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Pharmacological studies on prostanoid receptors in the rabbit isolated saphenous vein: a comparison with the rabbit isolated ear artery

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1 In isolated ring preparations of the rabbit saphenous vein, prostaglandin E_2 (PGE_2) caused well-defined, stable and concentration-dependent relaxations of KCl contracted tissues with a mean potency (p[A_{50}]) of 9.39.

2 The prostanoid EP-receptor agonists, PGE_1, 11-deoxy PGE_1, 16,16-dimethyl PGE_2 and misoprostol were all full agonists in this preparation. The EP_2-receptor selective agonists, butaprost and AH13205, and the EP_1/EP_3-receptor selective agonist, sulprostone, also relaxed this tissue but were at least 300 times less potent than PGE_2.

3 Prostaglandin D_2 (PGD_2), the DP-receptor agonist, BW245C, and the IP-receptor agonist, cicaprost, caused concentration-dependent relaxations of the rabbit saphenous vein but were at least 60 times less potent than PGE_2.

4 The selective EP_4-receptor antagonist, AH23848B (30 μM), antagonized the PGE_2 concentration-effect (E/[A]) curves yielding a pA_2 estimate of 4.96. The EP_1/DP-receptor antagonist, AH6809 (10 μM), had no effect on the location of PGE_2 E/[A] curves.

5 The stable thromboxane A_2-mimetic, U46619, elicited concentration-dependent contractions of the rabbit saphenous vein (p[A_{50}] = 8.01) however, PGE_2 and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) were unable to produce a contractile response. The response to U46619 was competitively antagonized by the TP-receptor antagonist, GR32191B, yielding a pK_B estimate of 7.08.

6 In the rabbit isolated ear artery, PGE_2, misoprostol and AH13205 relaxed tissues pre-contracted with phenylephrine. PGE_2 (p[A_{50}] = 7.04) and misoprostol were equipotent, whereas AH13205 was some 40 fold less potent. AH23848B (30 μM) and AH6809 (1 and 10 μM) caused no significant shift in the location of PGE_2 E/[A] curves.

7 These data suggest that the rabbit isolated saphenous vein contains prostanoid, EP-, DP-, IP- and TP-receptors. Based on antagonist affinity information and agonist potency orders, the rabbit saphenous vein contains an inhibitory prostanoid EP-receptor different from that in the rabbit ear artery, but comparable to the recently described EP_4-receptor.

Keywords: Rabbit isolated saphenous vein; prostanoid EP_4-receptors; receptor classification

Introduction

Prostaglandin and thromboxane receptors are members of the seven transmembrane-domain superfamily of G protein-coupled, cell surface receptors. They have been categorized into five main classes on the basis of their sensitivity to naturally occurring prostanoid agonists. Each prostanoid is at least one order of magnitude more potent then the others at one receptor and these have been termed DP- (PGD_2), EP- (PGE_2), FP- ($PGF_{2\alpha}$), IP- (PGI_2) and TP- (thromboxane A_2) receptors (Kennedy *et al.*, 1982; Coleman *et al.*, 1984). This initial classification was hampered by the paucity of selective agonists and antagonists, and the fact that most prostanoid agonists display measurable activity at a number of these receptors. However, conclusive evidence for this classification was provided by the recent cloning and expression of cDNA encoding pharmacologically characterized DP- (Hirata *et al.*, 1994), EP- (Honda *et al.*, 1993; Sugimoto *et al.*, 1992; Watabe *et al.*, 1993), FP- (Sugimoto *et al.*, 1994), IP- (Namba *et al.*, 1994) and TP-receptors (Hirata *et al.*, 1991).

With the recent description of an 'atypical' prostanoid EP-receptor (Coleman *et al.*, 1994), there would appear to be at least four, pharmacologically distinct, EP-receptors differing in their ligand binding profiles and signal transduction pathways. EP_1-receptors are functionally coupled to an increase in intracellular calcium (Funk *et al.*, 1993; Watabe *et al.*, 1993) and generally mediate contraction of smooth muscle (Coleman & Kennedy, 1985). EP_2-receptor effects include bronchial, vascular and gastrointestinal smooth muscle relaxation (Nials *et al.*, 1993) and are mediated via stimulation of adenylyl cyclase activity (Regan *et al.*, 1994). Four isoforms of the EP_3-receptors have been identified and are produced by alternative splicing of the mRNA C-terminus (Irie *et al.*, 1993; Namba *et al.*, 1993; Sugimoto *et al.*, 1993). EP_3-receptors subserve a wide range of pharmacological effects including smooth muscle contraction (Ahluwalia *et al.*, 1988), inhibition of neurotransmitter release (Coleman *et al.*, 1987) and inhibition of fat cell lipolysis (Strong *et al.*, 1992). EP_3-receptor isoforms are able to couple to different G-proteins and cause increases in intracellular calcium or inhibition of adenylyl cyclase activity (An *et al.*, 1994). To our current knowledge, no group has described studies on a recombinant EP_4-receptor; however, the receptor identified by Honda *et al.* (1993) and Bastien *et al.* (1994) as an EP_2-receptor, displays characteristics typical of an EP_4-receptor. PGE_2 binds with a nanomolar affinity, the EP_2-selective ligand butaprost does not displace radiolabelled PGE_2, and consistent with studies in the naive chinese hamster ovary cell (CHO) line (Milne *et al.*, 1994a), the receptor is positively coupled to adenylyl cyclase.

