ELSEVIER

European Journal of Pharmacology 378 (1999) 99–108

ejp

www.elsevier.nl/locate/ejphar

Inhibitory prostanoid EP receptors in human non-pregnant myometrium

Catherine J. Hillock [a], Denis J. Crankshaw [a,b,*]

[a] *Honours Biology and Pharmacology Programme, McMaster University, Hamilton, Ontario, Canada*
[b] *Department of Obstetrics and Gynecology, McMaster University, Hamilton, Ontario, Canada*

Received 22 March 1999; received in revised form 10 June 1999; accepted 15 June 1999

Abstract

Prostanoid EP receptor agonists relaxed cloprostenol-stimulated contraction of human non-pregnant myometrium in vitro with pEC_{50} values of ($n = 4$): prostaglandin E_2, 7.8 ± 0.2 > 1-OH prostaglandin E_1, 7.2 ± 0.3 > misoprostol, 6.6 ± 0.1 > 16,16-dimethyl prostaglandin E_2, 6.3 ± 0.7 > butaprost, 5.7 ± 0.3 > 11-deoxy prostaglandin E_1, 5.5 ± 0.2 = AH13205 ((±)-*trans*-2-[4-(1hydroxyhexyl)phenyl]-5-oxocyclopentaneheptanoic acid), 5.5 ± 0.2. The EP_4 receptor antagonist AH23848B ([1α(z), 2β5α]-(±)-7-[5-[[(1,1′-biphenyl)-4-yl]methoxy]-2-(4-morpholinyl)-3-oxo-cyclopentyl]-4-heptenoic acid) (29 μM) had no effect on concentration–effect curves to the EP receptor agonists. The mixed prostanoid receptor antagonist AH6809 (6-isopropoxy-9-oxaxanthene-2-carboxylic acid) competitively antagonised prostaglandin E_2 with a pA_2 of 5.6 ± 0.2. AH6809 (42 μM) antagonised misoprostol, 11-deoxy prostaglandin E_1, and the prostanoid DP receptor agonist BW245C (5-(6-carboxyhexyl)-1-(3-cyclohexyl-3-hydroxypropyl)hydantoin) with apparent pA_2 values of 5.6 ± 0.3, 5.1 ± 0.9 and 5.9 ± 0.4 ($n = 4$), respectively, but was ineffective against the IP receptor agonist cicaprost ($n = 4$). The prostanoid DP receptor antagonist BW A868C (3-benzyl-5-(6-carboxyhexyl)-1-(2-cyclohexyl-2-hydroxyethylamino)hydantoin) (50 nM) had no effect on responses to prostaglandin E_2 or misoprostol. The presence of an AH6809-sensitive, AH23848B- and BW A868C-insensitive mechanism is consistent with the hypothesis that inhibitory EP receptor agonists cause relaxation of human non-pregnant myometrium by an EP_2 receptor-mediated process.

Keywords: Myometrium; (Human); Prostanoid; Prostanoid EP_2 receptor; Prostanoid EP_4 receptor; AH23848B; AH6809

1. Introduction

Prostanoids inhibit the contractility of human non-pregnant myometrium by acting at DP receptors (Senior et al., 1992; Fernandes and Crankshaw, 1995), IP receptors (Senior et al., 1992) and inhibitory EP receptors (Senior et al., 1991; Brown and Crankshaw, 1995; Brown et al., 1997). The inhibitory EP receptors involved are thought to belong to the EP_2 subtype. This notion is based mainly upon the potency of the EP_2 receptor-selective agonist butaprost in a superfusion assay (Senior et al., 1991). Subsequent to the characterization of EP receptors in human myometrium (Senior et al., 1991), a novel prostanoid receptor, the EP_4 receptor, was identified pharmacologically (Coleman et al., 1994a). Cloning of the human EP_2 receptor by Regan et al. (1994) demonstrated that the human EP receptor previously cloned by Bastien et al. (1994) had been incorrectly identified. This earlier clone (Bastien et al., 1994) was subsequently identified as the EP_4 receptor (Toh et al., 1995). Both Northern blots of human uterus (Bastien et al., 1994) and reverse transcription-polymerase chain reaction (RT-PCR) of human myometrium (Senchyna and Crankshaw, 1995) revealed the presence of EP_4 receptor mRNA, but there is as yet no functional evidence for the presence of this receptor in human myometrium.

Although originally characterized as a TP receptor antagonist, the compound AH23848B is also an EP_4 receptor antagonist, with no antagonist activity at other EP receptors (Coleman et al., 1994a). On the other hand, AH6809 blocks DP and EP_1 receptors and these properties have been used extensively in prostanoid receptor classification (Coleman et al., 1994b), but it has recently been shown to

* Corresponding author. Department of Obstetrics and Gynecology, McMaster University, HSC 3N52; 1200 Main Street West, Hamilton, Ontario, Canada, L8N 3Z5. Tel.: +1-905-525-9140/22228; fax: +1-905-524-2911; E-mail: cranksha@mcmaster.ca

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00442-2

antagonise the recombinant human EP_2 receptor (Woodward et al., 1995). Therefore, both AH23848B and AH6809 may be helpful in fully characterizing the inhibitory EP receptors mediating relaxation of human myometrium.

In the present study, we have determined the potencies of a number of inhibitory EP receptor agonists on human non-pregnant myometrium in vitro using equilibrium methods. Tissues were pre-stimulated with the prostanoid FP receptor agonist cloprostenol (Coleman et al., 1994b). We have investigated the effects of AH23848B and AH6809 on prostanoid-induced relaxation.

