Anti-inflammatory and relaxatory effects of prostaglandin E₂ in myometrial smooth muscle

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The onset of human labour is complex and involves multiple mediators, prostaglandins, cytokines and chemokines. However, whilst prostaglandins are routinely used for labour induction and inhibitors of prostaglandin synthesis are used to prevent pre-term labour, these practices are not invariably successful, and the rationale for their use is equivocal. As COX-2 and prostaglandin E₂ (PGE₂) production is increased towards term, we have investigated the effect of PGE₂ and other cAMP-elevating agents on events associated with labour induction. Time-dependent increases in granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-8 (IL-8) release were observed following treatment of primary human myometrial smooth muscle (HMSM) cells with IL-1β, via mechanisms that required de novo transcription and translation. Prior treatment with PGE₂ (1 μM) produced 86 and 80% decreases in GM-CSF and IL-8 release, respectively. Similarly, the cAMP analogue, 8-bromo-cAMP (8Br-cAMP) and the phosphodiesterase-4 (PDE4) inhibitor, rolipram, also repressed GM-CSF and IL-8 release. In addition, PGE₂, 8Br-cAMP, rolipram and salbutamol all had a dose-dependent inhibitory effect on spontaneous myometrial contractions in vitro. In this study, PGE₂ reduced the release of factors associated with cervical ripening and attenuated force development in myometrial smooth muscle, raising the possibility that in myometrium, PGE₂ may act to down-regulate some of the processes that contribute to the onset of human labour and may be beneficial in helping to maintain pregnancy towards term.

Key words: contractility/cytokine/myometrial smooth muscle/prostaglandins

Introduction

Despite the introduction of numerous tocolytic interventions since the mid-1950s, delivery, following premature labour, remains the largest cause of neonatal mortality and morbidity worldwide (Goldenberg et al., 2003) observed in human myometrial tissue with labour, suggests there is a co-ordinate up-regulation of genes for the increased output of PGE₂ associated with labour. Indeed, there is mounting evidence in other systems that specific PGES isoforms co-ordinately regulate with other systems that specific PGES isoforms co-ordinately regulate with

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Introduction

Despite the introduction of numerous tocolytic interventions since the mid-1950s, delivery, following premature labour, remains the largest cause of neonatal mortality and morbidity worldwide (Goldenberg et al., 2003). Indeed, these are the desired clinical effects following administration of PGE analogues in obstetric practice. However, despite widespread use, the induction of labour with prostaglandins can be unsuccessful with a significant proportion of women requiring a caesarean section because of the failure of the induction process (Rayburn and Zhang, 2002; Ben-Haroush et al., 2004; Vrouenraets et al., 2005).

Conversely, inhibitors of prostaglandin synthesis, especially COX inhibitors, have been utilized to prevent, or more usually delay pre-term labour (Zuckerman et al., 1974; Sawdy et al., 1997, 2004). However, the use of these compounds does not invariably delay the onset of labour and is not without an increased risk of serious fetal side effects (Hendricks et al., 1990; Norton et al., 1993; Eronen et al., 1994; Holmes and Stone, 2000). It is now clear that prostaglandins also have a fundamental role in normal fetal growth and development (Mitchell and Olson, 2004).

Prostaglandin synthesis first involves the release of arachidonic acid, which is converted to prostaglandin H₂ (PGH₂) by one of two cyclooxygenase enzymes, COX-1 or COX-2. The conversion of PGH₂ into biologically active prostaglandins is mediated by specific synthase enzymes. Prostaglandin F₂α (PGF₂α), in particular, is produced in large quantities by fetal membranes, and postulated roles include cervical ripening and the stimulation of uterine contractions (Skinner and Challis, 1985; Challis et al., 2000). Indeed, these are the desired clinical effects following administration of PGE analogues in obstetric practice. However, despite widespread use, the induction of labour with prostaglandins can be unsuccessful with a significant proportion of women requiring a caesarean section because of the failure of the induction process (Rayburn and Zhang, 2002; Ben-Haroush et al., 2004; Vrouenraets et al., 2005).

