In vitro characterization of prostanoid FP-, DP-, IP- and TP-receptors on the non-pregnant human myometrium

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1 Prostaglandin F (PGF), PGD, PGI and thromboxane A₂ (TXA₂) receptors have been pharmacologically characterized on the non-pregnant human myometrium *in vitro* in accordance with the receptor classification proposed by Coleman *et al.* (1984). The tools for the classification include both natural prostanoids, synthetic, selective analogues and antagonists where available.

2 The potent excitatory actions of the natural FP-receptor prostanoid, PGF_{2α}, and the synthetic analogue, fluprostenol, indicate the presence of FP-receptors mediating contraction on the human myometrium.

3 PGD₂ produced a biphasic response consisting of excitation followed by relaxation of spontaneous activity of the myometrium. The selective DP-receptor agonists, BW245C, produced purely inhibitory responses illustrating the presence of inhibitory DP-receptors in this tissue. The inhibitory responses of both PGD₂ and BW245C were antagonized by the competitive DP-receptor antagonist, BWA 868C, providing conclusive evidence for the existence of DP-receptors.

4 PGI₂ produced a biphasic response similar to PGD₂. Iloprost, the EP₁/IP-receptor agonist also produced a biphasic response, whilst the IP-receptor selective agonist, cicaprost, caused inhibition only, suggesting that inhibitory IP-receptors exist in the non-pregnant human myometrium.

5 The TXA₂-mimetic, U46619, produced marked stimulation of the non-pregnant human myometrium and was approximately equipotent to PGF_{2α} and fluprostenol in this effect. The actions of U46619 were competitively antagonized by the TP-receptor antagonist GR32191 showing that excitatory TP-receptors exist in this tissue.

6 All prostanoids tested, both natural and synthetic, had activity on the non-pregnant human myometrium *in vitro*, supporting the existence of a heterogeneous population of prostanoid receptors in this tissue. If the results from the present study are combined with those previously reported for EP-receptor agonists (Senior *et al.*, 1991), it may be concluded that excitation may occur through FP-, TP-, EP₃- and few EP₁-receptors, whereas inhibition may occur through DP-, IP- and EP₂-receptors.

Keywords: Human myometrium; non-pregnant; prostanoids; synthetic analogues; antagonists; prostanoid receptors

Introduction

Initial evidence for the existence of myometrial prostanoid receptors dates back to 1967, when Pickles proposed that up to four different types existed. Since then, extensive work has led to a systematic prostanoid receptor classification. The present prostanoid receptor classification is a simple systematic working hypothesis whereby each of the natural prostanoids has its own receptor termed a P receptor where it is at least ten times more potent than any of the other natural prostanoids. Thus the prostaglandin E₂ (PGE₂)-sensitive receptors are termed the EP-receptors, PGF_{2α}, FP-receptors, PGD₂, DP-receptors, PGI₂, IP receptors and the thromboxane A₂ (TXA₂)-sensitive receptors, TP-receptors (Kennedy *et al.*, 1982; Coleman *et al.*, 1984). Furthermore, EP-receptors are subdivided into the EP₁-, EP₂- and EP₃-receptor subtypes on the basis of selectively acting agonists and antagonists (Coleman *et al.*, 1987a,b,c).

The existence of high and low affinity PGE₂ and PGF binding sites has been demonstrated on human myometrium (Wakeling & Wyngarden, 1974; Bauknech *et al.*, 1981; Giannopoulos *et al.*, 1985; Crankshaw *et al.*, 1986; Chegini *et al.*, 1986; Adelantado *et al.*, 1988), and the binding of these prostanoids is considered an essential step in the mechanism

of their action. Some workers have investigated the occurrence of binding sites at different stages of the menstrual cycle, and found no change in PGE and PGF binding (Hofmann *et al.*, 1983; Adelantado *et al.*, 1988). Studies employing natural agonists are limited because of the low specificity of such agents. The use of [³H]-sulprostone, which is a synthetic receptor-selective prostanoid, has demonstrated the presence of EP₁- and or EP₃-binding sites on human myometrium (Schillinger *et al.*, 1979).

