treated (0.871 ± 0.186 mN; $n=16$) animals. Further contractions induced by 8-iso $PGF_{2\alpha}$ (10^{-9}–10^{-4} M), U46619 (10^{-10}–3×10^{-4} M), or PGE_2 (10^{-8}–10^{-4} M) were therefore calculated as increases above that induced by L-NAME. In separate experiments, one pulmonary artery ring from each animal was left untreated, whilst ICI 192605 at either 1×10^{-6} M, 1×10^{-5} M or 1×10^{-4} M (dissolved in DMSO) was added to the other three for 30 min. Cumulative concentration-response curves were then obtained by the addition of 8-iso $PGF_{2\alpha}$. In each case, 8-iso $PGF_{2\alpha}$, U46619 or PGE_2 were dissolved, as recommended by the supplier, to the highest concentration possible (1×10^{-2} M) in ethanol. Stocks were then diluted in distilled water before being added to the organ baths. Due to the direct actions (contraction) of higher levels of ethanol on pulmonary arteries, we were limited to a maximum concentration of 1×10^{-4} M for contraction studies and 3×10^{-5} M for dilatation studies for each prostanoid. All other drugs were similarly used at concentrations where their respective vehicles had no direct effect on vascular tone.

Protocol design for dilator responses

Rat pulmonary artery does not acquire significant 'endogenoue tone' *in vitro* under the conditions used in our experiments. Thus, in order to observe any dilator function, tone must first be applied to the vessel by use of pharmacological agonists. For dilatation experiments the rings were equilibrated, exposed to KCl (4×10^{-2} M) and washed as above. Tissues were then contracted to approximately 5.9 mN tension by the addition of a single administration of U46619 (1×10^{-6} M), which produced stable increases in tone for the duration of the experiment. After a stable contractile response to U46619 was obtained either sodium nitroprusside (SNP; 10^{-10}–3×10^{-5} M), ACh (10^{-10}–3×10^{-4} M), 8-iso $PGF_{2\alpha}$ (10^{-10}–10^{-4} M) or PGE_2 (10^{-10}–3×10^{-5} M) was added in a cumulative fashion. In some experiments L-NAME (1×10^{-4} M) or indomethacin (3×10^{-5} M) were added to the tissue 30 min before U46619. In experiments designed to characterize the involvement of TP receptors in the dilator actions of 8-iso $PGF_{2\alpha}$, phenylephrine (1×10^{-4} M) was used to contract the vessels as U46619 was shown to be antagonized by ICI 192605 (data not shown). In these experiments tissues were treated as above and the dilator actions of 8-iso $PGF_{2\alpha}$ compared in the presence or absence of ICI 192605 (1×10^{-4} M). In comparable experiments, the vehicles used to dissolve each drug were tested for their vasoactive properties on pulmonary arteries.

Materials

All chemicals were obtained from Sigma (Poole, Dorset, U.K.), except 8-iso $PGF_{2\alpha}$ which was purchased from Cayman Chemical (Ann Habor, MI, U.S.A.). ICI 192605 was a kind gift from Zeneca Pharmaceuticals (Cheshire, U.K.).

Statistical analysis

Results are expressed as means ± s.e.mean. Values were compared by unpaired *t* test or two-way analysis of variance where appropriate (GraphPAD INSTAT, GraphPAD Software, San Diego, CA and GraphPAD PRISM, version 2). A P value of less than 0.05 was taken as significant and illustrated in the appropriate figures by an asterisk.

Results

Relative potencies of 8-iso $PGF_{2\alpha}$, U46619 and PGE_2 as contractile agents in rat pulmonary arteries from control and LPS-treated rats

All three prostanoids tested caused concentration-dependent contractions (Figures 1–3) in pulmonary arteries from control rats with the following order of potency; U46619 > 8-iso $PGF_{2\alpha}$ > PGE_2 (Table 1). Neither the rank order of potency, nor the concentration-response curves (two-way ANOVA) of the eicosanoids tested were affected by LPS pretreatment *in vivo* (Table 1). However the maximum contraction induced by 8-iso $PGF_{2\alpha}$ was reduced by LPS treatment *in vivo* (Table 1). Similarly, a significant reduction ($P<0.05$, unpaired *t* test) in the contraction induced by 4×10^{-2} M KCl was observed in

