British Journal of Pharmacology (1995) 116, 3035-3041

Identification of a prostanoid FP receptor population producing endothelium-dependent vasorelaxation in the rabbit jugular vein

J. Chen, M.L. Champa-Rodriguez & [1]D.F. Woodward

Department of Biological Sciences, Allergan, Inc., 2525 Dupont Drive, Irvine, CA 92713-9534, U.S.A

1 Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and its synthetic analogue, fluprostenol, potently relaxed the precontracted isolated jugular vein of the rabbit (RJuV). The vasorelaxant activity of $PGF_{2\alpha}$ and fluprostenol was dependent upon an intact vascular endothelium. Although removal of the vascular endothelium abolished activity associated with $PGF_{2\alpha}$-like agonists, it did not significantly alter the relaxant effects of prostaglandin E_2 (PGE_2).

2 The nitric oxide synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME), at 100 μM significantly inhibited the endothelium-dependent relaxations induced by $PGF_{2\alpha}$. Lower doses (1 μM, 10 μM) of L-NAME had little or no effect. The relaxant effects of PGE_2 were not affected by L-NAME (1-100 μM). D-NAME at 100 μM was without effect on the vasorelaxant responses to either $PGF_{2\alpha}$ or PGE_2.

3 The potassium (K)-channel blockers tetraethylammonium (TEA, 1 mM), barium (1 mM) and quinine (100 μM), each tested in the presence of the inactive enantiomer D-NAME (100 μM) did not significantly affect the response to $PGF_{2\alpha}$. Unexpectedly, both TEA and barium significantly and partially reversed the inhibitory effects of 100 μM L-NAME, whereas quinine had no effect. In similar studies, none of the three potassium channel blockers had any effect on relaxations elicited by PGE_2 when given with D-NAME or L-NAME.

4 These results indicate that the $PGF_{2\alpha}$-sensitive prostanoid receptors found in the vascular endothelium of the rabbit jugular vein are of the FP-receptor subtype. Nitric oxide (NO) appears to be the predominant messenger involved in $PGF_{2\alpha}$-induced relaxation of the rabbit jugular vein. Potassium channels may have a minor role in mediating the vasorelaxation response to $PGF_{2\alpha}$. When both NO synthesis and K-channels are simultaneously blocked, inhibition of $PGF_{2\alpha}$-induced vasorelaxation by L-NAME is opposed by K-channel blockers. This diminution of the inhibitory effect of L-NAME by TEA and barium suggests that K-channels may possibly serve a compensatory role via the NO pathway.

Keywords: FP receptor; prostaglandin $F_{2\alpha}$; fluprostenol; prostaglandin E_2; rabbit jugular vein; endothelium-dependent vasorelaxation; vascular endothelium; nitric oxide; L-NAME; potassium channels

Introduction

The classification of prostanoid receptors that is currently widely accepted is based on the hypothesis that distinct receptor subtypes exist for each of the natural prostanoids. Those receptors preferentially stimulated by prostaglandin D_2 (PGD_2), prostaglandin E_2 (PGE_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), prostacyclin (PGI_2), and thromboxane A_2 (TxA_2) were designated DP, EP, FP, IP and TP, respectively (Coleman *et al.*, 1982; 1984). This classification was confirmed by the recent cloning of prostanoid receptor subtypes (Hirata *et al.*, 1991; Funk *et al.*, 1993; Honda *et al.*, 1993; Narumiya *et al.*, 1993; Namba *et al.*, 1993; 1994; Abramovitz *et al.*, 1994; Regan *et al.*, 1994; Sugimoto *et al.*, 1994), which revealed that they are members of the G-protein coupled superfamily of receptors.

The synthetic analogue of $PGF_{2\alpha}$, fluprostenol, is a potent and selective agonist for the FP-receptor, and, in the absence of selective antagonists, has been widely used in the pharmacological characterization of FP-receptors (Crossley, 1975; Coleman *et al.*, 1994). Contraction of various smooth muscle tissues such as the iris sphincter (Coleman *et al.*, 1984; Woodward *et al.*, 1989), uterus (Senior *et al.*, 1992) and colon (Eglen & Whiting, 1988) has been well documented as mediated by the FP-receptor. The vasorelaxant effects of $PGF_{2\alpha}$, however, have not been studied with selective FP-receptor agonists. $PGF_{2\alpha}$ was reported to produce biphasic responses comprising relaxation followed by contraction in human endothelium-intact perfused umbilical cord veins and arteries (Bjøro & Stray-Pedersen, 1986; Haugen & Hovig, 1992), dog isolated cerebral arteries (Toda *et al.*, 1988), and monkey isolated cerebral arteries (Hayashi *et al.*, 1985; Kawai & Ohhashi, 1991). In human isolated hand veins, the vasorelaxation to $PGF_{2\alpha}$ was partly endothelium-dependent (Arner *et al.*, 1994). These $PGF_{2\alpha}$-mediated vasorelaxant effects were not attributed to stimulation of the FP-receptor but were suggested to occur by stimulation of the recognized relaxant prostanoid receptors such as the DP, EP_2 and IP receptor subtypes (Giles *et al.*, 1989; 1990; Nials *et al.*, 1991; Lawrence & Jones, 1992) as well as an endothelium-derived relaxing factor.

We sought to investigate further the vasorelaxation induced by $PGF_{2\alpha}$ and determine whether this response can be attributed to FP-receptor stimulation by using the potent and selective FP-receptor agonist, fluprostenol. The isolated external jugular vein of the rabbit was used because the rabbit was a readily available laboratory animal and relaxant EP, DP and IP receptors, but not FP receptors, have been reported in the vascular smooth muscle (Giles *et al.*, 1989; 1990; Lawrence & Jones, 1992). The present studies were performed with the endothelium of the rabbit jugular vein (RJuV) left intact or removed. Since endothelium-dependent relaxations have been associated with nitric oxide and endothelium-derived hyperpolarizing factor (Furchgott & Zawadzki, 1980; Furchgott, 1984; Palmer *et al.*, 1987; Ignarro *et al.*, 1987; Lansman, 1988;

[1] Author for correspondence.

Taylor & Weston, 1988; Cook & Quast, 1990; Moncada *et al.*, 1991; Moncada & Higgs, 1993; Cowan *et al.*, 1993), we investigated the effects of a nitric oxide synthase inhibitor and potassium channel blockers on the vasorelaxation induced by $PGF_{2\alpha}$.

