ELSEVIER

European Journal of Pharmacology 283 (1995) 73–81

ejp

Functional characterization of the prostanoid DP receptor in human myometrium

Bernadette Fernandes [a], Denis Crankshaw [a,b,*]

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

Received 16 February 1995; revised 2 May 1995; accepted 9 May 1995

Abstract

Spontaneous contractile activity of strips of human myometrium obtained from non-pregnant donors at the time of hysterectomy was inhibited by the selective prostanoid DP receptor agonists BW 245C (5-(6-carboxyhexyl)-1-(3-cyclohexyl-3-hydroxypropyl)hydantoin) and ZK110841 ((5*Z*,13*E*)-(9*R*,11*R*,15*S*)-9β-chlor-15-cyclohexyl-11,15-dihydroxy-16,17,18,19,20-pentanor-5,13-prostadienoic acid) with pEC_{50} values of 8.4 and 7.3 respectively but prostaglandin D_2 produced a biphasic effect. In the presence of the TP receptor antagonist L670596 ((−)-6,8-difluoro-9-*p*-methylsulfonyl benzyl-1,2,3,4-tetrahydrocarbazol-1-yl-acetic acid), contractile activity induced by the FP receptor agonist, cloprostenol ([1*R*-[1α(*Z*),2β(1*E*,3*R**),3α,5α]]-7-[2-[4-(3-chlorophenoxy)-3-hydroxy-7-butenyl]-3,5-dihydroxycyclopentyl]-5-heptenoic acid), was inhibited by BW 245C ($pEC_{50} = 7.5$), ZK110841 ($pEC_{50} = 6.7$) and prostaglandin D_2 ($pEC_{50} = 6.3$). Under these conditions both prostaglandin J_2 and 9α,11β-prostaglandin F_2 were inhibitory partial agonists. All compounds were antagonized by the selective DP receptor antagonist BW A868C (3-benzyl-5-(6-carboxyhexyl)-1-(2-cyclohexyl-2-hydroxyethylamino)hydantoin), but the pK_B values were both concentration-dependent (pK_B versus BW 245C at 10 nM = 9.1, at 50 nM = 8.3) and agonist-dependent (pK_B at 10 nM versus BW 245C = 9.1, versus ZK110841 = 7.4). Both agonist and antagonist potencies support the existence of DP receptors in human myometrium. The concentration and agonist dependence of the action of BW A868C suggests that putative DP receptor agonists relax human myometrium by more than one mechanism. These observations may be explained by the existence of subtypes of DP receptor in human myometrium.

Keywords: Prostanoid DP receptor; Myometrium, human; Prostaglandin D_2; BW A868C; Smooth muscle

1. Introduction

Prostaglandin D_2 has been shown to produce a biphasic response consisting of excitation followed by relaxation of human myometrium from both pregnant and non-pregnant donors (Senior et al., 1992, 1993). The hydantoin derivative BW 245C (5-(6-carboxyhexyl)-1-(3-cyclohexyl-3-hydroxypropyl)hydantoin), which has been suggested to act selectively at the DP receptor (Town et al., 1983) potently inhibits human myometrial contractility (Sanger et al., 1982; Senior et al., 1992, 1993). Furthermore, the selective DP receptor antagonist BW A868C (3-benzyl-5-(6-carboxyhexyl)-1-(2-cyclohexyl-2-hydroxyethylamino)hydantoin) (Giles et al., 1989) antagonizes BW 245C and the inhibitory effects of prostaglandin D_2 on human myometrium (Senior et al., 1992,1993). These observations have been taken as providing conclusive evidence for the presence of DP receptors in human myometrium (Senior et al., 1992).

We have attempted to determine if the human myometrial DP receptor is pharmacologically identical to the DP receptor in human platelets (Giles et al., 1989, 1991; Ito et al., 1990a) and rabbit jugular vein (Giles et al., 1989, 1991). To do this we have studied the concentration-effect relationships for two other putative DP receptor agonists, ZK110841 ((5*Z*,13*E*)-(9*R*,11*R*,15*S*)-9β-chlor-15-cyclohexyl-11,15-dihydroxy-16,17,18,19,20-pentanor-5,13-prostadienoic acid) (Thierauch et al., 1988) and 9α,11β-prostaglandin F_2 (Giles et al., 1991) as well as the dehydration product of prostaglandin

* Corresponding author. Department of Obstetrics and Gynecology, McMaster University, 1200 Main St. West, Hamilton, Ontario, Canada L8N 3Z5. Tel. (+1)905-525-9140 ext. 22228, fax (+1)905-524-2911.

SSDI 0014-2999(95)00288-X

D_2, prostaglandin J_2 (Fukushima, 1992). We have examined the interactions between the antagonist BW A868C and the agonists prostaglandin D_2, BW 245C, ZK110841, 9α,11β-prostaglandin F_2 and prostaglandin J_2.

