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Characterization of the prostanoid receptors mediating constriction and relaxation of human isolated uterine artery

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1 This study was undertaken to characterize pharmacologically the prostanoid receptor subtypes mediating constriction and relaxation of human isolated uterine artery.

2 U-46619 was a potent constrictor agonist on human uterine artery (EC_{50} [95% CL] = 3.5 [1.8-6.7] nM). Prostaglandin E_2 (PGE_2), $PGF_{2\alpha}$, PGD_2 and PGI_2 only weakly constricted the uterine artery, being at least 100 times less potent than U-46619. The PGE_2 and PGI_2 constrictor effects may be modified by the potent dilator effects of these compounds. A number of agonists which show selectivity for FP-, DP- and EP-receptors including ICI 81008, BW 245C, sulprostone, rioprostil and butaprost, failed to cause any constriction at concentrations up to 30 μM.

3 Constrictor responses induced by all agonists tested were reduced or abolished by the TP-receptor blocking drugs, GR 32191 and EP 092. pA_2 estimates for both antagonists versus U-46619 were 8.50, values which are consistent with their affinities at TP-receptors.

4 In preparations pre-constricted with phenylephrine (1 μM) both PGI_2 and PGE_2 were potent relaxant agonists. The selective IP-receptor agonists, cicaprost and iloprost, also dilated human uterine artery and were approximately 10 fold more potent than PGI_2. The EP_2-receptor agonists, butaprost and rioprostil and the selective DP-agonist, BW 245C, were at least 100 fold weaker than PGI_2 and PGE_2 suggesting that neither DP- nor EP_2 receptors were involved.

5 We conclude that TP-receptors mediate constriction, whereas IP- and possibly EP_4-receptors mediate relaxation of human uterine artery.

Keywords: Prostanoid receptors; human uterine artery; TP-receptors and constriction; IP-receptors and relaxation

Introduction

Prostanoid receptors comprise five major subtypes, including DP-, FP-, IP-, TP- and EP-receptors, named in accordance with their selectivity for the natural prostanoids, prostaglandin D_2 (PGD_2), $PGF_{2\alpha}$, PGI_2, TXA_2 and PGE_2, respectively (Coleman *et al.*, 1990). EP-receptors have been further subdivided into EP_1, EP_2, EP_3 and recently, EP_4 subtypes (Coleman *et al.*, 1990; 1994).

Prostanoid receptors are present on a variety of vascular preparations where they mediate both contraction and relaxation. By use of a range of prostanoid receptor ligands possessing varied selectivity, it has been possible to characterize those receptors mediating vascular smooth muscle contraction as TP- or EP_3-, and those mediating relaxation as either IP-, EP_2- or EP_4-receptors (see Coleman *et al.*, 1994). On human uterine artery excised from both pregnant and non-pregnant donors, PGE_2 and $PGF_{2\alpha}$ have been reported to cause constriction, and PGI_2 to cause relaxation (Wilhelmsson *et al.*, 1981; Vincent *et al.*, 1983; Maigaard *et al.*, 1985). Whilst these data suggest prostanoid receptors are present in human uterine artery, the relatively promiscuous activity of the 'natural' prostanoids used in these studies precludes any explicit characterization of the prostanoid subtypes present. In this regard, a single report disclosing the low potency of highly selective, synthetic TP-receptor agonist, U-46619 (Dong & Wadsworth, 1985) may imply that TP-receptors do not mediate the contractile response to prostanoids in this preparation.

We have previously characterized the prostanoid receptors present on human myometrial smooth muscle (Senior *et al.*, 1991; 1992; 1993); however, changes in uterine blood flow can also affect myometrial activity and *vice versa*. The aim of the present study, therefore, was to evaluate the actions of both natural and synthetic prostanoids to obtain a more detailed pharmacological characterization of the prostanoid receptors present.

A preliminary account of some of these data has been given to the British Pharmacological Society (Baxter *et al.*, 1989).