Methods

Isolated external jugular vein preparation of rabbit

New Zealand white rabbits of either sex, weighing 2–4 kg, were injected with 1000 u heparin into the marginal ear vein and then killed with CO_2 gas. The external jugular veins were cleaned of fat and adherent connective tissue and excised. The veins were transected and each ring of 4–5 mm length was suspended between two tungsten metal hooks. Smooth muscle tension of isolated tissues was measured isometrically with force displacement transducers (Grass FT-03) and recorded on a Grass Polygraph (Models 7G or 79E). The organ baths contained Krebs solution maintained at 37°C, gassed with 95% O_2/5% CO_2 to give a pH of 7.4. The Krebs solution had the following composition (mM): NaCl 118.0, KCl 4.7, KH_2PO_4 1.2, $CaCl_2$ 1.9, $MgSO_4$ 1.18, $NaHCO_3$ 25.0, glucose 11.7 and indomethacin 0.001. The tissues were equilibrated for 60 min under 0.5–0.75 g tension, which was readjusted as the tissues relaxed. Single doses of histamine, 10 μM then 2–3 μM, with washing after each dose, were given to contract the tissue and establish responsiveness. Since the RJuV contains contractile TP-receptors (Giles *et al.*, 1989), a selective TP-receptor antagonist with no antagonist activity at the FP-receptor, EP 092 (Armstrong *et al.*, 1985; Coleman *et al.*, 1994) and the purported TP-selective antagonist SQ 29548 (Ogletree *et al.*, 1985), were used to minimize this contractile influence. EP 092 at 2 μM or SQ 29548 at 1 μM, were applied for 5 min, then histamine at 2–3 μM was added to elicit the contractile response. After 30 min of pretreatment with histamine, the relaxant response was tested by adding cumulative doses of the test compounds, with 10^{-8} M to 10^{-7} M PGE_2 at the end of each dose-response curve to elicit maximal relaxation. In the studies with L-NAME, D-NAME, barium, quinine and TEA, these compounds were administered 20 min before precontracting the tissues with histamine. A recovery period of 30–50 min was allowed after wash-out of the tissues.

In the endothelium-denuded rings, the endothelial cells were removed by everting the rings (intimal surface outside) and gently rubbing the intimal surface with dampened cotton Q-tips for 30–60 s and then everting the rings (intimal surface inside). At the end of each experiment, the effectiveness of the rubbing procedure in removing the endothelial cells was demonstrated by the loss of relaxant response to acetylcholine in the histamine precontracted tissues (Cherry *et al.*, 1982).

Data analysis

Relaxant activity was determined as a percentage (%) of the control tone elicited by histamine and expressed as mean ± standard error (s.e.) of single values obtained from (n) preparations. Log concentration-response curves were graphed using the KaleidaGraph application on the Macintosh computer. The individual pEC_{50} values ($-\log EC_{50}$) were determined from the graph and the mean pEC_{50} ± s.e.mean for each curve was calculated. EC_{50} values are defined as the molar concentration of the prostaglandin agonist required to reduce the tone produced by histamine by 50%.

Statistical comparisons between treatments consisted of a preliminary F-test for significance, followed by the Student-Newman-Keuls' (S-N-K) test (Steel & Torrie, 1980) of pairwise comparison between means for all treatments using the PROC GLM procedure of SAS (Statistical Analysis System, Version 6.10) on an IBM-PC compatible computer. The S-N-K test was used to test the homogeneity of the paired treatment means and begins by comparing the maximum and minimum means of the treatments. If the range is not significant, the procedure is terminated and the set of means is considered homogeneous. If the difference between two means is significant, the procedure continues until all the pair-wise values are compared. Differences were considered statistically significant if the P-value was less than 0.05.

Materials

Prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) were purchased from Cayman Chemical Co. (Kalamazoo, MI). Fluprostenol (Na^+ salt) was purchased from Pittman-Moore (Berkhamsted). Prostaglandin solutions were prepared by adding 2% Na_2CO_3 followed by 0.9% normal saline. Stock solutions of EP 092 ([1α, 2β(Z), 3α, 4α]-, 7-[3-[1-[[(phenylamino) thioxomethyl] hydrazono] ethyl] bicyclo [2.2.1] hept-2-yl]-, 5-heptenoic acid; a gift from Dr R.L. Jones and the University of Edinburgh), SQ 29548 ([1S-[1α, 2α(Z), 3α, 4α]]-, 7-[3-[[2-[(phenylamino) carbonyl] hydrazino] methyl]-7-oxabicyclo [2.2.1] hept-2-yl]-, 5-hepenoic acid; purchased from Cayman Chemical) and quinine hydrochloride (purchased from Sigma, St. Louis, MO, U.S.A.) were prepared in 100% ethanol. Histamine, acetylcholine, tetraethylammonium chloride (TEA), barium chloride $2H_2O$ (purchased from Sigma), N^G-nitro-L-arginine methyl ester (L-NAME) and its D-isomer (D-NAME) (purchased from Biomol, Plymouth Meeting, PA, U.S.A.), were prepared in 0.9% normal saline. Indomethacin, purchased from Sigma, was dissolved in 2% Na_2CO_3.

Results

The activity of $PGF_{2\alpha}$ and fluprostenol in vascular endothelium intact and denuded rabbit jugular vein preparations is depicted in Figure 1. $PGF_{2\alpha}$ (pEC_{50} 8.24 ± 0.13) and fluprostenol (pEC_{50} 8.38 ± 0.17) were approximately equipotent in relaxing the intact, pre-contracted rabbit jugular vein (Figure 1a). In rabbit jugular vein segments, where the vascular endothelium had been removed, $PGF_{2\alpha}$ and fluprostenol produced little or no relaxant activity (Figure 1b).

The effects of acetycholine (ACh) and PGE_2 on the vascular endothelium intact and denuded rabbit jugular vein preparations were also examined for comparative purposes. ACh potently relaxed the intact rabbit jugular vein preparations (pEC_{50} 8.78 ± 0.21, Figure 2a), but lacked relaxant activity in preparations where the vascular endothelium had been removed (Figure 2b). A small contraction was apparent for high doses of ACh in the denuded preparations. PGE_2 was also a potent vasorelaxant and was active in both the endothelium-intact (pEC_{50} 9.41 ± 0.22, Figure 2a) and endothelium-denuded (pEC_{50} 8.89 ± 0.26, Figure 2b) preparations. Unlike the FP-receptor agonists and ACh, removal of the vascular endothelium had no statistically significant effect on PGE_2 activity.

