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Analysis of the pulmonary hypertensive effects of the isoprostane derivative, 8-iso-$PGF_{2\alpha}$, in the rat

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1 We analysed the pulmonary hypertensive effects of the F_2-isoprostane derivative, 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$), in comparison with those of the high efficacy thromboxane A_2/prostanoid (TP) receptor agonist, U-46619, in pentobarbitone-anaesthetized, open-chest rats ($n=4-15$ per group).

2 8-iso-$PGF_{2\alpha}$ produced dose-dependent increases in mean pulmonary arterial pressure, with an ED_{50} of 39.0 (31.4–50.6) μg kg^{-1}, i.v. (geometric mean with 95% confidence limits in parentheses) compared to 1.4 (1.1–2.3) μg kg^{-1}, i.v., for U-46619. The maximum responses evoked by U-46619 and 8-iso-$PGF_{2\alpha}$ were not statistically significantly different (21.0$\pm$1.0 and 25.8$\pm$1.9 mmHg at 10 μg kg^{-1} of U-46619 and 630 μg kg^{-1} of 8-iso-$PGF_{2\alpha}$, respectively).

3 The TP receptor antagonist, SQ 29,548 (0.63 mg kg^{-1}, i.v. + 0.63 mg kg^{-1} h^{-1}) fully antagonised both U-46619 and 8-iso-$PGF_{2\alpha}$-induced pulmonary hypertensive responses.

4 Further experiments were carried out to determine whether 8-iso-$PGF_{2\alpha}$ antagonized the pulmonary hypertensive responses evoked by U-46619, or those induced by itself, as would be predicted for a partial agonist. However, ED_{10} or ED_{25} doses of 8-iso-$PGF_{2\alpha}$ (10 or 20 μg kg^{-1}, i.v.) failed to reduce the pulmonary hypertensive responses induced either by U-46619 or by itself.

5 The data suggest that in the pulmonary vascular bed of the rat, 8-iso-$PGF_{2\alpha}$ acts as an agonist of high intrinsic activity at SQ 29,548-sensitive (probably TP) receptors.

Keywords: F_2-isoprostane; pulmonary hypertension; TP receptor; 8-iso-$PGF_{2\alpha}$; U-46619; SQ 29,548

Introduction

8-Iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) is mainly produced in man and rats *in vivo* during oxidative stress by non-cyclo oxygenase-dependent, free radical catalyzed peroxidation of arachidonic acid-containing phospholipids in plasma and cell membranes as well as by oxidation of low density lipoprotein and plasma (Morrow *et al*., 1990; 1992a; Awad *et al*., 1993; Lynch *et al*., 1994; Moore *et al*., 1995a, b; Roberts & Morrow, 1995; Morrow & Roberts, 1996). Small quantities of 8-iso-$PGF_{2\alpha}$ may also be generated by the cyclo-oxygenase pathway (Pratico *et al*., 1995; Delanty *et al*., 1996; Patrignani *et al*., 1996). A number of studies have shown 8-iso-$PGF_{2\alpha}$ to be an extremely accurate marker of lipid peroxidation in animal models of oxidative stress (Morrow *et al*., 1990; Roberts & Morrow, 1995; Delanty *et al*., 1997). Measurement of 8-iso-$PGF_{2\alpha}$ production could therefore be used to explore the role of free radicals and oxidant stress in the pathophysiology of several human diseases (Roberts & Morrow, 1995; Delanty *et al*., 1997).

8-iso-$PGF_{2\alpha}$-induces vasoconstriction in rat preglomerular vessels (Takahashi *et al*., 1992), in rat and rabbit isolated lungs (Banerjee *et al*., 1992; Kang *et al*., 1993), in porcine and bovine coronary arteries (Kromer & Tippins, 1996) and in rat and guinea-pig aorta (Zhang *et al*., 1996) which in each case were antagonized by thromboxane A_2/prostanoid (TP) receptor antagonists. These findings strongly suggest that the vasoconstrictor effects of 8-iso-$PGF_{2\alpha}$ are mediated by TP receptors. However the possibility that 8-iso-$PGF_{2\alpha}$ elicits agonist responses via non-TP receptors has been raised (Fukunaga *et al*., 1993; Yura *et al*., 1995; Pratico *et al*., 1996).

Kromer and Tippins (1996) showed that 8-iso-$PGF_{2\alpha}$ and the high efficacy TP receptor agonist, U-46619, evoked contractile responses in porcine coronary arteries which were antagonized by the TP receptor antagonist SQ 29,548. In addition, the contractile responses evoked by U-46619 were dose-dependently reduced by 8-iso-$PGF_{2\alpha}$, thus demonstrating that 8-iso-$PGF_{2\alpha}$ acted as a partial agonist at TP receptors (Kromer & Tippins, 1996). Furthermore, 8-iso-$PGF_{2\alpha}$, in contrast to U-46619, failed to elicit agonist responses in ovine isolated coronary arteries, as TP receptor reserve was likely to be low in this preparation (Kromer & Tippins, 1996). Similarly, Morrow *et al*. (1992b) showed that 8-iso-$PGF_{2\alpha}$ acted as a partial agonist at TP receptors in both rat and human platelets.