2. Materials and methods

2.1. Materials

Prostaglandin D_2, 9α,11β-prostaglandin F_2, prostaglandin J_2 and U46619 ([1*R*-[1α,4α,5β(*Z*),6α-(1*E*,3*S**)]]-7-[6-(3-hydroxy-1-octenyl)-2-oxabicyclo [2.2.1]hept-5-yl]-5-heptenoic acid), were purchased from Cayman Chemical (Ann Arbor, MI, USA). Cloprostenol ([1*R*-[1α(*Z*),2β(1*E*,3*R**),3α,5α]]-7-[2-[4-(3-chlorophenoxy)-3-hydroxy-7-butenyl]-3,5-di-hydroxycyclopentyl]-5-heptenoic acid) (Estrumate) was from Coopers Agropharm (Willowdale, Ontario, Canada), indomethacin (1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indole-3-acetic acid) and D600 ((±)-α-[3-[[2-(3,4-dimethoxyphenyl)ethyl]methylamino]-propyl]-3,4,5-tri-methoxy-α-(1-methylethyl)-benzeneacetonitrile hydrochloride) from Sigma (St. Louis, MO, USA). BW 245C and BW A868C were gifts from the Wellcome Research Laboratories (Beckenham, UK). ZK110841 was a gift from Schering (Berlin, Germany). L670596 ((−)-6,8-difluoro-9-*p*-methylsulfonyl benzyl-1,2,3,4-tetrahydrocarbazol-1-yl-acetic acid) was a gift from the Merck Frosst Centre for Therapeutic Research (Pointe Claire, Québec, Canada). All other chemicals were from BDH (Toronto, Ontario, Canada).

Prostaglandin D_2, 9α,11β-prostaglandin F_2, BW 245C, BW A868C and ZK110841 were dissolved in 70% ethanol and stock solutions were stored at −20°C. L670596 was dissolved in dimethyl sulphoxide, prostaglandin J_2 and U46619 in methyl acetate and cloprostenol in aqueous solution as supplied by the manufacturer. Serial dilutions of all drugs except indomethacin into physiological salt solution (PSS) were made freshly on the day of each experiment and were kept on ice throughout. Indomethacin was prepared as described by Curry et al. (1982).

2.2. Human myometrial strips

Pieces of human myometrium were obtained from non-pregnant women aged 29–56 years undergoing hysterectomy for benign disorders such as menorrhagia, dysmenorrhea and fibroids. Pieces were removed from the uterine fundus and placed in Hepes-buffered PSS for transport to the laboratory. The Hepes-buffered PSS had the following compostion: 4.6 mM KCl, 1.16 mM $MgSO_4$, 1.8 mM $CaCl_2$, 150 mM NaCl, 11.1 mM *d*-glucose, 5 mM Hepes, pH 7.4. On arrival at the laboratory the samples were washed in oxygenated (95% O_2, 5% CO_2) indomethacin-PSS of the following composition: 4.6 mM KCl, 1.16 mM $MgSO_4$, 1.16 mM NaH_2PO_4, 2.5 mM $CaCl_2$, 115.5 mM NaCl, 21.9 mM $NaHCO_3$, 11.1 mM *d*-glucose and 10 μM indomethacin. Endometrium, serosa and sections containing fibroids were dissected away. Up to 16 longitudinal strips (10 × 1.5 × 0.5 mm) were cut from the muscle layer adjacent to the serosa and in a direction parallel to that of the serosa.

2.3. Recording isometric contractions

Tissue strips were tied at each end with silk thread and mounted longitudinally in individual 10 or 15 ml jacketed muscle baths containing oxygenated indomethacin-PSS at 37°C. An initial resting force of 25 mN was applied to each strip and measured via a Grass FT-03 force displacement transducer writing either to a Grass 7D polygraph or a custom-made amplifier writing directly to the data collection software, In Vitro Collection System Ver. 4.0 (J. Milton, Dundas, Ontario, Canada), running on a personal computer. The mean force developed by the individual muscle strips was used as a measure of their contractility. This technique has been used routinely in this laboratory (Dyal and Crankshaw, 1988; Wainman et al., 1988; Crankshaw and Dyal, 1994) and others (Cheuk et al., 1993) since it can be used to successfully quantify drug effects in tissues that develop significant spontaneous activity and that respond to stimulation by changes in both tonic and phasic activity (Crankshaw, 1990). Mean force was determined as described by Wainman et al. (1988) using the In Vitro Collection System software.

Effect of drugs on spontaneous contractile activity

Tissue strips were allowed to equilibrate for 2 h during which time the resting tension was readjusted to 25 mN and spontaneous contractile activity usually developed. At the end of the equilibration period the mean force developed during a 10 min control period was determined. Drugs were then added to the baths, in a cumulative fashion, by increments that would produce approximately one-half log unit changes in the bath concentration. Each addition was immediately followed by a 10 min period during which the mean contractile force was determined. The mean force recorded in the 10 min period immediately following agonist addition, minus the mean control force, was considered to be the force developed in response to that concentration of agonist. Additions were continued until further addition produced no further change in mean force. When antagonists were used in such experiments, they were added to the baths 1 h before the 10 min control period and were present throughout the experiment.

Effect of drugs on cloprostenol-induced contractile activity

As reported by others (Senior et al., 1992, 1993) the spontaneous activity developed by strips of human myometrium from non-pregnant donors was variable. Spontaneous contractility of time-matched control strips often decayed to zero during the course of an experiment making quantification of inhibitory drug effects in these circumstances impossible. We therefore adopted the strategy employed by Cheuk et al. (1993) of assessing relaxant activity against drug-induced rather than spontaneous contractile activity.

After 1 h of equilibration tissues were challenged with 2 μM cloprostenol which remained in contact with the tissues for the remainder of the experiment. After a further 1 h 50 nM L670596 was added. When cloprostenol had been in contact with the tissue for 2 h a concentration-effect experiment, as described above, was begun. When BW A868C was used, it was added to the baths 1 h after cloprostenol challenge and remained in contact with the tissues for the remainder of the experiment.