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The cellular effects of prostaglandins are dependent on the nature of the receptor and the second messenger system to which that receptor is coupled. PGE₂ exhibits a particularly wide spectrum of physiological actions as there are four known, pharmacologically distinct, PGE₂ receptor (EP) subtypes EP₁-₄ (Coleman et al., 1994; Negishi et al., 1995). All four EP receptors are present in pregnant human myometrium (Leonhardt et al., 2003; Astle et al., 2005).

In addition to increased PG synthesis, parturition also involves the production of inflammatory cytokines and chemokines, such as interleukin-8 (IL-8), IL-1 and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Romero et al., 1992; Bry et al., 1997; Elliott et al., 2000). These factors promote the recruitment, activation and maintenance of inflammatory cells, such as macrophages, important for preparation of the uterus and cervical ripening prior to labour onset (Bowen et al., 2002). Such events are also seen in pre-term labour and may be induced as a result of infection and subsequent release of pro-inflammatory cytokines, including IL-1 and IL-β (Osman et al., 2003). Furthermore, IL-1β induces the release of both IL-8 and PGE₂ in amnion (Elliott et al., 2001).

Whilst historically, increased prostaglandin synthesis has been viewed as pro-inflammatory, it is becoming increasingly clear that prostaglandins may be also beneficial in the resolution of inflammation and may act to relax smooth muscle and prevent inflammatory gene expression in other physiological settings (Gilroy et al., 1999; Clarke et al., 2004).

The aims of this study were to investigate the effect of PGE₂ on (i) the production of GM-CSF and IL-8 stimulated by IL-1β in human myometrial smooth muscle (HMSM) cells and (ii) spontaneously contracting term human myometrial strips in vitro.

Materials and methods

Tissue collection

Human myometrial samples were taken at term (38–40 weeks) elective caesar- ean section, from women not in labour, with no regular contractions, intact fetal membranes and without underlying disease, having given informed consent and with local ethical committee approval (CREC049/09/08). Indications for caesarean section delivery included breech presentation, previous section and maternal request.

Biopsies, taken from the lower edge of the upper part of the incision in the lower uterine segment, were utilized for isolation of myometrial smooth muscle cells or contractility studies.

Isolation and culture of HMSM cells

HMSM cells were isolated from term no labour (TNL) myometrial biopsies, as previously described (Tribe et al., 2000). Briefly, small segments of myometrium were washed in Hanks balanced salt solution (Sigma, Poole, UK) and then incubated at 37°C for 30 min in Dulbecco’s modified Eagle’s medium (DMEM) containing 1 mg/ml collagenase IA and 1 mg/ml collagenase XI (Sigma). Cells were dissociated by triturating through a sterile Pasteur pipette, filtered through a 45 μm sterile filter and further digestion stopped by adding 10% fetal calf serum (FCS) (Invitrogen Life Technologies, Paisley, UK) in DMEM. Following digestion, cells were washed in Hanks balanced salt solution (Sigma, Poole, UK) and then incubated at 37°C in 5% CO₂ for 30 min in Dulbecco’s modified Eagle’s medium (DMEM) supplemented 10% FCS, penicillin–streptomycin (100 U/ml) and amphotericin B (2 μg/ml) (Sigma). HMSM cells (2000–4000 cells/cm²) were cultured in 75 cm² culture flasks and incubated at 37°C in 5% air/5% CO₂ and maintained as a primary culture until confluent (initial culture took on average 7 days in keeping with other groups (Dalrymple et al., 2002; Ciontea et al., 2005). The culture medium was changed every 2 days. Cells were seeded on to 6-well plates and used at 95% confluence. Myometrial cells were identified by positive immunohisto-chemical staining with a monoclonal smooth muscle α-actin antibody. All experiments in this report were performed with myometrial cells between three and six passages. Prior to experiments, cells were serum deprived overnight (DMEM, 0.5% FCS) before changing to fresh serum-deprived media containing the cytokines and drugs. Initial studies included dose-response and time-course experiments (data not shown) to determine the parameters for subsequent studies. IL-1β (R&D System, Abingdon, Oxon, UK) was used at a concentration of 1 ng/ml; all other drugs were obtained from Sigma and were utilized at the following concentrations unless otherwise stated—actinomycin D (10 μg/ml), cycloheximide (10 μg/ml), PGE₂ (1 μM), 8-bromo-cAMP (8Br-cAMP) (1 mM), rolipram (30 μM), salbutamol (10 μM) and indomethacin (10 μM).