Methods

Samples of ascending uterine artery were obtained from pre-menopausal women (all patients gave written consent) undergoing hysterectomy for the treatment of benign disorders such as menorrhagia or fibroids. Patients with endometriosis or underlying malignancy were excluded from the study. The phase of the menstrual cycle was assessed by histological examination of the endometrium by the Pathology Department of St. Lukes Hospital, Bradford. After surgery, tissues were placed in ice cold Krebs solution and transported to the laboratory where experimental preparations were completed within a 60 min post-operative period. Arterial rings, 3 mm in diameter with a lumen diameter of approximately 2 mm, were suspended under 1 g tension in 10 ml tissue baths containing oxygenated (95% O_2/5% CO_2) Krebs solution (pH 7.4) at 37°C. The composition of the Krebs solution used in these studies was as follows (mM): NaCl 118, KCl 4.75, $NaHCO_3$ 25, glucose 11.1, K_2HPO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5 and indomethacin 0.003. This concentration of indomethacin did not affect the responsiveness of the tissue to phenylephrine or potassium chloride. A 2 h equilibration period was allowed and during this time the Krebs solution was changed every 15 min.

Responses were measured with isometric tension transducers (Dynamometer UF1) coupled to a Grass Polygraph (Model 7D) 8-channel chartpen recorder.

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Histological examination of the arterial rings showed that the endothelium remained intact during the dissection procedure.

Agonist potency

In order to study agonist-induced relaxation, tone had to be induced by the addition of a constricting agent. Several substances including histamine, potassium chloride, noradrenaline and phenylephrine were evaluated, all of which produced variable results. In many instances, tone was poorly maintained and spontaneous activity was often superimposed on an elevated, and fluctuating baseline. Although the stability of the constriction induced with phenylephrine was variable, it did appear to be the most reliable agonist tested and was therefore used to induce tone in human uterine artery for the evaluation of relaxant activity. Relaxant responses to prostanoids were not evaluated on tissues which showed poor stability of tone. Phenylephrine (1 μM) caused constriction of human uterine artery equal to approximately 50% of its maximum response. For evaluation of the relaxant potency of agonists, the TP-receptor antagonist, GR 32191 (1 μM) was included in Krebs solution (Baxter *et al.*, 1989), and PGI_2 was used as the standard agonist.

Two cumulative concentration-effect curves were constructed in each preparation. The first concentration-effect curve was generated with the 'standard' agonists, U-46619 (for constrictor potency) or PGI_2 (for dilator potency). The second concentration-effect curve was then constructed, 1 h later, with another prostanoid. Responses were expressed as a percentrage of the maximum response obtained with either U-46619 or PGI_2 (for comparison of contractile and relaxant potency respectively) achieved in the first, control concentration-effect curve. The relative potency of agonists was established by comparison of equi-effective molar concentration ratios (ECR) determined by dividing the EC_{50} (concentration required to evoke a 50% of maximal response) of the test agonist with the EC_{50} for U-46619 or PGI_2 obtained on the same preparation. On repetition, concentration-effect curves for the standard agonists, U-46619 and PGI_2 were reproducible and for this reason, correction factors were not routinely applied to account for changes in sensitivity between first and second concentration-effect curves.

Antagonist affinity

Affinity estimates for antagonists were expressed as pA_2 values, calculated according to the method of Arunlakshana & Schild (1959). Tissues were incubated with antagonists for a period of at least 1 h and concentration-ratios were determined by comparison of EC_{50}s for agonist concentration-effect curves constructed in the absence and presence of antagonists in the same preparation.

Compounds used

$PGF_{2\alpha}$ (Lutalyse, tromethamine salt) and PGE_2 (Dinoprostone) were obtained from Upjohn Pharmaceuticals, Kalamazoo. Sulprostone, iloprost and cicaprost were gifts from Schering AG, Berlin. PGI_2, phenylephrine and indomethacin were obtained from Sigma. Rioprostil was obtained from the Ortho Pharmaceutical Corporation, New Jersey. Butaprost was obtained from Bayer plc, Slough. ICI81008 (fluprostenol) was obtained from Zeneca Pharmaceuticals, Macclesfield. BW 245C (5-(6-carbohexyl)-1 (3-cyclohexyl-2-hydroxypropyl)-hydantoin) was obtained from Wellcome, Beckenham. PGD_2, U-46619 (11α, 9α, epoxymethano PGH_2) and GR 32191 {[1R-[1α(Z), 2β, 3β, 5α]-(+)-7-5-([1,1-biphenyl)-4-yl)methyloxyl}-3-hydroxy-2-(1-piperidinyl) cyclopentyl]-4-heptanoic acid hydrochloride) were synthesized by Glaxo Research and Development Ltd., Ware. EP 092 (9α,11α-ethano-ω-heptanor-13-methyl-13-phenyl-thio-carbamoy-hydrazino-prosta -5Z -enoic acid) was obtained from the Pharmacology Department, Edinburgh University. All of the compounds listed as 'obtained' were gifts from the drug companies indicated and we are grateful for their generosity. All stock solutions and vehicles were the same as those previously published (Senior *et al.*, 1991; 1992).

