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Variable responses to prostaglandin E_2 in human non-pregnant myometrium

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Abstract

Cumulative concentration–effect curves for prostaglandin E_2, sulprostone and butaprost were constructed in matched strips of human non-pregnant myometrium from 14 different donors. All samples were obtained from the mid-lateral wall of the uterus. Prostaglandin E_2 produced four types of concentration–effect curves: monophasic inhibitory ($n = 7$), monophasic excitatory ($n = 2$), biphasic consisting of an excitatory phase followed by an inhibitory phase ($n = 4$), and biphasic consisting of an inhibitory phase followed by an excitatory phase ($n = 1$). Sulprostone produced excitation of spontaneous contractile activity in all tissues (mean $pEC_{50} = 9.1 \pm 0.2$, range 8.1–10.1, $n = 14$). Butaprost produced relaxation of cloprostenol-stimulated contractile activity in all tissues (mean $pEC_{50} = 5.7 \pm 0.1$, range 5.0–6.9, $n = 14$). The mean pEC_{50} value for sulprostone was significantly higher in tissues where prostaglandin E_2 caused some excitation ($pEC_{50} = 9.4 \pm 0.2$, $n = 7$) compared to those where prostaglandin E_2 caused only inhibition ($pEC_{50} = 8.8 \pm 0.2$, $n = 7$). Mean pEC_{50} values for butaprost were not significantly different between these groups. These data suggest that (a) variability in EP receptor-mediated responses exists within a single anatomical site; (b) both excitatory and inhibitory EP receptor-mediated pathways are always operative in human non-pregnant myometrium, regardless of the type of tissue response to prostaglandin E_2; and (c) regulation of EP receptor-mediated responses occurs predominantly in the excitatory (EP_3 or EP_1 receptor) pathway rather than the inhibitory (EP_2 receptor) pathway.

Keywords: Myometrium; (Human); Prostaglandin E_2; Prostanoid EP receptor; Concentration–effect curve, Biphasic; Two-receptor model; Sulprostone; Butaprost

1. Introduction

In human myometrium, prostaglandin E_2 causes contraction through activation of EP_1 and EP_3 receptors and/or relaxation through activation of EP_2 receptors. The predominant excitatory EP receptor subtype in this tissue is EP_3 (Senior et al., 1991). Consequently, responses to prostaglandin E_2 in this tissue are often complex, and vary markedly between donors (Senior et al., 1991; Popat and Crankshaw, 1997). Different responses to prostaglandin E_2 may be explained by differences in the balance between the excitatory and inhibitory pathways, where tissues that are predominantly inhibited by prostaglandin E_2 exhibit EP_2 receptor dominance, while tissues excited by prostaglandin E_2 exhibit EP_3 and/or EP_1 receptor dominance (Crankshaw and Dyal, 1994).

Previous work has also shown differences in myometrial responses to some prostaglandins between tissues obtained from different anatomical sites of the uterus (Wikland et al., 1984). Alternatively, responses to prostanoid TP receptor activation in human non-pregnant myometrium are not influenced by site of origin of the tissue (Senchyna and Crankshaw, 1999).

Because several confounding variables are difficult to control, we asked a simple, testable question: is inter-donor variability in responses of human non-pregnant myometrium to prostaglandin E_2 observed if samples are obtained from a single anatomical site? Therefore, we studied the effects of prostaglandin E_2 on 14 donor tissues obtained from the mid-lateral wall of the uterus from

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non-pregnant donors. Our next objective was to determine if the variability we found was due to differences in receptor dominance between donor tissues. We investigated this issue by pharmacologically separating the excitatory and inhibitory EP receptor-mediated pathways. Because of the unavailability of selective EP receptor antagonists, we isolated each pathway through the use of sulprostone, an EP_3/EP_1 receptor selective agonist, and butaprost, an EP_2 receptor selective agonist (Coleman et al., 1994). In order to estimate potency values, monophasic responses were fit to a one-receptor model. Biphasic responses to prostaglandin E_2 were fit to the two-receptor model of Szabadi (1977) under the assumption that such complex responses result from stimulation of two functionally opposite receptor populations (Crankshaw and Popat, 1997).