Measurement of GM-CSF and IL-8

HMSM cells were incubated in serum-deprived media (control) or media containing drugs for 20 h. GM-CSF and IL-8 released into the culture supernatant was quantified by a sandwich enzyme-linked immunosorbent assay (ELISA) (human DuoSet®, R&D Systems Europe, Abingdon, Oxon, UK), according to the manufacturer’s instructions.

Measurement of myometrial contractile activity

Myometrial tissue was placed in Krebs–Henseleit solution (18 mM NaCl, 25 mM NaHCO₃, 4.8 mM KCl, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄ 11.1 mM glucose, 1.25 mM CaCl₂, pH7.4) and gassed with 5% CO₂ balanced air at 37°C. Longitudinal strips (1 × 1 × 10 mm), adjacent to the serosa, were cut and mounted in organ baths for isometric force recording under 2 × g of tension.

Force was measured with FT03C transducers (Grass Instrument Co. Quincy, MA, USA) and recorded digitally using MacLab running Chart software (AD Instruments, Hastings, UK). Strips that failed to spontaneously contract (<5%) during a 90 min equilibration period were discarded. Basal activity was recorded for 30 min prior to the cumulative addition of drugs at 30 min intervals. Time and vehicle controls were performed in parallel for each patient.

Activity integral (integrated area under the contraction-times curve), peak increase in tension and duration (measured at 20% peak amplitude) were analysed for individual contractions at each concentration and expressed as a percentage of the basal activity. Frequencies of contraction were also determined for each 30 min period.

RT–PCR of EP receptors

RT–PCR was used to determine which of the EP receptors were expressed in the HMSM cell cultures utilized. EP receptor expression was determined in non-stimulated (NS) cells and cells treated with IL-1β (1 ng/ml) for 6 h. In addition, as a positive control, RT–PCR was performed on RNA isolated from human myometrial tissue collected at delivery, as this tissue had previously been shown to express all four EP receptors (Leonhardt et al., 2003; Astle et al., 2005). For each sample analysed, a negative RT control reaction was also performed, in which the reverse transcriptase enzyme was absent, to control for possible contamination by genomic DNA.

RNA was isolated using a SV Total RNA Isolation System (Promega, Southamp ton, UK). RT–PCR was used for semiquantitative analysis of RNA expression. RT was carried out using random hexanucleotide primers, and the resultant cDNA was used as template for PCR, using gene-specific primers. Total RNA (100 ng) was first denatured at 70°C for 5 min, followed by RT with Superscript II ( Gibco BRL, Paisley, Scotland, UK) at 70°C for 60 min. The resultant cDNA was utilized for subsequent PCR amplification. PCR primers (5’-3’), annealing temperature and product sizes were EP₁ (accession no. NM000955) AGGACACTGTCTGCTGGGCTT (sense), TGGCCACATCCCTCTCAC (anti-sense) (57°C; 295 bp), EP₂ (accession no. NM000957) GCCATCCGCTGCTGCT (sense), TCAAAAGTGCCGGTGCCTTAC (anti-sense) (60°C; 1065 bp); generic EP (accession no. NM000957) CTGGTATGGCCACGATTGA (sense), TGAAGGCGGCGGAAACCT (anti-sense) (55°C; 521 bp); EP₃ (accession no. NM000958) TCTGACCTCGCTGTCACAAATCG (sense), TGGTGACTGGACGGCCTT (anti-sense) (60°C; 713 bp). Cycling parameters were denaturing, 94°C, 30 s, annealing 30 s at primer-specific annealing temperature; extension, 72°C, 30 s for 35 cycles and followed by a 72°C, 5 min extension. Following amplification, PCR products were analysed by agarose gel electrophoresis, subcloned into the pGEM-T Easy vector (Promega) and verified by sequencing.