Table 1 Comparison of the maximum response obtained (E_{max}) and apparent pD_2 values for 8-iso $PGF_{2\alpha}$, PGE_2, U46619 and ACh on pulmonary artery from untreated (control) and LPS-treated rats

	Control		*LPS*	
	Contraction			
	E_{max} (mN)	pD_2	E_{max} (mN)	pD_2
8-iso $PGF_{2\alpha}$	5.10±0.37	5.18 (5.57 to 4.81)	3.96±0.4	5.12 (5.60 to 4.60)
PGE_2	5.89±0.29	4.09 (4.19 to 3.98)	5.25±0.38	3.93 (4.06 to 3.80)
U46619	6.00±0.17	7.18 (7.37 to 6.99)	5.58±0.10	7.68 (7.74 to 7.62)
	Relaxation			
	%	pD_2	%	pD_2
8-iso $PGF_{2\alpha}$	26.80±3.83	5.47 (6.13 to 4.79)	26.93±2.46	6.77 (7.39 to 6.10)
PGE_2	13.85±0.48	7.34 (7.68 to 6.99)	19.46±0.81	7.19 (7.55 to 6.85)
ACh	59.67±2.19	6.65 (6.95 to 6.36)	46.15±1.61	6.42 (6.7 to 6.14)

The table shows contractile responses in mN and relaxation responses as percentage of induced tone. E_{max} and pD_2 values (with 95% confidence limits in parentheses) were calculated from concentration-response curves (1×10^{-10} to 1×10^{-4} M) by GraphPAD PRISM version 2.0. In all cases, except with U46619 and ACh, maximum responses could not be achieved. Thus, in the case of contractions induced by 8-iso $PGF_{a\alpha}$ ad PGE_2 the attributed E_{max} and pD_2 values are 'apparent'.

LPS-treated rats (control vs LPS, 3.63±0.19 mN vs 2.52±0.19 mN; $n=32$ and 40, respectively). In this study, the contractile responses of prostanoids are given as mN and not normalized to the KCl response in individual tissues. However, when data were normalized, no appreciable difference was seen in any of the protocols (not shown).

Effect of the cyclo-oxygenase inhibitor, indomethacin, on the contractile responses induced by 8-iso $PGF_{2\alpha}$, U46619 and PGE_2 in pulmonary arteries from control and LPS-treated rats

For contractile responses, neither the potency nor the efficacy of U46619 or PGE_2 in pulmonary arteries from either control or LPS-treated rats were affected by the presence of 3×10^{-5} M indomethacin (data not shown; $n=4-5$), a concentration known to inhibit both cyclo-oxygenase-1 and cyclo-oxygenase-2 (Mitchell *et al*., 1993). Similarly, the contractile effects of 8-iso $PGF_{2\alpha}$ in vessels from control animals were unaffected by indomethacin (Figure 1). By contrast, the effects of 8-iso $PGF_{2\alpha}$ in vessels from LPS-treated animals showed a trend towards inhibition by indomethacin, illustrated by a rightward shift in the concentration-response curve (pD_2 in the absence of indomethacin, 5.12: pD_2 in the presence of indomethacin, 4.18; $n=4-5$), with no change in the maximum (3.96±0.4 mN in the absence and 4.6±0.55 mN in the presence of indomethacin; $n=4-5$), although this did not reach statistical significance (two-way ANOVA).

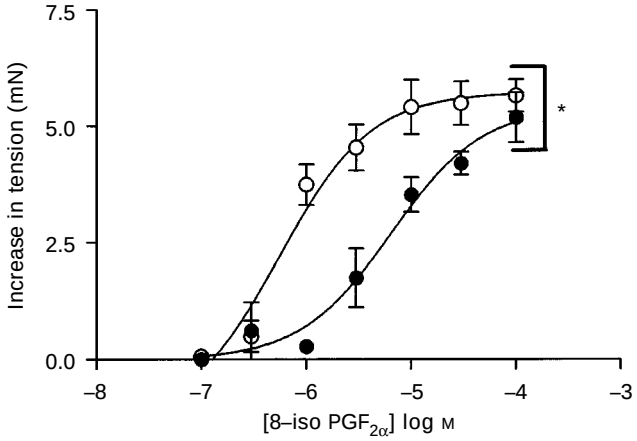

Figure 1 Contractile concentration-response curves of rat pulmonary arteries to 8-iso $PGF_{2\alpha}$. (●) Control; (○) plus 1×10^{-4} M L-NAME. The figure shows responses of pulmonary arteries from control rats, similar observations were obtained with vessels from LPS-treated rats. *Indicates a significant difference ($P<0.05$) between responses obtained in the presence and absence of L-NAME (two-way ANOVA; $n=4/5$). Vertical lines show s.e.mean.