Quantification of agonist responses

When relaxant effects were analysed two strips were used as time-matched controls to which no inhibitory agonist was added. At the end of the experiment the calcium-channel blocker D600 (Fleckenstein et al., 1969) was added to all baths to give a final concentration of 100 μM. After 15 min exposure to D600 a final data collection was performed. D600 reduced activity to basal tone and this was used to define zero contractility.

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:

$$E_\chi = \left((D_\chi - C_\chi)/-C_\chi\right) \times 100$$

where E_χ is the percentage inhibition produced by the drug at concentration χ, D_χ is the percentage of control period activity developed in the presence of concentration χ of the drug, and C_χ is the percentage of control period activity developed in the time-matched control tissues for the same time period. When stimulant effects were analysed, time-matched control tissues were not employed, and no corrections were made.

Concentration-effect curves (effect versus log molar agonist concentration) were constructed from the data obtained by fitting the equation:

$$E = E_{\min} + (E_{\max} - E_{\min})/1 + e^{-k(\log C - \log D)}$$

where E is the effect of the agonist, k is a power coefficient, C is the molar concentration of the agonist and D is the molar concentration of the agonist that produces a half-maximal response (EC_{50}). The value of $-\log D$ is equivalent to the pEC_{50}.

Normally the same agonist was tested on two muscle strips from the same donor and the average of the two pEC_{50} values obtained was used, thus n values represent the number of donors on which each drug was tested.

Quantification of antagonist effects

Only one concentration-effect experiment could be performed in each tissue strip. Therefore antagonist effects were investigated by incubating separate tissue strips from the same donor in the absence (control) or presence of various concentrations 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. Agonist EC_{50} values at each antagonist concentration were determined from pEC_{50} values calculated as described above. Antagonist pK_B values were then determined according to the equation:

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

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 some cases complete concentration-effect curves could not be obtained when the concentration of BW A868C was high, because of limited availability of agonist. In these circumstances pEC_{50} values were determined by extrapolation of the fitted curves.

2.4. Effect of cloprostenol on platelet aggregation

Blood was collected from healthy male and female volunteers who had not taken any drugs known to affect platelet function for 2 weeks prior to the experiment. The blood was collected into a 0.38% (w/v, final concentration) trisodium citrate solution. Citrated blood was centrifuged at $164 \times g$ for 20 min; the supernatant was platelet-rich plasma. All isolation steps were performed at room temperature and all glassware was treated with dimethyldichlorosilane.

Aggregation responses were measured in 1 ml aliquots of platelet-rich plasma maintained at 37°C and stirred at 800 rpm in a Payton Aggregation Module connected to a Linear 1200 pen recorder. Samples of platelet-rich plasma were incubated for 2 min, after which BW 245C alone or together with cloprostenol (final concentration of 2 μM) was added to the suspension and incubated for a further 2 min. Finally, U46619 at a final concentration of 10 μM was added. The response to U46619 alone was determined at intervals throughout the experiment. Concentration-effect curves for BW 245C on U46619-induced platelet aggregation

in the presence and absence of 2 μM cloprostenol were constructed from these data and fitted as described in Section 2.3.

2.5. Statistical analysis

All data are expressed as arithmetic means ± the standard deviation of the mean. The effect of L670596 on the response of human myometrium to BW 245C and the effect of cloprostenol on the response of human platelets to BW 245C were analysed by two-tailed paired *t*-test. All other comparisons used analysis of variance. Values of $P < 0.05$ were considered to indicate significant differences.

3. Results

3.1. Effect of prostaglandin D_2, BW 245C and ZK110841 on the spontaneous contractile activity of human myometrium

The concentration-effect relationship for prostaglandin D_2 was complex (Fig. 1), consisting of an initial excitatory component followed by an inhibitory component. There was considerable inter-donor variation in the concentration of prostaglandin D_2 that produced maximum excitation and in the extent of inhibition. Approaches to the analysis of complex concentration-effect curves such as that shown in Fig. 1 have been suggested (Szabadi, 1977; Pliška, 1994) but we were unable to make reliable estimates of EC_{50} values for the two components of the curve from our data. The TP receptor antagonist L670596 at 2.3 μM did not eliminate the excitatory response to prostaglandin D_2.

In contrast to prostaglandin D_2, both BW 245C and ZK110841 produced monophasic, inhibitory concentration-effect curves. These experiments yielded the following values: BW 245C $pEC_{50} = 8.4 \pm 0.7$ (3); ZK110841 $pEC_{50} = 7.3 \pm 0.4$ (5).

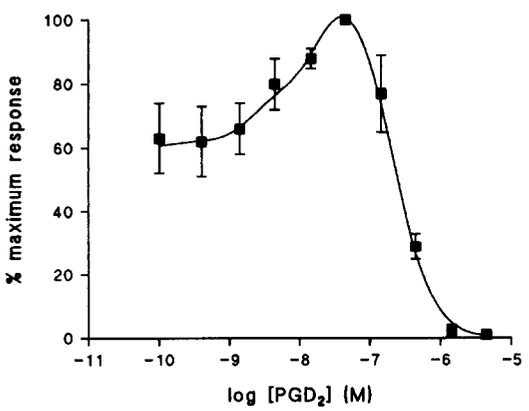

Fig. 1. Concentration-effect curve for prostaglandin D_2 on the spontaneous activity of human myometrium from a non-pregnant donor in vitro. The experiment was performed as described in the text. Points represent the means ± S.D. of results from four strips from the same donor. The line was fitted by eye.