Results

Approximately 40% of human uterine arteries exhibited spontaneous activity which almost always consisted of long (2–10 min duration) tonic contractions. Tissues which displayed significant levels of spontaneous activity were not used.

With the possible exception of responses to phenylephrine, agonist responses were generally slow both in onset and offset, especially with synthetic analogues. Typical sample traces showing the effect of (a) U-46619 and (b) PGI_2 are shown in Figure 1. Although the low sample numbers did not allow a detailed examination, it appeared qualitatively that neither the magnitude nor the profile of response to any agonist was related to the stage of the menstrual cycle.

Constriction of human uterine artery

U-46619 caused potent concentration-dependent constriction (EC_{50} = 3.5 (1.8–6.7) nM, n = 6). Responses to U-46619 were often slow to develop, especially at lower concentrations, and were slow to return to baseline after drug washout. PGE_2, PGD_2 and PGI_2 also evoked constriction, in most, but not all preparations; they were of low potency relative to U-46619, and did not achieve a similar maximum response at the maximum concentrations used (Figure 2, Table 1). Tissue responses evoked by these prostanoids tended to be more rapid in onset and offset than those to U-46619. Although $PGF_{2\alpha}$ was some 750 times less potent than U-46619, it did attain a simular maximum response. (Figure 2, Table 1).

The TP-receptor ligand, GR 32191, was a surmountable antagonist of U-46119-induced constriction (Figure 3) and caused concentration-dependent rightward displacements of concentration-effect curves to this agonist. EP 092 was also a potent antagonist of U-46619-induced constriction. Data from

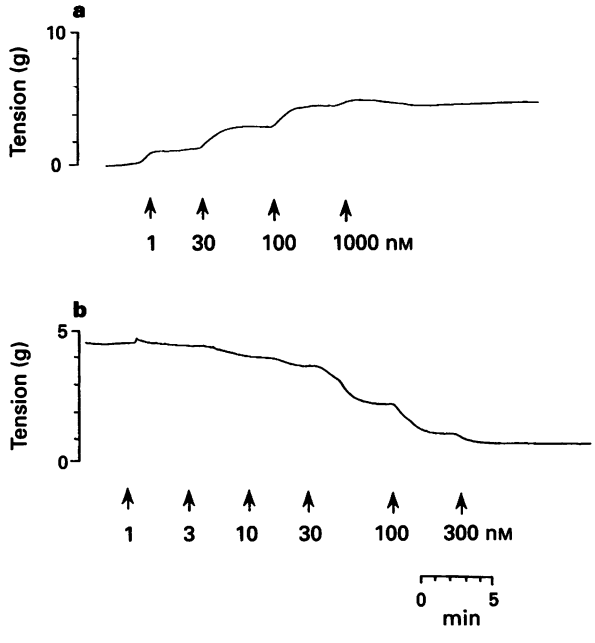

Figure 1 Typical sample traces showing dose-effect curves obtained with (a) U-46619 and (b) PGI_2 (in the presence of phenylephrine 1 μM) on human uterine arterial rings.

Schild regression analysis for both GR 32191 and EP 092 using concentration-ratio data from individual preparations is shown in Table 2. The pA_2 data means are from 4 experiments.

GR 32191 (1 μM) abolished constrictor responses obtained to $PGF_{2\alpha}$, PGE_2, PGD_2 and PGI_2 and in the presence of this antagonist ICI81008, BW 245C, sulprostone, rioprostil and butaprost evoked a constriction (at 30 μM, but not at lower concentrations) in human uterine artery (Table 1).