The vasorelaxant responses to $PGF_{2\alpha}$ (Figure 3a) and PGE_2 (Figure 3b) in the presence of L-NAME (1, 10, 100 μM) or D-NAME (100 μM) were compared in vascular endothelium-intact rabbit jugular vein preparations. The concentration-response curve for $PGF_{2\alpha}$ (Figure 3a) in the presence of D-NAME control (pEC_{50} 8.31 ± 0.22) or saline (pEC_{50} 8.07 ± 0.60; data not shown) was significantly shifted to the right by 100 μM L-NAME (pEC_{50} 6.76 ± 0.47; $P < 0.05$). Neither 1 μM L-NAME (pEC_{50} 8.65 ± 0.21) nor 10 μM L-NAME (pEC_{50} 8.36 ± 0.31) caused significant dextral shifts of the $PGF_{2\alpha}$ concentration-response curve. L-NAME at 10 μM diminished the maximal relaxation to $PGF_{2\alpha}$, but the difference did not achieve statistical significance. In contrast to the results obtained for $PGF_{2\alpha}$, L-NAME over the same concentration-range had no significant effects on the relaxations induced by PGE_2 (Figure 3b). L-NAME (100 μM) alone and with quinine, barium or TEA, administered for the 20 min pretreatment period before histamine dosing, caused small contractions of the RJuV (data not shown). D-NAME (100 μM) had no contractile effects.

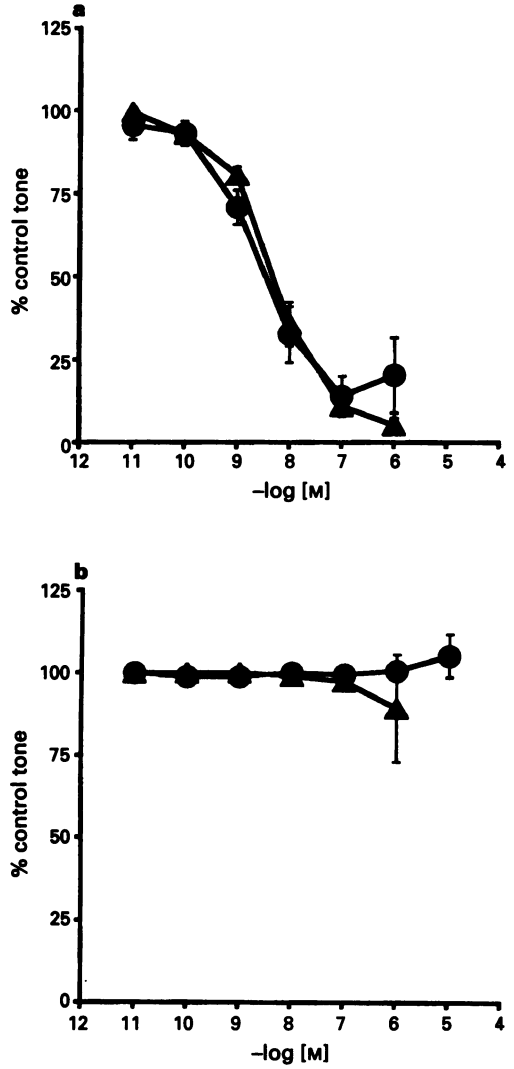

Figure 1 The activity of $PGF_{2\alpha}$ (▲, $n=6$) and fluprostenol (●, $n=5$) in histamine precontracted vascular endothelium (a)-intact and (b)-denuded rabbit jugular vein preparations. Results are expressed as mean ± s.e.mean.

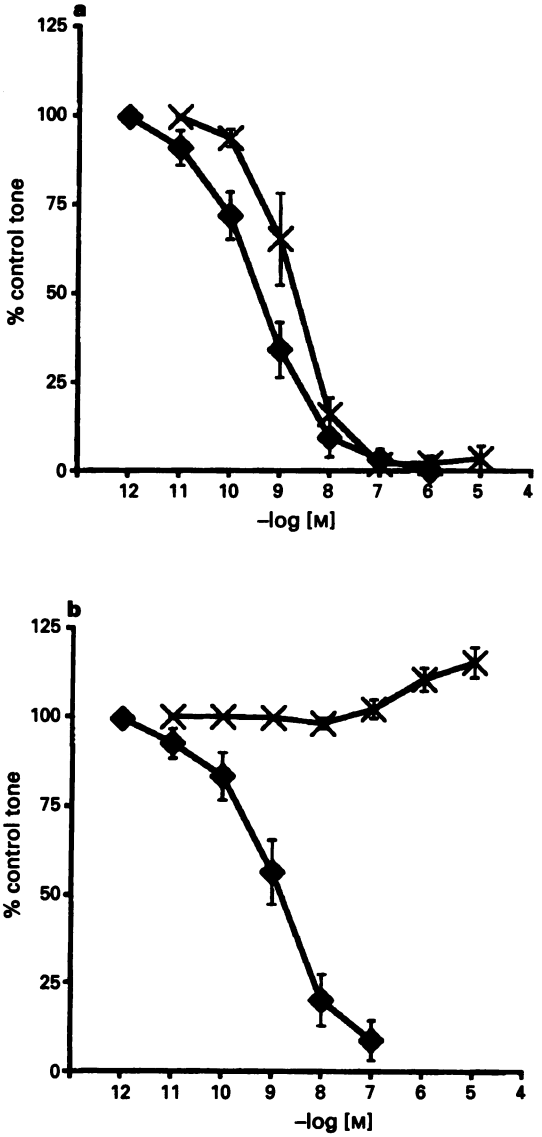

Figure 2 The effects of PGE_2 (◆) and acetylcholine (×) on (a) endothelium-intact and (b) endothelium-denuded rabbit jugular vein precontracted by histamine. Results are expressed as mean ± s.e.mean of 5–7 animals.

The potassium (K)-channel blockers were tested for their effects on the relaxations induced by $PGF_{2\alpha}$ (Figure 4a) and PGE_2 (Figure 4b) in the endothelium-intact rabbit jugular vein incubated with 100 μM D-NAME, the inactive enantiomer. None of the K-channel blockers studied (100 μM quinine, 1 mM barium, or 1 mM TEA) had any significant effects on the responses to either $PGF_{2\alpha}$ or PGE_2.

