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Coronary artery constriction by the isoprostane 8-epi prostaglandin $F_{2\alpha}$

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1 This study was undertaken to compare the effects of 8-epi prostaglandin $F_{2\alpha}$ (8-epi $PGF_{2\alpha}$) to those of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and U46619, a thromboxane mimetic, on ovine, bovine and porcine coronary arteries.

2 8-epi $PGF_{2\alpha}$ constricted porcine and bovine coronary arteries in a concentration-dependent manner with EC_{50} values of 689.0 ± 229.3 and 1361.0 ± 272.3 nM, respectively, but had no effect on ovine coronary arteries.

3 U46619 was a potent vasoconstrictor of porcine, ovine and bovine coronary arteries with EC_{50} values of 33.0 ± 23.5, 373.3 ± 69.7 and 254.1 ± 134.3 nM, respectively. E_{max} values were significantly greater than those obtained with 8-epi $PGF_{2\alpha}$.

4 $PGF_{2\alpha}$ constricted porcine and bovine coronary arteries in a concentration-dependent manner with EC_{50} values of 1631.0 ± 207.6 and 3644.0 ± 344.8 nM, respectively, but had no effect on ovine coronary arteries.

5 Concentration-dependent constriction to U46619 in porcine coronary arteries was competitively inhibited by SQ29548 (10^{-8} M to 10^{-7} M) and BM13505 (10^{-8} M to 10^{-6} M) with no decrease in maximal responses.

6 Concentration-dependent constriction to 8-epi $PGF_{2\alpha}$ in porcine coronary arteries was inhibited in a concentration-dependent manner by SQ29548 (10^{-8} M to 10^{-7} M) and BM13505 (10^{-8} M to 10^{-6} M). However, the inhibition was associated with a decrease in maximal response.

7 Maximal responses of porcine coronary artery to U46619 (1 μM) and 8-epi $PGF_{2\alpha}$ (30 μM) were inhibited in a concentration-dependent manner by SQ29548 with IC_{50} values 99 ± 12.36 nM and 46.5 ± 18.67 nM, respectively.

8 Although ovine coronary arteries did not constrict to 8-epi $PGF_{2\alpha}$, pre-incubation of these vessels with 8-epi $PGF_{2\alpha}$ caused a rightward shift of the U46619 response curve in a concentration-dependent manner.

9 Pre-incubation of porcine coronary arteries with 8-epi $PGF_{2\alpha}$ competitively inhibited responses to U46619 with a Schild slope of 0.99 and a pA_2 of 6.13.

10 We conclude that 8-epi $PGF_{2\alpha}$ is a vasoconstrictor within porcine and bovine coronary arteries, with a potency approximately twice that of $PGF_{2\alpha}$ but 5-20 times lower than U46619. The data suggest that 8-epi $PGF_{2\alpha}$ is acting as a partial agonist on the TP-receptor in the coronary vasculature.

Keywords: Isoprostane; ischaemia; oxidant stress; coronary artery disease

Introduction

Free radical mediated lipid peroxidation has been implicated in a number of coronary artery disease states including stable and unstable angina (Dubois Rande *et al.*, 1994), ischaemic heart disease (Chandra *et al.*, 1994) and the pathogenesis of atherosclerosis (Jayakumari *et al.*, 1992). High levels of lipid peroxides have been found during the progression of atherosclerosis (Stringer *et al.*, 1989) and strong evidence has accumulated suggesting that circulating peroxides play a pivotal role in the process of atherosclerotic lesion formation (Hennig & Chow, 1988).

Recently, a novel family of prostanoids, the isoprostanes, has been described. These prostanoids are unique in that they are produced *in vivo* by a non-enzymatic free radical peroxidation of arachidonic acid (Morrow *et al.*, 1994a). Isoprostanes, therefore, are produced in conditions of oxidant stress and, as they are stable within the circulation, have been quantified as a reliable measure of oxidant injury *in vivo* (Roberts & Morrow, 1994).