Statistical analysis

Results are expressed as means ± SEM. Statistical analysis was performed by analysis of variance (ANOVA) followed by a Bonferroni post-test. In each case, P < 0.05 was considered statistically significant. * is P < 0.05, ** is P < 0.01 and *** is P < 0.001.
Results

IL-1β induces a transcription- and translation-dependent release of GM-CSF and IL-8

Preliminary concentration-response analysis revealed a dose-dependent increase in the production of both GM-CSF and IL-8 from HMSM cells in response to 6 and 20 h treatment with IL-1β (data not shown). In each case, maximal responses were observed at 10 ng/ml IL-1β. Subsequently, 1 ng/ml, which was just sub-maximal, at 20 h, was used for all stimulation experiments. Treatment with IL-1β (1 ng/ml), significantly, induced the release of GM-CSF and IL-8 from cultured HMSM cells when compared with control. In each case, this response was prevented by co-treatment with either actinomycin D (**P < 0.01) or cycloheximide (*P < 0.05), indicating a requirement for de novo transcription and translation (Figure 1A and B). Cell viability was not affected at the time points utilized in these experiments (data not shown).

Effect of PGE2 and cAMP-elevating agents on IL-1β-induced cytokine release

As PGE2 levels are increased in amniotic fluid, during pregnancy and labour, we examined the effect of PGE2 on cytokine release from HMSM cells. Cells treated with PGE2 in the presence or absence of IL-1β revealed a profound inhibition of both GM-CSF (**P < 0.001) and IL-8 (***P < 0.005) release, whereas incubation with PGE2 alone was without effect (Figure 2A and B).

In airway smooth muscle, the ability of PGE2 to repress cytokine elaboration has been shown to occur via the cAMP-signalling pathway and to be mediated via the EP2 and EP4 prostanoid receptors (Clarke et al., 2004). HMSM cells were, therefore, treated with the non-hydrolysable cAMP analogue, 8Br-cAMP in the presence and absence of IL-1β. As with PGE2, 8Br-cAMP alone produced no effect on cells but did substantially repress the IL-1β-induced release of GM-CSF (**P < 0.001) and IL-8 (***P < 0.005) (Figure 2). An alternative means of raising intracellular cAMP levels is to inhibit the phosphodiesterase (PDE) enzymes that are responsible for the rapid degradation of intracellular cAMP. In this respect, inhibitors of PDE4 are known to repress the production of inflammatory cytokines, and previous studies have identified that PDE4 is the predominant PDE isofrom present in human pregnant myometrium (Leroy et al., 1994). Therefore, we tested the effect of the PDE4 inhibitor, rolipram, on IL-1β-induced GM-CSF and IL-8. In each case, a significant inhibition of cytokine release was observed (GM-CSF, **P < 0.005, and IL-8, *P < 0.005), and there was no effect on unstimulated cells (Figure 2). Finally, α2-adrenoceptor agonists elicit their biological effects via the α2-adrenoceptor, which is coupled via Gβγ to adenyl cyclase, and therefore cAMP elevation (Johnson, 1998). Salbutamol did not significantly affect the IL-1β-induced GM-CSF and IL-8 release (71.8 ± 20.9 and 51.8 ± 15.9%, respectively) (Figure 2).

No effect of indomethacin on GM-CSF and IL-8 expression

The above analysis clearly shows that PGE2 may repress IL-1β-induced expression of GM-CSF and IL-8. We were, therefore, interested to investigate the possibility that autocrine feedback by PGE2, or other prostanoids, released from HMSM cells was modulating GM-CSF or IL-8 expression. Cells were incubated in the presence or absence of indomethacin (10 μM) prior to stimulation with IL-1β. In each case, no obvious change in GM-CSF or IL-8 expression was observed, suggesting that autocrine feedback of prostanoids was not an issue in this system (Table I).
Effect of indomethacin on release of granulocyte-macrophage colony-stimulating factors (GM-CSFs) and interleukin-8 (IL-8) from primary human myometrial smooth muscle (HMSM) cells