In the presence of 100 μM L-NAME, the K-channel blockers did not have an additive inhibitory effect on the vasorelaxant responses to $PGF_{2\alpha}$ (Figure 5a). However, the inhibition of $PGF_{2\alpha}$-induced vasorelaxation by 100 μM L-NAME (pEC_{50} 6.76 ± 0.47) was significantly and partially reversed by 1 mM TEA (pEC_{50} 7.52 ± 0.27; $P<0.05$) and 1 mM barium (pEC_{50} 7.45 ± 0.43; $P<0.05$), but not 100 μM quinine (pEC_{50} 7.21 ± 0.13). Both TEA and barium caused left and downward shifts of the $PGF_{2\alpha}$ concentration-response curve which achieved statistically significant differences. In similarly performed studies with PGE_2 (Figure 5b), the relaxant response obtained in the presence of 100 μM L-NAME was not affected by quinine, barium or TEA.

Discussion

At present, the most widely documented effects of $PGF_{2\alpha}$ are its luteolytic activity in farm animals and its ability to contract smooth muscle. The stimulant effects of $PGF_{2\alpha}$ on smooth muscle may be mediated either by FP receptors or by alternative prostanoid receptor subtypes which can accept $PGF_{2\alpha}$ but preferentially interact with a different prostanoid. TP receptors appear to mediate the contractile effects of $PGF_{2\alpha}$ on respiratory (Coleman & Sheldrick, 1989; Featherstone *et al.*, 1990) and vascular smooth muscle (Jones *et al.*, 1982). The vasorelaxant effect of $PGF_{2\alpha}$ appears to be endothelium-dependent and has been attributed to stimulation of the prostanoid IP-receptor in the monkey cerebral arteries (Kawai & Ohhashi, 1991) and dog arteries (Toda *et al.*, 1988), and IP- or EP_2-receptors in human hand veins (Arner *et al.*, 1994) according to comparisons of the responses elicited by $PGF_{2\alpha}$ and other natural prostanoids. However, the biphasic response to $PGF_{2\alpha}$ in these isolated animal tissues suggests the presence of opposing stimulatory and inhibitory receptors which may obscure the vasorelaxant effect of $PGF_{2\alpha}$. It was necessary to use a TP-receptor antagonist in the present studies to minimize the involvement of contractile TP-receptors which are present in the RJuV vascular smooth muscle (Giles *et al.*, 1989; 1990; Lawrence & Jones, 1992). In the rabbit isolated external jugular vein preparation, we found that it potently relaxes in response to $PGF_{2\alpha}$ if the endothelium is left intact, but is virtually unresponsive to $PGF_{2\alpha}$ when the endothelial cells were removed. This was in contrast to the activity of PGE_2 in this

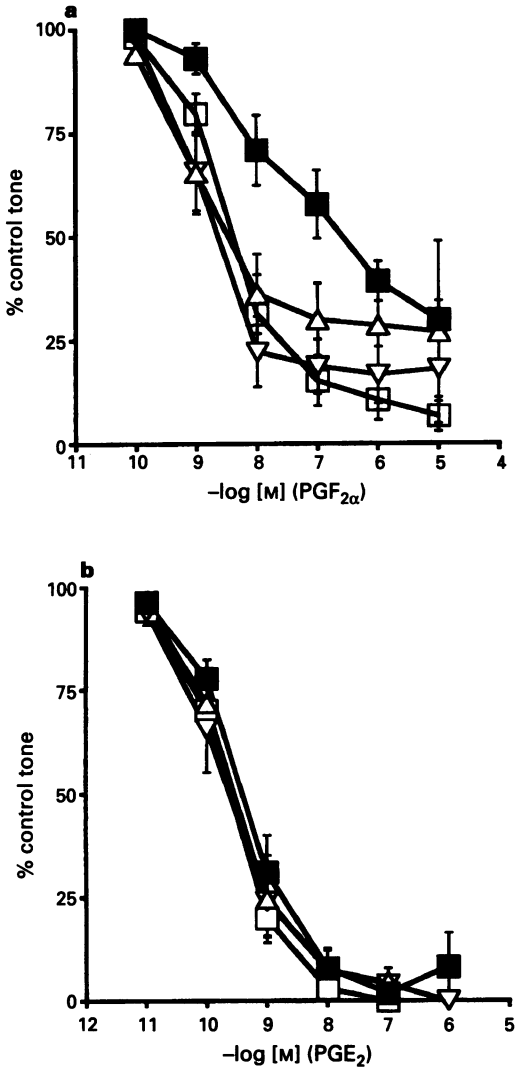

Figure 3 The effects of 100 μM D-NAME (□), and L-NAME (▽ 1, △ 10, ■ 100 μM) on relaxations to (a) $PGF_{2\alpha}$ and (b) PGE_2 in histamine precontracted endothelium-intact rabbit jugular vein preparations. Results are expressed as mean ± s.e.mean. $n = 4-7$.

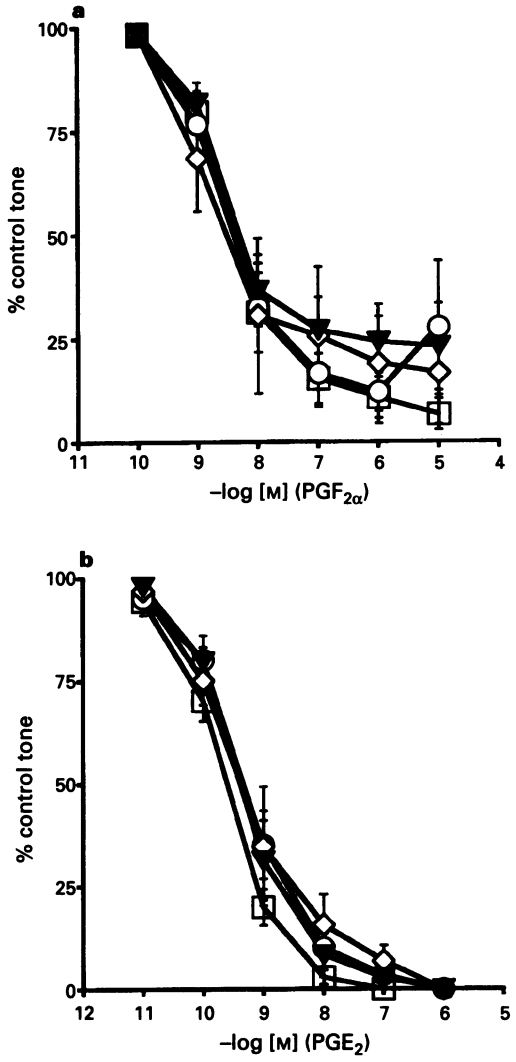

Figure 4 The relaxant effects of (a) $PGF_{2\alpha}$ and (b) PGE_2 on the endothelium-intact rabbit jugular vein were determined in the presence of 100 μM D-NAME control (□), 100 μM D-NAME with 100 μM quinine (○), 1 mM barium (◇), or 1 mM TEA (▼). Results are expressed as mean ± s.e.mean. $n = 4-5$.

