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Effects of the isoprostane, 8-epi-prostaglandin $F_{2\alpha}$, on the contractility of the human myometrium in vitro

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Abstract

8-epi-prostaglandin $F_{2\alpha}$ stimulated contraction of human myometrial strips obtained from five different donors at the time of hysterectomy with a pEC_{50} value of 6.3 ± 0.5. In paired strips from the same donors the pEC_{50} value for the selective TP receptor agonist U46619 ([1*R*-[1a,4a,5b(*Z*),6a(1*E*,3*S**)]]-7-[6-(3-hydroxy-1-octenyl)-2-oxabicyclo [2.2.1]hept-5-yl]-5-heptenoic acid) was 8.3 ± 0.4. In strips from four other donors 8-epi-prostaglandin $F_{2\alpha}$ was ineffective whereas the pEC_{50} for U46619 was 6.9 ± 0.3. Responses to 8-epi-prostaglandin $F_{2\alpha}$ were unaffected by the selective DP receptor antagonist BW A868C (3-benzyl-5-(6-carboxyhexyl)-1-(2-cyclohexyl-2-hydroxyethylamino)hydantoin) at 50 nM but were blocked by the selective TP receptor antagonist L670596 ((−)6,8-difluoro-9-*p*-methylsulfonyl benzyl-1,2,3,4-tetrahydrocarbazol-1-yl-acetic acid) at 50 nM. The pIC_{50} values obtained when the TP receptor antagonists GR 32191 ([1*R*-[1α(*Z*),2β,3β,5α]]-(+)-7-[5-[[(1,1'-biphenyl)-4-yl]methoxy]-3-hydroxy-2-(1-piperidinyl)cyclopentyl]-4-heptenoic acid), ICI D1542 ((4(*Z*)-6-[(2*S*,4*S*,5*R*)-2-[1-methyl-1-(2-nitro-4-tolyloxy)ethyl]-4-(3-pyridyl)-1,3-dioxan-5-yl]hex-4-enoic acid), ICI 192605 (4(*Z*)-6-[(2,4,5-*cis*)-2-(2-chlorophenyl)-4-(2-hydroxyphenyl)-1,3-dioxan-5-yl]hexenoic acid), L670596 and SQ 29548 ([1*S*-(1α,2β(5*Z*),3β,4α]]-7-[3-[[2-[(phenylamino)carbonyl]hydrazino]methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid) were added cumulatively to strips pre-contracted with an EC_{80} concentration of 8-epi-prostaglandin $F_{2\alpha}$ were not significantly different from those obtained when an EC_{80} concentration of U46619 was used. The effects of 8-epi-prostaglandin $F_{2\alpha}$ on strips pre-contracted with an EC_{80} concentration of U46619 were not different from those of U46619 itself. It is concluded that in the non-pregnant human myometrium 8-epi-prostaglandin $F_{2\alpha}$ is a medium potency contractile agonist acting predominantly at the TP receptor.

Keywords: Isoprostane; Myometrium, human; Contraction; Prostanoid TP receptor; U46619

1. Introduction

The isoprostanes are a recently discovered series of prostaglandin-like compounds (Morrow et al., 1990). Unlike the prostaglandins the isoprostanes are produced by free radical-catalyzed peroxidation of membrane-associated arachidonic acid, a mechanism independent of the cyclooxygenase pathway (Morrow et al., 1990). To date F, D and E ring products have been identified (Morrow et al., 1990, 1994b) and their formation in vitro confirmed (Morrow et al., 1994a,b). 8-epi-prostaglandin $F_{2\alpha}$ is a prominent member of the isoprostane family and has been shown to possess potent vasoconstrictor properties that are mediated by action at the TP receptor (Takahashi et al., 1992; Banerjee et al., 1992). In platelets 8-epi-prostaglandin $F_{2\alpha}$ appears to be a partial agonist at TP receptors (Morrow et al., 1992; Yin et al., 1994). More recently the existence of distinct F_2-isoprostane receptors has been proposed (Fukunaga et al., 1993).