Effect of the NO synthase inhibitor, L-NAME, on the contractile responses induced by 8-iso $PGF_{2\alpha}$, U46619 and PGE_2 in pulmonary arteries from control and LPS-treated rats

In the presence of the NOS inhibitor, L-NAME (1×10^{-4} M) there was an increase in the potency of 8-iso $PGF_{2\alpha}$ as illustrated by an increase in the apparent pD_2 value from 5.18 (control) to 6.3 (plus L-NAME) (Figure 1). Similarly L-NAME increased the pD_2 value for 8-iso $PGF_{2\alpha}$ on pulmonary artery from LPS-treated rats from 5.12 to 6.02. However, the maximum response to 8-iso $PGF_{2\alpha}$ on pulmonary artery from control (5.10±0.37 mN) animals was not affected by L-NAME (5.72±0.3 mN). Although L-NAME did increase the maximum response to 8-iso $PGF_{2\alpha}$ in pulmonary arteries from LPS-treated rats from 3.96±0.4 to 5.14±0.31 mN ($n=4-5$). In contrast, the inactive enantiomer, D-NAME (1×10^{-4} M) had no effect on the contractile responses of 8-iso $PGF_{2\alpha}$ (data not shown; $n=4$). Similar to its effects on 8-iso $PGF_{2\alpha}$, L-NAME potentiated the contractile effects of PGE_2 (Figure 3) resulting in an increase in its apparent pD_2 values on vessels from control animals of 4.09 (control) to 5.04 (plus L-NAME)

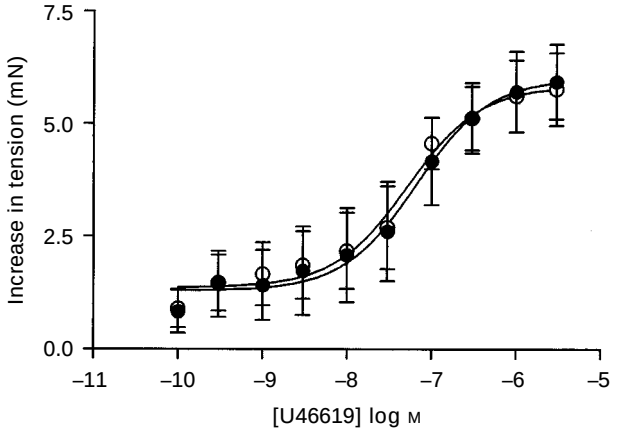

Figure 2 Contractile concentration-response curves of rat pulmonary artery to U46619. (●) Control; (○) plus 1×10^{-4} M L-NAME. The figure shows responses of pulmonary arteries from control rats, similar observations were obtained with vessels from LPS-treated rats. No significant difference was observed between the contractile responses induced by U46619 in the presence and absence of L-NAME (two-way ANOVA; $n=5/4$). Vertical lines show s.e.mean.

(Figure 3) and from LPS-treated animals of 3.93 to 5.17. In contrast to its effects on 8-iso $PGF_{2\alpha}$ and PGE_2, L-NAME did not effect the potency of U46619 in vessels from either control (Figure 2; $n=4-5$) or LPS-treated animals (pD_2 confidence intervals in the absence, 7.74 to 7.62 and in the presence, 8.33 to 7.85, of L-NAME; $n=4-5$). Paradoxically, L-NAME reduced the maximum contractile response to U46619 in vessels from LPS-treated animals from 5.58 ± 0.10 to 4.26 ± 0.14 mN ($n=4-5$).

Effect of the TP antagonist, ICI 192605, on the contractile responses induced by 8-iso $PGF_{2\alpha}$ in pulmonary arteries from control and LPS-treated rats

ICI 192605 (1×10^{-6}, 1×10^{-5} or 1×10^{-4} M) caused concentration-dependent inhibitions of the contractile responses induced by 8-iso $PGF_{2\alpha}$ (Figure 4). Maximum responses to 8-iso $PGF_{2\alpha}$ could not be achieved (see Methods) and so the nature of the antagonism by ICI 1292605 can only be estimated. However, from the data obtained, ICI 192605 would appear to be a competitive inhibitor of the contractile effects of 8-iso $PGF_{2\alpha}$.