However, there have been few other such binding studies, probably due to the lack of labelled selective ligands, and the actions of specific prostanoid analogues have been tested mostly by functional studies on human isolated myometrial preparations in order to evaluate the occurrence of receptors. By use of selective prostanoid analogues, it has been possible to demonstrate the presence of EP₁-, EP₂- and EP₃-receptor sites (Senior *et al.*, 1991).

Preliminary work with U46619 and fluprostenol has demonstrated TP- and FP-receptor sites respectively (Clayton *et al.*, 1986a,b). BW245C has been shown to relax human uterus (Sanger *et al.*, 1982); and cicaprost indicates relaxant responses in human myometrium (Dyal & Crankshaw, 1988).

The aim of the present study was to examine the effects of both natural and synthetic prostanoids and their antagonists, where available, by functional studies in order to characterize further the prostanoid receptors, other than the EP receptors, on the human myometrium *in vitro* from non-pregnant donors.

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Methods

Human myometrial samples were obtained from premenopausal non-pregnant women undergoing hysterectomy for benign disorders such as fibroids and dysfunctional menorrhagia. The specimens were taken from the anterior wall of the corpus uteri and then immediately placed in Krebs solution. Myometrial strips were set up within 60 min post-operative period and superfused with oxygenated Krebs solution (95% O₂/5% CO₂) at 37°C as previously described (Senior *et al.*, 1991). Agonists were administered as bolus doses immediately after a spontaneous contraction. It is accepted that this form of drug administration may be influenced by physiochemical and pharmacokinetic factors, and equilibrium studies are currently in progress in this laboratory. The reproducibility of the myometrial response to prostanoids has been assessed by means of time studies (Sangha, 1991), the results of which indicated that time was not a variable affecting tissue sensitivity to the agonist response.

The inherent myometrial activity is variable, which makes quantification of results difficult. Because of this, measurement of response and analysis of results were undertaken as detailed in the previous paper on EP receptors (Senior *et al.*, 1991). Briefly, excitatory responses were expressed as T/B ratios, i.e. ratio of the area of the excitatory response produced by a dose of agonist to the area of the previous spontaneous background contraction. Potency for excitatory agonists was expressed as an ED₁ value, i.e. the dose of agonist required to produce a T/B ratio equal to 1. The inhibitory actions of agonists were expressed in terms of how they modified the periodicity of spontaneous activity, measured in min. A measure of the potency of an agonist producing inhibition was obtained by calculating an inhibitory dose ID₄ value which was defined as the dose of agonist required to extend the normal period between two spontaneous background contractions by a period of 4 min.

Sample traces showing excitatory responses to PGF_{2α} and biphasic (excitatory followed by inhibitory) responses to PGD₂ are shown in Figure 1. The compounds used are shown in Table 1. ED₁ and ID₄ values were obtained from

individual dose-effect curves and expressed as geometric means with 95% confidence limits. Where antagonists were used, potency was expressed as the pA₂ value as determined by the method of Arunlakshana & Schild (1959). Statistical comparisons were made by an unpaired Student's *t* test (Snedecor & Cochran, 1979).

Figure 1 Sample traces showing the effect of a dose of (a) prostaglandin F_{2α} (PGF_{2α}, 1 × 10⁻⁹ mol) and (b) PGD₂ (3 × 10⁻⁸ mol) (at arrows) on the myogenic activity of human myometrium from non-pregnant donors.