The presence of TP receptors in human myometrium is supported by functional (Dyal and Crankshaw, 1988; Senior et al., 1992), radioligand binding (Senchyna and Crankshaw, 1994) and molecular biological data (Senchyna et al., 1994). It is therefore possible that 8-epi-prostaglandin $F_{2\alpha}$ has effects upon the contractility of the human myometrium that may serve as targets for the pharmacotherapy of disorders of uterine contractility such as dysmenorrhoea and

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pre-term labour. As a first step to testing this hypothesis I have examined the effects of 8-epi-prostaglandin $F_{2\alpha}$ on the contractility of the human myometrium in vitro.

2. Materials and methods

2.1. Materials

8-epi-prostaglandin $F_{2\alpha}$ and U46619 ([1*R*-[1a, 4a,5b(*Z*),6a(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). Indomethacin (1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indole-3-acetic acid) was from Sigma (St. Louis, MO, USA). The following compounds were gifts: BW A868C (3-benzyl-5-(6-carboxyhexyl)-1-(2-cyclohexyl-2-hydroxyethylamino)hydantoin) from Wellcome (Beckenham, Kent, UK); GR 32191 ([1*R*-[1α(*Z*),2β,3β,5α]]-(+)-7-[5-[[(1,1'-biphenyl)-4-yl]methoxy]-3-hydroxy-2-(1-piperidinyl)cyclopentyl]-4-heptenoic acid) from Glaxo Group Research (Ware, Herts, UK); ICI D1542 ((4(*Z*)-6-[(2*S*,4*S*,5*R*)-2-[1-methyl-1-(2-nitro-4-tolyloxy)ethyl]-4-(3-pyridyl)-1,3-dioxan-5-yl]-hex-4-enoic acid) and ICI 192605 (4(*Z*)-6-[(2,4,5-*cis*)-2-(2-chlorophenyl)-4-(2-hydroxyphenyl)-1,3-dioxan-5-yl]hexenoic acid) from Zeneca Pharmaceuticals (Alderley Park, Cheshire, UK), L670596 ((−)6,8-difluoro-9-*p*-methylsulfonyl benzyl-1,2,3,4-tetrahydrocarbazol-1-yl-acetic acid) from the Merck Frosst Centre for Therapeutic Research (Pointe Claire, Quebec, Canada); and SQ 29548 ([1*S*-(1α,2β(5*Z*),3β,4α]]-7-[3-[[2-[(phenylamino)carbonyl]hydrazino]methyl]-7-oxabicyclo[2.2.1]-hept-2-yl]-5-heptenoic acid) from Bristol-Myers Squibb (Princeton, NJ, USA). All other chemicals were purchased from BDH (Toronto, Ontario, Canada).

8-epi-prostaglandin $F_{2\alpha}$ and BW A868C were dissolved in 70% ethanol and U46619 in methyl acetate, stock solutions were stored at −20°C. Solvents for the TP receptor antagonists were as follows: GR 32191, 70% ethanol; ICI D1542, dimethylsulphoxide; ICI 192605, dimethylsulphoxide; L670596 dimethylsulphoxide; SQ 29548, 70% ethanol. Serial dilutions of all drugs except indomethacin into saline 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 pre-menopausal women undergoing hysterectomy for benign disorders such as menorrhagia, dysmenorrhoea and fibroids. Pieces were removed from the uterine fundus and placed in Hepes-buffered physiological salt solution (PSS) for transport to the laboratory. The Hepes-buffered PSS had the following composition: 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 sixteen 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. Reliable concentration-effect data are difficult to obtain from human myometrium, without manipulation of the ionic environment, because of the presence of significant spontaneous contractile activity and the fact that agonists can induce changes in both phasic and tonic activity (Crankshaw, 1990). Two quite different techniques (Wainman et al., 1988; Senior et al., 1991) have been developed to overcome this problem. In this study I employed the method of Wainman et al. (1988) in which the mean force developed by a tissue strip during a 10 min period is used as a measure of its contractility. The amplifier output was simultaneously sampled, digitalized and stored at a frequency of 2 Hz throughout the 10 min period. Each sample represented the force developed by the tissue at that particular instant; by adding all the samples in the period and dividing by the number of samples (1200), the mean force exerted during that time was obtained. In order to make direct comparisons between strips, mean forces were normalized to the cross-sectional area of each strip and expressed in terms of Newtons per square centimeter. Mean force is essentially equivalent to 'area under the curve' but has the additional advantage that direct comparisons can be made to conventional measurements from tissues that respond with only tonic changes. This technique has been used routinely in this laboratory (Wainman et al., 1988; Dyal and Crankshaw, 1988; Crankshaw and Dyal, 1994) and