The thromboxane A_2 (TxA_2) mimetic, U-46619, induces dose-dependent increases in mean pulmonary arterial pressure (MPAP) and variable effects upon mean systemic arterial pressure (MSAP), depending upon the dose, all of which are amenable to blockade by the silent TP receptor antagonist, SQ 29,548 (Bertolino *et al*., 1995a, b; Valentin *et al*., 1996). A role for TxA_2 and thus TP receptor activation in the development and/or maintainance of pulmonary hypertension in man has been proposed by the observation of elevated plasma levels of the stable TxA_2 metabolite, TxB_2 (Rubin, 1995). At the present time the respective pulmonary and systemic haemodynamic effects if 8-iso-$PFG_{2\alpha}$ *in vivo* have not yet been studied.

We therefore determined whether 8-iso-$PGF_{2\alpha}$ produced pulmonary hypertension *in vivo* and analysed its effects in comparison with those of the full agonist, U-46619. Experiments were carried out in anaesthetised, open-chest rats, a model we have previously shown to be highly sensitive to TP receptor agonists (Bertolino *et al*., 1995a, b; Valentin *et al*., 1996).

Methods

General procedure

In accordance with French law and the local ethical committee guidelines for animal research, male Sprague-Dawley rats (280–400g, OFA, Iffa-Credo, France) were housed in climate controlled conditions (21°C and 55% relative humidity with a 12 h light/dark cycle) and provided standard rat chow and

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water *ad libitum*. On the day of the experiment, animals were anaesthetized with an intraperitoneal injection of 60 mg kg^{-1} sodium pentobarbitone (Sanofi Laboratories, France) and placed on a heated table to maintain rectal temperature at 37 ± 0.5°C. They were prepared for acute experimentation as previously described (Bertolino *et al*., 1995a, b; Valentin *et al*., 1996). Animals underwent tracheotomy and were mechanically ventilated (60 cycles min^{-1}; 2.5 ml/cycle; Harvard apparatus, South Natick, MA) in order to maintain blood gases within the physiological range as previously described (Bertolino *et al*., 1995b). Catheters were inserted into a femoral vein and artery for infusing fluids and drugs and continuous measurement of arterial pressure (AP) via a Statham P10EZ pressure transducer (Viggo-Spectramed, Oxnard, CA) connected to a Gould amplifier (Gould Instruments, France) and a computerized data acquisition system (Acq*Knowledge*®, BIOPAC Systems Inc., Goleta, CA). A left thoracotomy was performed through the third intercostal space. The pulmonary artery was exposed and a curved 19-gauge needle, connected to a silastic tube (Dow Corning Corporation, Midland, MI), was inserted near the bifurcation of the artery from the right ventricle. Prompt return of arterial blood through the silastic tubing attached to the needle confirmed successful placement. The silastic catheter was secured to the exposed muscle layer of the animal, then the thorax was closed. PAP was recorded via a Statham pressure transducer connected to the computerized data acquisition system. Experiments were started 15–30 min after completion of surgical procedures.

Effect of U-46619 and 8-iso-$PGF_{2\alpha}$ on haemodynamic parameters

The vehicle of SQ 29,548 was administered intravenously (Na_2CO_3, 2 mM; see below) to 32 rats. Five minutes after initiation of the infusion, they received 5 successive increasing doses of either U-46619 (0.16, 0.63, 2.5, 10 and 40 $\mu g\ kg^{-1}$; $n=9$), 8-iso-$PGF_{2\alpha}$ (2.5, 10, 40, 160 and 630 $\mu g\ kg^{-1}$; $n=8$) or their respective vehicles (Na_2CO_3, 2 mM, $n=7$; NaCl 0.9% plus ethanol, 10%; 5 injections; $n=8$). Each dose of drug or vehicle was administered as a 1 ml kg^{-1} solution over 5 min when PAP had returned toward baseline values or had stabilized for serveral minutes.

In separate experiments PAP was determined in 32 open-chest anaesthetized rats which received a single intravenous injection of 8-iso-$PGF_{2\alpha}$, at one of the following doses: 2.5, 10, 40 or 160 $\mu g\ kg^{-1}$ ($n=8$ in each group). Each rat received one dose of 8-iso-$PGF_{2\alpha}$ as a 1 ml kg^{-1} solution over 2 min.

Effect of TP receptor blockade on the responses to U-46619 and 8-iso-$PGF_{2\alpha}$

Three groups of 6 animals each received SQ 29,548 at the dose of 0.63 mg kg^{-1}, i.v. + 0.63 mg $kg^{-1}\ h^{-1}$ followed 5 min later by 5 successive increasing doses of either U-46619 (0.16, 0.63, 2.5, 10 and 40 $\mu g\ kg^{-1}$), 8-iso-$PGF_{2\alpha}$ (2.5, 10, 40, 160 and 630 $\mu g\ kg^{-1}$) or the vehicles.

Effect of 8-iso-$PGF_{2\alpha}$ on the responses elicited by U-46619

Six rats received 8-iso-$PGF_{2\alpha}$ (approximate ED_{10}; 10 $\mu g\ kg^{-1}\ h^{-1}$) followed 5 min later by 5 successive increasing doses of U-46619 (0.16, 0.63, 2.5, 10 and 40 $\mu g\ kg^{-1}$) whereas 12 rats received 8-iso-$PGF_{2\alpha}$ (approximate ED_{25}; 10 $\mu g\ kg^{-1} + 10\ \mu g\ kg^{-1}\ h^{-1}$) followed by either U-46619 ($n=6$) or the vehicle ($n=6$).