Table 1 Compounds used in this study

Compound	Stock vehicle	Dilutions	Source
PDG ₂	1% NaHCO ₃ in 0.9% normal saline	0.9% normal saline	Glaxo
PGE ₂	1% NaHCO ₃ in 0.9% normal saline	0.9% normal saline	Upjohn
Dinoprostone	1% NaHCO ₃ in 0.9% normal saline	0.9% normal saline	Upjohn
PGF _{2α} tromethamine salt, Lutalyse	1% NaHCO ₃ in 0.9% normal saline	0.9% normal saline	Upjohn
PGI ₂ Prostacyclin	Tris buffer pH 9 at 37°C	Tris buffer pH 8	Sigma
U46619 11α 9α-epoxymethano PGH ₂	1% NaHCO ₃ in 0.9% normal saline	0.9% normal saline	Bayer
BW245C 5-(6-carboxyhexyl)-1 (3-cyclohexyl-2-hydroxypropyl)-hydantoin	Ethanol	0.9% normal saline	Wellcome
ICI81008 m-trifluoromethyl-16-phenoxy 17, 18, 19 20-tetranor-PGF _{2α} Fluprostenol	0.9% normal saline	0.9% normal saline	ICI
Iloprost ZK 36 374 16 methyl-18-yn carbacyclin	0.9% normal saline	0.9% normal saline	Schering
Cicaprost ZK 96 480 ([1S, 5S, 6R, 7R]-3-(5-carboxy-3-Oxa-1E-penty-1 dieno)-7-hydroxy-6-(35-hydroxy-4S-methyl-nona-1,6-dinyl)- bi cyclo [3.3.0] octane)	0.9% normal saline	0.9% normal saline	Schering
BW A868C3-benoxyl-5-(6-carboxy hexyl)-1-(2-cyclohexyl-2-hydroxyethylamino) hydantoin	Ethanol	Distilled water	Wellcome
GR32191 ([1R-[1α (Z) 2β, 3β, 5α)]-(+)-7-5-([1, 1-biphenyl)-4-yl] methoxy)-3-hydroxy-2-(1-piperidinyl) cyclopentyl]-4-heptanoic acid hydrochloride	Distilled water	Distilled water	Glaxo
Indomethacin (1-[p-chlorobenzoyl]-5-methoxy-2-methylindole-3-acetic acid	1% NaHCO ₃ in 0.9% normal saline		Sigma

Results

The effect of FP receptor agonists

PGF_{2α} produced only an excitatory response starting at 0.014 nmol, and this increased to a maximum around 1.4 nmol. The response then declined after 14 nmol, giving the dose-response curve to PGF_{2α} a bell-shaped appearance (Figure 2). The response to PGF_{2α} was rapid and consisted of an increase in baseline tonus with frequent phasic contractions superimposed on this effect. The potency of PGF_{2α} was similar to that previously reported for PGE₂ (ED₁, 0.04 and 0.02 nmol respectively) and the maximum effects observed with both agonists were also similar (Table 2). The synthetic FP-receptor agonist, fluprostenol (ICI 81008) produced a response similar to PGF_{2α}, the excitation was dose-related between 0.011–0.11 nmol, after which a further increase in dose produced little change in effect (Figure 2).

The effect of DP-receptor agonists and antagonists

PGD₂ produced a biphasic response similar to that seen with PGE₂. However, the former was less potent for both excitation and inhibition estimated by ED₁ and ID₄ values (Table 2). The excitatory component was dose-related between 0.28–14.0 nmol, whereas the inhibitory component was dose-related in the higher range of 1.4–140 nmol as shown in Figure 3a. The selective DP-receptor agonist BW245C produced only inhibition which was dose-dependent between 0.014–14 nmol (Figure 3b). Consideration of ID₄ values showed BW245C to be the most potent inhibitory agonist listed in this series of compounds (Table 2). However, it should be noted that a greater maximum effect is produced by the EP₂-receptor agonist, butaprost (Senior *et al.*, 1991).

The selective DP-receptor antagonist BW 868C (Giles *et al.*, 1989) abolished the weak inhibitory effect of PGD₂ at a concentration of 10⁻⁷ M. However, at the lower concentration of 10⁻⁸ M BW868C had no effect on the inhibitory dose-effect curve for PGD₂ (Figure 3a).

Parallel rightward displacement of the BW245C dose-response curve was observed at all concentrations of the

antagonist tested 10⁻⁸, 10⁻⁷ and 10⁻⁶ M (Figure 3b). Mean data from Schild analysis are shown below:

Schild regression parameter	Value
pA ₂ (range)	8.3 (8.2–8.7)
Slope (range)	0.7 (0.6–0.84)
Regression coefficient (range)	0.97 (0.96–0.99)

All values are the geometric mean of *n* = 5 determinations.