others (Cheuk et al., 1993) to successfully quantify drug effects.

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.

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}.

Quantification of antagonist effects

The relatively low potency of 8-epi-prostaglandin $F_{2\alpha}$ precluded the determination of pA_2 values for antagonists (Arunlakshana and Schild, 1959) because of the inability to achieve sufficiently high bath concentrations of agonist when concentration-effect curves were shifted significantly. Therefore antagonist pIC_{50} values were determined instead.

After 1 h of equilibration tissues were challenged with 30 nM U46619 or 3 μM 8-epi-prostaglandin $F_{2\alpha}$ which remained in contact with the tissues for the rest of the experiment. These represented approximately EC_{80} concentration of each agonist. When the agonist

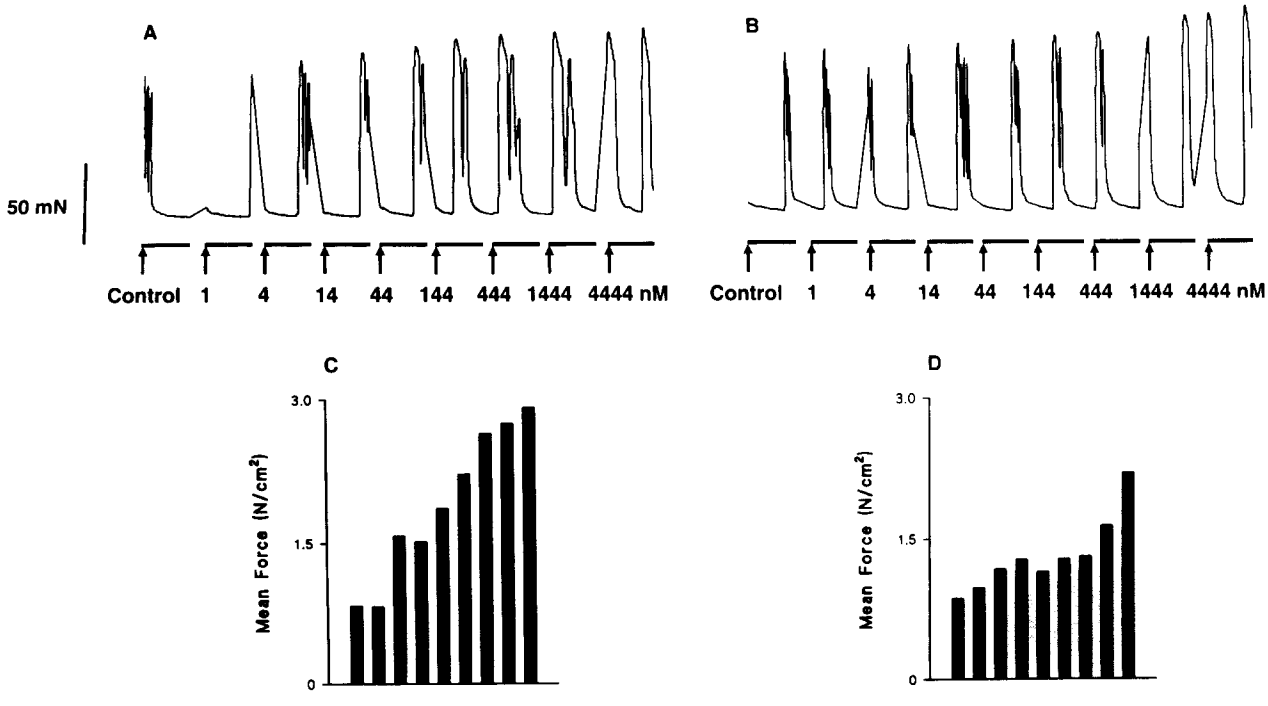

Fig. 1. The effect of cumulative addition of 8-epi-prostaglandin $F_{2\alpha}$ to strips of myometrium from a non-pregnant donor, in the absence (A,C) and the presence (B,D) of 50 nM L670596. Analogue traces of the experiments are shown in A and B where the horizontal bars indicate 10 min collection periods, the first of which is the control. Thereafter 8-epi-prostaglandin $F_{2\alpha}$ was added at the points indicated by the arrows to give the cumulative concentrations shown. The mean contractile force is represented graphically in C and D where each vertical 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 are as shown in A and B.