Characterization of the dilator actions of 8-iso $PGF_{2\alpha}$ on rat pulmonary artery from control and LPS-treated animals

8-iso $PGF_{2\alpha}$ (1×10^{-10} to 3×10^{-5} M and 1×10^{-10} to 3×10^{-7} M) caused concentration-dependent relaxations of pulmonary arteries contracted with U46619 (Figure 5; Table 1) or phenylephrine (Figure 6), respectively. The ability of 8-iso $PGF_{2\alpha}$ to relax pulmonary artery was greater when tissues were precontracted with U46619 (E_{max} $26.8\pm3.83\%$ induced tone) than with phenylephrine ($20.00\pm3.12\%$ induced tone). The vehicle for 8-iso $PGF_{2\alpha}$ caused a small relaxation of tissues contracted by U46619 (Figure 5) and had no effect (one-way ANOVA) on tissues contracted by phenylephrine ($n=4$; not shown). At higher concentrations (greater than 3×10^{-5} M for U46619 and greater than 3×10^{-7} M for phenylephrine), 8-iso $PGF_{2\alpha}$ began to reverse the dilatation induced by its cumulative addition at lower concentrations. The apparently contractile effects of 8-iso-$PGF_{2\alpha}$ in the presence of phenylephrine

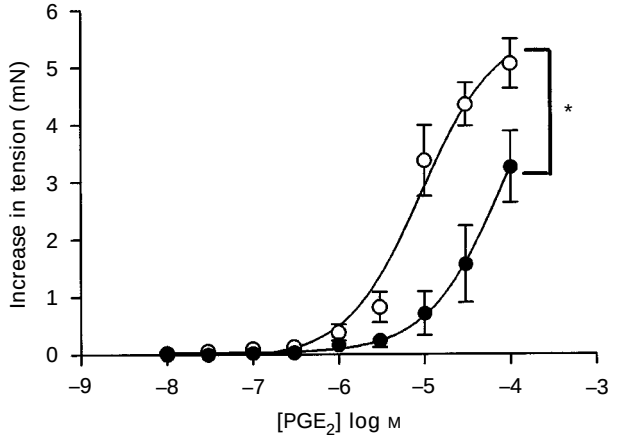

Figure 3 Contractile concentration-response curves of rat pulmonary artery to PGE_2. (●) Control; (○) plus 1×10^{-4} M L-NAME. The figure shows responses of pulmonary arteries from control rats, similar observations were obtained with vessels from LPS-treated rats. *Indicates significant difference ($P<0.05$) between responses obtained in the presence and absence of L-NAME (two-way ANOVA; $n=5/6$). Vertical lines show s.e.mean.

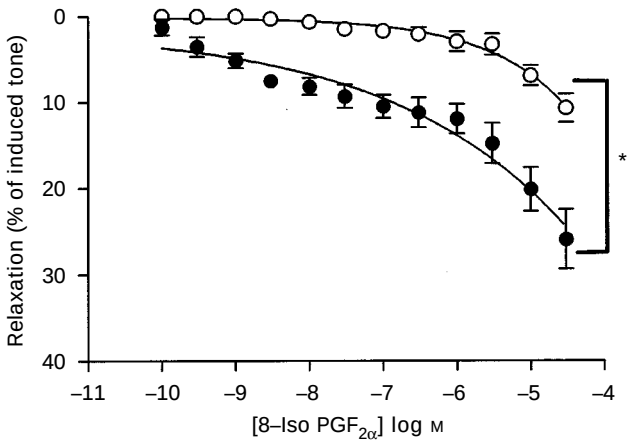

Figure 5 Dilator concentration-response curves of rat pulmonary artery to 8-iso $PGF_{2\alpha}$. (●) Control; (○) plus 1×10^{-4} M L-NAME. Vessels were precontracted with U46619 (1×10^{-6} M) in order to unmask the dilator action of 8-iso $PGF_{2\alpha}$. *Indicates significant difference between the two curves (two-way ANOVA; $n=6$). Vertical lines show s.e.mean.