8-epi Prostaglandin $F_{2\alpha}$ (8-epi $PGF_{2\alpha}$), one of the F_2-isoprostanes that has been shown to be produced in large quantities *in vivo*, has also been shown to be an extremely potent renal vasoconstrictor with activity in the low nanomolar concentration range (Morrow *et al.*, 1990). 8-epi $PGF_{2\alpha}$ has also been shown to be a potent vasoconstrictor of the pulmonary vascular bed (Banerjee *et al.*, 1992). Interestingly, the vasoconstrictor properties of this prostanoid have been shown to be inhibited by selective thromboxane receptor (TP-) antagonists (Morrow *et al.*, 1992), suggesting a mechanism of action via the TP-receptor. However, a unique isoprostane receptor has also been suggested (Fukunaga *et al.*, 1993).

Since the isoprostanes are produced *in vivo* under oxidant stress conditions, we hypothesized that they may be synthesized in coronary artery disease and have an effect on the coronary vasculature. Therefore, in this study we examined the pharmacology of 8-epi $PGF_{2\alpha}$ on coronary arteries from different species and attempted to elucidate a mechanism of action by use of selective thromboxane receptor antagonists.

Preliminary accounts of the work described in this study have been presented to the British Pharmacological Society (Kromer & Tippins, 1995; 1996).

Methods

Pig, sheep and cow hearts were obtained from an abattoir immediately following slaughter and washed in ice-cold Tyrode solution (composition, mM: NaCl 136.9, KCl 2.68,

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$CaCl.2H_2O$ 1.8, $MgCl_2.6H_2O$ 0.1, $NaH_2PO_4.2H_2O$ 0.42, $NaHCO_3$ 11.9 and glucose 5.55) that previously had been gassed with 100% O_2. The hearts were then transferred to fresh oxygenated Tyrode solution and transported to the laboratory.

The left anterior descending (LAD) coronary artery was identified and excised away from the heart leaving the circumflex attached. The vessel was classified according to the American Heart Association grading committee guidelines into segments 6, 7 and 8 (Austen *et al.*, 1975) and cut into 3 mm rings. Three rings from each segment were mounted on stainless steel wires, 0.25 mm in diameter, and immersed in 5 ml organ baths containing Krebs solution (composition in mM: NaCl 118, KCl 4.8, $CaCl.2H_2O$ 2.5, $MgSO_4.7H_2O$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 24 and glucose 11) maintained at 37°C and continuously gassed with 95% O_2 and 5% CO_2.

Responses were measured with isometric tension transducers (Lectromed UF1) coupled to three BBC (Model SE120) 3 channel recorders. Tissues were stretched to an optimal tension (Mulvany & Halpern, 1977) and allowed to equilibrate for 30 min. The vessels were constricted to a single dose of KCl (40 mM), and all subsequent responses presented as a percentage of the response to KCl. Initially vessels were tested for functional endothelium by their ability to relax to acetylcholine (10^{-6} M) when pre-constricted with noradrenaline (10^{-6} M), although no attempt was made to remove the endothelium.

The agonists U46619, 8-epi $PGF_{2\alpha}$ and $PGF_{2\alpha}$ were added in a cumulative dose fashion, allowing either ten minutes between doses or until a stable response was established. The thromboxane antagonists (SQ29548 or BM13505), indomethacin or, when tested for antagonist activity, 8-epi $PGF_{2\alpha}$, were added to the bath at the concentration required and left to equilibrate for a period of 15 min before the concentration-dependent contraction to agonists. Alone, neither the thromboxane receptor antagonists nor indomethacin had any effect on the tissue. After each concentration-response curve had been constructed the vessels were washed and left for 30 min or until a stable baseline was obtained.