2. Materials and methods

2.1. Materials

2.1.1. Drugs

Cloprostenol (Estrumate®) was purchased from Coopers Agropharm (Willowdale, ON, Canada); prostaglandin E_2, fluprostenol and U46619 were purchased from Cayman Chemical (Ann Arbor, MI, USA). Sulprostone, L670596 ((−)6,8-difluoro-9-*p*-methylsulfonylbenzyl-1,2,3,4-tetrahydrocarbazol-1-yl-acetic acid) and butaprost were received as gifts from Schering (Berlin, Germany), the Merck Frosst Centre for Therapeutic Research (Pointe Claire, PQ, Canada) and Dr. H. Kluender, Bayer (West Haven, CT, USA), respectively. Indomethacin, [Arg^8]-vasopressin and D600 ((±)-α-[3-[[2-(3,4-dimethoxyphenyl) ethyl] methylamino] propyl]-3,4,5-trimethoxy-α-(1-methylethyl)-benzeneacetonitrile hydrochloride) were obtained from Sigma (St. Louis, MO, USA).

Stock solutions of prostaglandin E_2, fluprostenol and U46619 were made in ethanol and stored at −20°C; butaprost was in ethanol or ethyl acetate at −20°C; sulprostone was in ethyl acetate at 4°C; cloprostenol, [Arg^8]-vasopressin and D600 were in aqueous solutions at 4°C; L670596 was in dimethylsulphoxide at 4°C. Serial dilutions of drugs were made freshly into double-distilled water and kept on ice throughout the experiment. Indomethacin was prepared as described by Curry et al. (1981). All other chemicals were from BDH (Toronto, ON, Canada).

2.1.2. Solutions

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

2.2. Tissue collection and preparation

Human myometrial samples were obtained from pre- or peri-menopausal non-pregnant women undergoing hysterectomy for benign disorders as previously described (Senchyna and Crankshaw, 1996). All samples were obtained from the mid-lateral wall of the uterus. Immediately following surgery, samples were placed in oxygenated (95% O_2, 5% CO_2) PSS. Tissues were normally used immediately, however, some were stored in oxygenated PSS at room temperature for up to 18 h post-operatively. Maintenance of tissue viability when stored in this manner has been demonstrated previously (Fernandes and Crankshaw, 1995; Senchyna and Crankshaw, 1996; Hillock and Crankshaw, 1999). The collection protocol was approved by the Research Advisory Group, McMaster University.

Sixteen myometrial tissue strips (15 × 2 × 3 mm) from each donor were set up as previously described (Fernandes and Crankshaw, 1995) and mounted longitudinally in individual 5- or 10-ml jacketed muscle baths containing oxygenated PSS at 37°C.

2.3. Recording isometric contractions

Contractions were recorded as described by Senchyna and Crankshaw (1999). An initial resting force of 25 mN, which is optimal for human myometrium of the size we used (Crankshaw and Dyal, 1994), was applied to each strip. The mean force developed by the individual muscle strips was used as a measure of their contractility (Wainman et al., 1988; Cheuk et al., 1993). Mean force was determined during 10-min epochs as described by Wainman et al. (1988). The force generated by each muscle strip was sampled at a frequency of 2 Hz. All samples taken over the 10-min period were added (this value corresponds to the area under the contraction curve). The total was then divided by the number of samples taken (1200) to give the mean force exerted over the 10-min period.

2.3.1. Effect of prostaglandin E_2 and sulprostone on spontaneous contractile activity

Tissue strips from fourteen separate donors were allowed to equilibrate for 1 h during which time they were washed three times with PSS. During the equilibration the resting force was readjusted to 25 mN until spontaneous contractile activity developed. L670596 (50 nM) was added to the baths to prevent stimulation of TP receptors (Ford-Hutchinson et al., 1989). When this protocol is used, the mean force developed by human myometrium remains relatively constant for 2.5 h (Crankshaw and Dyal, 1994) allowing for determination of concentration–effect parameters. At the end of the equilibration period, the mean force developed during a 10-min control period was determined. Tissue strips were randomly assigned either prostaglandin E_2 or sulprostone treatment (duplicate strips from

the same donor were treated with the same agonist) and a cumulative concentration–effect experiment was performed, using approximately one-half log unit concentration increases. Each successive agonist concentration was in contact with the tissue for 3 min to allow equilibrium conditions to be reached before a 10-min data collection was taken. Agonist additions continued until bath concentrations spanned 5 log units. After the last drug collection, the baths were drained and the PSS was replaced with double distilled water. A final 10-min data collection was then taken from which the mean force generated in response to the resulting hypotonic shock was determined and used as a measurement of maximum contractile activity (Crankshaw and Popat, 1997).