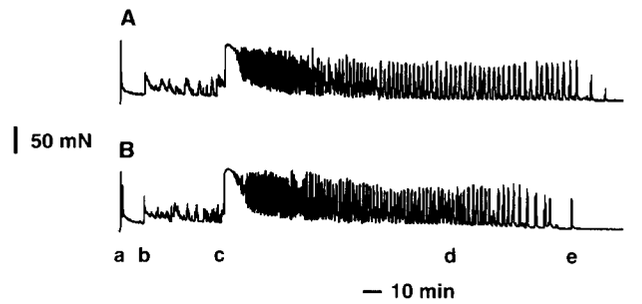

Fig. 2. Analogue trace of a complete experiment to determine the effect of BW 245C on cloprostenol-induced activity of human myometrium from a non-pregnant donor, (A) time-matched control tissue, (B) agonist-treated tissue. [a]Setting of initial resting tension. [b]Development of spontaneous contractility. [c]Addition of cloprostenol (2 μM). [d]Beginning of concentration-effect experiment proper. [e]Addition of D600 (100 μM).

3.2. Effect of DP receptor agonists on cloprostenol-induced activity of human myometrium

The analogue trace of a complete experiment on two strips of human myometrium from the same non-pregnant donor is shown in Fig. 2. One strip (Fig. 2A) served as a time-matched control, while the other strip was treated with BW 245C (Fig. 2B). Details of the concentration-effect portions of this experiment are shown in Fig. 3 and a graphical representation of the force developed is given in Fig. 4. The response to BW 245C was unaffected by the presence of 50 nM L670596 which was routinely included in subsequent concentra-

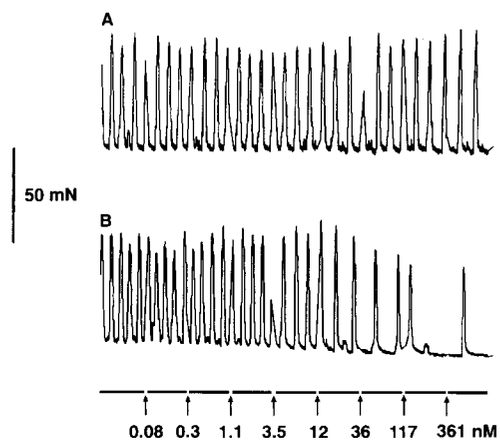

Fig. 3. Detail of Fig. 2 showing the effect of cumulative addition of BW 245C on cloprostenol-induced activity of human myometrium from a non-pregnant donor, (A) time-matched control tissue, (B) agonist-treated tissue. Horizontal bars indicate 10 min data collection periods, the first of which is the control. Thereafter BW 245C was added at the points indicated by the arrows to give the cumulative concentrations shown.

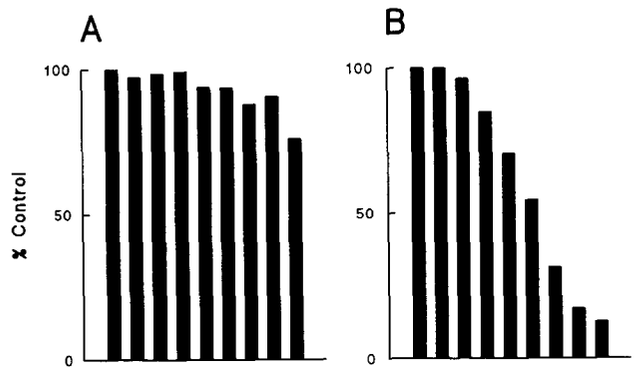

Fig. 4. The effect of cumulative addition of BW 245C on the mean contractile activity of cloprostenol-stimulated human myometrium from a non-pregnant donor, (A) time-matched control tissue, (B) agonist-treated tissue. Each bar represents the mean activity recorded during a 10 min period. First bar, activity during the control period, subsequent bars activity recorded immediately after drug addition. Cumulative concentrations were as shown in Fig. 3. The effect of the agonist was corrected for changes in the time-matched control tissue as described in the text.

tion-effect experiments to prevent TP receptor-mediated effects.

Concentration-effect curves to five putative DP receptor agonists, obtained from an experiment on myometrial strips from the same donor, are shown in Fig. 5. A summary of data obtained from all such experiments is given in Table 1.

3.3. Effect of BW A868C on responses to DP receptor agonists

The concentration-effect curve to BW 245C was displaced to the right by 10 nM and 50 nM concentrations of BW A868C (Fig. 6). Table 2 gives a summary of the effects of these two concentrations of the antagonist on responses to all the DP receptor agonists studied.

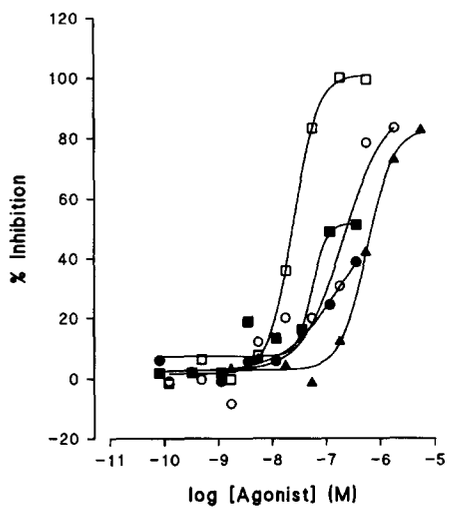

Fig. 5. Concentration-effect curves for putative DP receptor agonists on cloprostenol-stimulated activity of human myometrium from a non-pregnant donor in vitro. (□) BW 245C, (■) 9α,11β-prostaglandin F_2, (○) ZK110841, (●) prostaglandin J_2, (▲) prostaglandin D_2.