Drugs and solutions

SQ 29,548 ([1**S**-[1α,2α(5Z),3α,4α]]-7-[3-[[2-[(phenyl-amino)-carbonyl] hydrazino] methyl]-7-oxabicyclo [2.2.1]hept-2-yl]-5-heptenoic acid) and U-46619 (9, 11-dideoxy-9α-(methanoepoxy) $PGF_{2\alpha}$) were dissolved in Na_2CO_3 (2 mM). 8-Iso-$PGF_{2\alpha}$ was dissolved in NaCl 0.9% plus ethanol (10%). All compounds were purchased from the Cayman Chemical Company (Ann Arbor, MI). Drugs were maintained on ice after dissolution and were injected in $\mu g\ kg^{-1}$ base weight. U-46619 and 8-iso-$PGF_{2\alpha}$ were administered as 1 ml kg^{-1} solutions over 5 min whereas SQ 29,548 or its vehicle was administered as a 1 ml kg^{-1} solution over 2 min followed by a constant infusion at a rate of 20 $\mu l\ min^{-1}$ for 60 min.

Calculations and statistical analysis

Data are expressed as mean absolute maximal changes ± s.e.mean. One way analysis of variance followed by Dunnett's test was used to assess significance between groups (StatView®, Abacus Concepts Inc., Berkeley, CA). $P<0.05$ was considered the minimum level of significance. Dose-response curves were fitted by use of an operational sigmoid model (Marquardt, 1963); $ED_{10,25\ or\ 50}$ refers to the geometric mean agonist dose (with 95% confidence limits in parentheses) inducing 10, 25 or 50% of its maximal effect, respectively.

Results

Effects of U-46619 and 8-iso-$PGF_{2\alpha}$ on MSAP, MPAP and heart rate

No significant change in MSAP, MPAP or heart rate (HR) was detected in time control, vehicle-treated rats (Figure 1; Tables 1 and 2). Typical recordings of PAP following intravenous administration of U-46619 or 8-iso-$PGF_{2\alpha}$ are presented in Figure 1. MPAP increased promptly, within 2–3 min, after either U-46619 or 8-iso-$PGF_{2\alpha}$, then progressively returned to preinjection values within a few minutes at doses $\leqslant 2.5$ and 160 $\mu g\ kg^{-1}$, respectively. At higher doses, mortality of 5/9 and 9/9 rats at 10 and 40 $\mu g\ kg^{-1}$ of U-46619 and of 2/8 at both 160 and 630 $\mu g\ kg^{-1}$ of 8-iso-$PGF_{2\alpha}$ was noted. The increases in MPAP induced by 8-iso-$PGF_{2\alpha}$ were dose-dependent with an ED_{50} being 28 fold less potent than that of U-46619 (geometric mean agonist dose (with 95% confidence limits in parentheses): 39.0 (31.4–50.6) vs 1.4 (1.2–1.6) $\mu g\ kg^{-1}$, respectively; Figure 2). The maximum responses did not differ significantly between 8-iso-$PGF_{2\alpha}$ and U-46619 (21.0 ± 1.0 and 25.8 ± 1.9 mmHg at 10 $\mu g\ kg^{-1}$ of U-46619 and 630 $\mu g\ kg^{-1}$ of 8-iso-$PGF_{2\alpha}$, respectively; $P>0.05$; Figure 2, Table 2).

As shown in Table 2, over the same range of doses, U-46619-induced slight increases in MSAP at low doses and marked decreases at higher doses. A similar trend was observed following injection of 8-iso-$PGF_{2\alpha}$. Slight but not statistically significant increases in MSAP occurred at low doses ($\leqslant 160\ \mu g\ kg^{-1}$) and marked decreases at higher doses of 8-iso-$PGF_{2\alpha}$ (Table 2).

Slight increases in HR were detected at the highest, non lethal, doses of U-46619 and 8-iso-$PGF_{2\alpha}$ (Table 2).

We next determained whether the pulmonary hypertensive activities of U-46619 and 8-iso-$PGF_{2\alpha}$ were susceptible to blockade by the silent TP receptor antagonist SQ 29,548 (Bertolino *et al*., 1995a).

Effect of TP receptor blockade on the responses to U-46619 and 8-iso-$PGF_{2\alpha}$

As shown in Table 1, SQ 29,548 *per se* was devoid of any significant effect on MSAP, MPAP and HR compared to vehicle-infused animals.