2.3.2. Quantification of responses to prostaglandin E_2 and sulprostone

The mean force recorded in the 10-min period following each agonist addition, minus the mean force developed during the control period was considered to be the force generated in response to that concentration of agonist. Responses between tissue strips were normalized by expressing the mean force as a percentage of the force induced by hypotonic shock (Crankshaw and Popat, 1997).

We attempted to fit all responses to prostaglandin E_2 to both the one and two-receptor model of Szabadi (1977) using Eqs. (1) and (2), respectively. Data from paired strips were combined into a single analysis in order to fit curves significantly to the multi-parameter equation of the two-receptor model (Crankshaw and Popat, 1997).

In cases where the response to prostaglandin E_2 was monophasic, concentration–effect curves (percent response to hypotonic shock versus log molar agonist concentration) were constructed from the data obtained and fit to a one-receptor model using the following equation:

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

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

In cases where the response to prostaglandin E_2 was complex, appearing either biphasic or triphasic, an attempt was made to fit concentration–effect curves to a two-receptor model using the following equation:

$$E = \left[E_{\min_1} + \left(E_{\max_1} - E_{\min_1}\right)/\left(1 + e^{-k1^*(\log C_1 + p\mathrm{EC}_{501})}\right)\right] + \left[E_{\max_2} + \left(E_{\min_2} - E_{\max_2}\right)/\left(1 + e^{-k_2^*(\log C2 + p\mathrm{EC}_{502})}\right)\right] \quad (2)$$

where subscripts 1 and 2 represent the two opposing receptor populations.

Eq. (2) is derived from Eq. (1) using the Szabadi model (Eq. (3)) which proposes the total effect of an agonist in a pharmacological test system may be obtained by the algebraic summation of the two effects resulting from activation of two opposing receptor populations:

$$E_t = E_{(+)} + E_{(-)} \quad (3)$$

where E_t is the total effect of the agonist and the subscripts $(+)$ and $(-)$ are used to indicate the opposing nature of the of the two receptor populations, respectively.

Concentration–effect curves for sulprostone were constructed by fitting paired data to the one-receptor model using Eq. (1). A downturn in the concentration–effect curves for sulprostone was observed at high concentrations, giving the curves a bell-shaped appearance. However, the mechanism of this downturn remains unknown (Crankshaw and Popat, 1997). Therefore, use of the two-receptor model to fit such curves could not be justified. In lack of an appropriate model, data points falling below and to the right of the concentration–effect asymptote were not included in the determination of the $p\mathrm{EC}_{50}$ value. Such analyses of bell-shaped concentration–effect curves for prostanoid receptor agonists have been reported previously (Crankshaw and Gaspar, 1995; Senchyna and Crankshaw, 1996).

2.3.3. Effect of butaprost on cloprostenol-induced contractile activity

The effect of butaprost was assessed on separate strips from the same donors used in Section 2.3.1 using the cloprostenol-stimulated technique described by Fernandes and Crankshaw (1995). Tissue strips were allowed to equilibrate as described in Section 2.3.1. Strips were then challenged with 2 μM of the selective FP receptor agonist cloprostenol, which remained in contact with the tissues for the remainder of the experiment. After a 1-h incubation with cloprostenol, tissues were incubated for a further hour in 50 nM L670596.