The effect of IP-receptor agonists

The natural IP-receptor agonist, PGI₂, produced a biphasic response similar to and of similar potency to that seen with PGD₂ (Table 2). Again the excitatory component of the response occurred at lower doses than the inhibitory compo-

Figure 2 Mean stimulatory dose-effect curves to prostaglandin F_{2α} (PGF_{2α}, □) *n* = 5 and fluprostenol (◆) *n* = 7. Data are expressed as arithmetic means and vertical bars represent s.e.mean.

Table 2 Mean ED₁, ID₄ and maximum responses for excitation and inhibition by natural and synthetic prostanoids

Prostanoid	Excitation			Inhibition		
	ED ₁ (95% c.l.)	max (s.e.mean)	n	ID ₄ (95% c.l.)	max (s.e.mean)	n
PGE ₂ **	0.02 (0.001–0.58)	3.34 (1.69)	5	0.8 (0.2–2.9)	21.3 (4.2)	5
PGF _{2α}	0.04 (0.01–0.14)	4.3 (0.78)	5	> 140	NR	8
PGD ₂	3.56 (1.25–10.2)	1.9 (0.83)	10	> 1.7 (1.7–140)*	5.8 (1.5)	10
PGI ₂	3.65 (0.04–31.6)	2.2 (0.48)	5	6.4 (1.2–33)	11.7 (1.9)	5
U46619	0.05 (0.02–0.10)	2.69 (0.34)	8	> 140	NR	8
Fluprostenol	0.04 (0.013–0.1)	3.48 (0.75)	7	> 110	NR	7
BW245C	> 10	NR	7	0.05 (0.02–0.5)	39 (7)	7
Iloprost	2.74 (1.03–7.3)	2.3 (0.3)	7	0.94 (0.2–4.9)	7.6 (2.3)	7
Cicaprost	> 28	NR	5	0.2 (0.02–1.0)	14.3 (1.9)	5

*PGD₂ limits for ID₄ values expressed as range.

NR = no response.

ED₁ and ID₄ values are expressed as geometric means (nmol) with 95% confidence limits in parentheses.

Maximum responses for excitation are expressed as multiples of background activity (i.e. maximum T/B ratios) and maximum responses for inhibition are expressed in min.

**Senior *et al.* (1991).

Figure 3 Mean inhibitory dose-effect curves to (a) prostaglandin D_2 (PGD_2) and (b) BW245C alone ($\square$) and in the presence of BWA868C 10^{-8} M ($\blacklozenge$), 10^{-7} M ($\blacksquare$) and 10^{-6} M ($\diamond$). Data for both groups are expressed as arithmetic means of 5 determinations, and vertical bars represent s.e.mean.

ment. Like PGI_2 , the EP_1/IP -receptor agonist, iloprost produced a biphasic response as found previously (Senior *et al.*, 1991). The inhibition occurred at similar doses to the stimulation, i.e. between 0.14–14 nmol (Figure 4) and was dose-related within this range. As an inhibitory agonist, iloprost was more potent than PGI_2 (Table 2). In contrast, the selective IP -receptor agonist, cicaprost, produced only inhibition, similar to but less potent than BW245C. This effect of cicaprost produced a maximum inhibition of 14.3 min at 14 nmol which was the maximum dose available.

The effect of the TP-receptor agonist, U46619

The stable TP-receptor agonist U46619 produced purely contractile effects which, like those caused by $PGF_{2\alpha}$, consisted of an increase in baseline tonus with frequent phasic contractions superimposed on this effect. U46619 was a potent excitatory agonist (Figure 5) with a similar potency to both PGE_2 and $PGF_{2\alpha}$ (Table 2). The TP-receptor agonist GR32191 (Lumley *et al.*, 1989), at concentrations of 10^{-7} ,

Figure 4 Mean inhibitory dose-effect curves to prostacyclin (PGI_2 , $\blacklozenge$) $n = 5$, iloprost ($\square$) $n = 7$ and cicaprost ($\diamond$) $n = 5$. Data are expressed as arithmetic means and vertical bars represent s.e.mean.

Figure 5 Mean stimulatory dose-effect curves to U46619 alone ($\square$) and in the presence of GR32191 10^{-7} M ($\blacklozenge$), 3×10^{-7} M ($\square$) and 10^{-6} M ($\diamond$). Data are expressed as arithmetic means and vertical bars represent s.e.mean.