2. Materials and methods

2.1. Materials

2.1.1. Drugs

Cloprostenol (Estrumate®) was purchased from Coopers Agropharm (Willowdale, ON, Canada); indomethacin and D600 ((±)-α-[3-[[2-(3,4-dimethoxyphenyl)ethyl]methylamino]propyl]-3,4,5-trimethoxy-α-(1-methylethyl)-benzeneacetonitrile hydrochloride) from Sigma (St. Louis, MO, USA); prostaglandin E_2, 16,16-dimethyl prostaglandin E_2, 11-deoxy prostaglandin E_1 and 1-OH prostaglandin E_1 from Cayman Chemical (Ann Arbor, MI, USA). The following compounds were received as gifts: AH13205 ((±)-*trans*-2-[4-(1hydroxyhexyl)phenyl]-5-oxocyclopentaneheptanoic acid), AH23848B ([1α(z), 2β5α]-(±)-7-[5-[[(1,1′-biphenyl)-4-yl]methoxy]-2-(4-morpholinyl)-3-oxocyclopentyl]-4-heptenoic acid) and AH6809 (6-isopropoxy-9-oxaxanthene-2-carboxylic acid) from Glaxo (Stevenage, UK); butaprost from Dr. H. Kluender, Bayer (West Haven, CT, USA); BW A868C (3-benzyl-5-(6-carboxyhexyl)-1-(2-cyclohexyl-2-hydroxyethylamino)hydantoin) and BW245C (5-(6-carboxyhexyl)-1-(3-cyclohexyl-3-hydroxypropyl)hydantoin) from Wellcome (Beckenham, UK); cicaprost from Schering (Berlin, Germany); L670596 ((−)6,8-difluoro-9-*p*-methylsulfonyl benzyl-1,2,3,4-tetrahydrocarbazol-1-yl-acetic acid) from the Merck Frosst Centre for Therapeutic Research (Pointe Claire, PQ, Canada); misoprostol from Searle (Skokie, IL, USA).

Stock solutions of prostaglandin E_2, misoprostol, 1-OH prostaglandin E_1, 16,16-dimethyl prostaglandin E_2, BW245C and BW A868C were made in ethanol and stored at −20°C; butaprost was in ethyl acetate at −20°C; AH13205 and AH6809 were in 0.5% $NaHCO_3$ solution at −20°C; 11-deoxy prostaglandin E_1 in methyl acetate at −70°C; L670596 in dimethylsulphoxide at 4°C; cicaprost, cloprostenol and D600 were in aqueous solutions at 4°C. AH23848B was dissolved in 10% $NaHCO_3$ and used immediately. Serial dilutions of drugs were made freshly into double-distilled water and kept on ice throughout the experiment. Indomethacin was prepared as described by Curry et al. (1981). All other chemicals were from BDH (Toronto, ON, Canada).

2.1.2. Solutions

The buffered saline had the following composition (mM): *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic

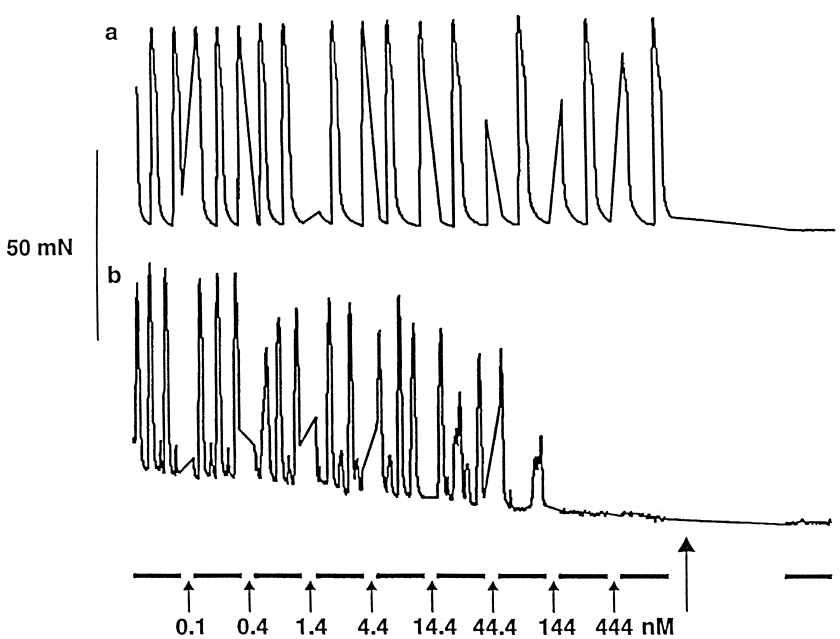

Fig. 1. Original tracing of the effect of cumulative addition of prostaglandin E_2 on cloprostenol-induced contractile activity of human myometrium from a non-pregnant donor. (a) Time-matched control strip, (b) agonist-treated strip. Horizontal bars indicate 10 min data collection periods, the first of which is the control and the last of which is zero contractile activity. Prostaglandin E_2 was added to the treated strip at the points indicated by the small arrows to give the cumulative concentrations shown. The larger arrow marks the point where D600 (final bath concentration 10 μM) was added to both control and agonist-treated strips.

acid 5.0, NaCl 154, pH 7.4. The physiological salt solution (PSS) was composed as follows (mM): KCl 4.6; $MgSO_4$ 1.16; NaH_2PO_4 1.16; $CaCl_2$ 2.5; NaCl 115.5; $NaHCO_3$ 21.9 and glucose 11.1 with indomethacin at 10 μM.