Drugs

8-epi $PGF_{2\alpha}$, U46619 (11α, 9α, epoxymethano PGH_2) and $PGF_{2\alpha}$ were purchased from Cascade Biochem Ltd. (Reading, U.K.). The thromboxane receptor antagonists SQ29548 ([1S-[1α,2β(5Z),3β,4α]]-7-[3-[[2-[(phenylamino) carbonyl hydrazino]methyl]-7-oxobicyclo[2.2.1]-hept-2-yl]-5-heptenoic acid) and BM13505 (4-[2-(4-chlorbenzene-sulphonamido)-ethyl]-phenylacetic acid) were obtained from Cayman Chemicals (Ann Arbor, MI, U.S.A.) and Boehringer Mannheim (Lewes, U.K.), respectively. All compounds were dissolved in ethanol and subsequently diluted in Krebs solution. The highest final ethanol concentration of 3.4% in the bath had no effect on the tissue. All other chemicals were of analytical grade and were purchased from either Sigma or BDH.

Statistical analysis

All results are expressed as means ± s.e.mean. Data analysis was performed by Student's unpaired *t* test. A P value <0.05 was taken as statistically significant.

Results

No significant difference was found between the responses of segments 6, 7 and 8 in any of the experiments. Therefore all results are expressed as mean ± s.e.mean for responses of all segments for any given tissue. Indomethacin (2.8) μM had no effect on any response obtained. A functional endothelium was absent in all tissues tested, as they all failed to give an endothelial-dependent relaxation to 10^{-6} M acetylcholine. Mean values of responses to KCl were 27.7 ± 1.4, 21.7 ± 1.3 and 10.2 ± 3.1 g for bovine, porcine and ovine vessels, respectively.

U46619 caused a potent concentration-dependent constriction in porcine coronary artery with an EC_{50} value of 33.0 ± 23.5 nM. The responses were slow in onset and slow to return to baseline after drug washout. 8-epi $PGF_{2\alpha}$ and $PGF_{2\alpha}$ constricted porcine coronary artery with lower potency than U46619. EC_{50} values were 689.8 ± 229 and 1631.0 ± 207.6 nM for 8-epi $PGF_{2\alpha}$ and $PGF_{2\alpha}$, respectively ($n=12$ vessels; Figure 1a), with the mean E_{max} value for 8-epi $PGF_{2\alpha}$ (93.8 ± 4.2 nM) being significantly lower than that for U46619 (117.3 ± 2.8 nM; $P=0.0002$; Student's *t* test).