As depicted in Figure 2, SQ 29,548 antagonized both U-46619 and 8-iso-$PGF_{2\alpha}$-induced pulmonary hypertension, although slight but statistically significant, increases in MPAP compared to vehicle were observed at the highest doses of U-46619 and 8-iso-$PGF_{2\alpha}$. Changes in MSAP evoked by U-46619 and 8-iso-$PGF_{2\alpha}$ were fully blocked by SQ 29,548. Furthermore, no mortality was detected at the highest doses of U-46619 and 8-iso-$PGF_{2\alpha}$. These results strongly suggest that the

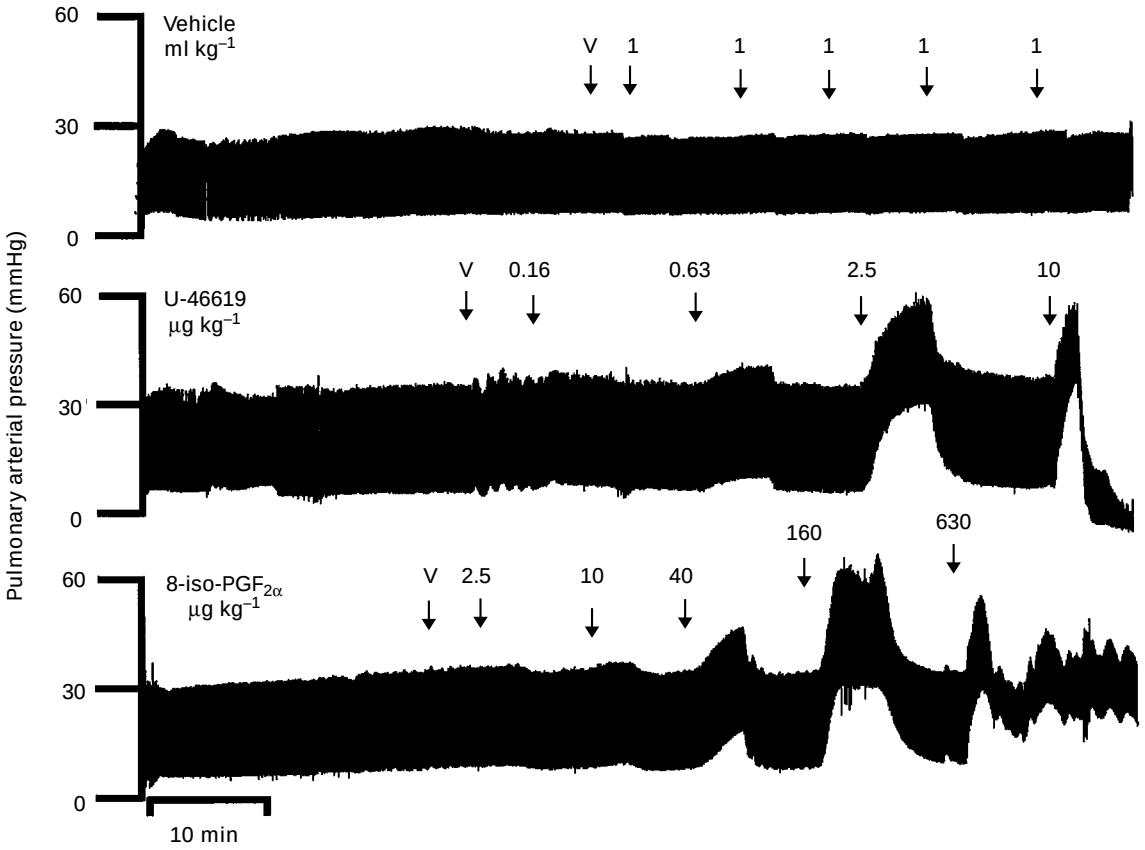

Figure 1 Typical recordings of pulmonary arterial pressure following injection of successive increasing doses of either U-46619 (0.16, 0.63, 2.5 and 10 μg kg^{-1}, i.v.), 8-iso-PGF$_{2\alpha}$ (2.5, 10, 40, 160 and 630 μg kg^{-1}, i.v.) or equal volumes of the vehicle (1). Drugs were administered as 1 ml kg^{-1} solution over 5 min as indicated by the arrows. V indicates the initiation of SQ 29,548 vehicle administration.

Table 1 Baseline values for haemodynamic parameters

Groups	*Pre-treatment*	*Dose* (μg kg^{-1} i.v. + μg kg^{-1} h^{-1})	*Treatment*	*Dose* (μg kg^{-1} i.v.)	n	*BW* (g)	*MPAP* (mmHg)	*MSAP* (mmHg)	*HR* (beats min^{-1})
1	Vehicle	1 ml kg^{-1} + 20 μl min^{-1}	Vehicle	5 × 1 ml kg^{-1}, i.v.	15	332 ± 7	18.1 ± 0.8	85 ± 5	346 ± 13
2	Vehicle	1 ml kg^{-1} + 20 μl min^{-1}	U-46619	0.16 to 40	9	352 ± 9	19.1 ± 0.7	84 ± 8	358 ± 17
3	Vehicle	1 ml kg^{-1} + 20 μl min^{-1}	8-Iso-PGF$_{2\alpha}$	2.5 to 630	8	351 ± 6	18.8 ± 0.7	88 ± 5	347 ± 16
4	SQ 29,548	630 + 630	Vehicle	5 × 1 ml kg^{-1}, i.v.	6	370 ± 6	19.4 ± 0.8	87 ± 5	346 ± 13
5	SQ 29,548	630 + 630	U-46619	0.16 to 40	6	349 ± 7	20.1 ± 0.6	81 ± 8	342 ± 6
6	SQ 29,548	630 + 630	8-Iso-PGF$_{2\alpha}$	2.5 to 630	6	358 ± 12	20.1 ± 0.6	87 ± 6	351 ± 15
7	8-Iso-PGF$_{2\alpha}$	10 + 10	Vehicle	5 × 1 ml kg^{-1}, i.v.	6	387 ± 8	22.6 ± 2.2	89 ± 7	366 ± 12
8	8-Iso-PGF$_{2\alpha}$	10 + 0	U-46619	0.16 to 40	6	362 ± 8	20.1 ± 0.6	87 ± 7	364 ± 13
9	8-Iso-PGF$_{2\alpha}$	10 + 10	U-46619	0.16 to 40	6	363 ± 16	21.1 ± 0.4	92 ± 7	391 ± 18