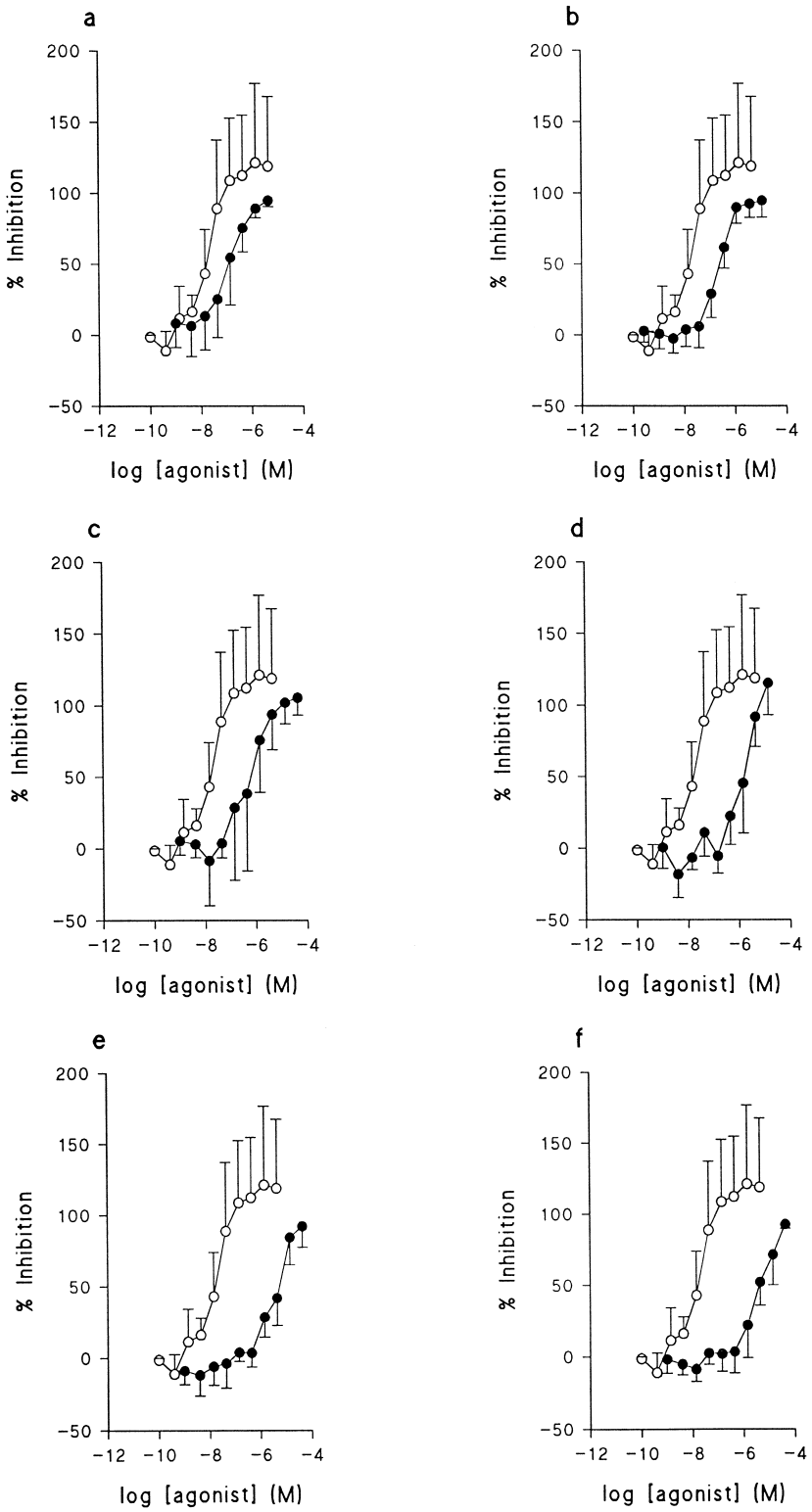

Fig. 2. Concentration–effect curves for inhibition of cloprostenol-stimulated activity of human non-pregnant myometrium in vitro by prostaglandin E_2 (○) and other putative inhibitory EP receptor agonists (●), (a) 1-OH prostaglandin E_1, (b) misoprostol, (c) 16,16-dimethyl prostaglandin E_2, (d) butaprost, (e) 11-deoxy prostaglandin E_1, (f) AH13205. The same data for prostaglandin E_2 are shown in each panel for comparison. Each point is the mean with S.D. mean of 4 determinations.

2.2. Tissue collection and preparation

Human myometrial samples were obtained from premenopausal, non-pregnant women undergoing hysterectomy for benign disorders such as fibroids, menorrhagia and uterine prolapse as previously described (Fernandes and Crankshaw, 1995; Senchyna and Crankshaw, 1996). At the time of surgery, none of the donors had used oral contraceptives or received any hormone therapy. Specimens were generally taken from the anterior wall of the corpus uteri. Immediately following surgery, samples were placed in buffered saline for transport to the laboratory, where they were transferred to, and maintained in oxygenated (95% O_2, 5% CO_2) PSS at room temperature for up to 18 h post-operatively.

Myometrial tissue strips were set up as previously described (Fernandes and Crankshaw, 1995). Whole tissue specimens were trimmed of endometrial, serosal, fat and fibrous tissue. Up to 16 strips of myometrium (15 mm × 2 mm × 3 mm) from the muscle layer directly adjacent to the serosa were cut in a direction parallel to that of the serosa, tied at each end with silk thread and mounted longitudinally in individual 10 or 15 ml jacketed muscle baths containing oxygenated PSS at 37°C.

2.3. Effect of drugs on cloprostenol-induced contractile activity

One end of each strip was anchored in the bath, the other was attached to an FT-03 force displacement transducer writing to either a 7D polygraph (Grass Instruments, Quincy, MA) or a custom-made amplifier writing to the data collection software, In Vitro Collection System Ver 4.0 (J. Milton, Dundas, ON, Canada), running on a personal computer. An optimum resting force of 25 mN was applied to each strip (Crankshaw and Dyal, 1993). The mean force developed by the individual muscle strips was used as a measure of their contractility (Wainman et al., 1988; Cheuk et al., 1993). Mean force was determined during 10 min epochs as described by Wainman et al. (1988) using the In Vitro Collection System software. The force generated by each muscle strip was sampled at a frequency of 2 Hz. All samples taken over the 10 min period were added (this value corresponds to the area under the contraction curve). The total was then divided by the number of samples taken (1200) to give the mean force exerted over the 10 min period.

2.3.1. Relaxant effects of prostanoid receptor agonists

Relaxant activity was assessed using the cloprostenol-stimulated technique described by Fernandes and Crankshaw (1995). Up to 16 tissue strips from the same donor were allowed to equilibrate for 1 h during which they were washed two to three times with PSS and the resting tension was readjusted to 25 mN. Strips were then challenged with 2 μM cloprostenol which remained in contact with the tissues for the remainder of the experiment. After 1 h incubation with cloprostenol, 50 nM L670596 was added to prevent stimulation of TP receptors (Ford-Hutchinson et al., 1989).

After 2 h exposure to cloprostenol, the mean force developed during a 10 min control period was determined. Two tissue strips were randomly assigned as temporal controls, and where appropriate, were treated with vehicle. The remainder of the strips were randomly assigned various drug treatments (usually duplicate strips from the same donor were treated with the same agonist) and a cumulative concentration–effect experiment was performed, using approximately half log unit concentration increases. Each successive agonist concentration was in contact with the tissue for 3 min before a 10 min data collection was taken. Agonist additions continued until bath concentrations spanned 5 log units or until further addition produced no further change in mean force. At the completion of agonist additions and without washing the baths, all tissue strips were treated with 10 μM of the Ca^{2+} channel blocker D600 (Fleckenstein et al., 1969). After tissues had been exposed to D600 for 15 min, a final 10 min recording was taken. D600 reduced activity to basal tone and this was used to define zero contractility.