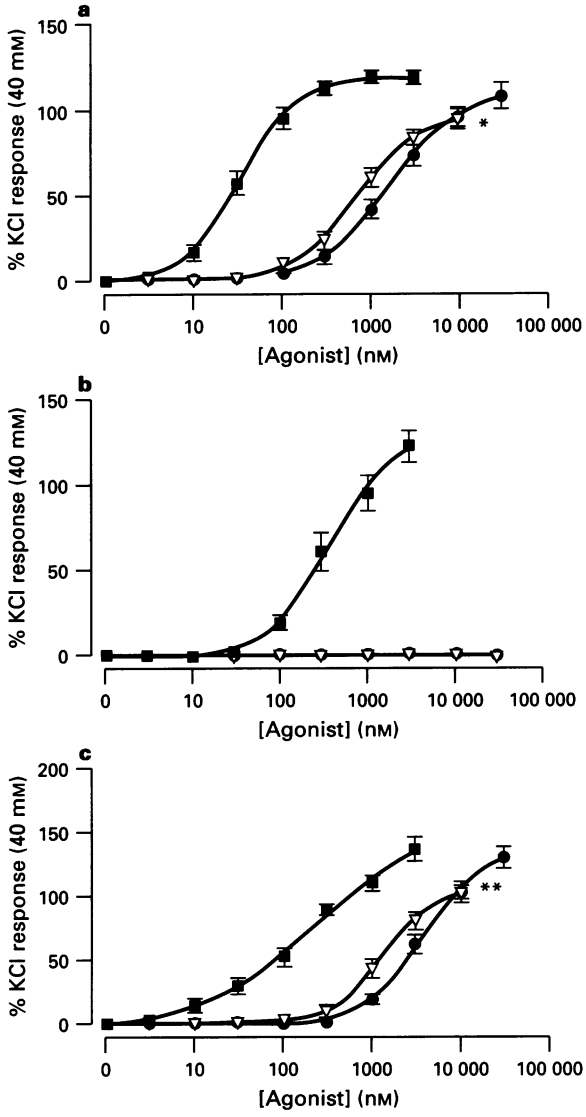

Figure 1 Mean concentration-effect curves to U46619 (■), 8-epi $PGF_{2\alpha}$ (▽) and $PGF_{2\alpha}$ (●) on (a) porcine ($n=12$), (b) ovine ($n=8$) and (c) bovine ($n=4$) coronary arteries. Values are mean and vertical lines show s.e.mean. $^{**}P<0.005$, $^{*}P<0.05$ (Student's *t* test), for comparison of E_{max} values of U46619 and 8-epi $PGF_{2\alpha}$.

U46619 constricted ovine coronary arteries but with a much lower potency than in porcine tissue (EC_{50} 373.3 ± 69.7 nM, $n=8$ vessels). However, 8-epi $PGF_{2\alpha}$ and $PGF_{2\alpha}$ had no effect on ovine coronary arteries, even at concentrations up to 10 μM (Figure 1b).

U46619, 8-epi $PGF_{2\alpha}$ and $PGF_{2\alpha}$ constricted bovine coronary arteries in a concentration-dependent manner with EC_{50} values of 254.1 ± 134.3, 1361.0 ± 272.3 and 3644.0 ± 344.8 nM respectively ($n=5$ vessels; Figure 1c), again with the mean E_{max} value for 8-epi $PGF_{2\alpha}$ (105.5 ± 7.6 nM) being significantly lower than that for U46619 (136.8 ± 9.8 nM; $P=0.0216$; Student's *t* test).

SQ29548 and BM13505 both caused concentration-dependent rightward shifts in the EC_{50} of U46619 on porcine tissue (33.0 ± 23.5 nM control ($n=12$); 259.7 ± 46.4 ($n=4$),

456.9±228.8 ($n=4$) and 5156.0±1445.0 nM ($n=4$), in the presence of 10^{-8}, 3×10^{-8} and 10^{-7} M SQ29548, respectively; 46.3±27.5 ($n=4$), 205.2±40.8 ($n=4$), 236.9±27.8 ($n=4$) and 1113.0±251.5 nM ($n=4$), in the presence of 10^{-8}, 10^{-7} M, 3×10^{-7} and 10^{-6} M BM13505, respectively) with no decrease in E_{max} values at any concentration (Figure 2a and c).

The responses to 8-epi $PGF_{2\alpha}$ in porcine coronary arteries were also inhibited in a concentration-dependent manner by both SQ29548 and BM13505. However, as can be seen in Figure 2b and d, the effect of both compounds was associated with a decrease in E_{max} values. The TP-receptor antagonist SQ29548 was also able to completely inhibit the maximal constriction by both 8-epi $PGF_{2\alpha}$ (30 μM) and U46619 (1 μM) with IC_{50} values of 46.5±18.67 nM and 99±12.36 nM, respectively (Figure 3).

Although 8-epi $PGF_{2\alpha}$ alone had no constrictor effect on ovine tissue, pre-incubation with the isoprostane caused a concentration-dependent rightward shift of the concentration-response curves to U46619 (Figure 4). In this tissue full concentration-response curves in the presence of 8-epi $PGF_{2\alpha}$ could not be achieved, because of the very large quantities of U46619 that would be required. A similar effect was also observed in porcine coronary arteries, where 8-epi $PGF_{2\alpha}$ caused a concentration-dependent contraction and also competitively inhibited the response to U46619 (Figure 5). When dose-ratios of responses equivalent to 110% of the KCl response were used, a Schild regression of this data was linear with a slope of 0.99; this gave a pA_2 of 6.13 (Figure 5).