Values are mean ± s.e.mean. Baseline values were obtained before treatment with U-46619, 8-iso-PGF$_{2\alpha}$ or vehicle. *n*, number of rats; BW, body weight; MPAP, mean pulmonary arterial pressure; MSAP, mean systemic arterial pressure; HR, heart rate.

pulmonary hypertensive responses evoked by U-46619 and 8-iso-PGF$_{2\alpha}$ are mediated by TP receptors.

We next determined whether 8-iso-PGF$_{2\alpha}$ pretreatment reduced pulmonary hypertension induced by either U-46619 or by itself as would be predicted by receptor theory for a partial agonist (Kenakin, 1993).

Effect of 8-iso-PGF$_{2\alpha}$ on the responses elicited by U-46619 and by itself

Baseline MPAP values were slightly, but significantly, higher in rats pretreated with ED$_{25}$ (10 μg kg^{-1} bolus + 10 μg kg^{-1} h^{-1}), but not the ED$_{10}$ (10 μg kg^{-1} h^{-1}), of 8-iso-PGF$_{2\alpha}$ compared to those receiving the vehicle (MPAP: 21.8 ± 1.0 vs 18.5 ± 0.4 mmHg in 8-iso-PGF$_{2\alpha}$ (ED$_{25}$) and vehicle-pretreated animals, respectively; $n = 12$ and 32, respectively; $P < 0.05$ between groups). Neither MSAP or HR differed significantly between vehicle and 8-iso-PGF$_{2\alpha}$-pretreated groups (Table 1).

Pretreatment with 8-iso-PGF$_{2\alpha}$ at a threshold pulmonary hypertensive dose (ED$_{10}$) and at a dose equal to the ED$_{25}$ did not antagonize the pulmonary hypertensive responses evoked by U-46619 (Figure 3; Table 3). In fact a propensity to potentiate pulmonary hypertensive responses to U-46619 was noted following pretreatment with the ED$_{10}$ of 8-iso-PGF$_{2\alpha}$ (Table 3). The maximal responses evoked by U-46619 in the presence of 8-iso-PGF$_{2\alpha}$ were slightly higher than those evoked by U-46619 alone (21.0 ± 1.0 vs 27.6 ± 2.6 and

Table 2 Mean maximal changes in haemodynamic parameters

Groups	Pretreatment	Treatment	Doses 1	2	3	4	5	Doses 1	2	3	4	5	Doses 1	2	3	4	5
			Mean pulmonary arterial pressure (mmHg)					*Mean systemic arterial pressure* (mmHg)					*Heart rate* (beats min^{-1})				
1	Vehicle	Vehicle	0.7±0.2	0.4±0.1	0.3±0.1	0.6±0.1	0.7±0.2	4.2±1.4	4.9±1.0	3.8±1.1	3.6±0.8	4.2±1.0	6.8±4.2	12.7±3.0	13.3±4.6	7.9±3.6	17.4±3.9
2	Vehicle	U-46619	1.5±0.3	4.2±0.4	15.1±0.9	21.0±1.0		4.9±1.3	12.0±3.2	13.6±3.9	−54.9±5.3		0.8±1.5	8.5±3.9	14.5±5.7	41.8±13.6	
3	Vehicle	8-iso-PGF$_{2\alpha}$	2.4±1.6	2.5±0.4	13.2±1.9	22.9±1.7	25.8±1.9	4.3±1.2	7.9±1.8	11.1±3.5	−41.5±9.4	−60.7±12.4	0.6±1.3	6.8±2.9	20.2±6.2	25.8±9.8	53.9±8.0
1 vs 2				****	****	****	ND		*	*	****	ND				*	ND
1 vs 3				***	****	****	****				****	****					****
2 vs 3				*			ND					ND					ND
4	SQ 29,548	Vehicle	0.7±0.3	0.5±0.3	0.7±0.2	1.2±0.4	1.1±0.3	4.8±2.1	5.9±2.3	2.6±1.6	3.7±2.5	1.5±2.3	5.1±4.6	7.3±1.3	6.1±2.4	3.9±2.8	1.3±3.5
5	SQ 29,548	U-46619	−1.0±1.6	0.1±1.9	−0.4±1.8	2.5±0.4	7.2±1.9	2.6±1.3	3.6±1.6	4.4±1.0	−2.1±1.0	−12.3±4.1	5.1±4.6	4.7±1.7	3.9±1.3	16.2±7.4	19.8±4.8
6	SQ 29,548	8-iso-PGF$_{2\alpha}$	0.8±0.2	1.4±0.4	1.9±0.4	3.5±0.8	10.1±3.9	6.9±1.9	5.3±1.5	5.3±1.8	−5.0±1.1	−10.0±3.1	7.0±2.9	9.5±3.3	9.4±2.8	8.7±3.3	12.2±4.1
4 vs 5							*										****
4 vs 6							****										*
2 vs 5			*	****	****	****	ND		*		****	ND					ND
3 vs 6					****	****	****				****	****					****
7	8-iso-PGF$_{2\alpha}$ (ED$_{25}$)	Vehicle	0.9±0.4	0.5±0.2	0.8±0.2	1.4±0.2	2.0±0.5	2.2±0.8	3.6±1.3	7.3±1.8	10.2±4.2	6.8±2.7	0.6±1.0	−0.7±1.1	3.1±2.9	7.8±4.2	9.7±3.5
8	8-iso-PGF$_{2\alpha}$ (ED$_{10}$)	U-46619	2.6±0.5	5.8±0.5	20.7±1.3	27.6±2.6		12.4±4.4	24.1±6.6	28.5±9.4	−69.5±12.6		8.8±6.0	24.0±6.2	34.4±5.0	39.2±17.9	
9	8-iso-PGF$_{2\alpha}$ (ED$_{25}$)	U-46619	1.2±0.3	2.8±0.6	17.5±2.8	23.4±3.3		6.7±1.7	8.3±3.3	15.7±3.2	−70.2±7.3		0.8±1.5	2.4±2.7	17.9±4.3	45.6±32.6	
7 vs 9				*	****	****	ND				****	ND					ND
2 vs 8					***	***	ND	**		***		ND		***	**		ND
2 vs 9							ND					ND					ND
8 vs 9				***			ND		***	*		ND		***			ND