Table 1
Inhibitory effects of putative DP receptor agonists on cloprostenol-induced activity of human non-pregnant myometrium in vitro

Agonist	pEC_{50}		% Maximum inhibition
BW 245C	7.5±0.3	(5)	100
ZK110841	6.7±0.6	(8)	100
Prostaglandin D_2	6.3±0.3	(5)	90±10
Prostaglandin J_2	7.4±0.2	(4)	40±20
9α,11β-Prostaglandin F_2	7.0±0.6	(5)	40±20

Values are means±S.D. of (n) independent experiments. Maximum inhibition was that produced by 100 μM D600.

Table 2
The effect of BW A868C on the inhibition of cloprostenol-stimulated activity of human non-pregnant myometrium in vitro by putative DP receptor agonists

Agonist	pK_B BW A868C 10 nM		50 nM	
BW 245C	9.1±0.3[a]	(5)	8.3±0.3[b]	(3)
ZK110841	7.4±0.4[c]	(3)	7.3[c]	(2)
Prostaglandin D_2	7.4±0.7[c]	(3)	7.7±0.2[c]	(5)
Prostaglandin J_2	8.6±0.4[b]	(3)	Could not be determined	(4)
9α,11β-Prostaglandin F_2	Could not be determined	(4)	Could not be determined	(4)

pK_B values were determined as described in Materials and methods. Values are means±S.D. of (n) independent experiments. Values with different superscripts are significantly different ($P < 0.05$, Student Newman-Keuls test). Where values could not be determined BW A868C produced variable results; the most common effect was an unmasking of excitatory effects of the agonist that precluded determination of inhibitory parameters.

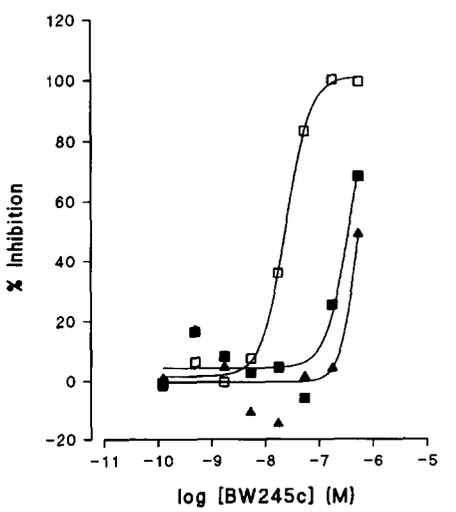

Fig. 6. Effect of BW A868C on the response of cloprostenol-stimulated human myometrium from a non-pregnant donor to BW 245C in vitro. (□) control, (■) 10 nM BW A868C, (▲) 50 nM BW A868C.

3.4. Effect of cloprostenol on the inhibition of platelet aggregation by BW 245C

Cloprostenol (2 μM) had no effect upon BW 245C-mediated inhibition of U46619-induced aggregation in human platelet-rich plasma. The pEC_{50} for BW 245C was 8.2 ± 0.5 in the presence and 8.5 ± 0.5 in the absence of cloprostenol ($n = 3$).

4. Discussion

When strips of human myometrium from both pregnant and non-pregnant donors were superfused with a solution containing prostaglandin D_2, a biphasic response consisting of excitation followed by relaxation of spontaneous activity was observed (Senior et al., 1992, 1993). In contrast, a monophasic, excitatory concentration-effect relationship was found for prostaglandin D_2; when equilibrium studies employing an essentially identical technique to the one used in this study were performed on pregnant human myometrium, the pEC_{50} was 6.1 (Crankshaw and Dyal, 1994). In the present study the concentration-effect relationship for prostaglandin D_2 was clearly biphasic and both components were concentration-dependent (Fig. 1), but we were unable to determine location parameters for the two components of the curve. Very similar effects were found in a washed human platelet suspension (Giles et al., 1989). Prostaglandin D_2 has significant potency at TP receptors ($pEC_{50} = 6.2$ in guinea pig aorta, Giles et al., 1991), but the failure of a high concentration of the potent TP receptor antagonist L670596 (Ford-Hutchinson et al., 1989) to abolish the excitatory responses to prostaglandin D_2 in human myometrium suggests that TP receptors do not play a major role in this process. Other likely sites for prostaglandin D_2's excitatory actions are the FP and EP_3 receptors (Senior et al., 1992). Prostaglandin D_2 appears to be a highly potent FP receptor agonist (Coleman et al., 1990) and in agreement with this prostaglandin D_2 has only a 2.5-fold lower affinity for the recombinant human FP receptor than does prostaglandin $F_{2\alpha}$ (Abramovitz et al., 1994) whereas its affinity for the recombinant human EP_3 receptor is from 500-to 2200-fold less than that of prostaglandin E_2 (Adam et al., 1994), so action of prostaglandin D_2 at FP receptors is most likely in this case. Unfortunately, no selective antagonists for FP or EP_3 receptors are available, so we were unable to pharmacologically isolate the inhibitory response to prostaglandin D_2 in order to study it in spontaneously active tissues.