3×10^{-7} and 10^{-6} M, produced parallel rightward displacement of the U46619 dose-response curve. Mean data from Schild analysis gave the results shown below:

Schild regression parameter	Value
pA_2 (range)	7.1 (6.9–7.2)
Slope (range)	0.95 (0.71–1.08)
Regression coefficient (range)	0.95 (0.89–1.0)

All values are the geometric mean of $n = 5$ determinations.

Discussion

All the prostanoids tested, both natural and synthetic, had some effect on the activity of myometrial strips. Previous studies, on other isolated tissues, have provided evidence that each of the natural prostanoids is at least ten times more potent at its own receptor than at any of the other pros-

tanoid receptors (Coleman, 1987). If this finding is applied to the present studies, then the equal potency of PGE_2 , $\text{PGF}_{2\alpha}$ and U46619 as excitatory agonists gives a preliminary indication that EP, FP and TP receptors exist on the human myometrium. The low potency of PGI_2 and PGD_2 which are over 100 times less potent than PGE_2 , for excitation suggests that their effect may be mediated by interaction with one other prostanoid receptor. The contractile effect produced by PGE_2 , PGD_2 and PGI_2 was not affected by morphine (10^{-6} M), suggesting that this effect is a direct receptor-mediated event. Indeed, the data obtained with the synthetic selective agonists confirms these suggestions and will be discussed individually. In so far as inhibition is concerned, PGE_2 was the most potent of the natural prostanoids in this effect, i.e. compared to PGD_2 and PGI_2 . However, all these prostanoids produced a biphasic effect, which suggests that the inhibitory responses may well be masked to some extent by the initial stimulation of the tissue by the compounds. These data suggest separate receptors for the natural prostanoids, and results with selective agonists have confirmed this.

The effect of the natural EP-receptor prostanoid, PGE_2 , and selective synthetic EP-receptor prostanoids as well as the EP_1 -receptor antagonist AH6809 (Coleman *et al.*, 1985) have been discussed previously (Senior *et al.*, 1991). In summary, the results of these studies indicated the existence of both contractile EP_1 and EP_3 receptors and inhibitory EP_2 receptors in the non-pregnant human myometrium *in vitro*.

$\text{PGF}_{2\alpha}$ was a potent excitatory agonist like PGE_2 . This indicates that FP-receptors mediating excitation may occur in non-pregnant human myometrium but is not conclusive because of the cross-reactivity that exists between the natural prostanoids and the various receptors. In a range of tissues, $\text{PGF}_{2\alpha}$ has been shown to interact with EP and TP receptors (Coleman *et al.*, 1984). Previous studies have demonstrated that the TP-receptor antagonist, GR32191, has no effect on the $\text{PGF}_{2\alpha}$ response on the human isolated non-pregnant myometrium which indicates at most a minor involvement of TP-receptors in the $\text{PGF}_{2\alpha}$ response (Baxter, 1989). The use of the 16-phenoxy analogue of $\text{PGF}_{2\alpha}$, fluprostenol, gave support for FP-mediated contraction. Fluprostenol was as effective as $\text{PGF}_{2\alpha}$ ($\text{ED}_{50} = 0.04\text{ nmol}$ for both agonists) on this tissue. Fluprostenol was originally produced from structural modification of $\text{PGF}_{2\alpha}$ (Crossley, 1975) and it proved to have a potent contractile action on FP-receptor-containing preparations such as dog and cat iris sphincter muscle, where it is equipotent with $\text{PGF}_{2\alpha}$, but it is more selective than the parent compound, having no demonstrable activity at EP -, DP -, IP - and TP -receptors (Coleman *et al.*, 1987c). Due to this high selectivity of action, fluprostenol is useful in identifying FP-receptors in mixed receptor populations. Therefore, the data obtained with $\text{PGF}_{2\alpha}$ and, in particular, fluprostenol are consistent with the presence of FP-receptors in non-pregnant human myometrium. This work could not be supported by the use of antagonists because, although a wide range of compounds have been reported to antagonize various actions of $\text{PGF}_{2\alpha}$, no selective FP receptor antagonist has been developed to date. Hence the occurrence of FP-receptors in a mixed population of prostanoid receptors can only be examined by the use of selective agonists such as fluprostenol.