After a 2-h exposure to cloprostenol, the mean force developed during a 10-min control period was determined. Two strips were randomly assigned as temporal controls, and were treated with vehicle in order for us to be able to correct for the combined effects of time and of vehicle upon cloprostenol-induced activity. A cumulative concentration–effect experiment for butaprost was then performed on two strips, using the same technique as described in Section 2.3.1. Additions of butaprost continued until bath concentrations spanned 4 log units. After the last drug addition, all four tissue strips were treated with 10 μM of the calcium channel blocker D600 (Fleckenstein et al., 1969). After tissues had been exposed to D600 for 15 min, a final 10-min recording was taken. D600 reduced contractile activity to basal tone and this was used to define zero contractility.

2.3.4. Quantification of responses to butaprost

Responses to butaprost were quantified as described by Hillock and Crankshaw (1999). Concentration–effect

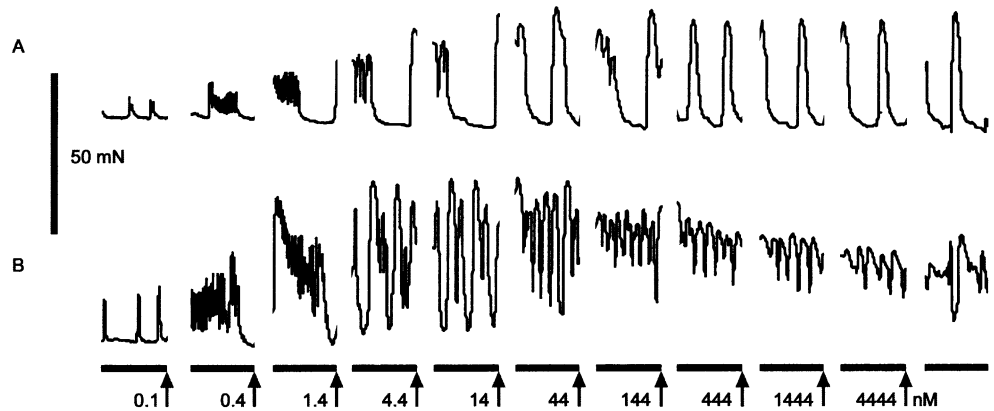

Fig. 1. Original tracing of the effect of cumulative addition of (A) prostaglandin E_2 and (B) sulprostone on contractile activity of human myometrium from a non-pregnant donor. Horizontal bars indicate 10-min data collection periods, the first of which is the control. Agonists were added to the baths at the points indicated by the arrows to give the cumulative concentrations shown. The time-base of the recordings is exactly as collected. However, the 3-min equilibration times have been removed from the traces in order to clarify the drug-induced effects.

curves for butaprost (% inhibition versus log molar agonist concentration) were constructed by fitting data to Eq. (1). The average of the two pEC_{50} values obtained from the two tissues strips treated with butaprost was used, thus n values represent the number of donors on which butaprost was tested.

2.4. Statistics

All values reported are arithmetic means ± the standard error of the mean (S.E.M.). Sulprostone and butaprost concentration–effect curve parameters were compared using the Students' t-test, values of $P < 0.05$ were considered significant.

Data from paired strips were combined when fitting concentration–effect curves for prostaglandin E_2 and sulprostone. The applicability of the one- or two-site model was assessed using the F-test (Motulsky and Ransnas, 1987). Values of $P < 0.05$ were considered significant.

3. Results

Fig. 1 shows a sample trace of the effect of cumulative addition of prostaglandin E_2 and sulprostone on human myometrial contractility. Fig. 2 shows a sample trace of the effect of cumulative addition of butaprost compared to a time-matched control obtained in a parallel strip from the same donor.

Responses to prostaglandin E_2 showed considerable variability between donor tissues. Fig. 3 shows representative concentration–effect curves for the four types of prostaglandin E_2 responses observed. The biphasic response shown in Fig. 3D only occurred in one donor