BW 245C has been previously shown to potently inhibit human myometrial contractility (Sanger et al., 1982; Senior et al., 1992, 1993) but this is the first report of an equilibrium study from which a pEC_{50} has been determined. In contrast, effects of ZK110841 on human myometrium have not been previously reported; its inhibitory activity taken together with evidence supporting an action at DP receptors (Schulz et al., 1990) add further support to the claim that DP receptors are present in human myometrium from non-pregnant donors (Senior et al., 1992). Our results from spontaneously active tissue indicate that BW 245C is approximately 10-fold more potent than ZK110841. We are unaware of any other functional study in smooth muscle in which the potencies of the two compounds have been directly compared.

Minimal requirements for the functional definition of a receptor must include the determination of antagonist affinities, establishment of a potency ratio for agonists and estimation of the relative intrinsic efficacies of agonists (Kenakin et al., 1992). In order to attempt to fulfill these requirements we needed to develop a system in which the inhibitory actions of a range of putative DP receptor agonists could be quantified. The use of cloprostenol-stimulated tissues allowed us to do this since our results from study with human platelets suggest that cloprostenol has no significant activity at the DP receptor. The most plausible explanation for the conversion of the prostaglandin D_2 response from biphasic in spontaneously contracting tissues to monophasic inhibitory in cloprostenol-stimulated tissues is that prostaglandin D_2's excitatory effects are mediated via the FP receptor and the concentration of cloprostenol that we used is sufficient to prevent any action of prostaglandin D_2 at this site. The potency ratios (EC_{50} drug/EC_{50} BW 245C) for full agonists on cloprostenol-stimulated activity were 1:6:16 (BW 245C : ZK110841 : prostaglandin D_2). The potency ratio BW 245C : prostaglandin D_2 was between 1:8 and 1:1 when the drugs were tested for their ability to inhibit ADP-induced platelet aggregation in plasma from several species (Whittle et al., 1983; Narumiya and Toda, 1985) and was 1:15 and 1:3 for cyclic AMP elevation in rat mast cells and relaxation of rabbit stomach strip respectively (Narumiya and Toda, 1985). The same ratio was 1:79 for stimulation of human platelet adenylyl cyclase (Trist et al., 1989). Prostaglandin D_2 and ZK110841 were equipotent in stimulating adenylyl cyclase in bovine embryonic tracheal fibroblasts (Ito et al., 1990b) and in inhibiting ADP-induced aggregation of human platelets (Schulz et al., 1990).

In both human platelet and rabbit jugular vein $9\alpha,11\beta$-prostaglandin F_2 was a full agonist of DP receptor-mediated responses, with a potency 30-to 60-fold less than that of prostaglandin D_2 (Giles et al., 1991). In comparison to prostaglandin D_2, $9\alpha,11\beta$-prostaglandin F_2 was a weak ocular hypotensive which, unlike the parent compound, did not cause conjunctival in-

flammation in rabbits (Woodward et al., 1990). In our study 9α,11β-prostaglandin F_2 was a partial agonist. Prostaglandin J_2 was also a partial agonist in our study whereas this compound mimicked the effects of prostaglandin D_2 on short-circuit current of canine colonic epithelium, though it was much less effective than the parent prostanoid (Rangachari and Betti, 1993). Prostaglandin J_2 had no significant effect on intraocular pressure, nor did it elicit a microvascular permeability response in the conjunctiva of rabbits (Woodward et al., 1990).

Whilst they give some general useful information concerning the nature of the prostanoid-receptive systems in human myometrium, the agonist potency ratios that we have determined are probably unreliable for receptor classification purposes since we cannot, through lack of appropriate tools, rule out action of any of these compounds at receptors other than the one of interest. Our conclusion must therefore be limited to one of general agreement between the potency ratios we obtained and those in the literature. The shift of 9α,11β-prostaglandin F_2 from full to partial agonism (Giles et al., 1991 compared to this study) may simply be explained by different receptor numbers in the two systems.

BW A868C had a pA_2 of 8.3 against BW 245C when tested over a concentration range of 10 nM to 1 μM on human myometrium from non-pregnant donors (Senior et al., 1992). This value is identical to the pK_B we determined at 50 nM BW A868C, but different from our pK_B at 10 nM. Our results suggesting a non-linearity in the interaction between BW A868C and BW 245C in non-pregnant human myometrium may in fact be consistent with those of Senior et al. (1992) who reported a Schild plot slope of 0.7. These same investigators observed a clear non-linearity in the interaction between BW A868C and prostaglandin D_2; at 10 nM BW A868C had no effect on prostaglandin D_2-mediated inhibition whereas at 100 nM the response was abolished. We did not observe this phenomenon with the concentrations and conditions that we used. Unlike in myometrial contractions, the interaction between BW A868C and BW 245C in washed human platelet aggregation produced a Schild plot with unit slope (Giles et al., 1989) and the pK_B of 9.26 agrees well with what we found at 10 nM BW A868C, as does the pK_B of 9.1 determined on human platelet adenylyl cyclase (Trist et al., 1989). However, there were two phases to the agonism of both prostaglandin D_2 and BW 245C on platelet adenylyl cyclase, one that was and one that was not sensitive to BW A868C (Trist et al., 1989). Similarly there were complexities in the interactions between BW A868C and both BW 245C and prostaglandin D_2 in rabbit jugular vein, resulting in curvilinear Schild plots (Giles et al., 1989). These latter data were consistent with a model in which each agonist acts at two receptors, only one of which is sensitive to the antagonist. However, more recently BW A868C has been shown to have significant but weak antagonistic effects at EP_4 receptors in rabbit saphenous vein (Lydford et al., 1994). This observation suggests an alternative model of the agonist acting at two receptors for which the antagonist has different but significant affinity. Unfortunately, the experimental design that we had to use to accurately quantify agonist effects does not allow us to perform the experiments necessary to differentiate between the two models in human myometrium with the tools available. Certainly our results with BW 245C suggest a one-agonist, two-receptor model. There is currently no evidence concerning the presence of EP_4 receptors in human myometrium, but the recent description of selective EP_4 receptor antagonists (Coleman et al., 1994) would make the hypothesis that some DP receptor agonists also relax human myometrium by an EP_4 receptor-mediated mechanism easily testable.