had been in contact with the tissue for 1 h a concentration-effect experiment was begun. The procedure was exactly as described above, except that antagonists were added and the response was a decline in agonist-induced activity. In all experiments eight tissue strips from the same donor were challenged with the same agonist, two strips served as time-matched controls, no antagonist being added during the concentration-effect experiment. The remaining six strips were treated with antagonist, usually two strips per compound. At the end of the experiment ICI D1542 was added to all baths to give a final concentration of 1 μM. After a 15 min exposure to ICI D1542 a final data collection was performed. The high concentration of ICI D1542 was expected to eliminate all remaining TP receptor-mediated contractility and therefore define zero agonist-induced 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.

Concentration-effect curves were constructed as described above, except that the value determined was the antagonist pIC_{50}. The average pIC_{50} value from each donor was determined, thus n values represent the number of donors on which each pIC_{50} was determined.

2.4. Statistical analysis

All data are expressed as arithmetic means ± the standard deviation of the mean. Antagonist pIC_{50} values against 8-epi-prostaglandin $F_{2\alpha}$-induced and U46619-induced contractions were compared using an unpaired t-test. Values of $P < 0.05$ were considered to indicate significant differences.

3. Results

3.1. Effects of 8-epi-prostaglandin $F_{2\alpha}$ and U46619 on the spontaneous contractile activity of human myometrium

Graded excitatory responses to the cumulative addition of U46619 were obtained in myometrial strips