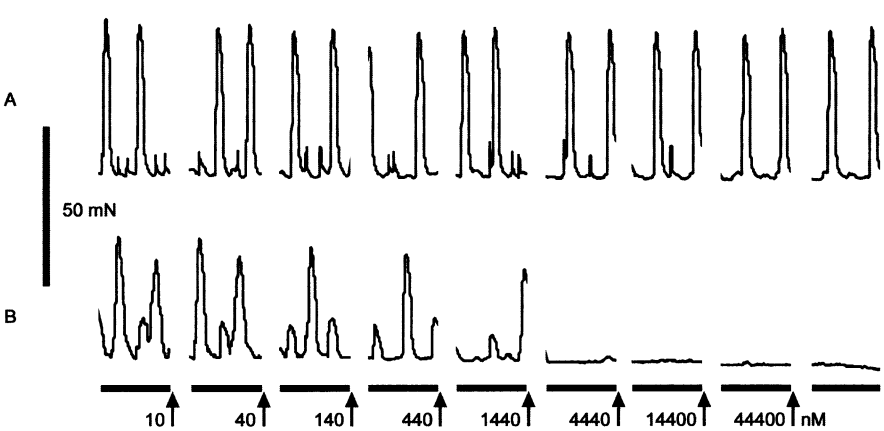

Fig. 2. Original tracing of the effect of cumulative addition of butaprost on cloprostenol-induced contractile activity of human myometrium from a non-pregnant donor. (A) Time-matched control strip, (B) agonist-treated strip. Horizontal bars indicate 10-min data collection periods, the first of which is the control. Butaprost was added to the treated strip at the points indicated by the arrows to give the cumulative concentrations shown. The time-base of the recordings is exactly as collected. However, the 3-min equilibration times have been removed from the traces in order to clarify the drug-induced effects.

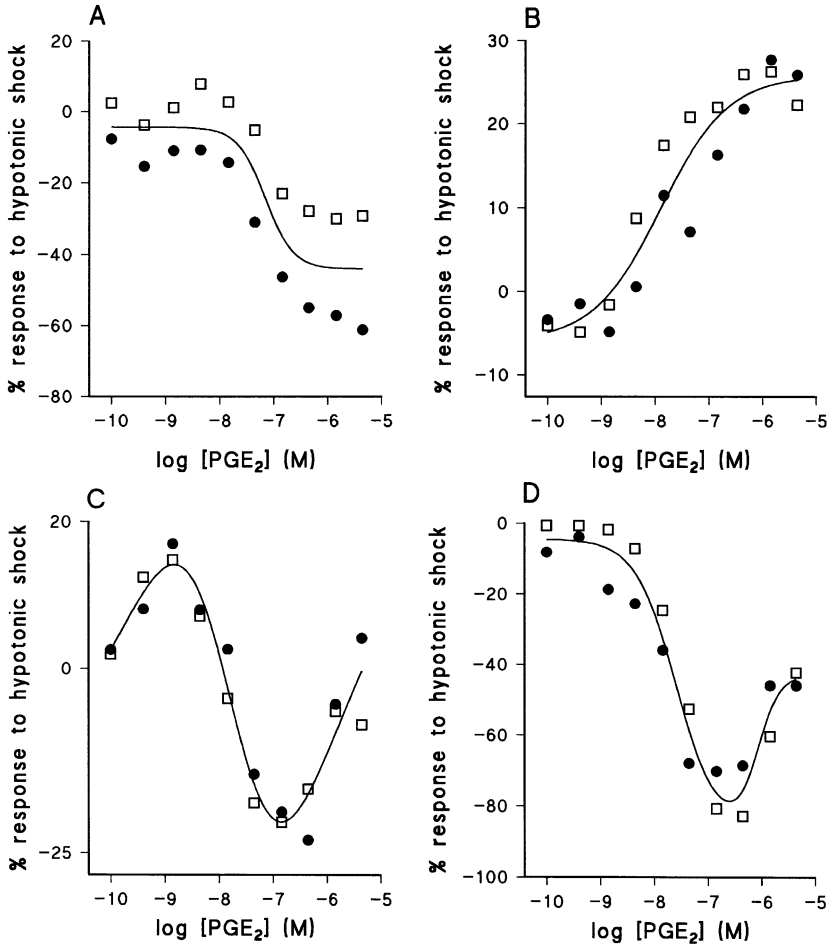

Fig. 3. Representative concentration–effect curves for prostaglandin E_2 on contractile activity of paired strips (● and □) of human non-pregnant myometrium. Four types of responses were observed between donors: (A) monophasic inhibitory; (B) monophasic excitatory; (C) biphasic$^{(E/I)}$ (excitation followed by inhibition); and (D) biphasic$^{(I/E)}$ (inhibition followed by excitation). The biphasic response shown in (D) occurred in only one donor tissue. Monophasic and biphasic curves were fit to Eqs. (1) and (2), respectively. Each curve represents data obtained from one donor.

tissue. The types of responses seen in Fig. 3C appear to have three components, but are best fit by a two component model. Monophasic responses significantly fit a one-receptor model, whereas most biphasic responses significantly fit the two-receptor model of Szabadi (1977). Mean concentration–effect curve parameters for the four types of prostaglandin E_2 responses are shown in Table 1.