preparation, where removal of the endothelium only modestly affected its relaxant potency. PGE_2 and PGD_2, which respectively exhibit potent and moderate vasodilator properties in the RJuV (Giles *et al.*, 1989; 1991; Milne *et al.*, 1994), do not require an intact vacular endothelium to exert their relaxant responses. In order to verify the presence of an FP receptor subtype, we examined the effect of the highly selective agonist fluprostenol, which lacks appreciable activity at EP-, DP-, IP- and TP-receptors (Crossley, 1975; Coleman *et al.*, 1982; 1990; 1994). Fluprostenol and $PGF_{2\alpha}$ were essentially equipotent in relaxing preparations with an intact vascular endothelium, but like $PGF_{2\alpha}$, fluprostenol was inactive in preparations in which the vascular endothelium had been removed. Fluprostenol, by virtue of its documented FP receptor selectivity, pharmacologically supports the presence of an FP receptor in the RJuV. Thus, the vasorelaxant receptor associated with the vascular endothelium of the RJuV appears to be FP and not EP or TP type. We provide no evidence regarding FP receptor heterogeneity from the results presented here, since selective competitive antagonists and a wide range of selective FP agonists are not available for generating potency ratio information.

The vasorelaxant responses associated with the $PGF_{2\alpha}$-sensitive FP receptor identified in our studies are endothelium-dependent and, therefore, may be mediated by endothelial vasoactive substances such as nitric oxide (NO) and/or endothelium-derived hyperpolarizing factor (EDHF) (Furchgott & Zawadzki, 1980; Palmer *et al.*, 1987; Ignarro *et al.*, 1987; Taylor & Weston, 1988; Nelson *et al.*, 1990; Moncada *et al.*, 1991; Edwards *et al.*, 1992). FP-receptor stimulation is associated with phosphotidylinositol breakdown with resultant release of calcium (Ca^{2+}) from intracellular stores (Macphee *et al.*, 1984; Woodward *et al.*, 1990; Nakao *et al.*, 1993; Woodward & Lawrence, 1994; Sugimoto *et al.*, 1994). Likewise, acetylcholine, carbachol and shear stress on endothelial cells evoke increases in intracellular Ca^{2+} (Lansman, 1988; Sato *et al.*, 1990; Rusko *et al.*, 1992; Moncada & Higgs, 1993). In the role of a second messenger, Ca^{2+} activates the constitutive nitric oxide synthase (cNOS) in the endothelial cells, resulting in the conversion of L-arginine to NO (Lückhoff *et al.*, 1988; Sato *et al.*, 1990; Moncada *et al.*, 1991). In stimulated endothelial cells, the opening of calcium-activated potassium (K)-channels and hyperpolarization leads to increased calcium influx into the endothelial cells, which may also contribute to the NO-mediated vasorelaxation (Lückhoff & Busse, 1990; Rusko *et al.*, 1992). The hyperpolarization of endothelial cells, evoked by agonists or the influx of Ca^{2+}, may stimulate the release of EDHF and this is another mechanism for relaxation of the underlying vascular smooth muscle (Taylor & Weston, 1988; Nelson *et al.*, 1990; Edwards *et al.*, 1992; Cowan *et al.*, 1993). The potential role of these mechanisms in mediating $PGF_{2\alpha}$-induced vasorelaxation of the RJuV was investigated as follows. N^G-nitro-L-arginine methyl ester (L-NAME), a potent

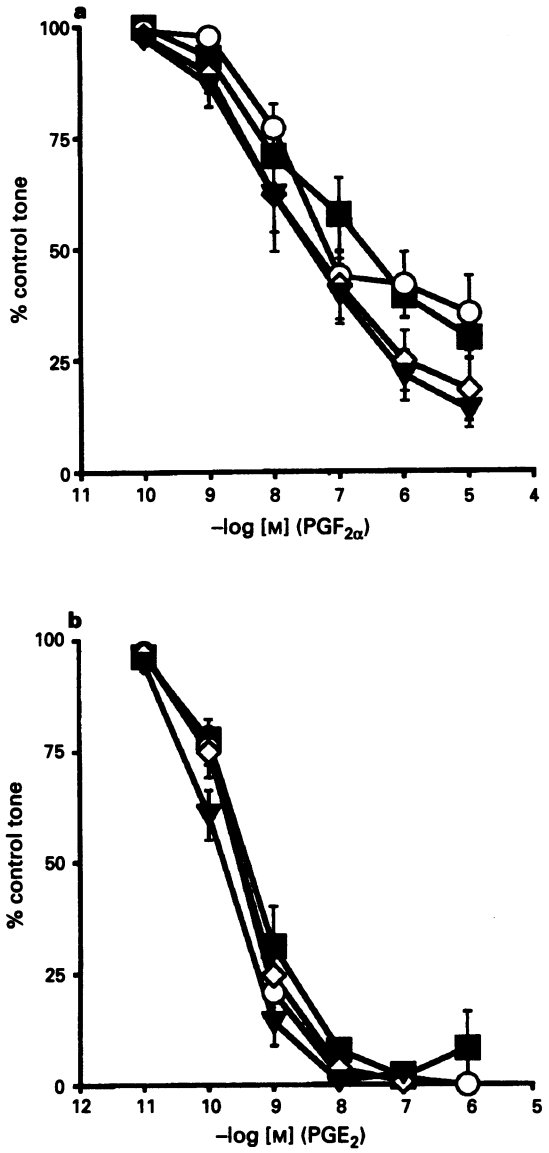

Figure 5 The relaxant effects of (a) $PGF_{2\alpha}$ and (b) PGE_2 on the endothelium intact rabbit jugular vein were determined in the presence of 100 μM L-NAME (■) alone, 100 μM L-NAME with 100 μM quinine (○), 1 mM barium (◇), or 1 mM TEA (▼). Results are expressed as mean ± s.e.mean. $n = 4-5$.

inhibitor of nitric oxide synthase in the vascular endothelium (Rees *et al.*, 1990), was used to determine the contribution of NO to the vasorelaxant response. Its D-enantiomer (D-NAME) was inactive, and thus was used as a control. The role of K-channels in modulating endothelium-dependent $PGF_{2\alpha}$-induced vasorelaxation was studied using three K-channel blockers with some differences in blocking activity. Externally applied TEA at 1 mM has a relatively high affinity for calcium-activated K-channels; externally applied barium at 1 mM has higher affinity for the ATP-sensitive K-channel than the calcium-activated K-channel; and quinine at 100 μM has blocking activity at K-channels as well as calcium and sodium channels (Nelson *et al.*, 1990; Cook & Quast, 1990; Ishikawa & Cook, 1993).