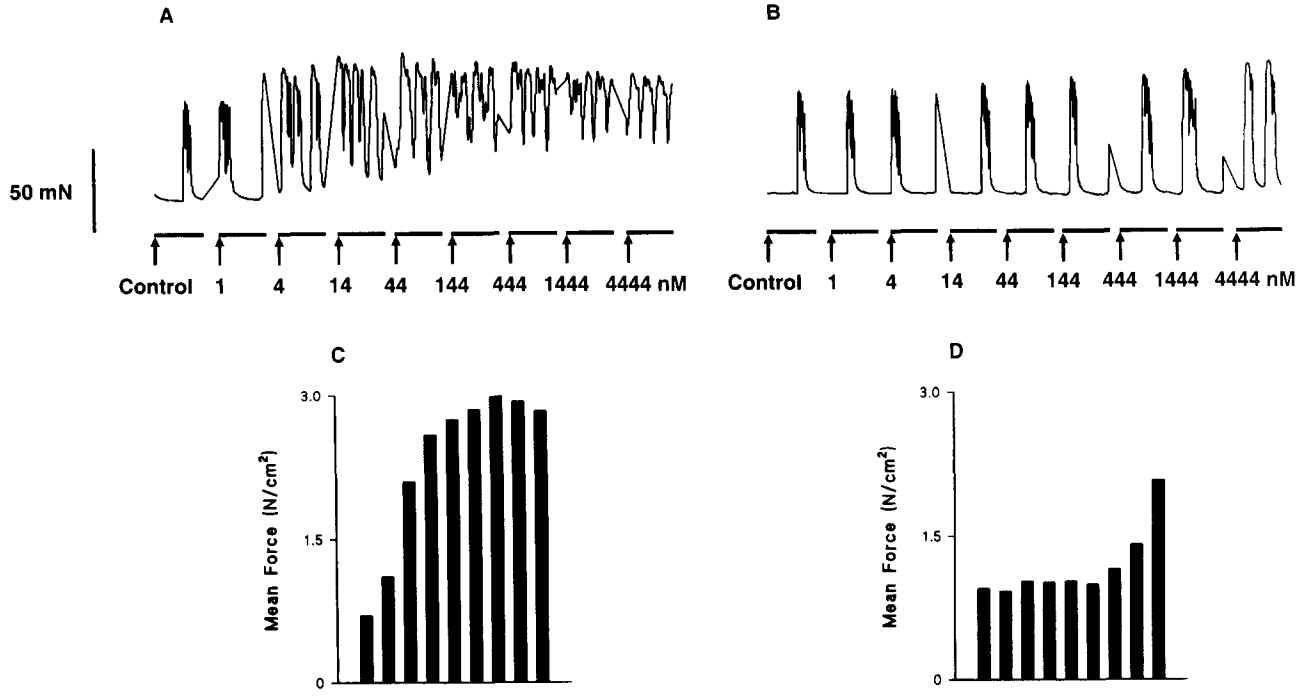

Fig. 2. The effect of cumulative addition of U46619 to strips of myometrium from a non-pregnant donor, in the absence (A,C) and the presence (B,D) of 50 nM L670596. Analogue traces of the experiments are shown in A and B where the horizontal bars indicate 10 min collection periods, the first of which is the control. Thereafter U46619 was added at the points indicated by the arrows to give the cumulative concentrations shown. The mean contractile force is represented graphically in C and D where each vertical 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 are as shown in A and B.

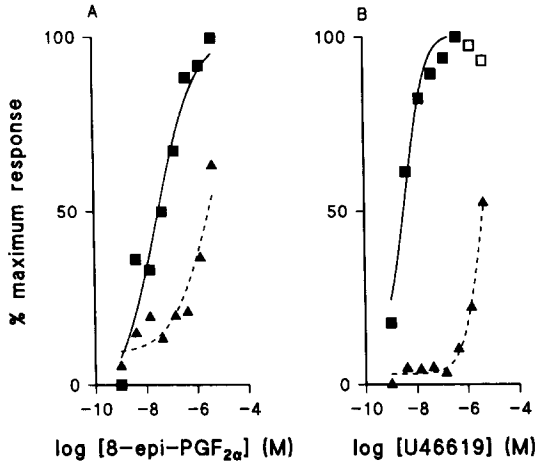

Fig. 3. The effects of (A) 8-epi-prostaglandin $F_{2\alpha}$ and (B) U46619 in the absence (■,□) and the presence (▲) of 50 nM L670596. Concentration-effect curves derived from the experiment shown in Figs. 1 and 2. Points representing the downturn of a biphasic concentration-effect curve (□) were not included in the determination of the pEC_{50} value.

from nine different donors. Matched strips from five of these donors also produced graded excitatory responses to 8-epi-prostaglandin $F_{2\alpha}$, whereas strips from the other four did not respond to 8-epi-prostaglandin $F_{2\alpha}$. The selective TP receptor antagonist L670596 (Ford-Hutchinson et al., 1989) at 50 nM antagonised both 8-epi-prostaglandin $F_{2\alpha}$ (Fig. 1; Fig. 3) and U46619 (Fig. 2; Fig. 3) whereas the selective DP receptor antagonist BW A868C (Giles et al., 1989) at 50 nM was without discernable effects.