Discussion

In this study U46619 is a potent constrictor of all coronary vessels tested, whilst both 8-epi $PGF_{2\alpha}$ and $PGF_{2\alpha}$ constricted porcine and bovine coronary arteries but had no effect on ovine coronary arteries. Responses to 8-epi $PGF_{2\alpha}$ were observed at concentrations approximately half the equipotent concentrations of $PGF_{2\alpha}$ in both porcine and bovine coronary arteries. This is of interest, since basal levels of the isoprostane in the circulation have been found to be an order of magnitude higher than those of the cyclo-oxygenase derived prostaglandins. In addition, plasma levels of isoprostanes have been shown to increase as much as 100 fold in conditions of lipid peroxidation. Therefore, in conditions of oxidant stress such as those found in ischaemic heart disease, levels of isoprostanes may rise from basal (35±6 pg ml^{-1}; Morrow & Roberts, 1996) such that they are capable of producing pathophysiological effects. The vasoactive properties of isoprostanes within the coronary arteries may therefore exacerbate the ischaemic condition. A comparable situation has been suggested in hepatorenal syndrome, where 8-epi $PGF_{2\alpha}$ levels have been shown to increase and the prostanoid has a highly potent vasoconstrictor action upon renal arteries (Morrow *et al.*, 1993).

There is substantial evidence that the selective thromboxane antagonist SQ29548 can inhibit 8-epi $PGF_{2\alpha}$-elicited responses, suggesting a mechanism of action via the activation of TP-receptors (Morrow *et al.*, 1992; 1994b; Longmire *et al.*, 1994; Morrow & Roberts, 1996). 8-epi $PGF_{2\alpha}$ has also been shown to be a partial agonist at the TP-receptor, being able to induce shape change in platelets, but primarily acting as an antagonist of U46619-induced aggregation (Morrow *et al.*, 1992). However, 8-epi $PGF_{2\alpha}$ has been shown to be more potent at constricting renal arteries that U46619, suggesting that isoprostanes may act at a receptor related to, but distinct from, the TP-receptor (Morrow & Roberts, 1996). At present only one thromboxane receptor gene has been cloned (Hirata *et al.*,

