Loss of prostaglandin F_{2α}, but not thromboxane, responsiveness in pregnant human myometrium during labour

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Abstract

Prostaglandins (PG) E₂, PGF_{2α} and thromboxane (TX) mediate uterine contractility by targeting prostanoid EP, FP and TP receptors respectively. The aim of this study was to elucidate the function of these receptors in isolated human myometrium taken at term gestation prior to and following labour onset. Lower segment myometrial strips were immersed in organ baths in oxygenated Krebs' solution at 37 °C and connected to isometric force transducers. After equilibration, spontaneous activity and concentration responses to PGE₂, PGF_{2α} and U46619 (a stable TX mimetic) were measured as area under the curve and expressed as a percentage of the final contraction induced by hypotonic shock. Results were expressed as arithmetic means ± S.E.M. and analysed using

two-way ANOVA with Bonferroni's *post hoc* test. Myometrium excised at late gestation displayed the greatest spontaneous activity compared with the tissues taken during labour ($P < 0.001$). Excitation evoked by PGF_{2α} ($P < 0.01$) and PGE₂ at 10⁻⁵ mol/l were attenuated after labour onset. U46619 consistently stimulated concentration-dependent contractions ($P < 0.001$) and selective antagonists confirmed TP-mediated effects. The maintained responses to TX indicate crucial roles for TP receptors in the muscular tonus of the parturient uterus. This receptor and its secondary messenger system represent effective myometrial targets for tocolytic agents in both pregnancy and labour-associated disorders.

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Introduction

The genomic and biochemical mechanisms underlying the transition from uterine quiescence to activation are not fully understood. Elucidation of these mechanisms may result in better intervention to reduce the high incidence of premature births (Lumley 2003). Prostanoids appear to be critical in human parturition due to their actions on myometrial contractility and cervical ripening (Hertelendy & Zakr 2003). In late pregnancy, enhanced prostaglandin (PG) E₂ and F_{2α} biosynthesis by intrauterine tissues precedes labour onset (Gibb 1998), whilst clinical applications of PGE analogues are widely used for labour induction, cervical effacement and to maintain patency of the ductus arteriosus. Moreover, in terms of tocolysis, many prostanoid synthesis inhibitors can prolong gestation via temporary suppression of myometrial contractility (Vermillion & Landen 2001).

Functional studies on the human myometrium have characterised heterogeneous DP, EP, FP, IP and TP prostanoid receptor subtypes in both non-pregnant (Senior *et al.* 1991, 1992, Hillock & Crankshaw 1999) and term pregnant donors (Senior *et al.* 1993). These seven transmembrane G-protein-coupled receptors are classified according to their respective

affinities for the five primary prostanoids PGD₂, PGE₂, PGF_{2α}, PGI₂ and thromboxane (TX; Coleman *et al.* 1994, IUPHAR 2006). Individual genes encode a further four EP subtypes, which differ in structure, signal transduction pathways and pharmacological action. G_{αs}-coupled DP, EP₂, EP₄ and IP receptors mediate uterine relaxation via adenylyl cyclase and cyclic AMP (cAMP). By contrast, activation of EP₁, EP₃, FP and TP receptors by uterotonins either reduces intracellular cAMP or mobilises calcium to facilitate contraction (Coleman *et al.* 1994). Thereby, variations in prostanoid receptor expression and distribution throughout gestation and labour may contribute to total uterine function.

To maintain human pregnancy, the lower segment of the myometrium primarily expresses inhibitory EP₂ receptors (Senior *et al.* 1993, Brodt-Eppley & Myatt 1999) whilst EP₃ and FP receptor expression appear substantially reduced compared with uterine muscle from non-pregnant (Matsumoto *et al.* 1997) and labouring donors (Brodt-Eppley & Myatt 1999). The expression of TP receptors remains unaltered during human pregnancy (Friel *et al.* 2005a). Prior to parturition, a reduction in cAMP indicates the loss of inhibitory pathways (Europe-Finner *et al.* 1994), with reduced myometrial EP₂

receptor expression and withdrawal of PGI synthase contributing to a successful fetal delivery (Giannoulas *et al.* 2002, Astle *et al.* 2005). Even so, the functional dynamics of the prostanoid receptors have not yet been elucidated in the myometrium at parturition.