Regardless of the type of prostaglandin E_2 response, sulprostone responses were excitatory and butaprost responses were inhibitory in all donor tissues. Fig. 4 shows mean concentration–effect curves for sulprostone and butaprost. A downturn in the concentration–effect curves constructed for sulprostone was often observed at high concentrations. In lack of an appropriate model, data points

Table 1
Concentration–effect curve parameters for the four types of prostaglandin E_2 responses observed in human non-pregnant myometrium in vitro

Prostaglandin E_2 response	Excitatory phase			Inhibitory phase		
	$p\mathrm{EC}_{50}$	k	E_{max}	$p\mathrm{EC}_{50}$	k	E_{max}
Monophasic inhibitory ($n=7$)	–	–	–	7.7 ± 0.2	-7.6 ± 1.9	-30 ± 8
Monophasic excitatory ($n=2$)	8.5 ± 0.6	2.4 ± 0.8	38 ± 12	–	–	–
Biphasic$^{(E/I)}$ ($n=4$)	7.8 ± 0.2	1.9 ± 0.6	120 ± 23	7.5 ± 0.1	-3.0 ± 0.5	-113 ± 16
Biphasic$^{(I/E)}$ ($n=1$)	6.1	4.9	61	7.6	-2.7	-66

Monophasic and biphasic responses were fit to a one-receptor and two-receptor model, respectively, as described in Section 2. E_{max} values are expressed as a percentage of the tissue's contractile response to hypotonic shock. $p\mathrm{EC}_{50}$ is the negative log of the molar concentration of the agonist that produces a half-maximal response, k is the power coefficient and E_{max} is the maximum agonist-induced effect. Data are means ± S.E.M.

within this component of the curve were excluded in the determination of pEC_{50} values. We performed similar experiments, using the same protocol, but different agonists, on tissues from different donors from those used in Section 2.3.1. Similar types of responses to those produced by sulprostone were obtained to the excitatory agonists fluprostenol, arginine vasopressin and U46619 which had pEC_{50} values of 8.6 ± 0.3, 8.6 ± 0.1 and 7.0 ± 0.2 and E_{max} values of $48 \pm 14\%$, $74 \pm 3\%$, and $65 \pm 8\%$ of that to hypotonic shock, respectively ($n = 3$).

Tissue sensitivities to sulprostone and butaprost were variable between the 14 donor tissues. The pEC_{50} values for sulprostone ranged from 8.1 to 10.1, while the pEC_{50} values for butaprost ranged from 5.0 to 6.9. Table 2 shows a comparison of the mean concentration–effect curve parameters for sulprostone and butaprost between tissues