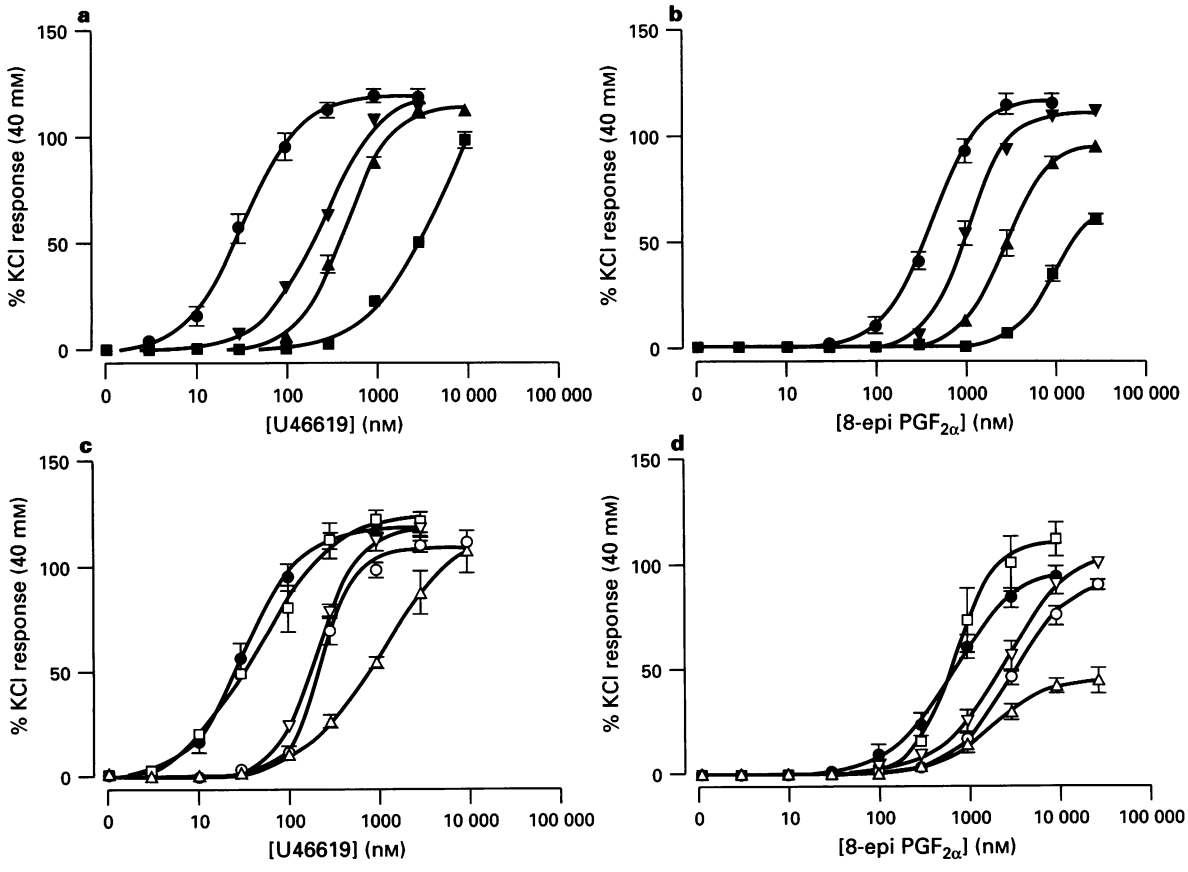

Figure 2 Mean concentration-effect curves to (a) and (c) U46619 and (b) and (d) 8-epi $PGF_{2\alpha}$ in porcine coronary artery, in the absence (●) $n=12$ and presence of (a) and (b) SQ29548 10^{-8} M (▼) $n=4$, 3×10^{-8}M (▲) $n=4$, 10^{-7}M (■) $n=4$ and (c) and (d) BM13505 10^{-8}M (□) $n=4$, 10^{-7}M (▽) $n=4$, 3×10^{-7}M (○) $n=4$ and 10^{-6}M (△) $n=4$. Values are mean and vertical lines show s.e.mean.

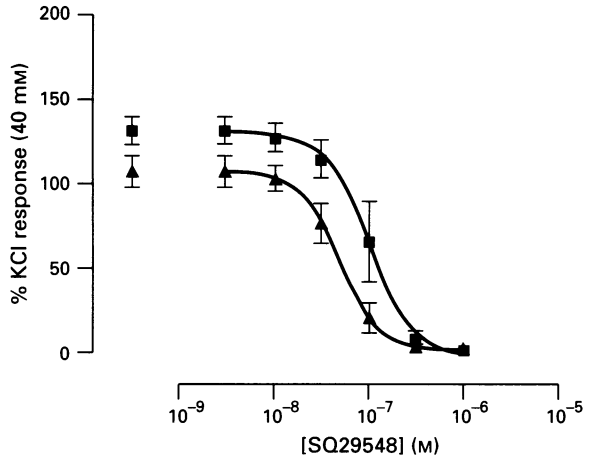

Figure 3 Mean concentration-effect curves to SQ29548 in porcine coronary artery to show the antagonist effect on U46619 (■; $n=4$, 1 μM) and 8-epi $PGF_{2\alpha}$ (▲; $n=4$, 30 μM). The two points adjacent to the ordinate scale are responses to U46619 and 8-epi $PGF_{2\alpha}$ in the absence of SQ29548. Values are mean and vertical lines show s.e.mean.