<table>
<thead>
<tr>
<th>Treatments</th>
<th>GM-CSF (ng/ml)</th>
<th>IL-8 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No stimulation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No indo</td>
<td>7.2 ± 5.2</td>
<td>0</td>
</tr>
<tr>
<td>+ indo</td>
<td>7.9 ± 4.9</td>
<td>174.7 ± 72.6</td>
</tr>
<tr>
<td>IL-1β</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No indo</td>
<td>493.8 ± 132.0</td>
<td>170.6 ± 68.8</td>
</tr>
<tr>
<td>+ indo</td>
<td>381.4 ± 89.1</td>
<td></td>
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</tbody>
</table>

Cells were pretreated for 60 min with indomethacin (10 μM) before either no stimulation or stimulation with IL-1β (1 ng/ml). After 20 h, supernatants were harvested, and the enzyme-linked immunosorbent assay (ELISA) performed for GM-CSF and IL-8. Data (n = 5) are given as means ± SEM.

**Effect of 8Br-cAMP, rolipram and salbutamol on spontaneous myometrial contractions**

Cumulative addition of 8Br-cAMP produced a concentration-dependent inhibition of peak tension and activity integral to 8 ± 3% (P < 0.05) and 9 ± 4% (P < 0.05) of the original values, respectively, produced with 1 mM (Figure 4). The apparent effects on duration and frequency failed to reach a level of significance (64 ± 22%, P > 0.05, and 54 ± 21%, P > 0.05, respectively). Similarly, the cumulative addition of rolipram caused a concentration-dependent inhibition of the peak tension and activity integral to 39 ± 15% (P < 0.05) and 32 ± 11% (P < 0.05) of the original values, respectively, at 10 μM (Figure 5A). Likewise, the repression of peak tension and activity integral elicited by salbutamol reached 27 ± 12% and (P < 0.05) 12 ± 5% (P < 0.05), respectively, at 10 μM (Figure 5B). Effects on duration or frequency for both rolipram and salbutamol failed to reach significance (data not shown).

**EP receptor expression in HMSM cells**

The data above strongly support multiple functional roles for the Gs-linked PGE2 receptors, EP2 and EP4 in HMSM. Our HMSM cells were, therefore, tested for the expression of EP1-4 using RT-PCR. Analysis using primers that are selective for the generic forms of EP1-4 revealed that all four receptor subtypes were present in HMSM cells following treatment with IL-1β for 6 h, whereas in untreated control cells only expression of EP2–EP4 could be detected (Figure 6). No products were observed in lanes corresponding to the reverse transcriptase negative control indicating that there was no contamination with genomic DNA.

**Discussion**

This study examined the possible functional significance of increased PGE2 treatment of HMSM cells results in a significant and reproducible time-dependent increase, in both GM-CSF and IL-8 release, by a mechanism requiring de novo transcription and translation. However, prior incubation with PGE2 substantially repressed the IL-1β-stimulated GM-CSF and IL-8 release by the HMSM cells, suggesting that PGE2 acting on the HMSM cells may have an anti-inflammatory action. This anti-inflammatory effect of PGE2 has not previously been described in myometrial smooth muscle cells. We have also shown using in vitro contractility studies that PGE2 inhibited various parameters of spontaneous contractility in terms of non-laboured myometrial strips, suggesting that the administration of PGE2 may be expected to relax the myometrium.