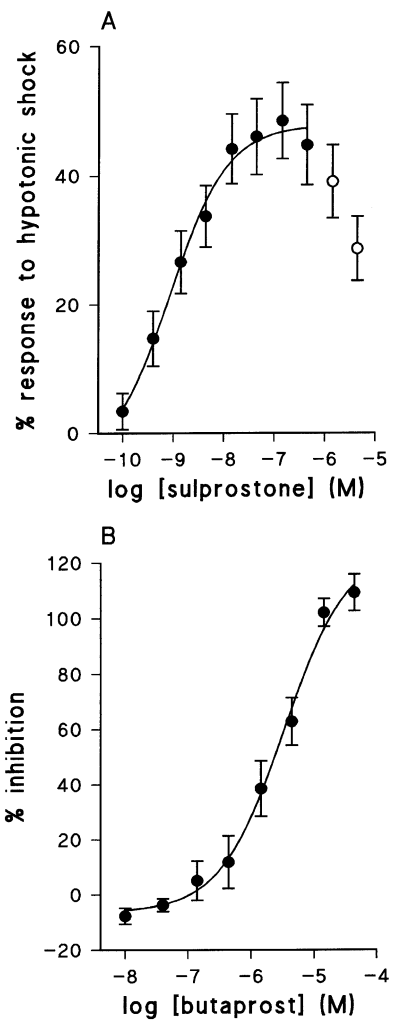

Fig. 4. Concentration–effect curves for (A) sulprostone on spontaneous contractile activity and (B) butaprost on cloprostenol-stimulated activity of human non-pregnant myometrium in vitro. Each point is the mean with S.E.M. of 14 determinations. Data points within the downturn of concentration–effect curves for sulprostone (○) were not included in the determination of pEC_{50} values.

Table 2
Effects of sulprostone and butaprost in tissues responding differently to prostaglandin E_2 in human non-pregnant myometrium

PGE_2 response	Sulprostone			Butaprost		
	pEC_{50}	k	E_{max}	pEC_{50}	k	E_{max}
Inhibitory ($n = 7$)	8.8 ± 0.2[a]	2.4 ± 0.4	50 ± 12	5.8 ± 0.2	5.8 ± 1.8	111 ± 7
Excitatory ($n = 7$)	9.4 ± 0.2[a]	2.1 ± 0.4	54 ± 6	5.5 ± 0.1	4.0 ± 0.3	115 ± 13

Sulprostone E_{max} values are expressed as a percentage of the tissue's contractile response to hypotonic shock. Butaprost E_{max} values are expressed as percentage inhibition as described in Section 2. pEC_{50} is the negative log of the molar concentration of the agonist that produces a half-maximal response, k is the power coefficient and E_{max} is the maximum agonist-induced effect. Data are means ± S.E.M.

[a] Significantly different, Student's t test ($P < 0.05$).

where prostaglandin E_2 caused an excitatory effect and tissues where prostaglandin E_2 caused only inhibition. The mean pEC_{50} value for sulprostone was significantly greater in tissues that responded to prostaglandin E_2 with excitation. Mean pEC_{50} values for butaprost were not significantly different between these groups.

4. Discussion

Variable responses to agonists may be ascribed to differences in tissue sensitivity that coincide with anatomical orientation (Kenakin, 1984). Such anatomical gradients of responsiveness have been demonstrated in a variety of tissues, including the human myometrium (Wikland et al., 1984). Wikland et al. (1984) showed differences in myometrial responses to prostaglandins between strips obtained from upper and lower uterine segments during active, spontaneous labour. Inter-donor variability in responses to prostaglandin E_2 in human non-pregnant myometrium has also been reported previously (Senior et al., 1991; Popat and Crankshaw, 1997) and might be explained by differences in the anatomical location from which myometrial samples were obtained. The primary objective of the present study was to determine whether tissue variability in responses to prostaglandin E_2 in human non-pregnant myometrium occurs independently of anatomical site. Therefore, we studied the effects of prostaglandin E_2 on 14 donor tissues obtained from the mid-lateral wall of the uterus. Our observation of four different types of responses to prostaglandin E_2 indicates that factors regulating agonist sensitivity vary between donor tissues and are operative within a single anatomical site. These results are in contrast to those obtained for the TP receptor in the same preparation (Senchyna and Crankshaw, 1999) where responses were constant within the same site.

Our next objective was to determine whether the observed monophasic responses to prostaglandin E_2 were due to the absence of either the excitatory or inhibitory EP

receptor-mediated pathway. For example, a tissue responding to cumulative additions of prostaglandin E_2 in a monophasic inhibitory fashion may lack expression of excitatory EP receptor subtypes (Crankshaw and Dyal, 1994). Similarly, monophasic excitatory responses to prostaglandin E_2 may arise from the absence of the inhibitory EP receptor subtype. To investigate this issue it was necessary to isolate pharmacologically the excitatory (EP_1 and EP_3) and inhibitory (EP_2) pathways. Because of the lack of sufficiently selective antagonists, the pathways were isolated using selective agonists. Sulprostone stimulates both EP_3 and EP_1 receptors but produces excitatory concentration–effect curves that are best fit by a single site model, suggesting that sulprostone does not discriminate between EP_1 and EP_3 receptors in non-pregnant human myometrium. Butaprost is a selective EP_2 receptor agonist. Our observation that sulprostone produced contraction and butaprost produced relaxation in all tissues suggests both excitatory and inhibitory EP receptor-mediated pathways are always operative in human non-pregnant myometrium. Therefore, monophasic prostaglandin E_2 responses do not indicate the presence of only one receptor pathway, and the simple hypothesis that monophasic responses arise from tissues operationally expressing a single receptor subtype is untenable.