The rabbit isolated saphenous vein has been extensively used as a bioassay in the investigation of many receptor sys-

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tems including 5-HT_1-like receptors (Martin *et al.*, 1988), P_{2x}-purinoceptors and α-adrenoceptors (MacDonald *et al.*, 1992). The main aim of the present study was to characterize fully the prostanoid receptor(s) subserving smooth muscle effects in this tissue and specifically, to ascertain if the predominant inhibitory receptor was similar to the EP_4-receptor subtype in the piglet saphenous vein (Coleman *et al.*, 1994). A preliminary account of these findings has been presented to the British Pharmacological Society (Lydford & McKechnie, 1994a).

Methods

Isolated preparations

Male New Zealand white rabbits (2.5–3.8 kg) were killed by intravenous administration of pentobarbitone sodium (60 mg kg^{-1}) into a marginal ear vein. A small section of the saphenous vein (RbSV) or ear artery (RbEA) was exposed and cannulated *in situ* with a lightly scored polypropylene cannula (RbSV: 1.02 mm o.d., RbEA: 0.75 mm o.d.). This effectively removed the majority of the endothelium in both the RbSV (demonstrated by the absence of relaxant responses to 10 μM acetylcholine on pre-contracted tissues, $n=5$, data not shown) and RbEA (O'Connor *et al.*, 1990). The tissues, mounted on their cannulae were dissected free, cleared of fat and adhering connective tissue, cut into 5 mm rings and mounted in 10 ml organ baths containing modified Krebs buffer (pH 7.4). The buffer (composition, mM: NaCl 117.56, KCl 5.36, $CaCl_2$ 2.55, $MgSO_4$ 1.18, NaH_2PO_4 1.15, $NaHCO_3$ 25.00 and glucose 11.10) was maintained at 37 ± 0.5°C and aerated with 5% CO_2 in oxygen. Indomethacin (2.8 μM) was added to the buffer to prevent the release of endogenous cyclo-oxygenase products.

Tissues were suspended between two parallel wire hooks (0.25 mm o.d.), one attached to an Ormed beam isometric transducer and the other to a stationary support in the organ bath. Tissue responses were measured as changes in isometric force on 2-channel Advance Bryans flat bed chart recorders.

Experimental protocols

General: An initial force of 3 g (RbSV) or 1.5 g (RbEA) was applied to each tissue; this was adjusted over a 60 min period to achieve a final force of approximately 1 g during which time the bathing fluid was replaced three times. With the exception of the U46619 studies, 10 μM GR32191B was added to the buffer 30 min before constructing agonist concentration-effect (E/[A]) curves in order to eliminate possible TP-receptor mediated contractile effects. In all cases E/[A] curves were constructed by cumulative additions of the agonist at 0.5 $\log_{10}$ unit intervals; the volume of drug solutions added did not exceed 5% of the total buffer volume.

Rabbit saphenous vein: To reduce inter-tissue variations, relaxant responses were measured by a paired curve experimental design. Tissues were initially exposed to 40 mM KCl (previous experiments having shown that this concentration represented an $[A_{80}]$ for KCl in this preparation, data not shown) to obtain a stable contraction. First curves were obtained to the standard agonist, PGE_2, and following a 60 min washout period, second curves were constructed to PGE_2 as a time matched control or to one of the test agonists. Antagonists were added to pre-contracted tissues to check for the possibility of additional pharmacological effects. Data were expressed as a percentage of the maximum relaxation of PGE_2.

Experiments to determine the effects of GR32191B on U46619 contractions followed a single curve design due to variations between first and second curve data. Tissues were initially exposed to 40 mM KCl to obtain a reference contraction, washed and allowed to re-stabilize. GR32191B (1, 3 or 10 μM) was added to the appropriate baths and incubated for 60 min before constructing cumulative U46619 E/[A] curves. To measure the contractile effects of other prostanoids, a similar protocol was followed with the inclusion of 10 μM GR32191B in the Krebs buffer.

Rabbit ear artery: Preparations were challenged with 1 μM phenylephrine (PhE) to ascertain tissue viability. After a 30 min washout period, tissues were pre-contracted with PhE (0.3 or 1 μM, these concentrations represent an approximate $[A_{50}]$ for PhE in this preparation, data not shown) and a single cumulative E/[A] curve to PGE_2 or one of the test agonists obtained from each preparation. Antagonists were added to pre-contracted tissues prior to the construction of agonist E/[A] curves. Data were expressed as percentage inhibition of the PhE induced tone.