The aim of this study was to investigate the direct uterine responsiveness to PGE₂, PGF_{2α} and the stable TX mimetic U46619 in lower segment-isolated human myometrial samples taken at term pregnancy and during early (3–8.5 cm dilated) and late (9–10 cm dilated) labour. Furthermore, understanding functional PG receptor profiles during labour may enhance development of effective tocolytics.

Materials and Methods

Tissue collection

Human myometrial samples from term pregnant donors (38–40 weeks gestation) were taken from the upper margin of the uterine incision during caesarean section at the Bradford Royal Infirmary. For ethical reasons, sampling could only take place from the lower segment. Caesareans were performed because of fetal distress, breech presentation, previous section, placenta praevia, maternal request or failure to progress into labour. Myometrium was collected from women (aged 20–39 years) who were not in labour ($n=10$) or in spontaneous labour ($n=18$). Labour was defined as the presence of regular uterine contractions with early and late stages determined by cervical dilation at 3–8.5 and 9–10 cm respectively. Although objectively assessed and variable between groups, the duration of labour was relatively prolonged in fully dilated donors. Pharmacological augmentation of labour with syntocinon was only used in 3 of the 28 patients. This investigation had the approval of the Local Regional Ethics Committee (Bradford Hospital NHS Trust) and the University of Bradford Ethics Committee and all patients gave informed written consent.

Prior to immersion assays, Krebs'–Heinseleit physiological salt (Krebs') solution was freshly prepared at the following composition (mmol/l): NaCl 118.9; KCl 4.7; KH₂PO₄ 1.2; MgSO₄ 1.2; CaCl₂ 2.5, NaHCO₃ 25.0, glucose 10.0; oxygenated with 95% O₂ and 5% CO₂.

Immediately following surgery, uterine samples were placed in Krebs' solution for transport to the laboratory. The tissues were normally set-up for immersion within a 60-min post-operative period; however, some samples were stored in oxygenated Krebs' solution at room temperature for up to 18 h. Maintenance of tissue viability has been previously demonstrated in this laboratory and in others (Hillock & Crankshaw 1999, Popat & Crankshaw 2001, Hutchinson 2005) and due to similar functional myogenic responses, data from fresh and stored tissues were pooled and collectively analysed.

Immersion

Myometrial samples, free from decidua or serosa, were dissected into strips (10×3×3 mm) and mounted longitudinally in individual 8 ml water-jacketed organ baths containing oxygenated Krebs' solution (95% O₂ and 5% CO₂) at 37 °C (Hutchinson 2005). Once immersed, strips were attached to isometric force transducers and a resting tension of 2 g was applied (Senior *et al.* 1991). Samples were equilibrated for 2 h or until the development of regular spontaneous contractions.

The prostanoid agonists PGE₂, PGF_{2α} and U46619 were administered into each organ bath at 30-min intervals with concentration–effect curves (10^{−9}–10^{−5} mol/l) achieved in a cumulative manner. Time-matched vehicle studies were performed in parallel for each patient and only one concentration–effect curve was completed per tissue strip. Each n value represents data obtained from different individual donors. Responses to U46619 were also investigated in the presence of either TP receptor antagonist SQ29548 (10^{−6} mol/l) or GR32191B (10^{−6} mol/l; Moore *et al.* 2002). The immersion technique aimed to mimic *in vivo* uterine conditions.

At the end of the experiments, Krebs' solution in the organ baths was displaced by distilled water, inducing a large contraction unique to each myometrial strip (Popat & Crankshaw 2001). This hypotonic shock was used as a reference contraction, achieving reproducible contractions without activating G-protein-coupled receptors.