PGE2 exhibits a wide spectrum of physiological actions depending on the distribution and subtype of EP receptors present (Breyer et al., 2001). All four PGE2 receptors EP1-4 are present in human myometrial tissue during pregnancy, with distinct cellular patterns of expression being observed. Expression of EP1-4 receptor proteins are localized to vascular smooth muscle, vascular endothelium, glandular epithelium and decidual components of myometrial tissue. Myometrial smooth muscle cells also express EP2 and EP4, whereas EP1 protein expression has variously been described as relatively low (Astle et al., 2005) or absent (Leonhardt et al., 2003). It has also been suggested that EP receptor mRNA expression may be temporally expressed with respect to gestation and labour onset in human (Matsumoto et al., 1997; Brodt-Eppley and Myatt, 1999; Leonhardt et al., 2003). It has also been suggested that EP receptor mRNA expression may be temporally expressed with respect to gestation and labour onset in human (Matsumoto et al., 1997; Brodt-Eppley and Myatt, 1999; Leonhardt et al., 2003; Astle et al., 2005). It has also been suggested that EP receptor mRNA expression may be temporally expressed with respect to gestation and labour onset in human (Matsumoto et al., 1997; Brodt-Eppley and Myatt, 1999; Leonhardt et al., 2003). It has also been suggested that EP receptor mRNA expression may be temporally expressed with respect to gestation and labour onset in human (Matsumoto et al., 1997; Brodt-Eppley and Myatt, 1999; Leonhardt et al., 2003). However, there is no general consensus as to whether or not there is a definitive up-regulation of excitatory EP1 and EP3 or a down-regulation of EP2 or EP3 in association with labour. Erkinheimo et al. (2000) previously found that EP2 and EP4 mRNAs were expressed in HMSM cultures but that EP3 expression was observed only after stimulation with IL-1β. They were unable to detect EP1 or EP3 by northern analysis. Similar to this, we show EP3 mRNA expression, in...
Anti-inflammatory effects of PGE₂

HMSM cells, increased with IL-1β treatment, compared with untreated cells. In contrast, we show EP₁ and EP₃ mRNA expression, these observed differences may be because of sensitivity of the assay as RT–PCR is more sensitive compared with northern analysis.

Functionally, EP₁ and EP₃ couple to G proteins, G_qα or G_iα, and promote calcium influx or the inhibition of adenylate cyclase, respectively (Breyer et al., 2001; Bos et al., 2004). The stimulation of EP₁ and EP₃ would, therefore, result in the contraction of smooth muscle or a potentiation of inflammatory outputs. By contrast, EP₂ and EP₄ couple to G_sα to increase adenylyl cyclase activity and intracellular cAMP levels, leading to smooth muscle relaxation and repressed the expression of many inflammatory cytokines (Breyer et al., 2001; Regan, 2003). Indeed, observations in our studies suggest that the EP₂ and/or EP₄ rather than the EP₁ and/or EP₃ pathways predominate, at

Figure 3. Effect of prostaglandin E₂ (PGE₂) on spontaneous myometrial contractions. Spontaneously contracting strips of myometrium were treated with increasing concentrations of PGE₂. (A) Representative tracing of tension generated against time showing the effect of cumulative addition of PGE₂ or appropriate vehicle control. (B) Data (peak tension, integral activity, duration and frequency) from experiments conducted in strips from six patients were plotted as a percentage of the response at the start of the experiment (without PGE₂ present) and expressed as means ± SEM. For each patient, strips were incubated in parallel with (●) and without (○) PGE₂ (*P < 0.05).
least in myometrial tissue and HSM cultures isolated from non-laboured term patients and in respect of the function responses measured in this study. This mode of action is supported by our findings that the cAMP analogue, 8Br-cAMP, the PDE4 inhibitor, rolipram, and salbutamol, which also couples to G\(\alpha\) and elevates cAMP, inhibited spontaneous contractions of in vitro myometrial strips. These results are consistent with those of Bardou et al. (1999). Indeed as rolipram was the more potent inhibitor of myometrial contractions in this previous study (Bardou et al., 1999), it is possible that PDE4 inhibitors alone or in combination with \(\beta_2\)-adrenoreceptor agonists may be potentially useful as tocolytics for preterm labour. In addition to the effects on myometrial contractility, 8Br-cAMP and rolipram also repressed the IL-1\(\beta\)-induced expression of IL-8 and GM-CSF, in HSM cultures, again suggesting a functionally relevant role for EP\(_2\) and EP\(_4\) signalling.