Doses 1 to 5 refer to 0.16, 0.63, 2.5, 10 and 40 μg kg^{-1} of U-46619 and to 2.5, 10, 40, 160 and 630 μg kg^{-1} of 8-iso-PGF$_{2\alpha}$. Values are mean±s.e.mean. *, **, *** and **** $P<0.05$, 0.02, 0.01 and 0.001 between groups; ND, not determined due to mortality.

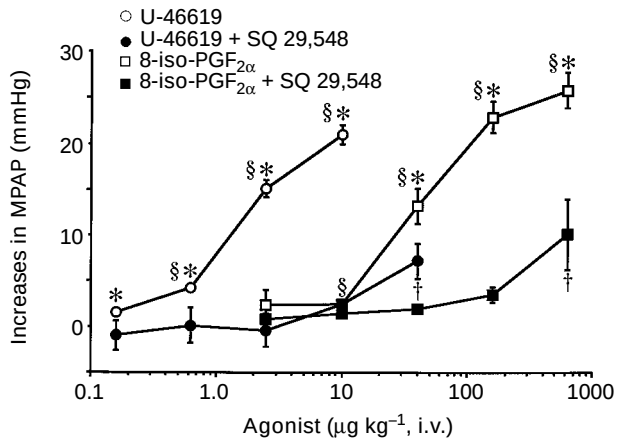

Figure 2 Effect of TP receptor blockade on the mean maximal changes in mean pulmonary arterial pressure (MPAP) induced by U-46619 ($n=15$) and 8-iso-PGF$_{2\alpha}$ ($n=14$). *$P<0.05$ between U-46619 or 8-iso-PGF$_{2\alpha}$ in the absence ($n=17$) vs presence of SQ 29,548 ($n=12$). §$P<0.05$ vs vehicle group. †$P<0.05$ vs SQ 29,548 alone.

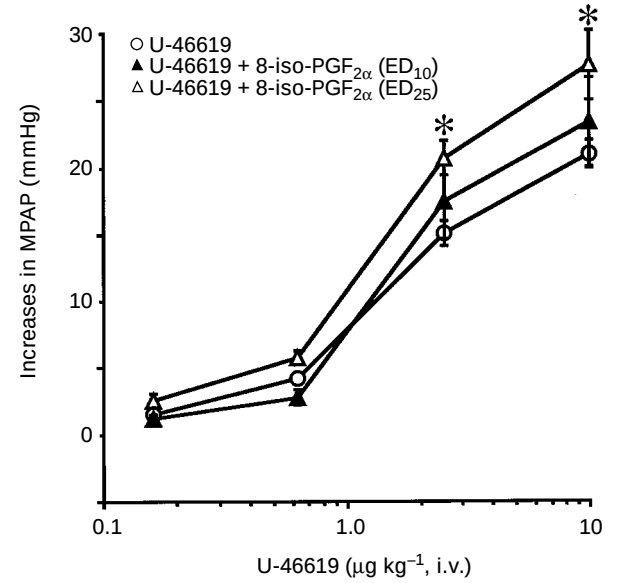

Figure 3 Effect of pretreatment with 8-iso-PGF$_{2\alpha}$ on the mean maximal changes in mean pulmonary arterial pressure (MPAP) induced by U-46619. 8-Iso-PGF$_{2\alpha}$ was administered at 10 μg kg^{-1} h^{-1} (ED$_{10}$; $n=6$) or 10 μg kg^{-1} i.v. followed by 10 μg kg^{-1} h^{-1} (ED$_{25}$; $n=6$). The effects of U-46619 alone are also presented ($n=9$). *$P<0.05$ between U-46619 alone vs in presence of high dose of 8-iso-PGF$_{2\alpha}$.