In the present studies, the NO signal transduction pathway appears to play the predominant role in $PGF_{2\alpha}$-induced vasodilatation. L-NAME at 100 μM, alone or in the presence of K-channel blockers, increased the tone of the endothelium-intact RJuV and subsequently, augmented the histamine-induced contraction. This effect indicates that L-NAME is inhibiting NO synthesis in this preparation (Rees *et al.*, 1990; Ralevic *et al.*, 1991; Moncada *et al.*, 1991). The endothelium-dependent vasorelaxant effect of $PGF_{2\alpha}$ was inhibited by 100 μM L-NAME, which suggests that NO synthesis and release is responsible for the vasodilator effect. Although the $PGF_{2\alpha}$-induced response was significantly inhibited by 100 μM L-NAME, it was not abolished. Martin *et al.* (1992) who showed similar results with L-NAME in the rabbit isolated jugular vein with acetylcholine used as the agonist, were not able to show involvement of endothelial-derived relaxing factors other than NO in the vasorelaxant response. Since agonist and tissue differences appear to affect the efficacy of L-NAME (Rees *et al.*, 1990; Martin *et al.*, 1992), partial inhibition by L-NAME does not necessarily imply the involvement of other endothelial-derived vasoactive substances.

In tissues where hyperpolarization of the endothelial cell contributes to the agonist-induced vasodilatation, potassium (K)-channel blockers augment the inhibition produced by L-NAME (Cowan *et al.*, 1993). Our results suggest that endothelial cell hyperpolarization has only a minor role in $PGF_{2\alpha}$-induced vasorelaxation of the rabbit jugular vein. Firstly, the potassium-channel blockers had no significant effects on $PGF_{2\alpha}$-induced vasorelaxant responses by themselves and did not result in additive inhibition when given in conjunction with L-NAME, which indicates that K-channel activation is not a back-up mechanism for NO in the vascular preparation studied herein. Secondly, the unexpected reduction of the inhibitory effect of L-NAME by TEA and barium, but not quinine, suggests only a possible compensatory role for K-channels, in particular calcium-activated K-channels, in the modulation of vascular tone. We may postulate that in the presence of both a cNOS inhibitor and a K-channel blocker, the agonist-evoked rises in intracellular Ca^{2+} may be prolonged and higher because K-efflux is blocked (Edwards *et al.*, 1992). Any resultant increase in intracellular Ca^{2+} may possibly overcome the cNOS blockade in the endothelial cells.

In summary, an FP receptor population has been found in the vascular endothelium of the rabbit external jugular vein which mediates vasorelaxation. The endothelium-dependent nitric oxide signal transduction pathway, with a possible compensatory role by K-channels, appears responsible for $PGF_{2\alpha}$-induced vasodilatation in this preparation.

We would like to thank Dr James C. Wang, Director, Biostatitistics, Allergan, Inc. for statistical analysis of the data.

References

ABRAMOVITZ, M., BOIE, Y., NGUYEN, T., RUSHMORE, T.H., BAYNES, M.A., METTERS, K.M., SLIPETZ, D.M. & GRYGORCZYK, R. (1994). Cloning and expression of a cDNA for the human prostanoid FP receptor. *J. Biol. Chem.*, **269**, 2632–2636.

ARMSTRONG, R.A., JONES, R.L. & WILSON, N.H. (1985). Effect of the thromboxane receptor antagonist EP 092 on endotoxin shock in the sheep. *Prostaglandins*, **29**, 703–713.

ARNER, M., USKI, T. & HÖGESTÄTT, E.D. (1994). Endothelium dependence of prostanoid-induced relaxation in human hand veins. *Acta Physiol. Scand.*, **150**, 267–272.

BJØRO, K. & STRAY-PEDERSEN, S. (1986). Effects of vasoactive autacoids on different segments of human umbilicoplacental vessels. *Gynecol. Obstet. Invest.*, **22**, 1–6.

CHERRY, P.D., FURCHGOTT, R.F., ZAWADZKI, J.V. & JOTHIANANDAN, D. (1982). Role of endothelial cells in relaxation of isolated arteries by bradykinin. *Proc. Natl. Acad. Sci. U.S.A.*, **79**, 2106–2110.

COLEMAN, R.A., HUMPHREY, P.P.A. & KENNEDY, I. (1982). Prostanoid receptors in smooth muscle: further evidence for a proposed classification. *Trends Autonom. Pharmacol.*, **3**, 35–49.

COLEMAN, R.A., HUMPHREY, P.P.A., KENNEDY, I. & LUMLEY, P. (1984). Prostanoid receptors: the development of a working classification. *Trends Pharmacol. Sci.*, **5**, 303–306.

COLEMAN, R.A., KENNEDY, I., HUMPHREY, P.P.A., BUNCE, K. & LUMLEY, P. (1990). Prostanoids and their receptors. In *Comprehensive Medicinal Chemistry. The Rational Design, Mechanistic Study and Therapeutic Application of Chemical Compounds.* Vol. 3, ed. Emmett, J.C. pp. 643–714. Oxford: Pergamon Press.

COLEMAN, R.A. & SHELDRICK, R.L.G. (1989). Prostanoid-induced contraction of human bronchial smooth muscle is mediated by TP-receptors. *Br. J. Pharmacol.*, **96**, 688–692.

COLEMAN, R.A., SMITH, W.L. & NARUMIYA, S. (1994). VII. International union of pharmacology. Classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes. *Pharmacol. Rev.*, **46**, 205–229.

COOK, N.S. & QUAST, U. (1990). Potassium channel pharmacology. In *Potassium Channels. Structure, Classification, Function and Therapeutic Potential.* ed. Cook, N.S. pp. 181–231. Chichester: Ellis Horwood Limited.

COWAN, C.L., PALACINO, J.J., NAJIBI, S. & COHEN, R.A. (1993). Potassium channel-mediated relaxation to acetylcholine in rabbit arteries. *J. Pharmacol. Exp. Ther.*, **266**, 1482–1489.

CROSSLEY, N.S. (1975). The synthesis and biological activity of potent selective, luteolytic prostaglandins. *Protaglandins*, **10**, 5–9.