Recording isometric contractions

The activity of myometrial strips was measured via isometric force transducers (Grass Instrument Co., Quincy, MA, USA) linked to PowerLab hardware (AD Instruments Pty Ltd, Chalgrove, Oxfordshire, UK) and a PC (Dell Inc., Bracknell, Bucks, UK). Microsoft Chart 5 software (AD Instruments Pty Ltd) displayed traces and enabled tension changes in the tissue to be measured as an integrated area under the contraction curve. To normalise the data, a 30-min period of myogenicity after drug administration was expressed as a percentage of 30 min hypotonic shock; thereby units represented an integral of the force–time relationship.

Data analysis and statistical procedures

Data were first tested for normality using a Kolmogorov–Smirnov test. To examine the relationship between agonist concentrations and treatment, contractile activity of myometrial strips was compared using repeated measures ANOVA in a mixed model. *Post hoc* comparisons were performed using Bonferroni's adjustment. Estimates of maximal effect (E_m) and curve mid-point (EC₅₀) were calculated for U46619 and selective TP antagonists at different states of pregnancy and parturition (GraphPad Prism 4.0, San Diego, CA, USA). Results were expressed as the arithmetic mean ± S.E.M. and significance attributed at $P<0.05$.

Drugs, chemical reagents and other materials

PGE₂, PGF_{2α}, U46619 (9, 11-dideoxy-11α, 9α-methanoepoxy PGF_{2α}) and SQ29,548 ([1S-[1α,2α(Z),3α,4α]]-7-[3-[[2[(phenylamino)carbonyl]hydrazine]methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid) were obtained from Cayman Chemical (distributed by Alexis Corporation Ltd, Bingham, Notts, UK). GR32191B ([1R-[1α(z),2β,3β,5α]]-(+)-7-[5[[1,1'-biphenyl]-4-yl]methoxy]-3-hydroxy-2-(1-piperidinyl)cyclopentyl)-4-heptenoic acid, hydrochloride) was obtained from GlaxoSmithKline. PGE₂, PGF_{2α} and SQ29,548 were dissolved in ethanol, U46619 in methyl acetate and GR32191B in distilled water. Dilutions were made with 0.9% (w/v) normal saline and vehicles, matched for solvent, caused no effect on myogenicity. The agonist concentration range (10⁻⁹–10⁻⁵ mol/l) was adjusted to encompass full concentration–effect curves.

Results

Myogenic activity at term pregnancy and labour

Spontaneous contractions varied markedly between donor tissues taken at different stages of pregnancy and labour (Fig. 1). The greatest myogenic activity was exhibited by myometrial samples taken at term (39.3 ± 0.4 weeks gestation) from pregnant non-labouring donors. Myogenicity subsequently declined by 2.1- and 2.8-fold in tissue collected during the early ($P < 0.01$) and late ($P < 0.001$) stages of labour, observed by a reduction in both the frequency and amplitude of contractions with no change in baseline muscle tone. Responses to hypotonic shock were consistent regardless of gestational state (Table 1).