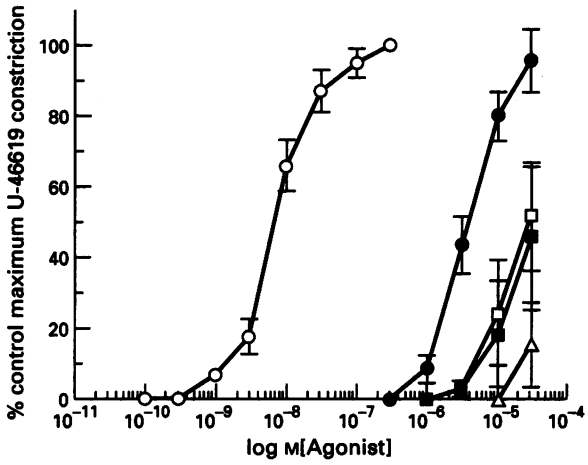

Figure 2 Mean constrictor concentration-effect curves to U-46619 (○) $n=6$, $PGF_{2\alpha}$ (●) $n=5$, PGE_2 (□) $n=5$, PGD_2 (■) $n=6$ and PGI_2 (△) $n=5$. Values are mean ± s.e.mean.

Table 1 Comparison of constrictor potency of prostanoids in human uterine arterial rings

Prostanoid	*ECR* (95% CL)	n
U-46619	1 (EC_{50} = 3.5(1.8–6.7) nM)	6
$PGF_{2\alpha}$	743(207–2667)	5
PGE_2	>3800(29–196)	5
PGD_2	>900(198–1211)	6
PGI_2	>2850	5
ICI 81008	>8500*	2
BW 245C	>8500*	2
Sulprostone	>8500*	4
Rioprostil	>8500*	6
Butaprost	>8500*	6

*At concentrations up to 30 μM in the presence of 1 μM GR 32191.

Relaxations of human uterine artery

PGI_2 was a potent dilator agonist, causing concentration-dependent dilator responses (EC_{50} = 12.7 (4.8–34.0) nM, $n=6$). Successive concentration-effect curves to PGI_2 showed no significant change in sensitivity. PGE_2 was of similar potency and intrinsic activity to PGI_2 (Table 3). Responses to PGD_2 were variable, and in two out of three experiments, it was at least 150 times less potent, but on the other occasions was only 7 times less potent than PGI_2. $PGF_{2\alpha}$ (up to 30 μM) did not evoke dilatation of human uterine artery.

The prostacyclin-mimetics, iloprost and cicaprost, were both highly potent agonists producing concentration-related dilatation over the range 0.1–30 nM (Figure 4). Whilst both prostanoids were approximately 10 times more potent than PGI_2 itself (Table 3), the relaxant responses induced by both these analogues tended to develop more slowly. These results may be an overestimate since PGI_2 is known to be an unstable compound.

Table 2 Antagonist affinity estimates for GR 32191 and EP 092 against U-46619 in human uterine arterial rings

Antagonist	pA_2 (95% CL)	*Slope* (95% CL)	n
GR 32191	8.5(8.4–8.5)	0.94(0.89–1.05)	4
EP 092	8.5(8.4–8.7)	0.9(0.86–1.08)	4

Table 3 Comparison of dilator potency of prostanoids in human uterine arterial rings

Prostanoid	*ECR* (95% CL)	n
PGI_2	1 (EC_{50}-12.7(4.8–34.0)nM)	6
PGE_2	0.8(0.6–1.5)	6
PGD_2	58(6.7–200)	5
Iloprost	0.07(0.04–0.12)	6
Cicaprost	0.05(0.02–0.17)	6
Butaprost	251(148–478)*	3
Rioprostil	110(70–210)*	4
BW 245C	181(181–>2360)*	4

*Range

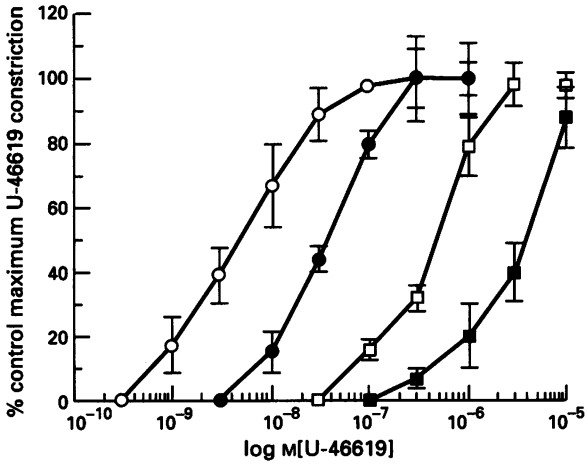

Figure 3 Mean constrictor concentration-effect curves to U-46619 in the absence (○) $n=5$ and presence of GR32191 10^{-8}M (●) $n=4$, 10^{-7}M (□) $n=4$, and 10^{-6}M (■) $n=4$. Values are mean ± s.e.mean.