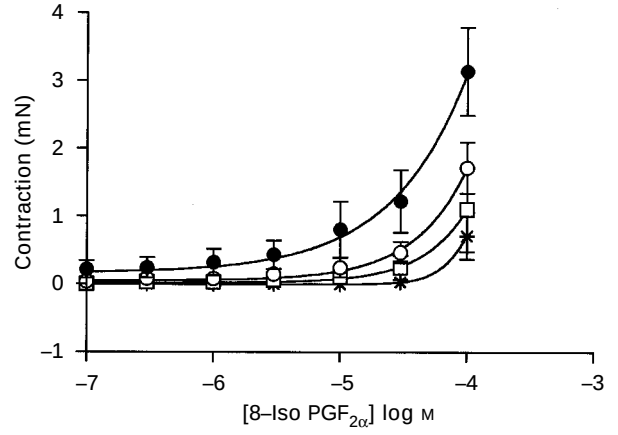

Figure 4 Effect of ICI 192605, 1×10^{-6} (○), 1×10^{-5} (□) and 1×10^{-4} M (*) on the contractile actions of 8-iso $PGF_{2\alpha}$ ($n=3-4$). The figure shows responses from control rats, similar observations were obtained with vessels from LPS-treated rats. By use of two-way ANOVA, significant differences were noted between control responses and those obtained in the presence of each concentration of ICI 192605. Vertical lines show s.e.mean.

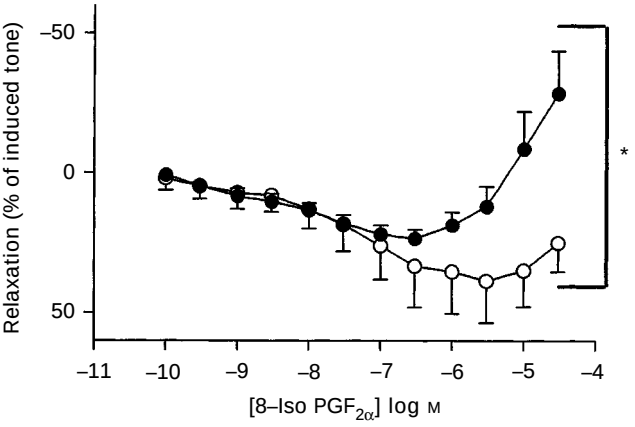

Figure 6 Effect of ICI 192605 (1×10^{-4} M) on the dilator actions of 8-iso $PGF_{2\alpha}$. (●) Control; (○) plus ICI 192605. Tissues were contracted with phenylephrine (1×10^{-4} M) in order to unmask the dilator action of 8-iso $PGF_{2\alpha}$. *Indicates significant difference between the two curves (two-way ANOVA; $n=9/5$). Vertical lines show s.e.mean.

were reversed when ICI 192605 (1×10^{-4} M; Figure 6) was added, and the dilatation induced by 8-iso $PGF_{2\alpha}$ was enhanced by the presence of the TP antagonist ICI 192605 (1×10^{-4} M; Figure 6; $P<0.05$ for relaxations induced by 8-iso $PGF_{2\alpha}$ at 3×10^{-6}, 1×10^{-5} and 3×10^{-5} M, in the absence versus the presence of ICI 192605, paired t test). No appreciable difference was seen in the ability of 8-iso-$PGF_{2\alpha}$ to relax vessels from control (Figure 6) or LPS-treated animals (Table 1). The dilator action of 8-iso-$PGF_{2\alpha}$ (E_{max} 26.80±3.83% induced tone; pD_2 5.47) was virtually abolished in the presence of L-NAME (1×10^{-4} M: E_{max} 14.00±2.11% induced tone; pD_2 4.98) (Figure 6) and was unaffected by indomethacin (3×10^{-5} M; data not shown; $n=5$) or D-NAME (1×10^{-4} M; data not shown; $n=4$). In a similar fashion to 8-iso-$PGF_{2\alpha}$, PGE_2 caused concentration-dependent relaxations of preconstricted pulmonary arteries (E_{max} 13.85±0.48% induced tone; pD_2 7.34) which were inhibited by L-NAME (E_{max} 10.50±0.4% induced tone; pD_2 7.07; Figure 7). The degree of inhibition of the dilator actions of 8-iso-$PGF_{2\alpha}$ and PGE_2 caused by L-NAME was similar to that observed with ACh (control: E_{max} 59.67±2.19% induced tone; pD_2 6.65; plus L-NAME: E_{max} 15±07, pD_2 % induced tone 6.8; Figure 8). No effect of L-NAME was seen on the dilator action of the endothelium-independent nitrovasodilator, SNP (1×10^{-10}–3×10^{-5} M; $n=5$; data not shown).