PGD_2 produced a biphasic response which was qualitatively similar to that evoked by PGE_2 . The low potency of PGD_2 as an excitatory agonist indicates that it is probably acting through one of the other prostanoid receptors to bring about this effect. Results with sulprostone and iloprost alone and in the presence of the EP_1 -receptor blocking drug AH6809, have previously shown that few EP_1 -receptors exist in this tissue (Senior *et al.*, 1991). The TP-receptor antagonist, GR32191 (10^{-6} M) (Baxter, 1989), had no effect on the contraction to PGD_2 . These data therefore suggest that EP_1 - and TP -receptors are unlikely to be involved in the stimulatory response of PGD_2 . PGD_2 does have moderately

potent activity at FP- (Beasley *et al.*, 1989) and TP-receptors (Hamid-Bloomfield & Whittle, 1986), as well as some degree of action at EP - and IP -receptors (Coleman, 1987). As far as the non-pregnant human myometrium is concerned, PGD_2 could contract this tissue by acting at EP_3 - or FP-receptors. The selective DP-receptor agonist, BW245C (Giles & Leff, 1988) produced an inhibition of myometrial activity which indicates that the inhibition demonstrated by PGD_2 is DP-receptor-mediated. BW245C is a highly selective DP-agonist which appears to have little or no agonist activity at EP -, FP -, IP - or TP -receptors (Town *et al.*, 1983; Coleman *et al.*, 1984; Tynan *et al.*, 1984). In addition to its selectivity, BW245C is about thirty times more potent than PGD_2 at DP-receptors (Coleman, 1987), thereby making it a valuable tool in the identification of DP-receptors in a heterogeneous receptor population. The observation that BW245C is potent in relaxing human myometrium agrees with the data of Sanger *et al.* (1982). Indeed, the present studies reveal a high degree of potency of BW245C, supporting the existence of DP-receptors in non-pregnant human myometrium. The use of the novel, highly potent selective DP-receptor antagonist BW A868C (Giles *et al.*, 1989) adds further weight to the suggestion that relaxant DP-receptors are present in non-pregnant human myometrium. BW245C is specific in that it has no actions at the other prostanoid receptors at concentrations up to 1000 times higher than the DP-receptor affinity. Although the Schild slope was less than unity, the antagonism by BWA868C of the BW245C response appears to have some characteristics of a competitive interaction as demonstrated by the apparently parallel rightward displacements of the BW245C dose-effect curves. The inhibitory action of PGD_2 on non-pregnant myometrium was also antagonized by BW A868C at 10^{-7} M , this effect was associated with some potentiation of the contractile event. This unmasking provides further support for the contractile effect being mediated through a different receptor type. At the lower concentration of 10^{-8} M , BW A868C had no observable effect on the inhibitory dose-effect curve of PGD_2 . The data obtained with PGD_2 , BW 245C and BW A868C provide evidence that DP-receptors mediating inhibition of uterine activity in the non-pregnant human myometrium exist in this preparation.

Like PGD_2 , PGI_2 exhibited a weak contractile effect followed by an inhibition of activity. As with PGD_2 , the excitation with PGI_2 is believed to occur through another prostanoid receptor, PGI_2 is particularly active at EP -receptors (Coleman, 1987). The TP-receptor blocking drug, GR32191, at 10^{-6} M had no effect on stimulatory dose effect curves to PGI_2 (Baxter, 1989), implying that TP-receptors are not involved in this effect. The contraction could therefore be mediated through the contractile EP - or FP -receptors. As an inhibitory agonist, PGI_2 was the least potent of all those prostanoids tested that mediated this effect, and this could reflect the rapid metabolism of the compound, although in a superfused system the washout rate is high. In other tissues, PGI_2 is a potent inhibitor of platelet aggregation, and it relaxes smooth muscle (Moncada *et al.*, 1976). *In vivo* experiments in non-pregnant ovariectomized sheep have demonstrated inhibition of myometrial activity with PGI_2 (Lye & Challis, 1982). Biphasic activity has also been recorded from *in vitro* studies on non-pregnant human uterus and fallopian tubes (Omini *et al.*, 1978); this biphasic effect induced by PGI_2 was also observed when the tonus of the organ was pharmacologically increased with $\text{PGF}_{2\alpha}$.