Data analysis

Individual sets of E/[A] curve data were fitted to a form of the Hill equation:

$$E = \frac{\alpha[A]^m}{[A_{50}]^m + [A]^m} \quad (1)$$

where α, $[A_{50}]$ and m are the asymptote, location (expressed as $-\log_{10}[A_{50}]$) and slope parameters respectively. All agonist E/[A] curves were fitted on a Macintosh Centris 650 computer using the data analysis package 'KaleidaGraph', relative potencies were calculated as $[A_{50}]$ test agonist/$[A_{50}]$ PGE_2 and a geometric mean obtained. Statistical differences were assessed using the 'Instat' biostatistics package and were considered significant at the level of $P<0.05$.

Agonist data: For paired curve data, the differences (Δ values) between the computer generated estimates of α, m and $p[A_{50}]$ for each E/[A] curve data set were calculated. For multiple comparisons, one-way analysis of variance was subsequently performed on the Δ values from all treatment groups, followed by Tukey-Kramer's multiple comparison test. Student's *t* test was used to compare individual paired curve data sets. With single curve data, the same comparisons were made using the computed estimates of α, m and $p[A_{50}]$.

Antagonists - paired curve data: If one-way analysis of variance on the differences between α and m estimates for each pair of E/[A] curves was not significantly different, pA_2 values were calculated from the equation:

$$\log_{10}(r-1) = \log_{10}[B] + pA_2 \quad (2)$$

where $r=[A_{50}]$ agonist curve in the presence of the antagonist/$[A_{50}]$ agonist control curve and [B] is the concentration of antagonist. In antagonism studies in the RbSV, AH23848B displayed significant relaxant activity and it was necessary to take this into account during data analysis. The method of Pöch & Holzmann (1980) was used to quantify the effect of this response on the position of PGE_2 E/[A] curves. This analysis involves the comparison of experimental E/[A] curves with those predicted to apply when two agonists interact in the same system, and permits examination of any properties that the ligand may have over and above its agonist properties. Rightward deviation of the experimental E/[A] curve from the theoretical E/[A] curve for one agonist in the presence of the other, assuming this is merely a dose-additive effect, is indicative of antagonism.

Antagonists - single curve data: To test for simple competition, one way analysis of variance was performed on α and m estimates between treatment groups. If these parameters were not significantly different, $[A_{50}]$ data were fitted to the following form of the Schild equation (Black *et al.*, 1985):

$$\log_{10}[A_{50}] = \log_{10}[A_{50}]^c + \log_{10}(1 + [B]^n/K_B) \quad (3)$$

where $[A_{50}]$ is the midpoint location of the treatment curve, $[A_{50}]^c$ is the estimated control $[A_{50}]$ value, [B] is the concentration of the antagonist, K_B is the antagonist equilibrium constant and n is equivalent to the Schild plot slope parameter (unity for simple competition). If n was not significantly different from unity, it was constrained to this value and a pK_B estimate made.

Compounds

PGE_1, PGE_2, $PGF_{2\alpha}$, U46619 (11α,9α-epoxymethano prostaglandin H_2) and misoprostol ((±)-11, 16-dihydroxy-16-methyl-9-oxoprost-13-en-1-oic acid methyl ester) were purchased from Cascade Biochem, Reading, Berks. PGD_2 was obtained from Ultrafine Chemicals, Manchester. Potassium chloride, phenylephrine hydrochloride, acetylcholine bromide, indomethacin and (±)-isoprenaline hydrochloride were purchased from Sigma, Poole, Dorset. Sulprostone (16-phenoxy-ω-tetranor prostaglandin E_2 methylsulphonylamide) and cicaprose ([2-[hexahydro-5-hydroxy-4-(3-hydroxy-4-methyl-1,6-nonadinyl)-2-(1H)-pentalenylidene]ethoxy] acetic acid) were gifts from Schering AG, Berlin, Germany. Butaprost (methyl-3-hydroxy-2-[4-hydroxy-4-(1-propylcyclobutyl)-1-butenyl]-5-oxo-cyclopentane heptanoate) was a gift from Bayer, Stokes Poges, Bucks. AH23848B ([1α(Z),2β,5α]-(±)-7-[5-[[(1,1'-biphenyl)-4-yl]methoxy]-2-(4-morpholinyl)-3-oxo-cyclopentyl]-4-heptenoic acid) and GR32191B ([1R-[lα(Z),2β,3β,5α]]-(+)-7-[5-([1,1'-biphenyl]-4-ylmethoxy)-3-hydroxy-2-(1-piperidnyl) cyclopentyl]-4-heptenoic acid hydrochloride salt) were gifts from Glaxo, Ware, Herts. AH6809 (6-isopropoxy-9-oxaxanthene-2-carboxylic acid) and AH13205 (*trans*-2-[4-(1-hydroxyhexyl)phenyl]-5-oxocyclo pentane-heptanoic acid adamantanamine salt) were synthesized in the Medicinal Chemistry Department, Astra Charnwood, Loughborough, Leicestershire. BW245C (5-(6-carboxyhexyl)-1-(3-cyclohexyl-3-hydroxypropyl)-hydantoin) was a gift from Wellcome Research Laboratories, Beckenham, Kent.