The mean pEC_{50} value for 8-epi-prostaglandin $F_{2\alpha}$ obtained from the five responding donors was 6.3 ± 0.5, the mean pEC_{50} value for U46619 from these same donors was 8.3 ± 0.4. The rank orders of sensitivity of donors to 8-epi-prostaglandin $F_{2\alpha}$ and to U46619 were identical. The mean pEC_{50} for U46619 in donors who did not respond to 8-epi-prostaglandin $F_{2\alpha}$ was 6.9 ± 0.3.

3.2. Effects of TP receptor antagonists

Challenge with 3 μM 8-epi-prostaglandin $F_{2\alpha}$ produced sustained contractile activity in tissue strips from

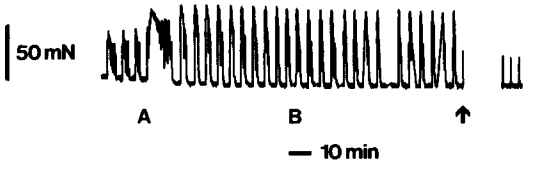

Fig. 4. The effect of a single concentration of 8-epi-prostaglandin $F_{2\alpha}$ on the contractility of a strip of human myometrium from a non-pregnant donor. 8-epi-prostaglandin $F_{2\alpha}$ (3 μM) was added at A and data collection began at B. The TP antagonist ICI D1542 (1 μM) was added at the arrow.

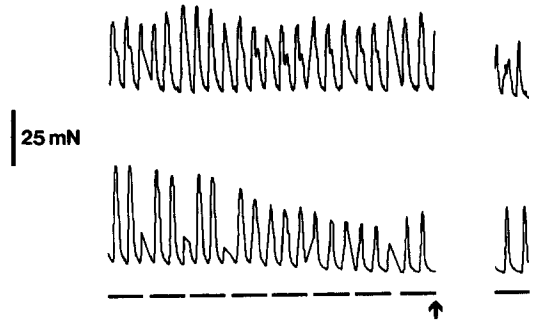

Fig. 5. The effect of time (upper trace) and cumulative addition of SQ 29548 (lower trace) on the contractility of strips of human myometrium from a non-pregnant donor induced by a single concentration of 8-epi-prostaglandin $F_{2\alpha}$. The horizontal bars indicate 10 min collection periods, the first of which is control. Thereafter SQ 29548 was added at each break to give final concentrations of 1 nM to 4 μM. At the arrow 1 μM ICI D1542 was added to eliminate all TP receptor-induced activity.

12 out of 19 donors, an example is shown in Fig. 4. The sustained activity was inhibited in a graded manner by TP receptor antagonists in 8 out of 12 cases. In contrast U46619 produced sustained contractile activity in strips from 12 out of 14 donors and this activity was inhibited in a graded manner by TP receptor antagonists in 10 cases. In both cases where U46619-induced activity was resistant to TP receptor antagonists, matched strips stimulated with 8-epi-prostaglandin $F_{2\alpha}$ were also resistant. In one case the 8-epi-prostaglandin $F_{2\alpha}$ response was resistant but the U46619 response was sensitive and in the final case only 8-epi-prostaglandin $F_{2\alpha}$ was tested.