Table 1
Effect of AH23848B (29 μM) on prostanoid-induced inhibition of human myometrial activity in vitro
Concentration–effect curve parameters were determined in the absence (Control) and presence (Treated) of AH23848B in paired myometrial strips as described in Section 2.
Data are means ± S.D. mean; $n = 4$; no significant difference in any parameter compared to control.

Compound	Control			Treated		
	pEC_{50}	k	E_{max}	pEC_{50}	k	E_{max}
Prostaglandin E_2	7.8 ± 0.2	4 ± 1	120 ± 50	7.5 ± 0.2	4 ± 1	94 ± 8
1-OH prostaglandin E_1	7.2 ± 0.3	3 ± 1	96 ± 4	7.1 ± 0.3	4 ± 1	100 ± 10
Misoprostol	6.6 ± 0.06	4 ± 1	100 ± 10	6.8 ± 0.1	4 ± 1	98 ± 5
16,16-dimethyl prostaglandin E_2	6.3 ± 0.7	5 ± 2	110 ± 10	6.1 ± 0.3	5 ± 2	95 ± 7
Butaprost	5.7 ± 0.3	5 ± 2	100 ± 20	5.5 ± 0.1	5 ± 2	100 ± 10
11-deoxy prostaglandin E_1	5.5 ± 0.2	4 ± 3	100 ± 20	5.8 ± 0.2	4 ± 2	130 ± 60
AH13205	5.5 ± 0.2	3 ± 1	97 ± 5	5.2 ± 0.3	3 ± 1	110 ± 20

2.3.2. Quantification of prostanoid receptor agonist-induced responses

The zero contractility value was subtracted from all preceding values, which were then expressed as a percentage of the control period values. Drug effects were then calculated according to the following equation:

$$E_x = \left(\left(D_x - C_x\right) / - C_x\right)100$$

where E_x is the percentage inhibition produced by the drug at concentration x, D_x is the percentage of control period activity developed in the presence of concentration x of the drug, and C_x is the percentage of control period activity developed in the time-matched control tissues for the same time period. If C_x was less than 50% immediately before the addition of D600, the experiment was rejected. Concentration–effect curves were constructed from the data obtained by fitting the following equation:

$$E = E_{\min} + \left(E_{\max} - E_{\min}\right) / \left(1 + e^{-k^*(\log C + \mathrm{pEC}_{50})}\right)$$

where E is the effect of the agonist, C is the molar concentration of the agonist, k is a power coefficient and pEC_{50} is the negative log of the molar concentration of the agonist that produces a half-maximal response.

2.3.3. Assessment of prostanoid receptor antagonist activity

Only one concentration–effect experiment could be performed reliably on each tissue strip. Therefore, antagonists' effects were investigated by incubating separate tissue strips from the same donor in the absence or in the

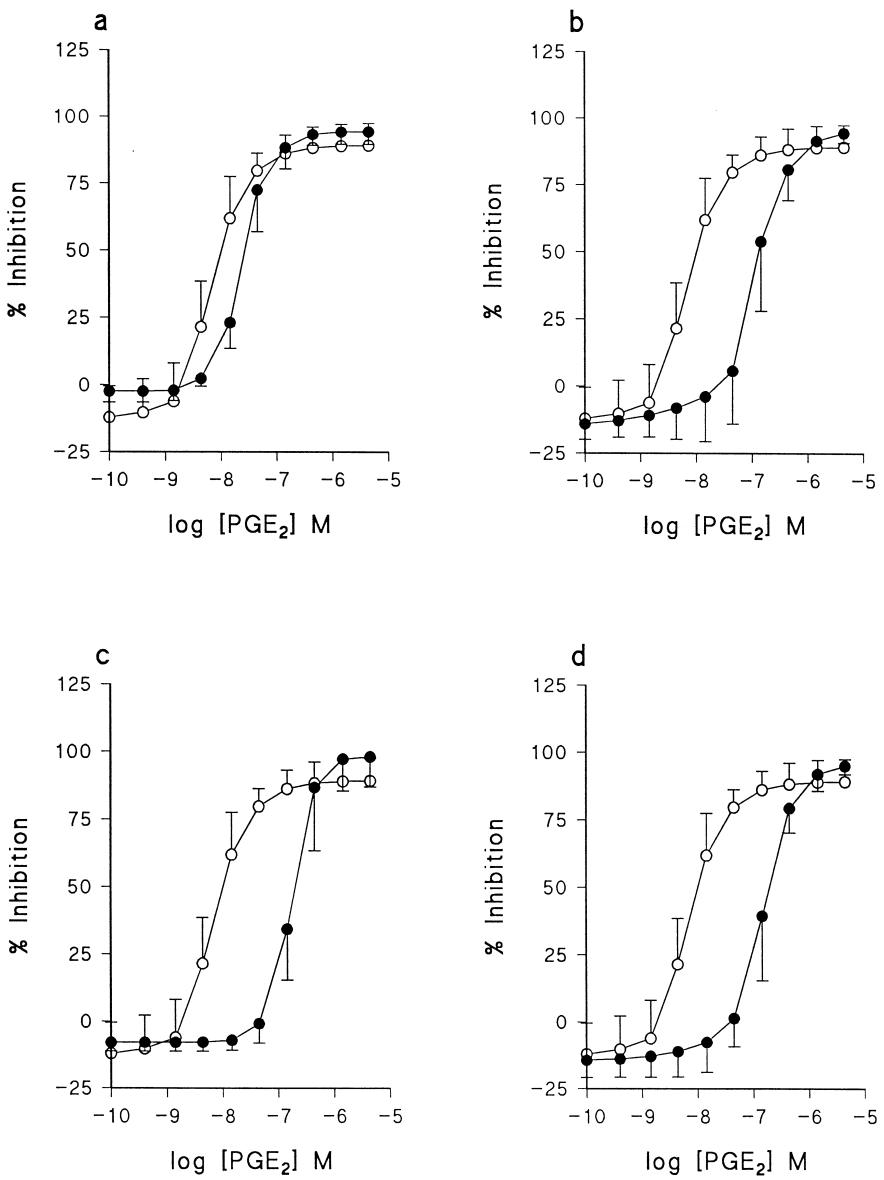

Fig. 3. Antagonism of prostaglandin E_2 by AH6809 in cloprostenol-stimulated human non-pregnant myometrium in vitro. Concentration–effect curves for prostaglandin E_2 in control (○) and AH6809-treated tissues (●), (a) 8.4 μM AH6809, (b) 16.8 μM AH6809, (c) 25.2 μM AH6809, (d) 42 μM AH6809. Each point is the mean with S.D. mean of 3 determinations.