Prostaglandins were stored at −20°C as either ethanolic or methyl acetate stocks and diluted in Krebs buffer. Indomethacin was dissolved in 10% w/v Na_2CO_3 whilst acetylcholine, phenylephrine, potassium chloride and GR32191B were dissolved in distilled water. Isoprenaline hydrochloride was dissolved and diluted in 100 μM ascorbate solution. AH23848B was dissolved in 1% bicarbonate in 0.9% saline. Sulprostone was diluted in 0.01% Tween 80 in 0.9% saline.

Results

Rabbit saphenous vein

Relaxant effects of PGE_2 and related analogues Pre-contracted tissues were relaxed by PGE_2 in a well-defined, concentration-dependent manner (Figure 1). First and second curve data were not significantly different, estimated $p[A_{50}]$s being 9.37±0.05 and 9.39±0.06 respectively (s.e., $n=28$, $P=0.56$). Figure 2 shows the inhibitory effects of PGE_2 and related analogues on the rabbit saphenous vein. With the exception of PGE_1, all of the agonists tested were less potent than PGE_2 and in the presence of 10 μM GR32191B, caused similar maximal degrees of relaxation ($P=0.14$). Due to its low potency, it was not possible to obtain complete E/[A] curves for sulprostone. The rank order of potencies for the EP-receptor agonists was; $PGE_2=PGE_1>$ 11-deoxy $PGE_1=$ 16,16-dimethyl $PGE_2>$ misoprostol > butaprost > AH13205 > > sulprostone. $p[A_{50}]$ values and relative potencies are shown in Table 1.

Relaxant potencies of other prostanoid agonists Cicaprost, BW245C and PGD_2 also caused concentration-dependent relaxations of the rabbit saphenous vein (Figure 3). Each of these agonists was at least 60 times less potent than PGE_2, with E/[A] curves parallel to, and with similar asymptotes to PGE_2. The data for these compounds are shown in Table 1.

Effect of antagonists on relaxant responses The EP_4-receptor antagonist, AH23848B (30 μM), caused significant dextral shifts of PGE_2 E/[A] curves ($P<0.01$), however at this concentration, AH23848B displayed agonist activity equivalent to 19.1±2.5% of the maximal relaxation to PGE_2. Figure 4a shows the influence of 30 μM AH23848B on PGE_2 responses in the RbSV. Comparison of the experimental E/[A] curve for PGE_2 in the presence of AH23848B ($p[A_{50}]=8.73$) to the theoretical dose-additive curve ($p[A_{50}]=9.29$) shows a significant rightward displacement, and a pA_2 estimate of 4.96±0.08 (s.e., $n=10$) was obtained (Figure 4). The same concentration of AH23848B failed to change significantly the location of AH13205 ($n=5$, $P=0.37$) or misoprostol ($n=7$, $P=0.19$) E/[A] curves, but did cause a similar relaxant response (data not shown). Smooth muscle relaxations produced by isoprenaline acting at β_2-adrenoceptors were unaffected by 30 μM AH23848B ($n=2$, data not shown).

The EP_1/DP-receptor antagonist, AH6809 (10 μM), was without significant effect on PGE_2 E/[A] curves ($n=5$, $P=0.56$) but did cause a small (7.5±1.5%) relaxation of pre-contracted tissues (data not shown).

Antagonism of U46619 by GR32191B The TP-receptor agonist, U46619, elicited concentration-dependent contractions of this tissue with a mean $p[A_{50}]$ of 8.01±0.07 (s.e., $n=7$). GR32191B produced rightward displacement of these agonist curves which, according to analysis of variance, did not significantly deviate from parallelism (Figure 5a). Analysis of the computed $[A_{50}]$ values by equation (3) indicated that the interaction conformed to simple competition. Figure 5b shows

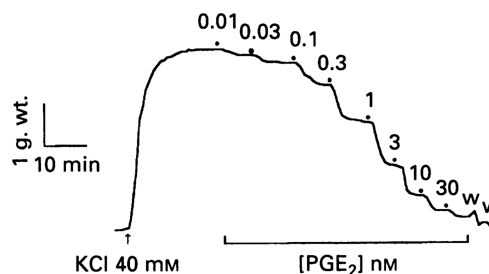

Figure 1 Diagrammatic representation of a typical E/[A] curve to PGE_2 in the pre-contracted rabbit saphenous vein.

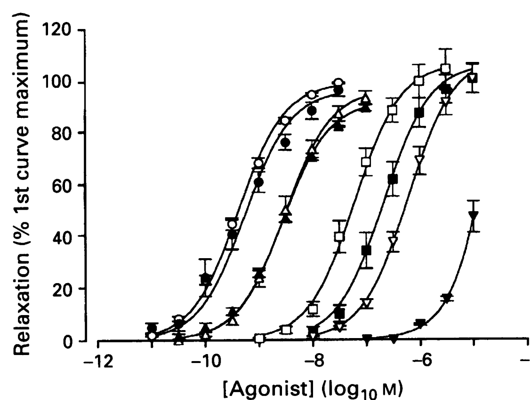

Figure 2 Concentration-effect curves for relaxation of the KCl-contracted rabbit saphenous vein by PGE_2 and related analogues in the presence of 10 μM GR32191B: PGE_2 (○, $n=28$), PGE_1 (●, $n=4$), 11-deoxy PGE_1 (△, $n=5$) 16,16-dimethyl PGE_2 (▲, $n=4$), misoprostol (□, $n=5$), butaprost (■, $n=5$), AH13205 (▽, $n=7$) and sulprostone (▼, $n=4$). The figure shows second curve data, with s.e.mean indicated.