23.4±3.3 mmHg in the vehicle, ED$_{10}$ and ED$_{25}$ of 8-iso-PGF$_{2\alpha}$-pretreated animals; $P<0.05$ and $P>0.05$, respectively).

As shown for MPAP, increases in MSAP and HR evoked by U-44619 in the presence of the ED$_{10}$ of 8-iso-PGF$_{2\alpha}$ were slightly greater than those induced by U-46619 alone. Changes in MSAP and HR evoked by U-44619 in the presence of the ED$_{25}$ of 8-iso-PGF$_{2\alpha}$ were not significantly different from those induced by U-46619 alone.

In separate experiments, the administration of single doses of 8-iso-PGF$_{2\alpha}$ was associated with increases in MPAP of 2.3±0.7, 5.5±1.2, 14.7±2.2 and 20.5±3.5 mmHg at 2.5, 10, 40 or 160 μg kg^{-1}, i.v. These increases were not different from those observed when the same doses were administered successively (2.4±1.6, 2.5±0.4, 13.2±1.9 and 22.9±1.7 mmHg at 2.5, 10, 40 or 160 μg kg^{-1}, i.v., respectively).

These results demonstrate that 8-iso-PGF$_{2\alpha}$ failed to attenuate the responses evoked by U-46619 or by itself suggesting that 8-iso-PGF$_{2\alpha}$ does not act as an antagonist at TP receptors in the rat pulmonary vasculature at the doses employed.

Discussion

The results of the present study show that 8-iso-PGF$_{2\alpha}$ evoked dose-dependent pulmonary hypertensive responses of similar amplitude, but lower potency, compared to those of the high efficacy TP receptor agonist U-46619. Both U-46619 and 8-iso-PGF$_{2\alpha}$-evoked responses were inhibited by the TP receptor antagonist, SQ 29,548. Furthermore, pretreatment with 8-iso-PGF$_{2\alpha}$ failed to antagonize the pulmonary hypertensive responses evoked either by U-46619 or by the isoprostane itself. Collectively, these findings indicate that in the pulmonary vascular bed of the rat *in vivo*, 8-iso-PGF$_{2\alpha}$ appears to act as an agonist of high intrinsic activity and moderate potency at TP receptors, but do not exclude the possibility that it may act as a partial TP receptor agonist in other organs and/or species.

Intrinsic activity of 8-iso-PGF$_{2\alpha}$

Systemically administered 8-iso-PGF$_{2\alpha}$ dose-dependently increased MPAP, with similar maximum increases but lower potency compared to the TP receptor agonist, U-46619. Pulmonary hypertensive responses evoked by 8-iso-PGF$_{2\alpha}$ have previously been shown in rabbit lungs perfused *in situ* (Banerjee *et al*., 1992) and in the rat isolated lung, in which bronchoconstriction was also seen (Kang *et al*., 1993). 8-iso-PGF$_{2\alpha}$ was 28 fold less potent than U-46619 in inducing pulmonary hypertension in the present study. Interestingly, 8-iso-PGF$_{2\alpha}$ was found to be equi (Crankshaw, 1995) or less (Kinsella *et al*., 1997; Kawikova *et al*., 1996; Kromer & Tippins, 1996; Zhang *et al*., 1996) potent than U-46619 in inducing functional responses such as calcium mobilization in TP receptor transfected HEK 293 cells (Kinsella *et al*., 1997), or in mediating contractions of (a) human and guinea-pig airways (Kawikova *et al*., 1996), (b) porcine and bovine coronary arteries (Kromer & Tippins, 1996), (c) rat and guinea-pig aorta (Zhang *et al*., 1996), and (d) non-pregnant human myometrium (Crankshaw, 1995). Finally, 8-iso-PGF$_{2\alpha}$ in contrast to U-46619 failed to evoke contractions of ovine coronary arteries, but antagonized the contractile responses evoked by U-46619 (Kromer & Tippins, 1996). Although the plasma concentrations of U-46619 and 8-iso-PGF$_{2\alpha}$ attained in the present experiments are likely to be well above the physiological range of TxA$_2$ and 8-iso-PGF$_{2\alpha}$ concentrations, the pharmacological effects observed appear to be relevant to pathological situations in which high local concentrations of TxA$_2$ (Gresele *et al*., 1991), and possibly 8-iso-PGF$_{2\alpha}$ could be produced.

In the present experiments, the efficacy of 8-iso-PGF$_{2\alpha}$, as assessed by the mean maximal increases in MPAP, was sufficient to raise MPAP to a similar extent to that of U-46619. In fact, 8-iso-PGF$_{2\alpha}$ was found to possess similar intrinsic activity compared to U-46619 in rat aorta (Zhang *et al*., 1996) and in the human myometrium (Crankshaw, 1995) or lower intrinsic activity in guinea-pig aorta (Zhang *et al*., 1996) and bovine, porcine and ovine coronary arteries (Kromer & Tippins, 1996). Interestingly, we observed that 8-iso-PGF$_{2\alpha}$ evoked slight increases in haematocrit in the rat but to a lesser extent than U-46619 (unpublished observation). Furthermore, the 8-iso-PGF$_{2\alpha}$-induced mortality was lower at doses producing pulmonary equihypertensive responses compared to U-46619.