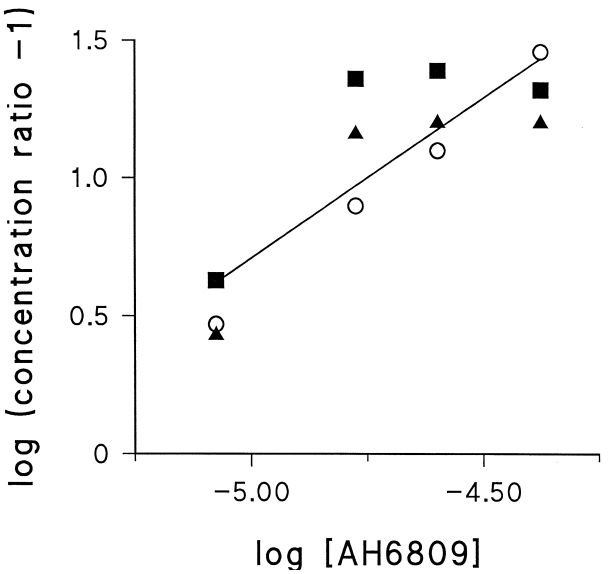

Fig. 4. Schild plot for the antagonism of prostaglandin E_2 by AH6809 in cloprostenol-stimulated human non-pregnant myometrium in vitro. Data from three independent experiments on tissues from different donors are represented by the three different symbols. The line is the best-fit linear regression to the mean data ($r^2 = 0.88$).

presence of antagonist for 1 h prior to and throughout the duration of an agonist concentration–effect experiment. Normally, two strips were used at each concentration of antagonist and two strips served as controls. pEC_{50} values were calculated as described above. Antagonist pA_2 values were then determined according to the equation:

$$pA_2 = \log\left((EC_{50A}/EC_{50C}) - 1\right) - \log[B]$$

where EC_{50A} is the agonist EC_{50} in the presence of antagonist, EC_{50C} is the control agonist EC_{50} and $[B]$ is the molar concentration of antagonist. In the case of AH6809 against prostaglandin E_2, the value was determined graphically from multiple paired curves whereas in other cases, an apparent pA_2 was calculated from single antagonist concentration experiments.

2.4. Statistics

Values of pEC_{50}, k, and E_{max} from individual donors were the averages from paired strips. All reported values are arithmetic means ± the standard deviation of the mean. Effects of antagonists on concentration–effect curve parameters were compared using a paired Student's *t*-test, values of $P < 0.05$ were considered significant.

3. Results

Under the conditions of our experiments the inhibitory EP receptor agonists prostaglandin E_2, 1-OH prostaglandin E_1, misoprostol, 16,16-dimethyl prostaglandin E_2, butaprost, 11-deoxy prostaglandin E_1, and AH13205 all caused concentration-dependent inhibition of human myometrial contractility. Fig. 1 shows a sample trace of the effect of cumulative addition of prostaglandin E_2 compared to a time-matched control obtained in a parallel strip from the same donor. Fig. 2 shows mean concentration–effect curves for all agonists. Concentration–effect curve parameters determined from the data in Fig. 2 are shown in Table 1 along with the concentration–effect parameters obtained in matched strips in the presence of 29 μM AH23848B. AH23848B had no effect on responses to any of the agonists tested. AH23848B had no direct effect on contractility, untreated tissues developed a mean contractile force of 28 ± 3 mN during the ten min control period whereas the corresponding value in matched, treated strips was 27 ± 6 mN ($n = 19$).

The concentration–effect curve to prostaglandin E_2 was shifted rightwards by AH6809 in a concentration-dependent manner (Fig. 3). Individual Schild plots for three independent experiments yielded a pA_2 of 5.6 ± 0.2 with a slope of 1.2 ± 0.2. A Schild plot of all the data is shown in Fig. 4. At a concentration of 42 μM, AH6809 also shifted the concentration–effect curves to two other inhibitory EP receptor agonists, misoprostol and 11-deoxy prostaglandin E_1, as well as to the DP receptor agonist BW245C (Town et al., 1983); but it did not shift the curve to the IP receptor agonist cicaprost (Coleman et al., 1990) (Table 2). AH6809 had no direct effect on contractility, untreated tissues developed a mean contractile force of 24 ± 9 mN during the 10 min control period, whereas the corresponding value in matched, treated strips was 23 ± 10 mN ($n = 23$).

Table 2
Effect of AH6809 (42 μM) on prostanoid-induced inhibition of human myometrial activity in vitro
Concentration–effect curve parameters were determined in the absence (Control) and presence (Treated) of AH6809 in paired myometrial strips as described in Section 2.
Data are means ± S.D. mean; $n = 4$.

Compound	Control			Treated			Apparent pA_2
	pEC_{50}	k	E_{max}	pEC_{50}	k	E_{max}	
Misoprostol	6.9 ± 0.3	4.5 ± 2.2	105 ± 13	5.6 ± 0.2*	3.8 ± 1.3	105 ± 5	5.6 ± 0.3
11-deoxy prostaglandin E_1	5.9 ± 0.7	4 ± 6	106 ± 35	5.0 ± 0.2*	6 ± 6	95 ± 22	5.1 ± 0.9
Cicaprost	7.2 ± 0.2	2.6 ± 0.1	70 ± 32	7.2 ± 0.4	2.0 ± 0.3*	79 ± 27	–
BW245C	8.1 ± 0.8	4.6 ± 3.1	100 ± 17	6.6 ± 0.5*	3.7 ± 1.1	102 ± 19	5.9 ± 0.4

*$P < 0.05$ compared to control.

Table 3
Effect of BW A868C (50 nM) on prostanoid-induced inhibition of human myometrial activity in vitro
Concentration–effect curve parameters were determined in the absence (Control) and presence (Treated) of BW A868C in paired myometrial strips as described in Section 2.
Data are means ± S.D. mean; $n = 3$; no significant difference in any parameter compared to control.