Table 1 Agonist potency orders in the rabbit isolated saphenous vein

Compound	*p[A₅₀]*	*Relative potency*	*95% confidence limits*	n
EP-receptor agonists				
PGE_2	9.39 ± 0.06	1.0	–	28
PGE_1	9.20 ± 0.08	1.1	0.6–2.3	4
11-deoxy PGE_1	8.58 ± 0.06	4.9	3.2–7.9	5
16,16-dME_2	8.50 ± 0.03	6.8	3.1–15.5	4
Misoprostol	7.25 ± 0.13	96	42–220	5
Butaprost	6.72 ± 0.13	382	157–929	5
AH13205	6.26 ± 0.07	1,496	840–2,667	7
Sulprostone	< 5.5	15,572	9,177–26,422	4
DP/IP-receptor agonists				
BW245C	7.58 ± 0.10	61	36–103	8
PGD_2	6.98 ± 0.10	248	131–469	5
Cicaprost	7.50 ± 0.14	92	58–146	6

Relative potencies > 1 indicate that the agonist is less potent than PGE_2. Abbreviations: 16,16-dME_2 = 16,16-dimethyl PGE_2.

the $[A_{50}]$ data in Clark plot form (Stone & Angus, 1978). The Schild slope parameter was not significantly different from unity (1.09 ± 0.15) and the resulting pK_B estimate was 7.08 ± 0.10.

Contractile effects of other prostanoid agonists In the presence of 10 μM GR32191B to block excitatory TP-receptor effects, PGE_2 and $PGF_{2\alpha}$ were devoid of contractile activity in this preparation at concentrations up to 10 μM (data not shown, $n \geq 2$).

Rabbit ear artery

Relaxant effects of PGE_2 and related analogues In the presence of 10 μM GR32191B, PGE_2 caused complete relaxation of this preparation with a mean $p[A_{50}]$ of 7.04 ± 0.09 (s.e., n = 12). Figure 6 shows the relaxant effects of PGE_2 and four analogues. Misoprostol, 16,16-dimethyl PGE_2, 11-deoxy PGE_1 and AH13205 concentration-effect curves were all well-defined and reached similar asymptotes to PGE_2. The following rank order of potencies for the EP-receptor agonists was obtained; PGE_2 = misoprostol > 16,16-dimethyl PGE_2 = 11-deoxy PGE_1 > AH13205. The mean $p[A_{50}]$s and relative potencies are compared with other EP-receptor containing tissues in Table 2.

Effect of antagonists on EP-receptor mediated responses In contrast to the results obtained in the RbSV, AH23848B (30 μM) did not cause relaxation of PhE contracted tissues, or significantly antagonize responses to PGE_2 (n = 7, P = 0.8) in this preparation. This is shown in Figure 4b.

AH6809 displayed no significant antagonist activity in the RbEA ($n \geq 4$, P = 0.63); however, it did cause relaxations of 6.4 ± 2.8% and 39.9 ± 5.4% at concentrations of 1 and 10 μM respectively (data not shown).

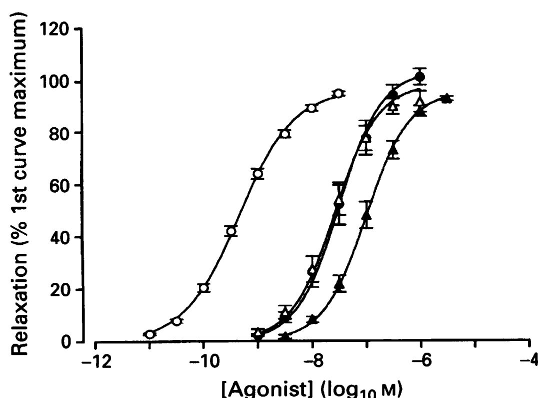

Figure 3 Concentration-effect curves for relaxation of the KCl-contracted rabbit saphenous vein by PGE_2 (○, n = 28), cicaprost (●, n = 5), BW245C (△, n = 8) and PGD_2 (▲, n = 6). The figure shows second curve data, with s.e.mean indicated.