Based on the evidence that both excitatory and inhibitory EP receptor populations are present, we attempted to fit all prostaglandin E_2 responses to the two-receptor model. This theoretical model, presented by Szabadi (1977), provides an applicable and comprehensive treatment of one-agonist-two-receptor systems and has proven very useful in the interpretation and analysis of bell-shaped and biphasic curves (Leff, 1994). In our study, biphasic prostaglandin E_2 responses were significantly fit to the Szabadi model. By using this model, we were able to estimate concentration–effect curve parameters for both the contractile and relaxant components of the curve by separating the excitatory and inhibitory phases. The estimated E_{max} values for biphasic prostaglandin E_2 curves appear inflated, and whether they reflect the true maximal responses within the tissue's capability is questionable, particularly since E_{max} values for sulprostone concentration–effect curves were relatively constant. The E_{max} values for monophasic excitatory and monophasic inhibitory prostaglandin E_2 responses were much lower compared to the estimated E_{max} values for biphasic prostaglandin E_2 responses. These depressed E_{max} values may reflect the activity of the opposing receptor population, which is separated when estimating parameters using the theoretical two-receptor model.

In cases where the response to prostaglandin E_2 was monophasic, we were unable to achieve a statistically significant fit to the two-receptor model. While our sulprostone and butaprost data clearly indicate the presence of two opposing receptor populations, the inability to fit monophasic prostaglandin E_2 curves to the two-receptor model suggests limitations in the resolving power of our experimental design and/or analysis. A significant fit to the two-receptor model might be achieved if we were able to obtain more data points for monophasic concentration–effect curves. However, the length of an experiment required to achieve such resolution would sacrifice the viability of the tissue and therefore is not possible logistically.

Since our data indicate the presence of both receptor pathways, monophasic responses to prostaglandin E_2 may result from differences in the relative number of EP receptor subtypes or differences in the efficiency of receptor–effector coupling (Crankshaw and Dyal, 1994; Europe-Finner et al., 1994). To investigate this possibility, we compared the mean potencies of sulprostone and butaprost between tissues that responded differently to prostaglandin E_2. The significant difference in the potency of sulprostone, but not of butaprost, between tissues that responded to prostaglandin E_2 with some excitation compared to only inhibition, suggests that EP receptor-mediated responses are regulated through the excitatory (EP_1 and EP_3) pathway (Popat and Crankshaw, 1997). Similar findings were reported by Senior et al. (1991, 1993) who showed sulprostone to be 10 times more potent in myometrium from non-pregnant donors compared to pregnant donors, with no difference in the potency of butaprost. However, in our study there was a wide range in both butaprost and sulprostone pEC_{50} values between the 14 separate donors. Although there was no significant difference in the potency of butaprost between the two groups of tissues that responded differently to prostaglandin E_2, the overall variability in butaprost potency may indicate some subtle regulation within the inhibitory pathway. Such regulation of EP receptor-mediated responses may be governed by clinical parameters such as phase of the menstrual cycle, parity, and age of the donor. Further studies are required to determine if these factors influence the variability in responses to prostaglandin E_2.

In conclusion, inter-donor variability in responses to prostaglandin E_2 in non-pregnant human myometrium occurs independently of the anatomical site of the tissue sample. Both excitatory and inhibitory EP receptor-mediated pathways are always operative, regardless of how the tissue responds to prostaglandin E_2. Regulation of EP receptor-mediated events appears to predominate in the excitatory (EP_3 or EP_1 receptor) pathway rather than the inhibitory (EP_2 receptor) pathway. The exact site at which this regulation occurs awaits further investigation.

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