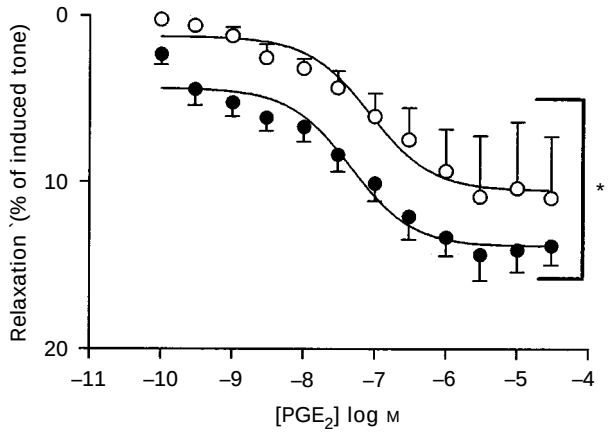

Figure 7 Dilator concentration-response curves of rat pulmonary artery to PGE_2. (●) Control; (○) plus 1×10^{-4} M L-NAME. The figure shows response from control rats, similar observations were obtained with vessels from LPS-treated rats. *Indicates a significant difference between the two curves (two-way ANOVA; $n=5/6$).

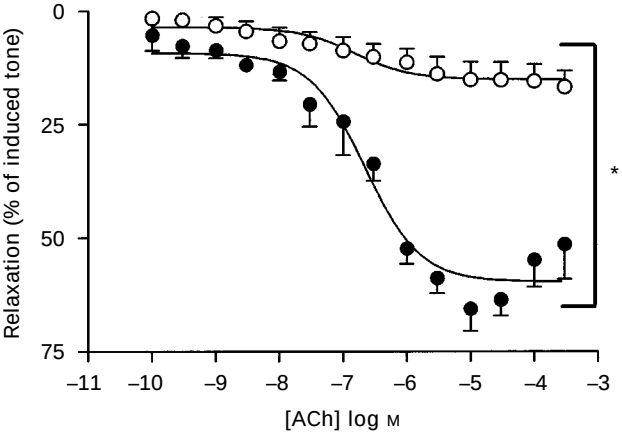

Figure 8 Dilator concentration-response curves of rat pulmonary artery to ACh. (●) Control; (○) plus 1×10^{-4} M L-NAME. The figure shows responses from control rats, similar observations were obtained with vessels from LPS-treated rats. *Indicates significant difference between the two curves (two-way ANOVA; $n=4$). Vertical lines show s.e.mean.

Discussion

In this study, the isoprostane, 8-iso $PGF_{2\alpha}$ was shown to have both constrictor and dilator functions in rat pulmonary arteries. The contractile actions of 8-iso $PGF_{2\alpha}$ have been described in several smooth muscle preparations and attributed to TP receptor activation (Morrow *et al*., 1990b; Takabashi *et al*., 1992; Banerjee *et al*., 1992; Kang *et al*., 1993; Crankshaw, 1995; Kawikova *et al*., 1996). In this study, the TP receptor antagonist, ICI 192605, inhibited the contractile action of 8-iso $PGF_{2\alpha}$ in pulmonary arteries from control and LPS-treated animals in a concentration-dependent manner. This observation is in agreement with previous studies showing that TP receptor antagonists inhibit the contractile action of 8-iso $PGF_{2\alpha}$ on the pulmonary vasculature in rat isolated lungs.

The relative potency of 8-iso $PGF_{2\alpha}$ and the thromboxane mimetic, U46619 differs in different preparations. Indeed, U46619 is more potent than 8-iso $PGF_{2\alpha}$ by between 9 and 100 fold as a constrictor of porcine and ovine coronary arteries (Kromer & Tippins, 1996a) and of human and guinea-pig trachea (Kawikova *et al*., 1996). Interestingly, the sensitivity of smooth muscle to 8-iso $PGF_{2\alpha}$ appears to alter after physiological changes. For example, in human myometrium from pregnant donors 8-iso $PGF_{2\alpha}$ is equipotent to U46619, whereas U46619 is more potent than 8-iso $PGF_{2\alpha}$ as a constrictor of myometrium from non-pregnant donors (Crankshaw, 1995). Similarly, in rat isolated, perfused hearts, where U46619 is a potent constrictor of coronary vessels, 8-iso $PGF_{2\alpha}$ is inactive. However, after a short period of 'low flow' ischaemia 8-iso $PGF_{2\alpha}$ becomes a potent constrictor of the coronary vasculature with no change observed in the efficacy of U46619 (Kromer & Tippins, 1996b). By contrast, we found no effects of experimental sepsis on the potency of 8-iso $PGF_{2\alpha}$ in rat pulmonary arteries.