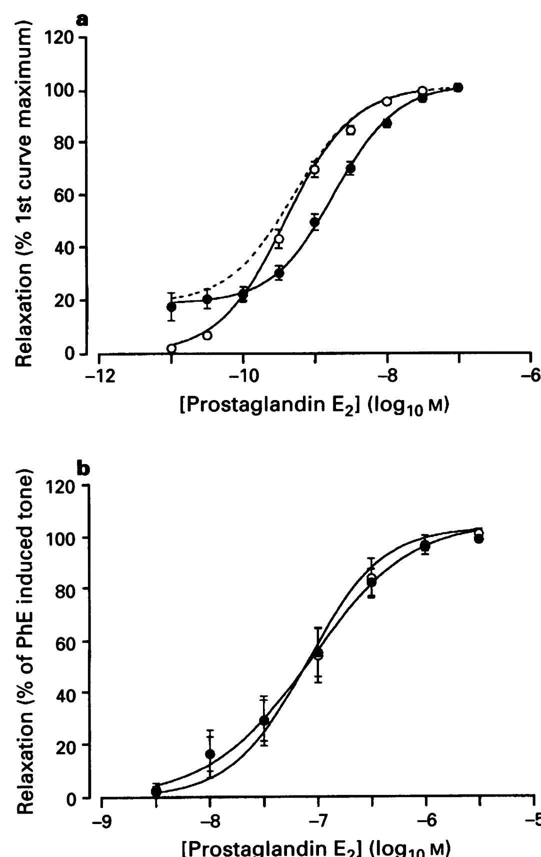

Figure 4 (a) Antagonism of PGE_2 E/[A] curves (○) in the rabbit saphenous vein by 30 μM (●) AH23848B. Each point is the mean of 10 determinations, the dotted line shows the theoretical dose-additive curve indicating the effects on PGE_2 curves of AH23848B acting simply as an agonist. (b) PGE_2 E/[A] curves in the rabbit ear artery in the absence (○) and presence (●) of 30 μM AH23848B (n = 7). Values are mean with s.e.mean.

Discussion

The objective of this study was to characterize fully the prostanoid receptors in the rabbit isolated saphenous vein, with specific reference to the EP-receptors mediating smooth muscle relaxation.

The high potency of PGE_2 ($p[A_{50}]=9.39$) in relaxing this tissue, considering that potent IP- and DP-receptor agonists were almost two orders of magnitude less potent than PGE_2, implies that the receptor subserving this response is an EP-receptor (Coleman *et al.*, 1984). Our finding that the EP_3/EP_1-receptor-selective agonist sulprostone was a low potency agonist, suggests little or no activity at these receptors. The lack of antagonism with AH6809 (EP_1/DP-receptor selective, Keery & Lumley, 1988) against PGE_2-mediated relaxations is further confirmation that EP_1-receptors are not involved. If the receptor mediating this inhibitory response was of the EP_2 subtype, then the selective agonists, butaprost and AH13205, should have been in the region of 10–100 times less potent than PGE_2, as is the case in the rabbit isolated ear artery (Humbles *et al.*, 1991). Comparison of the potency orders for the range of EP-receptor agonists in these two tissues reveals conspicuous differences. As shown in Figure 3, the EP_2-receptor agonists were at least 380 times less potent than PGE_2 in the rabbit saphenous vein and there was reversal in the positions of misoprostol (EP_2/EP_3-receptor selective) and 11-deoxy PGE_1 and 16,16-dimethyl PGE_2. Finally, in the rabbit ear artery PGE_2 had an absolute potency in the sub-micromolar range rather than the sub-nanomolar range as is the case in the rabbit saphenous vein. The agonist profile in the rabbit ear artery is representative of EP_2-receptor activity (Gardiner, 1986; Nials *et al.*, 1993) and suggests that the predominant prostanoid receptor eliciting smooth muscle relaxation in the rabbit saphenous vein, is not of the EP_2 subtype.

The extremely high potency of PGE_2 at EP-receptors, as in the rabbit saphenous vein, has been reported previously in tissues such as the rabbit jugular vein, hamster uterus and piglet saphenous vein (Lawrence & Jones, 1992; Yeardley *et al.*, 1992; Coleman *et al.*, 1994), and more recently, in the