Cumulative addition of antagonists produced graded inhibition of the agonist-induced activity (Fig. 5) from which concentration-effect curves were constructed (Fig. 6) and pIC_{50} values determined. The mean pIC_{50}

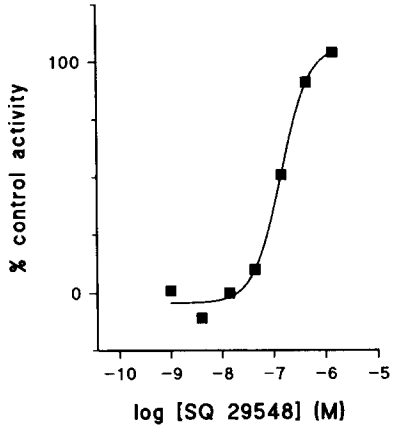

Fig. 6. The effect of SQ 29548 on 8-epi-prostaglandin $F_{2\alpha}$-induced contractility of strips of human myometrium from a non-pregnant donor. Concentration-effect curve derived from the experiment shown in Fig 4. The effect of time was corrected for as explained in the text.

Table 1
The effects of several TP receptor antagonists on 8-epi-prostaglandin $F_{2\alpha}$ and U46619-induced contractions of human myometrium from non-pregnant donors in vitro

Agonist	Antagonist				
	SQ 29548	ICI D1542	GR 32191	ICI 192605	L670596
8-epi-prostaglandin $F_{2\alpha}$	7.5 ± 0.8 (4)	7.4 ± 0.5 (6)	7.3 ± 0.3 (3)	6.5 ± 0.1 (3)	5.6 ± 0.2 (3)
U46619	7.6 ± 0.6 (6)	7.3 ± 0.2 (7)	7.4 ± 0.1 (3)	6.8 ± 0.3 (3)	5.7 ± 0.4 (3)

Values are pIC_{50} ± S.D., determined as described in the text. The number in parentheses represents the number of different donors from whom the mean values are derived. The pIC_{50} for each compound against 8-epi-prostaglandin $F_{2\alpha}$ was compared with its pIC_{50} against U46619 using an unpaired Student's *t*-test and a significance cut off of $P < 0.05$. In no case a significant difference was found.

values for five TP receptor antagonists against 8-epi-prostaglandin $F_{2\alpha}$ and U46619-induced contractions are shown in Table 1.

3.3. Effect of 8-epi-prostaglandin $F_{2\alpha}$ on responses to U46619

When 8-epi-prostaglandin $F_{2\alpha}$ was added cumulatively to a maximum concentration of 14 μM to tissues that had been pre-contracted with U46619, a slight (10–15%) inhibition of contractility was observed. This inhibition was no different from that produced by addition of equi-effective concentrations of U46619. This experiment was performed on strips from three different donors.

4. Discussion

The sensitivity of the human myometrium in vitro to selective TP receptor agonists such as U46619 has been shown to vary significantly from donor to donor (Crankshaw, 1992). This variability can be attributed in part to the menstrual status of the donor, but there remain unexplained inter-individual differences that cannot be attributed to differences in myometrial TP receptor numbers (Senchyna and Crankshaw, 1994). Consequently, the variability in sensitivity to U46619 observed in the present study was not unexpected.

That the rank orders of donor sensitivity to U46619 and 8-epi-prostaglandin $F_{2\alpha}$ were identical provides weak circumstantial support for the hypothesis that the two compounds act by the same mechanism. This is further strengthened by the observation that when the potency of U46619 was low, a response to 8-epi-prostaglandin $F_{2\alpha}$ could not be detected.

The potency of 8-epi-prostaglandin $F_{2\alpha}$ on the myometrium from non-pregnant donors was approximately 100 times less than that of U46619. In bovine coronary arteries the potency difference was 15 (Ogletree, 1992) whereas in rat and guinea pig aortae the differences were 176 and 183, respectively (Ogletree, personal communication). In all these cases the responses to 8-epi-prostaglandin $F_{2\alpha}$ were sensitive to TP receptor antagonists so it is possible to attribute the variability in the potency ratio to species differences in the TP receptor (Ogletree and Allen, 1991).