Compound	Control			Treated		
	pEC_{50}	k	E_{max}	pEC_{50}	k	E_{max}
Prostaglandin E_2	7.9 ± 0.2	4.0 ± 1.3	92 ± 15	7.9 ± 0.3	4.4 ± 1.4	88 ± 13
Misoprostol	6.8 ± 0.4	6.1 ± 3.5	92 ± 10	7.0 ± 0.3	4.0 ± 2.2	95 ± 4

Responses to prostaglandin E_2 and misoprostol were unaffected by the selective DP receptor antagonist BW A868C (Giles et al., 1989) at a concentration of 50 nM (Table 3).

4. Discussion

In this study, we sought to characterize the prostanoid EP receptors coupled to relaxation in the human myometrium using functional methods. The pharmacological tools available for such an enterprise are limited, in that most compounds have significant activity at more than one receptor. However, the compounds used in this study were among those used, in functional studies, to establish the current IUPHAR prostanoid receptor classification (Coleman et al., 1994b). Prostaglandin E_2, misoprostol, and 16,16-dimethyl prostaglandin E_2 produce potent excitation as well as inhibition of human myometrial contractility (Senior et al., 1991). In order to assess the inhibitory effects of these and other compounds, it was therefore necessary to employ a technique that would allow us to pharmacologically isolate the inhibitory component of the response. Because of the lack of sufficiently selective antagonists, we chose to use the cloprostenol-stimulation technique (Fernandes and Crankshaw, 1995). The concentration–effect curves in Fig. 2 show little sign of any agonist-induced excitation, and therefore suggest that our goal was attained. However, we may have underestimated the relaxant potencies of some compounds if their excitatory EP receptor effects opposed their relaxant action in a way that was not detectable by our methods.

The most likely explanation for the lack of effect of AH23848B on responses to any of the agonists we tested is that there is no significant role for EP_4 receptors in prostanoid-induced relaxation of human myometrium (Brown and Crankshaw, 1995). The possibility that our preparation of AH23848B was inactive was ruled out (Brown, 1996), since it produced antagonism of prostaglandin E_2-induced relaxation of the rat isolated trachea exactly as described by Lydford and McKechnie (1994). In light of a number of reports of AH23848B's antagonism of prostaglandin E_2 in human systems (De Vries et al., 1995; Blaschke et al., 1996; Mukhopadhyay et al., 1997), it also now seems unlikely that the human EP_4 receptor is sufficiently pharmacologically distinct from other species homologues to be AH23848B-insensitive. Piglet saphenous vein functionally expresses only the EP_4 subtype of the EP receptor, and in this preparation AH23848B has a pA_2 of 5.4 (Coleman et al., 1994a). Thus, if the EP_4 receptor-mediated the full response to any of the agonists we tested, AH23848B should have produced an 8-fold shift in the EC_{50} value for that agonist.

AH23848B has recently been shown to act as a partial agonist at the EP_4 receptor in rat neutrophils (Wise, 1998). The lack of effect of the compound on control period activity in the present study does not support any significant agonist activity for AH23848B at inhibitory receptors in human non-pregnant myometrium.

Since our results with AH23848B do not support the involvement of EP_4 receptors in prostanoid-induced inhibition of human myometrial contractility, some explanation must be found for the expression of EP_4 receptor mRNA in human myometrium (Senchyna and Crankshaw, 1995). One possibility is that the relative contribution of EP_4 receptors to inhibition is so small as to be undetectable by the functional studies we employed. Alternatively, EP_4 receptors may be expressed on cells other than myometrial smooth muscle, or, despite the presence of mRNA, not expressed at all. All of these possibilities await further experimentation.

The effect of AH6809 on the response to prostaglandin E_2 (Fig. 4) is consistent with competitive antagonism at a single site, although we cannot exclude a more complex action if prostaglandin E_2 caused significant EP_1 receptor activation in our preparation. Furthermore, its ability to shift the concentration–effect curves to misoprostol and 11-deoxy prostaglandin E_1 but not to the selective IP receptor agonist cicaprost (Coleman et al., 1990) (Table 2) suggests some selectivity of action. AH6809 also antagonised the DP receptor agonist BW245C (Table 2) consistent with its recognized property as a competitive DP receptor antagonist (Keery and Lumley, 1988). At a concentration of 50 nM the selective DP receptor antagonist BW A868C (Giles et al., 1989) produces a 30-fold shift in the EC_{50} value of BW245C in human non-pregnant myometrium (Fernandes and Crankshaw, 1995), so its lack of effect on responses to prostaglandin E_2 and misoprostol (Table 3) rule out the involvement of the DP receptor in the inhibition of myometrial contractility produced by

Table 4
Comparison of relative molar potencies of EP receptor agonists in various preparations

Agonist	Relative molar potencies, prostaglandin $E_2 = 1$		
	Pig saphenous vein	Cat trachea	Human myometrium
1-OH prostaglandin E_1	NT	NT	4
Misoprostol	NT	3.7[b]	16
16,16-dimethyl prostaglandin E_2	2.8[a]	20[b]	32
Butaprost	42[a]	17[c]	126
11-deoxy prostaglandin E_1	2.0[a]	13[d]	200
AH13205	3100[a]	29[e]	200

[a] Milne et al. (1995).
[b] Coleman et al. (1988).
[c] Gardiner (1986).
[d] Dong et al. (1986).
[e] Nials et al. (1993).
NT, not tested.

these compounds. We therefore conclude that AH6809, blocks the inhibitory actions of prostaglandin E_2, misoprostol and 11-deoxy prostaglandin E_1 on human non-pregnant myometrium by action at an inhibitory EP receptor.