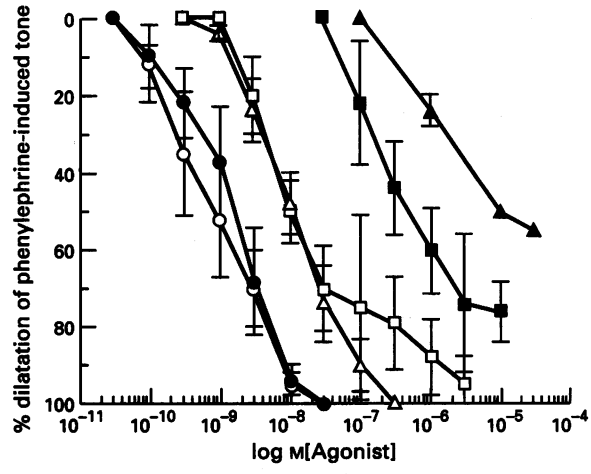

Figure 4 Mean concentration-effect curves to cicaprost (○) $n=6$, iloprost (●) $n=6$, PGI_2 (△) $n=6$, PGE_2 (□) $n=6$, PGD_2 (■) $n=5$ and butaprost (▲) $n=3$. Values are mean ± s.e.mean.

Butaprost was a weak dilator agonist in human uterine artery and was approximately 150–500 times less potent than PGI_2. Rioprostil was also seen to be active, and in experiments in which it was directly compared to PGI_2, it was found to be at least 100 times less potent. Dilator responses to both compounds were very slow in onset (e.g. discernible reaction time was 15.5 ± 2.2 min at 1 μM for butaprost compared to 3.3 ± 0.43 min for PGI_2 at 0.1 μM).

The selective DP-receptor agonist, BW 245C, did cause dilatation but was of low potency, being approximately 180 times less potent than PGI_2.

Discussion

There is an abundance of evidence in the literature that prostanoid-induced constrictor responses of isolated blood vessels appear to be mediated predominantly by TP- and EP_3-receptor subtypes and dilatation by IP-, EP_2- and EP_4-receptors (*see* Coleman *et al.*, 1994). Whilst the nature of the prostanoid receptor subtypes mediating vascular responses in animal models has been well established, the characteristics of those mediating responses in human vascular preparations, with few exceptions (Lumley *et al.*, 1989; Qian *et al.*, 1994) are not well defined.

In the present study, U-46619 was a potent constrictor agonist, suggesting TP-receptors are present in this preparation of human uterine artery. This view is supported by the sensitivity to the potent TP-receptor blocking drugs, GR 32191 and EP 092, and in this regard, the pA_2 for GR 32191 in this study was of the same order as that determined previously at TP-receptors in human pulmonary artery (Lumley *et al.*, 1989), and the pA_2 for EP 092 was similar to that found in rat aorta (Tymkewycz *et al.*, 1991). The natural prostanoids, $PGF_{2\alpha}$, PGD_2, PGE_2 and PGI_2, on the other hand were all weak contractile agonists on this preparation, and responses to these agonists were abolished by GR 32191. With regard to the constrictor results for PGE_2 and PGI_2, these may have been reduced by the very marked dilator effects of these prostanoids. The order of potency of the natural prostanoids as contractile agonists in human uterine artery is in general agreement with that reported in other vascular TP-receptor containing preparations such as rat aorta, in that U-46619 is at least 100 times more potent than the other naturally occurring prostanoids, $PGF_{2\alpha}$, PGD_2, PGE_2, or PGI_2 (Kennedy *et al.*, 1982). Our finding that ICI 81008, BW 245C, sulprostone, rioprostil and butaprost did not evoke constriction (at doses within the normal range for specific receptor activation) suggests that EP_1-, EP_2, EP_3-, FP- and DP-receptors mediating constriction do not occur in human uterine artery. In view of this substantial body of information in this report suggesting the presence of excitatory TP-receptors in human uterine artery, it is interesting that Dong & Wadsworth (1985) failed to demonstrate a potent contractile agonist action with U-46619 in similar preparations. These data, therefore, suggest that prostanoid-induced constrictor effects in human uterine artery are mediated by a homogeneous population of TP-receptors. The reason for the discrepancy between the present finding and those of Dong & Wadsworth (1985) is not known.