EDWARDS, G., DUTY, S., TREZISE, D.J. & WESTON, A.H. (1992). Effects of potassium channel modulators on the cardiovascular system. In *Potassium Channel Modulators. Pharmacological, Molecular and Clinical Aspects.* ed. Weston, A.H. & Hamilton, T.C. pp. 369–421. Blackwell Scientific Publications.

EGLEN, R.M. & WHITING, R.L. (1988). The action of prostanoid receptor agonists and antagonists on smooth muscle and platelets. *Br. J. Pharmacol.*, **94**, 591–601.

FEATHERSTONE, R.L., ROBINSON, C., HOLGATE, S.T. & CHURCH, M.K. (1990). Evidence for thromboxane receptor mediated contraction of guinea-pig and human airways *in vitro* by prostaglandin (PG) D_2, 9α, 11β-PGF_2 and $PGF_{2\alpha}$. *Naunyn-Schmied. Arch. Pharmacol.*, **341**, 439–443.

FUNK, C.D., FURCI, L., FITZGERALD, G.A., GRYGORCZYK, R., ROCHETTE, C., BAYNE, M.A., ABRAMOVITZ, M., ADAM, M. & METTERS, K.M. (1993). Cloning and expression of a cDNA for the human prostaglandin E receptor EP_1 subtype. *J. Biol. Chem.*, **268**, 26767–26772.

FURCHGOTT, R.F. (1984). The role of endothelium in the responses of vascular smooth muscle to drugs. *Annu. Rev. Pharmacol. Toxicol.*, **24**, 175–197.

FURCHGOTT, R.F. & ZAWADZKI, J.V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*, **288**, 373–376.

GILES, H., BOLOFO, M.L. & LEFF, P. (1990). Rabbit jugular vein contains three different relaxant prostaglandin receptors. *Br. J. Pharmacol.*, **99**, 202P.

GILES, H., BOLOFO, M.L., LYDFORD, S.J. & MARTIN, G.R. (1991). A comparative study of the prostanoid receptor profile of 9α11β-prostaglandin F_2 and prostaglandin D_2. *Br. J. Pharmacol.*, **104**, 541–549.

GILES, H., LEFF, P., BOLOFO, M.L., KELLY, M.G. & ROBERTSON, A.D. (1989). The classification of prostaglandin DP-receptors in platelets and vasculature using BW A868C, a novel, selective and potent competitive antagonist. *Br. J. Pharmacol.*, **96**, 291–300.

HAUGEN, G. & HOVIG, T. (1992). Studies of autacoid responsiveness and endothelium dependency in human umbilical arteries. *Scand. J. Clin. Lab. Invest.*, **52**, 141–149.

HAYASHI, S., PARK, M.K. & KUEHL, T.J. (1985). Relaxant and contractile responses to prostaglandins in premature, newborn and adult baboon cerebral arteries. *J. Pharmacol. Exp. Ther.*, **233**, 628–635.

HIRATA, M., HAYASHI, Y., USHIKUBI, F., YOKOTA, Y., KAGEYAMA, R., NAKANISHI, S. & NARUMIYA, S. (1991). Cloning and expression of cDNA for a human thromboxane A_2 receptor. *Nature*, **349**, 617–620.

HONDA, A., SUGIMOTO, Y., NAMBA, T., WATABE, A., IRIE, A., NEGISHI, M., NARUMIYA, S. & ICHIKAWA, A. (1993). Cloning and expression of a cDNA for mouse prostaglandin E receptor EP_2 subtype. *J. Biol. Chem.*, **268**, 7759–7762.

IGNARRO, L.J., BUGA, G.M., WOOD, K.S. & BYRNS, R.E. (1987). Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc. Natl. Acad. Sci. U.S.A.*, **84**, 9265–9269.

ISHIKAWA, T. & COOK, D.I. (1993). Effects of K^+ channel blockers on inwardly and outwardly rectifying whole-cell K^+ currents in sheep parotid secretory cells. *J. Membrane Biol.*, **133**, 29–41.

JONES, R.L., PEESAPATI, V. & WILSON, N.H. (1982). Antagonism of the thromboxane-sensitive contractile systems of the rabbit aorta, dog saphenous vein, and guinea-pig trachea. *Br. J. Pharmacol.*, **76**, 423–438.

KAWAI, Y. & OHHASHI, T. (1991). Prostaglandin $F_{2\alpha}$-induced endothelium-dependent relaxation in isolated monkey cerebral arteries. *Am. J. Physiol.*, **260**, H1538–H1543.

LANSMAN, J.B. (1988). Going with the flow. *Nature*, **331**, 481–482.

LAWRENCE, R.A. & JONES, R.L. (1992). Investigation of the prostaglandin E (EP-) receptor subtype mediating relaxation of the rabbit jugular vein. *Br. J. Pharmacol.*, **105**, 817–824.

LÜCKHOFF, A. & BUSSE, R. (1990). Activators of potassium channels enhance calcium influx into endothelial cells as a consequence of potassium currents. *Naunyn-Schmied. Arch. Pharmacol.*, **342**, 94–99.

LÜCKHOFF, A., POHL, U., MULSCH, A. & BUSSE, R. (1988). Differential role of extra- and intracellular calcium in the release of EDRF and prostacyclin from cultured endothelial cells. *Br. J. Pharmacol.*, **95**, 189–196.

MACPHEE, C.H., DRUMMOND, A.H., OTTO, A.M. & JIMENEZ DE ASUA, L. (1984). Prostaglandin $F_{2\alpha}$ stimulates phosphatidylinositol turnover and increases the cellular content of 1,2-diacylglycerol in confluent resting Swiss 3T3 cells. *J. Cell. Physiol.*, **119**, 35–40.

MARTIN, G.R., BOLOFO, M.L. & GILES, H. (1992). Inhibition of endothelium-dependent vasorelaxation by arginine analogues: a pharmacological analysis of agonist and tissue dependence. *Br. J. Pharmacol.*, **105**, 643–652.

MILNE, S.A., SALA TENNA, A.M., ARMSTRONG, R.A. & WOODWARD, D.F. (1994). Further investigation of prostanoid EP receptors in rabbit jugular and pig saphenous veins. *Br. J. Pharmacol.*, **111**, 79P.

MONCADA, S. & HIGGS, A. (1993). The l-arginine-nitric oxide pathway. *N. Engl. J. Med.*, **329**, 2002–2012.