In addition two selective synthetic IP receptor agonists, iloprost and cicaprost were tested in the present study. Iloprost also acts at EP_1 -receptors as discussed previously (Senior *et al.*, 1991), the inhibition of myometrial activity is of a similar potency to PGE_2 (ID_{50} 0.94 and 0.8 nmol respectively), although the maximum inhibition with the former is comparatively small. The action of iloprost at IP-receptors suggests that the inhibition is most likely to be mediated through a population of IP-receptors rather than through the

contractile EP₁ receptors in non-pregnant human myometrium.

This proposal is adequately supported by the action of the IP-receptor selective prostanoid, cicaprost, which produced a potent inhibition of myometrial activity and was the most effective agent after BW245C on comparison of the ID₅₀ values for all the prostanoids tested (ID₅₀ = 0.2 nmol). Cicaprost was originally developed after attempts were made to improve the metabolic stability of iloprost (Stürzebecher *et al.*, 1986). It is more potent than PGI₂ or iloprost as an inhibitor of platelet aggregation, as a relaxant of bovine coronary strip and as a hypotensive agent. Unlike iloprost, it has no demonstrable action at any of the other prostanoid receptors, thereby making cicaprost the most selective IP-receptor agonist yet described (Dong *et al.*, 1986). The results obtained with PGI₂, iloprost and cicaprost suggest that prostanoid receptors of the IP type are involved in the inhibition of myometrial activity in non-pregnant human myometrial strips.

TXA₂ was not used for the study of TP-receptors in non-pregnant human myometrium because, unlike the other natural prostanoids, TXA₂ is not commercially available due to its extreme instability. However, the stable TXA₂-mimetic (11 α , 9 α -epoxymethano PGH₂ or U46619) is commonly used to assess the presence of TXA₂-sensitive receptors. In the present study, U46619 was equipotent with PGE₂ and PGF_{2 α} as an excitatory agonist (ED₅₀ = 0.05, 0.02 and 0.04 nmol respectively). U46619 has been compared to TXA₂ on a range of smooth muscle preparations, including guinea-pig lung strip, dog saphenous vein, rat and rabbit aorta, which are thought to contain TP-receptors (Coleman *et al.*, 1981; Kennedy *et al.*, 1982). U46619 showed little or no activity at

other prostanoid receptors; thus, it is a valuable tool for the study and characterization of TP-receptors. Further support for the presence of TP-receptors was provided by the TP antagonist, GR32191. The potent contractile action of U46619 in the myometrial strips was antagonized in a competitive manner by GR32191. The pA₂ for GR32191 on this myometrial preparation was lower than that reported by many authors, although Cuthbert *et al.* (1990) found a low pK_B (6.9) value of rat lung strip, and suggested that this preparation together with human bronchial muscle may contain a subtype of the TXA₂ receptor population. The presence of this range of both excitatory and inhibitory prostanoid receptors on human myometrium suggests that control of myometrial activity by prostanoids is complex, and that further studies are needed to elucidate the effect of mixtures of prostaglandins. It is also of interest to note that despite investigation into many species, including primates, no animal model has been found which exhibits the same complexity of prostanoid receptors as does human myometrium (studies in this laboratory to be published). The knowledge of the prostanoid receptors present in human myometrium is important in clinical conditions involving myometrial dysfunction (e.g. dysmenorrhoea) and in the development of prostanoid-mimetics for the treatment of other conditions (e.g. peptide ulceration).

We thank all suppliers of prostanoid analogues and antagonists listed in Table 1. G.S.B. was supported by Glaxo Group Research Ltd., Ware and R.S. by the Royal Pharmaceutical Society of Great Britain. We are grateful to Dr R.A. Coleman of Glaxo Group Research Ltd., Ware and Professor R.L. Jones of The Chinese University of Hong Kong for discussion. Thanks are also due to Miss Annalie Green for preparing the manuscript.

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(Received January 21, 1992

Revised May 14, 1992

Accepted May 20, 1992)