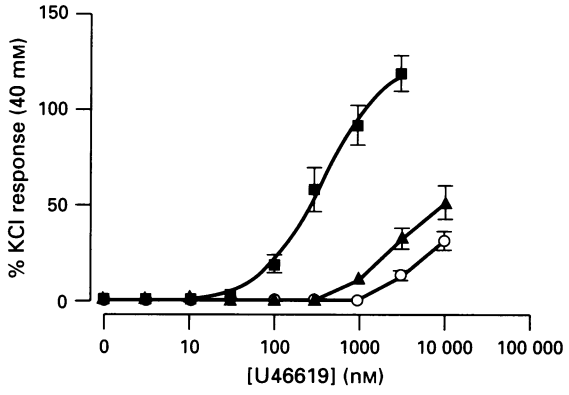

Figure 4 Mean concentration-effect curves to U46619 in ovine coronary artery, in the absence (■) $n=8$ and presence of 8-epi $PGF_{2\alpha}$ 10^{-7} M (▲) $n=4$ and 3×10^{-7} M (○) $n=4$. Values are mean and vertical lines show s.e.mean.

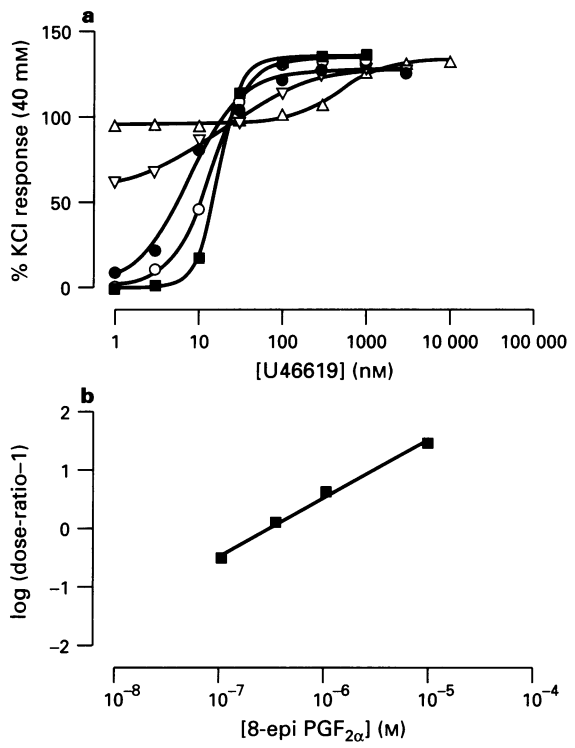

Figure 5 (a) Mean concentration-effect curves to show the antagonist effect of 8-epi $PGF_{2\alpha}$ on U46619-induced responses of porcine coronary artery. Control (■), 10^{-7} M (○), 3×10^{-7} M (●), 10^{-6} M (▽), 10^{-5} M (△) 8-epi $PGF_{2\alpha}$, all $n=4$. Error bars have been omitted for clarity. (b) Schild plot of the antagonist effect of 8-epi $PGF_{2\alpha}$ on U46619-induced responses of porcine coronary artery.

1991), but receptor subtypes have been suggested by the use of pharmacological techniques (Halushka *et al.*, 1995). The suggestion of a separate isoprostane receptor was originally made by Fukunaga who demonstrated that the ability of 8-epi $PGF_{2\alpha}$ to displace U46619 in rat isolated smooth muscle cells did not reflect the potency to induce receptor-mediated responses such as phosphoinositide turnover and calcium flux (Fukunaga *et al.*, 1993). Therefore, the precise mechanism of isoprostane action remains unclear.

In the present study, 8-epi $PGF_{2\alpha}$ constricted both porcine and bovine coronary arteries, but these responses did not reach the same maxima as those to the thromboxane mimetic U46619. If 8-epi $PGF_{2\alpha}$ is acting at the TP-receptor, this would be indicative of partial agonism.

Responses to 8-epi $PGF_{2\alpha}$ in porcine coronary arteries were inhibited by SQ29548 and BM13505. However, the concentration-dependent inhibition was also associated with a decrease in maximal response, unlike that of U46619. This suggests that isoprostane is acting at a novel receptor and is inhibited in a non-competitive manner by the TP-receptor antagonists. This would therefore indicate that these antagonists are not selective for the TP-receptor.