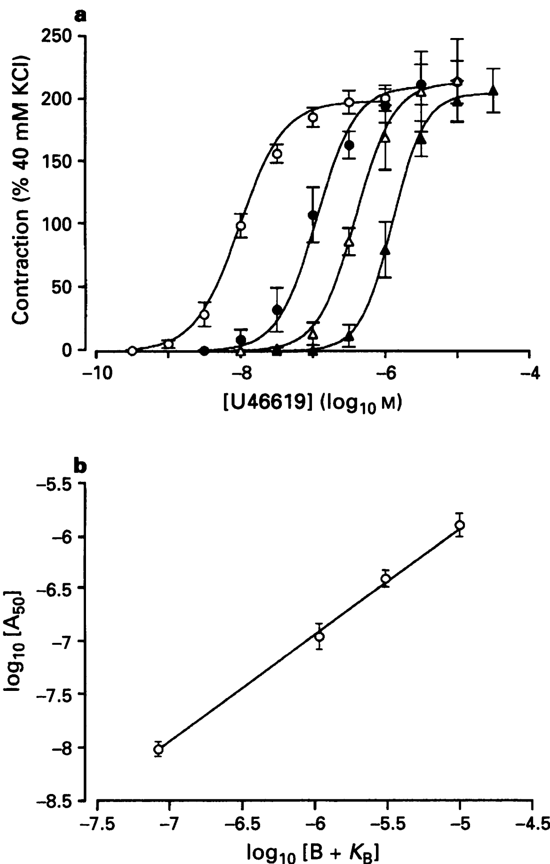

Figure 5 Effect of GR32191B on U46619 E/[A] curves in the rabbit saphenous vein: (a) shows the data obtained in the absence (○) of GR32191B and in the presence of the following concentrations (μM): 1(●), 3(△) and 10 (▲). Each point is the mean of at least 3 determinations, with s.e.mean indicated. (b) Clark plot for the antagonism of U46619 by GR32191B. The adherence of the data with the unit slope line drawn through them indicates consistency with simple competition.

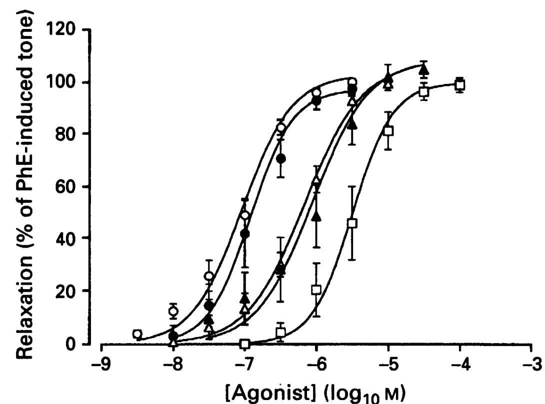

Figure 6 Concentration-effect curves for relaxation of the phenylephrine (PhE)-contracted rabbit ear artery by PGE_2 (○, $n=12$), misoprostol (●, $n=4$), 11-deoxy PGE_1 (△, $n=5$), 16,16-dimethyl PGE_2 (▲, $n=4$) and AH13205 (□, $n=5$). Values are mean with s.e.mean.

Table 2 Comparison of the agonist potencies of prostaglandin E_2 analogues in the rabbit isolated saphenous vein with other tissue containing relaxant EP-receptor

Agonist	*Rabbit saphenous vein*	*Rabbit jugular vein*	*Piglet saphenous vein*	*Rat trachea*	*Rabbit ear vein*
PGE_2 ($p[A_{50}]$)	9.39	9.34*	9.63†	7.81**	7.04
	1.0	1.0	1.0	1.0	1.0
11-deoxy PGE_1	4.9	2.1*	1‡	2.4**	11.9
16,16-dME_2	6.8	2.1*	1‡	2.8**	9.1
Misoprostol	96	8.3*	NT	16.1**	1.3
Butaprost	382	685*	3‡	NT	16#
AH13205	1,496	NT	11,040†	1,118**	37
Sulprostone	15,572	> 3000*	6,480†	Inactive**	> 770#

Published data from; † Coleman *et al.*, 1994; # Humbles *et al.*, 1991; * Lawrence & Jones, 1992; ** Lydford & McKechnie, 1994b; ‡ Milne *et al.*, 1994b.
NT, not tested.

rabbit ductus arteriosus (Smith *et al.*, 1994). Furthermore, the agonist potency order in the rabbit saphenous vein is very similar to that found in the aforementioned tissues, all of which contain so called 'atypical' EP-receptors. Evidence for the existence of a fourth class of prostanoid EP-receptors has been strengthened by the recent cloning of two pharmacologically different subtypes. Regan *et al.* (1994) isolated a clone (HuP-4) that, based on radioligand binding studies and cyclic AMP determinations, apparently encodes the human EP_2-receptor. The receptors cloned by Honda *et al.* (1993) and Bastien *et al.* (1994) amongst others, although classified as EP_2-receptors, probably represent EP_4-receptors based on their high affinities for PGE_2 and relative insensitivities to EP_2-receptor ligands.

According to Coleman *et al.* (1994), this unique profile with PGE_2 being much more potent than selective EP_1-, EP_2- and EP_3-receptor agonists, designates activity at EP_4-receptors. Confirmation of this activity is if the PGE_2-mediated response is susceptible to blockade with AH23848B. This compound, developed as a TP-receptor antagonist (Brittain *et al.*, 1985) is also a partial agonist at TP-receptors (Lumley, 1986) and more importantly, has been shown to be an antagonist at EP_4-receptors (Coleman *et al.*, 1994). In the present study we have shown that AH23848B was a weak antagonist of PGE_2-mediated responses in the rabbit saphenous vein, and the pA_2 estimate of 4.96 that we obtained is consistent with an activity at EP_4-receptors (Coleman *et al.*, 1994). Against AH13205 and misoprostol E/[A] curves, AH23848B (30 μM) was without significant effect. This suggests that these two compounds are acting purely at EP_2-receptors, and therefore the rabbit saphenous vein contains a mixed population of inhibitory EP-receptors. Although AH23848B has properties other than EP_4-receptor antagonism, this effect was prostanoid-specific as there was no effect on the $p[A_{50}]$ of isoprenaline E/[A] curves. Interestingly, the rabbit jugular vein which would appear to contain EP_4-receptors based purely on agonist data, is insensitive to AH23848B (Milne *et al.*, 1994b), whereas the rat trachea displays all the characteristics of an EP_4-receptor but without the sub-nanomolar potency for PGE_2 (Lydford & McKechnie, 1994b). Whether these receptors are of the EP_4-subtype and the differences due to species variation, or if they represent distinct receptor populations, has yet to be clarified.