Extensive functional studies with AH6809 in animal tissues have not heretofore demonstrated significant inhibitory EP receptor blockade (Coleman et al., 1990). Indeed, the compound has been used for receptor classification purposes as an EP_1 receptor antagonist (Coleman et al., 1994b). However, AH6809 had no affinity for the recombinant mouse EP_1 receptor and a K_i of 350 nM at the recombinant mouse EP_2 receptor (Kiriyama et al., 1997). AH6809 has antagonistic activity at a putative inhibitory EP receptor in human neutrophils with a pA_2 value of 7.0 (Li et al., 1995). It also blocks prostaglandin E_2-mediated inhibition of lipopolysaccharide-induced tumour necrosis factor-α generation by human monocytes with an apparent pA_2 of 5.4 (Meja et al., 1997). Furthermore, AH6809 is an antagonist of the recombinant human EP_2 receptor (Woodward et al., 1995) with a pA_2 that we estimate from the data provided to be 6.5. In the absence of compelling evidence to support the existence of an alternate inhibitory EP receptor, our demonstration of an AH6809-sensitive, AH23848B-insensitive mechanism suggest that the inhibitory EP receptors in the human isolated myometrium are predominantly of the EP_2 subtype. The discrepancy between the pA_2 value that we determined, and that found for the recombinant receptor is not surprising considering the inherent differences in the two techniques.

AH6809 blocks both inhibitory (this study) and excitatory (Senior et al., 1991) human EP receptors and is therefore an unsuitable tool for receptor classification in this species (Woodward et al., 1995; Brown et al., 1997).

Comparison of the relative molar potencies of inhibitory EP receptor agonists in human myometrium with those in pig saphenous vein, the archetype EP_4 receptor preparation (Coleman et al., 1994a; Milne et al., 1995), and cat trachea, an archetypal EP_2 receptor-containing preparation (Dong et al., 1986; Gardiner, 1986; Coleman et al., 1988; Nials et al., 1993) (Table 4) reveals no striking similarities. The lack of overall correlation could result from a possible under-estimation of the relaxant potency of mixed agonists in our experiments, together with the well-recognized inter-species differences in G-protein coupled receptors. However, the relative potencies of AH13205 and butaprost can be compared with some confidence since there is no evidence for any significant excitatory activity by either of these compounds. The relative potencies of AH13205 to butaprost are 1.6, 1.7 and 73 in human myometrium, cat trachea and pig saphenous vein, respectively. Thus, these limited agonist potency data also support the hypothesis that the inhibitory EP receptors in the human isolated myometrium are predominantly of the EP_2 subtype.

An isoform of the human EP_3 receptor has been shown to couple to the stimulation of adenylyl cyclase when over-expressed in surrogate cells (Kotani et al., 1995). If such a mechanism were operative in human myometrium, relaxation might be mediated by the EP_3 receptor. However, there is currently no evidence to support inhibition of human smooth muscle contractility by a wild-type EP_3 receptor-mediated mechanism. Furthermore, sulprostone (a mixed EP_3/EP_1 receptor agonist devoid of EP_2 and EP_4 receptor activity) produces purely excitatory responses in human myometrium (Senior et al., 1991). Therefore, the inhibitory responses that we observed are unlikely to be mediated by the EP_3 receptor.

In conclusion, EP receptor agonists relax the human isolated myometrium by an AH6809-sensitive, AH23848B- and BW A868C-insensitive mechanism. This suggests that the predominant receptor involved is the EP_2 subtype.

Acknowledgements

We are indebted to Jeanette Boersma and Jean Crankshaw for their skillful assistance with some of the

experiments and to R.A. Coleman for helpful comments on the manuscript. We thank all those who supplied compounds used in this study. This work was supported by the Medical Research Council of Canada.

References

Bastien, L., Sawyer, N., Grygorczyk, R., Metters, K.M., Adam, M., 1994. Cloning, functional expression, and characterization of the human prostaglandin E_2 receptor EP_2 subtype. J. Biol. Chem. 269, 11873–11877.

Blaschke, V., Jungermann, K., Püschel, G.P., 1996. Exclusive expression of the Gs-linked prostaglandin E_2 receptor subtype 4 mRNA in human mononuclear Jurkat and KM-3 cells and coexpression of subtype 4 and 2 mRNA in U-937 cells. FEBS Lett. 394, 39–43.

Brown, C.J., 1996. Inhibitory Prostanoid EP Receptors in Non-pregnant Human Myometrium. Undergraduate Thesis, McMaster University.

Brown, C.J., Crankshaw, D.J., 1995. Effects of AH23848B on prostanoid-induced relaxation of human myometrium in vitro. Br. J. Pharmacol. 116, 359P.

Brown, C.J., Boersma, J.I., Crankshaw, D.J., 1997. Effects of AH6809 on prostanoid-induced relaxation of human myometrium in vitro. Br. J. Pharmacol. 120, 338P.

Cheuk, J.M.S., Hollingsworth, M., Hughes, S.J., Piper, I.T., Maresh, M.J.A., 1993. Inhibition of contractions of the isolated human myometrium by potassium channel openers. Am. J. Obstet. Gynecol. 168, 953–960.

Coleman, R.A., Humphrey, P.P.A., Sheldrick, R.L.G., White, B.P., 1988. Gastric anti-secretory prostanoids: actions at different prostanoid receptors. Br. J. Pharmacol. 95, 724P.

Coleman, R.A., Kennedy, I., Humphrey, P.P.A., Bunce, K., Lumley, P., 1990. Prostanoids and their receptors. In: Hansch, C., Lammes, P.G., Taylor, J.B. (Eds.), Membranes and Receptors, Pergamon, Oxford, pp. 643–714.

Coleman, R.A., Grix, S.P., Head, S.A., Louttit, J.B., Mallett, A., Sheldrick, R.L.G., 1994a. A novel inhibitory prostanoid receptor in piglet saphenous vein. Prostaglandins 47, 151–168.

Coleman, R.A., Smith, W.L., Narumiya, S., 1994b. International Union of Pharmacology classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes. Pharmacol. Rev. 46, 205–229.

Crankshaw, D.J., Dyal, R., 1993. Effects of some naturally occurring prostanoids and some cyclooxygenase inhibitors on the contractility of the human lower uterine segment in vitro. Can. J. Physiol. Pharmacol. 72, 870–874.

Curry, S.H., Brown, E.A., Kuck, H., Cassin, S., 1981. Preparation and stability of indomethacin solutions. Can. J. Physiol. Pharmacol. 60, 988–992.

De Vries, G.W., Guarino, A., McGlaughlin, A., Chen, J., Andrews, S., Woodward, D.F., 1995. An EP receptor with novel pharmacological profile in the T-cell line Jurkat. Br. J. Pharmacol. 115, 1231–1234.