The action of naturally occurring prostanoids is not often confined to one receptor (Coleman et al., 1990), it would therefore be a mistake to assume a high degree of receptor selectivity for 8-epi-prostaglandin $F_{2\alpha}$. Unfortunately, studies on the pharmacology of 8-epi-prostaglandin $F_{2\alpha}$ are currently extremely limited and the compound has not been tested in systems that could clearly establish its activity at each prostanoid receptor. The human myometrium is inappropriate for such a study since pharmacological evidence suggests that it simultaneously expresses DP, EP_1, EP_2, EP_3, FP, IP and TP receptors (Senior et al., 1991, 1992) but selective antagonists that could be used to pharmacologically isolate each receptor in turn are for the most part unavailable. However I was able to demonstrate that the selective DP receptor antagonist BW A868C (Giles et al., 1989) had no effect on responses to 8-epi-prostaglandin $F_{2\alpha}$. This suggests that in human myometrium from non-pregnant donors 8-epi-prostaglandin $F_{2\alpha}$ possesses insignificant activity at the DP receptor. If there had been significant activity at the DP receptor I would have expected 8-epi-prostaglandin $F_{2\alpha}$ to produce a biphasic concentration-effect curve (excitatory/inhibitory) or for BW A868C to potentiate the effects of 8-epi-prostaglandin $F_{2\alpha}$.

The proportion of tissues that produced sustained contractile responses to single doses of each agonist was consistent with the proportion of tissues from which graded concentration-effect curves could be obtained, although the stability of the compounds during this long exposure was not determined. GR 32191 (Lumley et al., 1989), ICI D1542 (Brownrigg et al., 1992), ICI 192605 (Jessup et al., 1988), L670596 (Ford-Hutchinson et al., 1989) and SQ 29548 (Ogletree et al., 1985) are selective TP receptor antagonists. Thus the fact that for each of these compounds there was no difference in the IC_{50} against 8-epi-prostaglandin $F_{2\alpha}$ and the IC_{50} against U46619 supports the hypothesis that 8-epi-prostaglandin $F_{2\alpha}$ acts predominantly at the TP receptor in human myometrium from non-pregnant donors. If 8-epi-prostaglandin $F_{2\alpha}$ were acting at a distinct F_2-isoprostane receptor (Fukunaga et al., 1993)

I would have expected that at least one of the antagonists tested would be able to discriminate between the TP receptor and the F_2-isoprostane receptor and thus produce significantly different IC_{50} values. The reduction in contractility of U46619 pre-contracted strips by subsequent addition of U46619 is possibly due to desensitization of the TP receptor. The inability of 8-epi-prostaglandin $F_{2\alpha}$ to produce greater inhibition of contractile activity in tissues pre-contracted with U46619 than U46619 itself argues against 8-epi-prostaglandin $F_{2\alpha}$ being a partial agonist in these circumstances. Thus 8-epi-prostaglandin $F_{2\alpha}$ is a medium potency contractile agonist on isolated myometrium from non-pregnant donors. Its actions appear to be mediated predominantly at the TP receptor where its lower potency relative to U46619 is probably explained by a lower affinity for the receptor rather than a significantly lower intrinsic efficacy. The observation that 8-epi-prostaglandin $F_{2\alpha}$ was sometimes not antagonised in a graded manner by TP antagonists leaves open the possibility that 8-epi-prostaglandin $F_{2\alpha}$ can act at a site other than the TP receptor in some circumstances.

The processes of cellular breakdown occurring at the time of menstruation may well lead to the generation of free radicals that in turn catalyse the formation of isoprostanes. Action of isoprostanes on the myometrium could then cause contraction and pain in susceptible individuals. This pain would be unresponsive to non-steroidal anti-inflammatory agents, and the mechanism just described could explain why a significant number of women do not obtain relief from menstrual cramping from non-steroidal anti-inflammatories (Åkerlund, 1990). Under these circumstances, the evidence presented in this study suggests that the TP receptor would be a more appropriate target for therapy. Support for this hypothesis awaits confirmation that isoprostanes are formed in the uterus during menstruation.

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