The fact that under the conditions that we used the pK_B values determined using prostaglandin D_2 and ZK110841 were neither dependent upon the concentration of BW A868C nor equivalent to either of the pK_B values obtained using BW 245C suggests that these two compounds act by mechanisms different from that of BW 245C. For prostaglandin D_2 this is consistent with the observations that rabbit intraocular pressure was reduced by both prostaglandin D_2 and BW 245C and both were antagonized by BW A868C whereas prostaglandin D_2 but not BW 245C increased conjunctival microvascular permeability and was antagonized by BW A868C (Woodward et al., 1993). That prostaglandin D_2 and ZK110841 act differently from BW 245C contradicts all other previous evidence concerning the mechanism of action of these two compounds (Giles et al., 1991; Schulz et al., 1990) but might be explained if the intrinsic efficacies of both prostaglandin D_2 and ZK110841 at the DP receptor are significantly lower than that of BW 245C. In a situation where DP receptors are low, the effects of the two compounds on this system would be negligible and therefore a pK_B for the antagonist at the DP receptor would not be obtainable. The pK_B obtained under such circumstances would represent an interaction of both agonists with the antagonist at another site or sites. Such a possibility might be predicted for prostaglandin D_2 (Giles et al., 1989; Woodward et al., 1993; Lydford et al., 1994) but not for ZK110841 (Schulz et al., 1990). Whatever this site or sites might be, it is unlikely to be solely the EP_4 receptor since the pA_2 for BW A868C at this site is 4.9 (Lydford et al., 1994). An alternative explanation compatible with our observations is that there are two subtypes of DP receptor in human myometrium for which BW A868C has different affinities with BW 245C being selective for the high

affinity subtype and ZK110841 being selective for the lower affinity subtype.

Our previous caution against overinterpretation of agonist potency ratios is substantiated by these data and by our observation that BW A868C converted the effects of prostaglandin J_2 and $9\alpha,11\beta$-prostaglandin F_2 from inhibitory to excitatory.

In conclusion, we have presented evidence for an inhibitory receptor in non-pregnant human myometrium at which BW 245C is a potent agonist that is antagonized by BW A868C with a pK_B of 9.1. These are criteria for the classical DP receptor (Giles et al., 1989; Trist et al., 1989) and therefore support the hypothesis that DP receptors are present in human myometrium (Senior et al., 1992). Further support is lent to this hypothesis by the inhibitory effects of other putative DP receptor agonists. However, the dependence of the pK_B value for BW A868C on both the concentration of antagonist and on the agonist used suggests that most putative DP receptor agonists also relax human myometrium by action at a site or sites that are distinct from the classical DP receptor. The question of the existence of more than one BW A868C-sensitive DP receptor subtype (Woodward et al., 1993) remains open.

Acknowledgements

We are grateful to Dr. Karl-Heinz Thierauch for introducing us to the DP receptor, to Dr. Heather Giles for many helpful suggestions during the course of this work and to Merck Frosst, Schering and Wellcome for supplying compounds. This work was supported by MRC Canada.

References

Abramovitz, M., Y. Boie, T. Nguyen, T.H. Rushmore, M.A. Bayne, K.M. Metters, D.M. Slipetz and R. Grygorczyk, 1994, Cloning and expression of a cDNA for the human prostanoid FP receptor, J. Biol. Chem. 269, 2632.

Adam, M., Y. Boie, T.H. Rushmore, G. Müller, L. Bastien, K.T. McKee, K.M. Metters and M. Abramovitz, 1994, Cloning and expression of three isoforms of the human EP_3 prostanoid receptor, FEBS Lett. 338, 170.

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

Coleman, R.A., I. Kennedy, P.P.A. Humphrey, K. Bunce and P. Lumley, 1990, Prostanoids and their receptors, in: Comprehensive Medicinal Chemistry: The Rational Design, Mechanistic Study and Therapeutic Application of Chemical Compounds. eds. C. Hansch, P.G. Lammes and J.B. Taylor (Pergamon Press, Oxford) p. 643.

Coleman, R.A., S.P. Grix, S.A. Head, J.B. Louttit, A. Mallett and R.L.G. Sheldrick, 1994, EP_4-receptors and cyclic AMP in pig venous smooth muscle: evidence with agonists and the EP_4-antagonist, AH22921, in: 9th International Conference on Prostaglandins and Related Compounds, Florence, p. 27.

Crankshaw, D.J., 1990, Myometrial contractility in vitro: its role in an understanding of the mechanisms controlling uterine activity, in: Uterine Contractility Mechanisms of Control, ed. R.E. Garfield (Serono Symposia, Norwell, MA) p. 85.