Consistent with the fact that both contractile and relaxatory EP receptors are simultaneously expressed in myometrial tissue, the evidence that PGE\(_2\) elicits myometrial contractions is also equivocal. Thus,

In contrast, our present data suggest that the predominant PGE2-dependent responses are via relaxatory EP2 and/or EP4 pathways at least in term myometrium before labour onset.

The seemingly inconsistent nature of many of the above reports raises a number of important questions in respect of how to best explain these differences. In this study, all myometrial samples were taken at elective caesarean section from the lower segment before labour onset and may be expected to represent a relatively homogeneous sample group. These details are likely to be critical as changes in EP receptor expression and functional coupling have been reported during pregnancy and labour and may be location dependant. Thus, PGE2 acting on myometrial strips obtained during spontaneous labour evoked a stimulatory effect on upper segment strips, yet elicited a relaxatory response in lower segment strips (Wiqvist et al., 1985). Furthermore, whilst EP1 and EP3 can be shown to be functionally coupled to phospholipase C activation and rises in intracellular Ca2+ (Asboth et al., 1996), the expression of EP3 is down-regulated in 8–38 week human myometrium, and this may be expected to favour a non-contractile response to PGE2 (Matsumoto et al., 1997). In addition, EP2 is expressed at higher levels in the decidual component, compared with the myometrial cells (Leonhardt et al., 2003; Astle et al., 2005), and may, therefore, play an as yet undetermined role in pregnancy. Similarly, EP2 might be involved in mediating myometrial quiescence during pregnancy in rats, and decreased expression at term could release inhibition to promote induction of labour (Brodt-Eppley

![Figure 5. Effect of rolipram and salbutamol on spontaneous myometrial contractions. Spontaneously contracting strips of myometrium were treated with increasing concentrations of (A) rolipram or (B) salbutamol, and the effects on tension were monitored as in Figures 3 and 4. Data (peak tension and integral activity) from experiments conducted in strips from six patients were plotted as a percentage of the response at the start of the experiment (without drug present) and expressed as means ± SEM. For each patient, strips were incubated in parallel with (●) and without (○) drug present (*P < 0.05). In experiments without drug, an appropriate vehicle was added at successive time points.](image-url)
and Myatt, 1999). Conversely, our present study and others (Erkinheimo et al., 2000) document an up-regulation of EP$_{3}$ by IL-1β. This, given the increased expression of IL-1β in pregnancy, implies that an equivalent effect may also be observed in the uterus during pregnancy and would tend to promote G$_{s}$α-dependent responses. Another key factor in determining myometrial responsiveness may be alterations in the expression of G$_{s}$α itself (Europe-Finner et al., 1994). Thus, increased expression of G$_{s}$α during pregnancy may provide a mechanism to help maintain uterine quiescence, and the down-regulation of G$_{s}$α, plus possibly uncoupling from adenylyl cyclase, may provide a mechanism to unmask contractile responsiveness in labour (Europe-Finner et al., 1994). Taken together, these data imply that differential expression and localization of prostaglandin receptors may play a key role in mediating myometrial quiescence and contractility.

In conclusion, our data demonstrate that the effects of PGE$_{2}$ on human myometrium can be both anti-inflammatory and relaxatory. This contrasts with the conventional view that PGE$_{2}$ is primarily involved in labour onset and may provide an explanation for the variable response to the clinical use of prostaglandins and COX inhibitors. Whilst, PGE$_{2}$ is clinically administered to promote cervical ripening and induce labour, the data above suggest that at least some of the effects mediated by PGE$_{2}$, e.g. acting via inhibitory EP$_{3}$ and EP$_{4}$, would also be beneficial for maintenance of pregnancy. We, therefore, suggest that a more systematic approach to understanding of the mechanisms by which PGE$_{2}$ exerts its effects is required. This, in turn, may lead to the pharmacological isolation of ‘pro-labour’ actions of PGE$_{2}$ from ‘pro-pregnancy’ actions. In terms of improving labour induction and reducing the rate of deliveries by caesarean section, the potential benefits of such an approach are clear, and this could lead to the improved management of obstetric patients when compared with current treatments.

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