Inhibition of 8-iso-PGF$_{2\alpha}$ and U-46619-induced responses by SQ 29,548

As the pulmonary hypertensive responses evoked by both 8-iso-PGF$_{2\alpha}$ and U-46619 were antagonized by SQ 29,548, clearly the receptors mediating both U-46619 and 8-iso-PGF$_{2\alpha}$-

induced increases in MPAP recognize SQ 29,548, which does not lend support to the involvement of different receptors in the responses mediated by 8-iso-$PGF_{2\alpha}$ as claimed by some groups (Fukunaga *et al*., 1993; Yura *et al*., 1995; Pratico *et al*., 1996). Indeed, the present consensus is that constrictor responses evoked by 8-iso-$PGF_{2\alpha}$ are mediated by TP receptors. 8-Iso-$PGF_{2\alpha}$ induces smooth muscle constriction in a number of preparations which are fully prevented by TP receptor antagonists (Banerjee *et al*., 1992; Takahashi *et al*., 1992; Kang *et al*., 1993; Crankshaw, 1995; Kromer & Tippins, 1996; Zhang *et al*., 1996).

Effect of 8-iso-$PGF_{2\alpha}$ on the responses to U-46619 and itself

A further series of experiments was performed to examine whether 8-iso-$PGF_{2\alpha}$ pretreatment attenuated the pulmonary hypertensive responses evoked by U-46619 or itself as would be predicted by receptor theory for a partial agonist (Kenakin, 1993). Pretreatment of animals with 8-iso-$PGF_{2\alpha}$ failed to reduce U-46619-induced pulmonary hypertension.

8-Iso-$PGF_{2\alpha}$ failed to diminish its own pulmonary hypertensive responses as confirmed by similar increases in MPAP evoked by the successive administration of increasing doses of 8-iso-$PGF_{2\alpha}$ and those observed when single doses were administered only once per animal. As pretreatment of animals with doses of 8-iso-$PGF_{2\alpha}$ higher than the estimated ED_{10} or ED_{25} was not performed, we cannot exclude the possibility that antagonism of pulmonary hypertensive reponses evoked by U-46619 or by 8-iso-$PGF_{2\alpha}$ itself could occur under these conditions, in line with partial agonist properties of 8-iso-$PGF_{2\alpha}$ at TP receptors described elsewhere (Morrow *et al*., 1992b; Kromer & Tippins, 1996). However the fact that 8-iso-$PGF_{2\alpha}$ failed to attenuate its own pulmonary hypertensive responses strongly suggests that 8-iso-$PGF_{2\alpha}$ does not exert antagonist activity at pulmonary vascular TP receptors. This phenomenon can be explained when TP receptor reserve is taken into consideration.

The functional response to a particular agonist in a given preparation is dependent upon receptor density and coupling of these receptors to intracellular signalling (Kenakin, 1993). In theory, when receptor reserve is low, the response to a partial agonist will be reduced whereas a high efficacy agonist will still induce a maximal response. Thus in the pulmonary vasculature of the rat, TP receptor reserve is likely to be sufficiently high for 8-iso-$PGF_{2\alpha}$ to elicit similar maximal responses to those evoked by the high efficacy agonist, U-46619 assuming that 8-iso-$PGF_{2\alpha}$-induced responses are also mediated by TP receptors. This is corroborated by the fact that the pulmonary vascular bed of the rat is highly responsive to TP receptor agonists (Bertolino *et al*., 1995a, b; Valentin *et al*., 1996). Similarly, TP receptor reserve is likely to be relatively low in preparations in which U-46619 but not 8-iso-$PGF_{2\alpha}$ elicited agonist responses, such as ovine coronary arteries (Kromer & Tippins, 1996). We tentatively suggest that TP receptor reserve is the primary determinant of the degree of intrinsic activity exhibited by 8-iso-$PGF_{2\alpha}$ in a given preparation.

Thus, in the rat pulmonary vasculature 8-iso-$PGF_{2\alpha}$ appears to act as an agonist of high intrinsic activity and moderate potency at SQ 29,548-sensitive (probably TP) receptors. However, our data do not exclude, and are compatible with, the possibility that 8-iso-$PGF_{2\alpha}$ acts as a partial agonist at TP receptors in other organs and/or species in which TP receptor reserve is relatively low.

In conclusion, in anaesthetized, open-chest rats, the F_2-isoprostane derivative, 8-iso-$PGF_{2\alpha}$, elicited dose-dependent pulmonary hypertensive responses which were antagonized by the TP receptor antagonist SQ 29,548 strongly suggesting that these responses were mediated by TP receptors. Furthermore 8-iso-$PGF_{2\alpha}$ failed to attenuate its own pulmonary hypertensive responses or those evoked by U-46619. Thus, in the rat pulmonary vasculature 8-iso-$PGF_{2\alpha}$ appears to act as an agonist of high intrinsic activity and moderate potency at TP receptors.

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