Crankshaw, D.J. and R. Dyal, 1994, 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.

Curry, S.H., E.A. Brown, H. Kruck and S. Cassin, 1982, Preparation and stability of indomethacin solutions, Can. J. Physiol. Pharmacol. 60, 988.

Dyal, R. and D.J. Crankshaw, 1988, The effects of some synthetic prostanoids on the contractility of the human lower uterine segment in vitro, Am. J. Obstet. Gynecol. 58, 281.

Fleckenstein, A., H. Tritthart, B. Fleckenstein, A. Herbst and G. Grün, 1969, Eine neue Gruppe kompetitiver Ca^{++}-Antagonisten (Iproveratril, D 600, Prenylamin) mit starken Hemmeffekten auf die elektromechanische Koppelung im Warmblüter-Myokard, 35, Pflüg. Arch. Ges. Physiol. 307, R25.

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

Fukushima, M., 1992, Biological activities and mechanisms of action of PGJ_2 and related compounds: an update, Prostaglandins Leukotrienes Essent. Fatty Acids 47, 1.

Giles, H., P. Leff, M.L. Bolofo, M.G. Kelly and A.D. Robertson, 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.

Giles, H., M.L. Bolofo, S.J. Lydford and G.R. Martin, 1991, A comparative study of the prostanoid receptor profile of $9\alpha11\beta$-prostaglandin F_2 and prostaglandin D_2, Br. J. Pharmacol. 104, 541.

Ito, S., M. Negishi, K. Sugama, E. Okuda-Ashitaka and O. Hayaishi, 1990a, Signal transduction coupled to prostaglandin D_2, Adv. Prostaglandin Thromboxane Leukotriene Res. 21, 371.

Ito, S., E. Okuda, K. Sugama, M. Negishi and O. Hayaishi, 1990b, Evaluation of ZK110841 and AH6809, an agonist and an antagonist of prostaglandin DP-receptors on human platelets, with a PGD_2-responsive cell line from bovine embryonic trachea, Br. J. Pharmacol. 99, 13.

Kenakin, T.P., R.A. Bond and T.I. Bonner, 1992, Definition of pharmacological receptors, Pharmacol. Rev. 44, 351.

Lydford, S.J., K.C.W. McKechnie and P. Leff, 1994, Interaction of BW A868C with two receptor-subtypes in the rabbit saphenous vein, in: 9th International Conference on Prostaglandins and Related Compounds, Florence, p. 85.

Narumiya, S. and N. Toda, 1985, Different responsiveness of prostaglandin D_2-sensitive systems to prostaglandin D_2 and its analogues, Br. J. Pharmacol. 85, 367.

Pliška, V., 1994, Models to explain dose-response relationships that exhibit a downturn phase, Trends Pharmacol. Sci. 15, 178.

Rangachari, P.K. and P.-A. Betti, 1993, Biological activity of metabolites of PGD_2 on canine proximal colon, Am. J. Physiol. 264, G886.

Sanger, G.J., A. Jackson and A. Bennett, 1982, A prostaglandin analogue which potently relaxes human uterus but not gut muscle, Eur. J. Pharmacol. 81, 141.

Schulz, B.G., R. Beckmann, B. Müller, G. Schröder, B. Maass, K.-H. Thierauch, B. Buchmann, P.F.J. Verhallen and W. Fröhlich, 1990, Cardio-and hemodynamic profile of selective PGD_2-ana-

logues, Adv. Prostaglandin Thromboxane Leukotriene Res. 21, 591.

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

Senior, J., K. Marshall, R. Sangha and J.K. Clayton, 1993, In vitro characterization of prostanoid receptors on human myometrium at term pregnancy, Br. J. Pharmacol. 108, 501.

Szabadi, E., 1977, A model of two functionally antagonistic receptor populations activated by the same agonist, J. Theor. Biol. 69, 101.

Thierauch, K.-H., C. Sturzebecher and E. Schillinger, 1988, Stable 9β-or 11α-halogen-15-cyclohexyl-prostaglandins with high affinity to the PGD_2-receptor, Prostaglandins 35, 855.

Town, M.-H., J. Casals-Stenzel and E. Schillinger, 1983, Pharmacological and cardiovascular properties of a hydantoin derivative, BW 245 C, with high affinity and selectivity for PGD_2 receptors, Prostaglandins 25, 13.

Trist, D.G., B.A. Collins, J. Wood, M.G. Kelly and A.D. Robertson, 1989, The antagonism by BW A868C of PGD_2 and BW245C activation of human platelet adenylate cyclase, Br. J. Pharmacol. 96, 301.

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

Whittle, B.J.R., S. Moncada, K. Mullane and J.R. Vane, 1983, Platelet and cardiovascular activity of the hydantoin BW245C, a potent prostaglandin analogue, Prostaglandins 25, 205.

Woodward, D.F., S.B. Hawley, L.S. Williams, T.R. Ralston, C.E. Protzman, C.S. Spada and A.L. Nieves, 1990, Studies on the ocular pharmacology of prostaglandin D_2, Invest. Ophthalmol. Vis. Sci. 31, 138.

Woodward, D.F., C.S. Spada, S.B. Hawley, L.S. Williams, C.E. Protzman and A.L. Nieves, 1993, Further studies on ocular responses to DP receptor stimulation, Eur. J. Pharmacol. 230, 327.