In the presence of GR 32191 (1 μM), to preclude any action of prostanoids at TP-receptors, both PGI_2 and PGE_2 were found to be potent dilator agonists, observations consistent with the presence of both inhibitory IP- and EP-receptors in human uterine artery. This is in close agreement with findings of other workers (Maigaard *et al.*, 1985).

The presence of inhibitory IP-receptors is supported by the potent dilator activity of iloprost and cicaprost. Cicaprost is a highly potent and selective IP-receptor agonist (Dong *et al.*, 1986; Lawrence *et al.*, 1992), whereas iloprost, whilst a potent IP-agonist, also possesses agonist activity at EP_1-receptors (Sheldrick *et al.*, 1988). The potency of both compounds in the present study reflects that observed by other workers on other vascular preparations *in vitro* (Coleman, personal communication). While both cicaprost and iloprost appear to be of equal potency to PGI_2 at IP-receptors mediating inhibition of platelet aggregation (Mueller *et al.*, 1984), cicaprost appears to be at least 5 times more potent than the other two prostanoids at IP-receptors mediating inhibition of spontaneous contractions in rat colon (Dong & Jones, 1985). The lower activity of iloprost on this preparation may be due, in part, to the opposing contractile activity of iloprost.

It is interesting that the high relaxant potency of PGE_2 (relative to PGI_2) was not reflected by the EP_2-receptor selective analogues, butaprost or rioprostil. Indeed these compounds proved to be at least 100 times weaker than the natural prostanoid in provoking a relaxant response, and were extremely slow in onset. Rioprostil has been shown to be approximately three times, and butaprost 30 times less potent than PGE_2 on cat trachea, an EP_2-receptor containing preparation (Gardiner, 1986; Coleman *et al.*, 1987), and both compounds are at least equipotent with PGE_2 as inhibitors of spontaneous activity in non-pregnant human myometrium, an effect thought to be mediated by EP_2-receptors (Senior *et al.*, 1991). Thus the potency differences in human uterine artery do not equate with an action at EP_2-receptors. It is unlikely that these differences result from limited access of rioprostil and butaprost to EP-receptors, because even though the long onset of action may prevent detection of responses at lower concentrations, equilibrium was attained at higher levels, and was of a demonstrably lower magnitude than that obtained with equal or lower concentrations of PGE_2. It is unlikely that responses to PGE_2 are mediated via either IP-, or DP-receptors, as both PGI_2 and PGE_2 are of similar potency, and based on studies with the selective DP-receptor agonist, BW 245C, there appear to be few if any DP-receptors present in human uterine artery. It is therefore tempting to consider the possibility that EP_4-receptors mediate the potent dilator actions of PGE_2, as reported for piglet saphenous vein (Coleman *et al.*, 1994). In this regard, further studies with antagonists such as, AH22921X or AH23848, which show low but demonstrable affinity for EP_4-receptors (Coleman *et al.*, 1994), should provide clear evidence one way or the other regarding the involvement of EP_4-receptors.

In the light of the low potency of the DP-receptor-selective agonist, BW 245C, it appears that there are few if any DP-receptors mediating relaxation in human uterine artery. If this is correct, then the moderately potent activity of PGD_2 must, presumably, be mediated via a different receptor-type. It is unlikely that PGD_2 would be acting via IP-receptors, as studies in platelets have revealed that this prostanoid possesses low affinity for this site (Siegl *et al.*, 1979; Schafer *et al.*, 1979). PGD_2 also has rather low affinity for EP_2- and EP_4-receptors, being at least 100 times less potent than PGE_2 in EP_2-receptor containing preparations such as cat trachea or guinea-pig ileum circular muscle (Kennedy *et al.*, 1982; Coleman, 1983) and in EP_4-receptor containing preparations such as piglet saphenous vein and rabbit ductus arteriosus (Coleman *et al.*, 1994; Smith *et al.*, 1994). The site of action of PGD_2 therefore remains to be identified.

In summary, the findings in the present study are consistent with the presence of only TP-receptors mediating constriction, and IP- and possibly EP_4-receptors mediating dilatation of human isolated uterine artery. The actions of all other prostanoids tested appear to be mediated via these receptor types. These findings are important in relation to the contractility of the myometrium. Any constriction of the artery and subsequent myometrial ischaemia will serve to increase the effects of spasmogens on the uterus. Similarly, it should be noted that although the uterine artery contains only a limited number of prostanoid receptor subtypes, these may be activated by many different prostanoid receptor agonists, if present in appropriate concentrations.

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