A further point of curiosity was the agonism that we encountered in the RbSV whilst using AH23848B (Figure 4a). This phenomenon has not been reported before, possibly due to differences in experimental technique. Analysis of our experimental data according to the method of Pöch & Holzmann (1980) revealed that this agonism was not influencing our estimate of antagonist affinity. As to the nature of this agonism, it may represent EP_4-receptor partial agonism as preliminary experiments in this laboratory using naive CHO cells, revealed that AH23848B (30 μM) was able to increase cyclic AMP significantly above basal levels (unpublished observations). In the rabbit ear artery, AH23848B showed no agonist activity and did not antagonize PGE_2 E/[A] curves, therefore the agonism seen with AH23848B in the rabbit saphenous vein was unlikely to be mediated through EP_2-receptors. The relaxations caused by AH6809 in both tissues may be due to the compound's known cyclic AMP-phosphodiesterase inhibitory activity (Keery & Lumley, 1988). The difference in the magnitude of this response between the two vessels is possibly due to the concentrations of spasmogen used in each preparation ($[A_{80}]$ in the RbSV v $[A_{50}]$ in the RbEA), or the respective levels of endogenous phosphodiesterase activity.

The potencies of cicaprost, an IP-receptor agonist (Stürzbecher *et al.*, 1986), and the two DP-receptor agonists, PGD_2 and BW245C (Whittle *et al.*, 1983) are consistent with published *in vitro* data, confirming the presence of IP- and DP-receptors. We have previously demonstrated that BW245C is able to interact with BW A868C-sensitive DP-receptors in the rabbit saphenous vein, as well as EP_4-receptors (Lydford *et al.*, 1994). This activity at the latter receptor has been confirmed by Smith *et al.* (1994), who found that BW245C responses in the rabbit ductus arteriosus were antagonized by AH23848B, the EP_4-receptor antagonist. The absence of DP-receptors in the rabbit ductus arteriosus, as indicated by the insensitivity of BW245C relaxant responses to the DP-receptor antagonist BW A868C (Giles *et al.*, 1989), probably accounts for the low potency of BW245C in this preparation.

In addition to inhibitory prostanoid receptors, the results that we obtained for the synthetic thromboxane A_2-mimetic, U46619, suggest that the rabbit saphenous vein contains contractile TP-receptors. The competitive nature of receptor-blockade observed with GB32191B, a potent and selective TP-receptor antagonist (Lumley *et al.*, 1989), supports an action at contractile TP-receptors. The pK_B of 7.08 for GR32191B in this preparation, although lower than usually encountered, is similar to that obtained in the human myometrium (Senior *et al.*, 1993) and is within the reported range for this compound (Lumley *et al.*, 1989). Indeed, this description of TP-receptor antagonists displaying lower affinities in the rabbit has been documented in earlier studies (Tymkewycz *et al.*, 1991) and probably reflects a species difference rather than receptor heterogeneity. Our original classification of 16,16-dimethyl PGE_2 as a partial agonist at relaxant EP_4-receptors (Lydford & McKechnie, 1994a) may be due to this low affinity of GR32191B in the rabbit saphenous vein. Tymkewycz *et al.* (1991) demonstrated that 16,16-dimethyl PGE_2 was a full agonist at TP-receptors in various isolated preparations, including the rabbit aorta. In our preliminary studies the lower concentrations of GR32191B used ($1-3$ μM), may not have been sufficient to prevent the contractile TP-receptor effects of 16,16-dimethyl PGE_2 opposing its relaxant effects, leading to underestimation of its true asymptote.

The presence of contractile EP and FP-receptors was ruled out by the inactivities of PGE_2 and $PGF_{2\alpha}$ in tissues in which TP-receptors had been blocked.

In conclusion, the data presented demonstrate that the rabbit isolated saphenous vein contains a heterogeneous population of prostanoid receptors. On the basis of its high potency, the rank order of potency for a number of selected agonists and the affinity of AH23848B, PGE_2-induced relaxations are primarily mediated through EP_4-receptors. The presence of EP_2-receptors, as found in the rabbit ear artery, is suggested by the fact that the effects of the EP_2-receptor agonists butaprost and AH13205, are resistant to AH23848B. The potencies of cicaprost and BW245C suggest the presence of IP- and DP-receptors respectively, and the effect of GR32191B on U46619 responses indicates the involvement of excitatory TP-receptors. Whether the EP_4-receptors in the rabbit and piglet saphenous veins are identical to the 'EP_2-like' receptors found in other preparations remains to be established, and awaits the development of more potent and selective ligands.

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