MONCADA, S., PALMER, R.M.J. & HIGGS, E.A. (1991), Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.*, **43**, 109–142.

NAKAO, A., WATANABE, T., TANIGUCHI, S., NAKAMURA, M., HONDA, Z-I., SHIMIZU, T. & KUROKAWA, K. (1993). Characterization of prostaglandin $F_{2\alpha}$ receptor of mouse 3T3 fibroblasts and its functional expression in xenopus laevis oocytes. *J. Cell. Physiol.*, **155**, 257–264.

NAMBA, T., OIDA, H., SUGIMOTO, Y., KAKIZUKA, A., NEGISHI, M., ICHIKAWA, A. & NARUMIYA, S. (1994). cDNA cloning of a mouse prostacyclin receptor. *J. Biol. Chem.*, **269**, 9986–9992.

NAMBA, T., SUGIMOTO, Y., NEGISHI, M., IRIE, A., USHIKUBI, F., KAKIZUKA, A., ITO, S., ICHIKAWA, A. & NARUMIYA, S. (1993). Alternative splicing of c-terminal tail of prostaglandin E receptor subtype EP_3 determines G-protein specificity. *Nature*, **365**, 166–170.

NARUMIYA, S., HIRATA, N., NAMBA, T., HAYASHI, Y., USHIKUBI, F., SUGIMOTO, Y., NEGISHI, M. & ICHIKAWA, A. (1993). Structure and function of prostanoid receptors. *J. Lipid Med.*, **6**, 155–161.

NELSON, M.T., PATLAK, J.B., WORLEY, J.F. & STANDEN, N.B. (1990). Calcium channels, potassium channels, and voltage dependence of arterial smooth muscle tone. *Am. J. Physiol.*, **259**, C3–C18.

NIALS, A.T., COLEMAN, R.A., HARTLEY, D. & SHELDRICK, R.L.G. (1991). AH13205 – a novel selective prostanoid EP_2 agonist. *Br. J. Pharmacol.*, **102**, 24P.

OGLETREE, M.L., HARRIS, D.N., GREENBERG, R., HASLANGER, M.F. & NAKANE, M. (1985). Pharmacological actions of SQ29,548, a novel selective thromboxane antagonist. *J. Pharmacol. Exp. Ther.*, **234**, 435–441.

PALMER, R.M.J., FERRIGE, A.G. & MONCADA, S. (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*, **327**, 524–526.

RALEVIC, V., MATHIE, R.T., ALEXANDER, B. & BURNSTOCK, G. (1991). N^G-Nitro-L-arginine methyl ester attenuates vasodilator responses to acetylcholine but enhances those to sodium nitroprusside. *J. Pharm. Pharmacol.*, **43**, 871–874.

REES, D.D., PALMER, R.M.J., SCHULZ, R., HODSON, H.F. & MONCADA, S. (1990). Characterization of three inhibitors of endothelial nitric oxide synthase *in vitro* and *in vivo*. *Br. J. Pharmacol.*, **101**, 746–752.

REGAN, J.W., BAILEY, T.J., DONELLO, J.E., PIERCE, K.L., PEPPERL, D.J., ZHANG, D., KEDZIE, K.M., FAIRBAIRN, C.E., BOGARDUS, A.M., WOODWARD, D.F. & GIL, D.W. (1994). Molecular cloning and expression of human EP_3 receptors: evidence of three variants with differing carboxyl termini. *Br. J. Pharmacol.*, **112**, 377–385.

RUSKO, J., TANZI, F., VAN BREEMEN, C. & ADAMS, D.J. (1992). Calcium-activated potassium channels in native endothelial cells from rabbit aorta: conductance, Ca^{2+} sensitivity and block. *J. Physiol.*, **455**, 601–621.

SATO, K., OZAKI, H. & KARAKI, H. (1990). Differential effects of carbachol on cytosolic calcium levels in vascular endothelium and smooth muscle. *J. Pharmacol. Exp. Ther.*, **255**, 114–119.

SENIOR, J., SANGHA, R., BAXTER, G.S., MARSHALL, K. & CLAYTON, J.K. (1992). *In vitro* characterization of prostanoid FP-, DP-, IP- and TP-receptors on the non-pregnant human myometrium. *Br. J. Pharmacol.*, **107**, 215–221.

STEEL, R.G.D. & TORRIE, J.H. (1980). Multiple Comparisons. In *Principles and Procedures of Statistics. A Biometrical Approach.* 2nd Edition, ed. Napier C. & Maisel, J.W. pp. 172–194. New York: McGraw-Hill Book Company.

SUGIMOTO, Y., HASUMOTO, K-Y., NAMBA, T., IRIE, A., KATSUYAMA, M., NEGISHI, M., KAKIZUKA, A., NARUMIYA, S. & ICHIKAWA, A. (1994). Cloning and expression of a cDNA for mouse prostaglandin F receptor. *J. Biol. Chem.*, **269**, 1356–1360.

TAYLOR, S.G. & WESTON, A.H. (1988). Endothelium-derived hyperpolarizing factor: a new endogenous inhibitor from the vascular endothelium. *Trends Pharmacol. Sci.*, **9**, 272–274.

TODA, N., INOUE, S., OKAMURA, T. & OKUNISHI, H. (1988). Mechanism underlying relaxations caused by prostaglandins and thromboxane A_2 analog in isolated dog arteries. *J. Cardiovasc. Pharmacol.*, **11**, 354–362.

WOODWARD, D.F., BURKE, J.A., WILLIAMS, L.S., PALMER, B.P., WHEELER, L.A., WOLDEMUSSIE, E., RUIZ, G. & CHEN, J. (1989). Prostaglandin $F_{2\alpha}$ effects on intraocular pressure negatively correlate with FP-receptor stimulation. *Invest. Ophthalmol. Vis. Sci.*, **30**, 1838–1842.

WOODWARD, D.F., FAIRBAIRN, C.E., GOODRUM, D.D., KRAUSS, A.H-P., RALSTON, T.L. & WILLIAMS, L.S. (1990). Ca^{2+} transients evoked by prostanoids in Swiss 3T3 cells suggest an FP-receptor mediated response. *Adv. Prostaglandin Thromboxane Leukot. Res.*, **21**, 367–370.

WOODWARD, D.F. & LAWRENCE, R.A. (1994). Identification of a single (FP) receptor associated with prostanoid-induced Ca^{2+} signals in Swiss 3T3 cells. *Biochem. Pharmacol.*, **47**, 1567–1574.

(Received March 10, 1995
Revised August 7, 1995
Accepted August 11, 1995)