However, the antagonistic effect of 8-epi $PGF_{2\alpha}$ on U46619-induced responses in ovine and porcine coronary arteries provides support for the suggestion that isoprostane is acting as a partial agonist at the TP-receptor. In ovine coronary arteries 8-epi $PGF_{2\alpha}$ antagonised U46619 concentration responses in a competitive manner even though alone the isoprostane had no effect. The explanation for this could be that the receptor reserve is too low for the partial agonist to have a constrictor effect, but receptor occupancy by 8-epi $PGF_{2\alpha}$ reveals its competitive antagonistic affect on the full agonist U46619. In this tissue maximal responses to U46619 in the presence of 8-epi $PGF_{2\alpha}$ could not be obtained because of the large quantities of U46619 required. However, the data from porcine tissue agree with the theoretical effects of a partial agonist on responses produced by a full agonist (Kenakin, 1993). In this tissue, 8-epi $PGF_{2\alpha}$ had a dual effect. It produced an agonist effect and it shifted the U46619 concentration-response curve to the right in a concentration-dependent manner. The competitive nature of the effect of 8-epi $PGF_{2\alpha}$ was confirmed by the Schild regression that was linear and gave a slope of 0.99. The concentrations at which 8-epi $PGF_{2\alpha}$ had agonist and antagonist effects were also very similar and this suggests that 8-epi $PGF_{2\alpha}$ acts at a single receptor.

If 8-epi $PGF_{2\alpha}$ is acting solely as a partial agonist at the TP-receptor, then the results with the thromboxane antagonists have to be explained in this context. A possible explanation is that the action of 8-epi $PGF_{2\alpha}$ on the TP-receptor requires a high level of receptor occupancy. Initially the thromboxane antagonists act to inhibit responses to the isoprostane in a competitive manner, since with low concentrations of the antagonists the E_{max} is not depressed. With higher concentrations the receptor reserve is reduced and 8-epi $PGF_{2\alpha}$ then is unable to occupy a sufficient number of receptors to achieve this maximal effect.

It must be stressed that although our results suggest a partial agonism by 8-epi $PGF_{2\alpha}$ at TP-receptors in coronary vasculature, as in the case of platelets, the hypothesis of the isoprostane receptor cannot be ruled out. However, if this were the case then the selective TP-receptor antagonists would also

have to act at an isoprostane receptor and in a non-competitive manner; this would contradict previous suggestions that these antagonists are selective for the TP-receptor (Ogletree *et al.*, 1985). Another possibility is that 8-epi $PGF_{2\alpha}$ acts on both the TP-receptor and an isoprostane receptor in a similar manner to $PGF_{2\alpha}$, which has affinity for the TP-receptor as well as the $PGF_{2\alpha}$ (FP)-receptor. The more plausible explanation is that 8-epi $PGF_{2\alpha}$ is acting as a partial agonist on the TP-receptor, but further investigation is required to clarify this situation.

In summary, these results show that 8-epi $PGF_{2\alpha}$ is a vasoconstrictor in porcine and bovine coronary arteries with a constrictive potency twice that of $PGF_{2\alpha}$. The vasoconstrictor action of 8-epi $PGF_{2\alpha}$ can be inhibited by selective TP-receptor antagonists and the effect of U46619 can be inhibited by 8-epi $PGF_{2\alpha}$, suggestive of a partial agonist action for 8-epi $PGF_{2\alpha}$ on the TP-receptor. It should also be noted that although 8-epi $PGF_{2\alpha}$ is much less potent than U46619 in porcine and bovine coronary arteries, the production of isoprostanes, unlike thromboxane, is not inhibited by aspirin. Importantly therefore, isoprostanes may still exert their effects in coronary artery disease in the presence of aspirin therapy.

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