It therefore seems that under some inflammatory conditions, the potency of 8-iso $PGF_{2\alpha}$ can be modulated by as yet uncharacterized factors. One agent which conceivably may contribute to the actions of 8-iso $PGF_{2\alpha}$ is NO. Indeed, the release of NO may not only regulate the amount and stability of isoprostanes formed but also may antagonize their constrictor actions. We found that NO released by pulmonary arteries from control and endotoxaemic rats inhibited the constrictor actions of 8-iso $PGF_{2\alpha}$ but not of U46619. Indeed, the potency of 8-iso $PGF_{2\alpha}$ and PGE_2 but not U46619 were increased approximately 10 fold in the presence of L-NAME. Similar increases were seen in vessels from control and LPS-treated animals. The pronounced affects of L-NAME on the contractile actions of 8-iso $PGF_{2\alpha}$ but not U46619 suggest that this was not a non-specific effect of NO on all contractile agents and raised the possibility that 8-iso $PGF_{2\alpha}$ may have dilator actions via the relase of NO. Indeed, when 8-iso $PGF_{2\alpha}$ was added to rat pulmonary arteries, which had been constricted by either U46619 or phenylephrine, concentration-dependent reductions in tone were detectable. Similar results were obtained with pulmonary arteries from control and LPS-treated animals. Interestingly, we found that the ability of 8-iso $PGF_{2\alpha}$ to relax preconstricted pulmonary artery was greatest when U46619 was used as the contractile agent. This suggested to us that the occupation of TP receptors by U46619 may reduce that ability of 8-iso $PGF_{2\alpha}$ to contract by this common receptor. Indeed, when phenylephrine was used as the contractile agent and TP-receptors were antagonized by ICI 192605, 8-iso $PGF_{2\alpha}$ produced comparable relaxations to those seen with U46619. We found that 8-iso $PGF_{2\alpha}$ was approximately 50% effective as ACh as a dilator agent, producing a maximum reduction in tone of approximately 30%. However, L-NAME inhibited the vasodilator actions of 8-iso $PGF_{2\alpha}$ and ACh to a similar degree. Interestingly, of the eicosanoids tested, only 8-iso $PGF_{2\alpha}$ was affected by the presence of in-

domethacin, leading to a righward shift of the concentration-response curve. This shift appeared more pronounced in vessels from LPS-treated rats, as endotoxin can induce cyclo-oxygenase-2 enzyme expression within four hours.

By contrast to its contractile function, the vasodilator properties of 8-iso $PGF_{2\alpha}$ were not antagonized by ICI 192605. Indeed, the dilator action of 8-iso $PGF_{2\alpha}$ was enhanced in the presence of ICI 192605, probably by inhibition of the 'masking' contractile action of the isoprostane. Thus, in the rat pulmonary artery, 8-iso $PGF_{2\alpha}$ contracts vascular smooth muscle by activation of TP or TP-like receptors. Further 8-iso $PGF_{2\alpha}$ may act on non-TP receptors to cause the release of NO with resultant vasodilatation. The identity of this putative receptor is unclear. Nevertheless the possiblity exists that in addition to TP-receptors, 8-iso $PGF_{2\alpha}$ acts on discrete receptors to produce a variety of biological effects. Like 8-iso $PGF_{2\alpha}$, PGE_2 caused vasodilatation via the release of NO. Thus, 8-iso $PGF_{2\alpha}$ may activate putative Ep_1 receptors on endothelial cells leading to inositol phosphate formation (Thierauch *et al*., 1994) followed by increased intracellular calcium and a direct activation of endothelial NOS (Pollock *et al*., 1991)

In conclusion, 8-iso $PGF_{2\alpha}$ has both dilator and constrictor activity. The predominant action of 8-iso $PGF_{2\alpha}$ is therefore dependent on its concentration and the level of endogenously produced NO. These results imply that *in vivo* 8-iso $PGF_{2\alpha}$ is a dilator under normal conditions of a reducing environment with low oxidant stress, but may reach concentrations sufficient to produce vasoconstriction during sepsis and thus contribute to the pulmonary hypertension seen in critically ill patients.

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