Dong, Y.J., Jones, R.L., Wilson, N.H., 1986. Prostaglandin E receptor subtypes in smooth muscle: agonist activities of stable prostacyclin analogues. Br. J. Pharmacol. 87, 97–107.

Fernandes, B., Crankshaw, D., 1995. Functional characterization of the prostanoid DP receptor in human myometrium. Eur. J. Pharmacol. 283, 73–81.

Fleckenstein, A., Tritthart, H., Fleckenstein, B., Herbst, A., Grün, G., 1969. Eine neue gruppe kompetitiver Ca^{2+}-antagonisten (Ipoveratril, D 600, Prenylamin) mit starken hemmeffekten auf electromechanischer koppelung im warmblütter-myocard. Pflügers Arch. 307, R25.

Ford-Hutchinson, A.W., Girard, Y., Lord, A., Jones, T.R., Cirino, M., Evans, J.F., Gillard, J., Hamel, P., Leveille, C., Masson, P., Young, R., 1989. The pharmacology of L-670,596, a potent and selective thromboxane/prostaglandin endoperoxide receptor antagonist. Can. J. Physiol. Pharmacol. 67, 989–993.

Gardiner, P.J., 1986. Characterization of prostanoid relaxant/inhibitory receptors (ψ) using a highly selective agonist, TR4979. Br. J. Pharmacol. 87, 45–56.

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.

Keery, R.J., Lumley, P., 1988. AH6809, a prostaglandin DP receptor blocking drug on human platelets. Br. J. Pharmacol. 94, 745–754.

Kiriyama, M., Ushikubi, F., Kobayashi, T., Hirata, M., Sugimoto, Y., Narumiya, S., 1997. Ligand binding specificities of the eight types and subtypes of the mouse prostanoid receptors expressed in Chinese hamster ovary cells. Br. J. Pharmacol. 122, 217–224.

Kotani, M., Tanaka, I., Ogawa, Y., Usui, T., Mori, K., Ichikawa, A., Narumiya, S., Yoshimi, T., Nakao, K., 1995. Molecular cloning and expression of multiple isoforms of human prostaglandin E receptor EP_3 subtype generated by alternative messenger RNA splicing: multiple second messenger systems and tissue-specific distributions. Mol. Pharmacol. 48, 869–879.

Li, S.W., McKechnie, K., Griffiths, R.J., Boughton-Smith, N.K., Blackham, A., Leff, P., 1995. Prostanoid antagonist AH6809 antagonises PGE_2 inhibition of human neutrophil activation. Br. J. Pharmacol. 114, 82P.

Lydford, S.J., McKechnie, K., 1994. Characterization of the prostaglandin E_2-sensitive EP receptor in the rat isolated trachea. Br. J. Pharmacol. 112, 133–136.

Meja, K.K., Barnes, P.J., Giembycz, M.A., 1997. Characterization of the prostanoid receptor(s) on human blood monocytes at which prostaglandin E_2 inhibits lipopolysaccharide-induced tumour necrosis factor-α generation. Br. J. Pharmacol. 122, 149–157.

Milne, S.A., Armstrong, R.A., Woodward, D.F., 1995. Comparison of the EP receptor subtypes mediating relaxation of the rabbit jugular and pig saphenous veins. Prostaglandins 49, 225–237.

Mukhopadhyay, P., Geoghegan, T.E., Patil, R.V., Bhattacherjee, P., Paterson, C.A., 1997. Detection of EP_2, EP_4, and FP receptors in human ciliary epithelial and ciliary muscle cells. Biochem. Pharmacol. 53, 1249–1255.

Nials, A.T., Vardey, C.J., Denyer, L.H., Thomas, M., Sparrow, S.J., Shepherd, G.D., Coleman, R.A., 1993. AH13205, a selective prostanoid EP_2 receptor agonist. Cardiovasc. Drug Rev. 11, 165–179.

Regan, J.W., Bailey, T.J., Pepperl, D.J., Pierce, K.L., Bogardus, A.M., Donello, J.E., Fairbairn, C.E., Kedzie, K.M., Woodward, D.F., Gil, D.W., 1994. Cloning of a novel human prostaglandin receptor with characteristics of the pharmacologically defined EP_2 subtype. Mol. Pharmacol. 46, 213–220.

Senchyna, M., Crankshaw, D.J., 1995. Use of reverse transcription-polymerase reaction to identify prostanoid receptor mRNA in human myometrium. Br. J. Pharmacol. 116, 280P.

Senchyna, M., Crankshaw, D.J., 1996. Characterization of the prostanoid TP receptor population in human non-pregnant myometrium. J. Pharmacol. Exp. Ther. 279, 262–270.

Senior, J., Marshall, K., Sangha, R., Baxter, G.S., Clayton, J.K., 1991. In vitro characterization of prostanoid EP receptors in the non-pregnant human myometrium. Br. J. Pharmacol. 102, 747–753.

Senior, J., Sangha, R., Baxter, G.S., Marshall, K., Clayton, J.K., 1992. In vitro characterization of prostanoid FP-, DP-, IP- and TP-receptors in the non-pregnant human myometrium. Br. J. Pharmacol. 107, 215–221.

Toh, H., Ichikawa, A., Narumiya, S., 1995. Molecular evolution of receptors for eicosanoids. FEBS Lett. 361, 17–21.

Town, M.-H., Casals-Stenzel, J., Schillinger, E., 1983. Pharmacological and cardiovascular properties of a hydantoin derivative, BW245C, with high affinity and selectivity for PGD_2 receptors. Prostaglandins 25, 13–28.

Wainman, B.C., Burcea, I., Crankshaw, D.J., 1988. The effects of prostanoids on estrogen dominated rat myometrial longitudinal muscle. Biol. Reprod. 39, 221–228.

Wise, H., 1998. Activation of the prostaglandin EP_4 receptor subtype is highly coupled to inhibition of *N*-formyl-methionyl-leucyl-phenylalanine-stimulated rat neutrophil aggregation. Prostaglandins Leukotrienes Essent. Fatty Acids 58, 77–84.

Woodward, D.F., Pepperl, D.J., Burkey, T.H., Regan, J.W., 1995. 6-isoproxy-9-oxoxanthene-2-carboxylic acid (AH6809), a human EP_2 receptor antagonist. Biochem. Pharmacol. 50, 1731–1733.