Effects of PGE₂, PGF_{2α} and U46619 on myogenic activity

Non-labouring donors In lower segment uteri obtained from term pregnant, non-labouring donors, PGE₂ (10⁻⁹ mol/l to 10⁻⁵ mol/l) evoked a predominant inhibitory effect on myogenicity via a reduction in the amplitude of myometrial contractions ($F(1, 54) = 35.94$; $P < 0.01$; Fig. 2a). Despite the lack of interaction between PGE₂ concentration and vehicle ($F(4, 54) = 1.81$; ns), myogenic activity was attenuated by 40% and some excitation was observed at 10⁻⁵ mol/l. In parallel myometrial strips, the spasmogens PGF_{2α} and stable TP mimetic U46619-enhanced contractions to a much greater extent than vehicle (Fig. 2b and c). PGF_{2α} elicited an excitatory monophasic response, reaching $77.1 \pm 6.3\%$ hypotonic shock ($F(4, 54) = 5.46$; $P < 0.001$). The concentration–effect of U46619 was more potent than PGF_{2α} with response to U46619 maximal at 10⁻⁶ and 10⁻⁵ mol/l ($F(4, 54) = 15.89$; $P < 0.001$). U46619 appeared to mask the upregulated contractions evoked by PGF_{2α} when added in a cumulative manner to organ baths (data not shown). The TP antagonists SQ29,548 and GR32191B (10⁻⁶ mol/l) had no effect on myogenicity and caused similar parallel rightward displacement of U46619 concentration–effect curves in myometrium taken at term pregnancy and parturition. These data are summarised in Table 2.

Early and late stage labouring donors In myometrium taken from donors at the early and late stages of labour, PGE₂ fully inhibited myogenic activity in a monophasic concentration–related manner (10⁻⁹–10⁻⁵ mol/l). Compared with vehicle, the response to PGE₂ was only significant at 10⁻⁵ mol/l during early labour ($F(1, 45) = 22.42$; $P < 0.05$) but showed no interaction between each group ($F(4, 45) = 0.48$, ns; Fig. 3a and b). By contrast to term pregnancy, with the onset of labour,

Figure 1 Spontaneous activity of isolated human myometrial samples obtained from term pregnant, non-labouring ($n = 10$), early ($n = 9$) and late stage ($n = 8$) labouring donors. Myogenicity was measured as 30 min area under the curve expressed as a percentage of 30 min hypotonic shock. Results are expressed as arithmetic means ± S.E.M with statistical significance determined by univariate analysis using *post hoc* Bonferroni's test. *** $P < 0.001$ compared with pregnant donors not in labour. Representative traces show myogenic activity in samples taken from human term-pregnant donors (a) prior to labour onset compared with, (b) early and (c) late labour.

Table 1 Hypotonic shock in isolated human myometrium taken from donors at late pregnancy (*n*=10), early (*n*=10) and late stages of labour (*n*=8). By replacing the physiological solution with distilled water, the induced hypotonic shock was unique for each myometrial strip for use as a reference contraction. Results were measured for 30 min as integrated area under the curve (g.s) and expressed as arithmetic means±S.E.M.

	Term pregnancy	Early labour	Late labour
Hypotonic shock (g.s)	2161.1±388.0	2115.5±367.4	1777.4±185.3

PGF_{2α} did not evoke a significant increase in myogenicity in analogous myometrial strips taken at early (*F* (4, 45)=2.07; ns) or late (*F* (4, 45)=0.49; ns) stages of labour. However, during early labour, two-way ANOVA showed significant effects of U46619 (*F* (4, 45)=10.70; *P*<0.001), concentration (*F* (1, 45)=42.67; *P*<0.001) and treatment–concentration interaction (*F* (4, 45)=9.91; *P*<0.001). U46619-induced substantial tissue excitation enhancing contractility to 90.8±13.3% hypotonic shock at 10^{−6} mol/l compared with vehicle (*P*<0.001). The contractions were attenuated at 10^{−5} mol/l (Fig. 3a). The concentration–effects of U46619 were weaker in myometrium from donors at late stages of labour with excitation only reaching 42.7±6.6% hypotonic shock at

10^{−5} mol/l. Even so activity, in response to U46619 (*F* (4, 45)=4.64; *P*<0.01), concentration (*F* (1, 45)=18.16; *P*<0.001) and treatment–concentration interaction (*F* (4, 45)=5.09; *P*<0.01), was greatly augmented in relation to the low amplitude and frequency of spontaneous contractions (Fig. 3b).

Discussion

In the gravid human uterus before term, low-grade epochs of myometrial activity predominate; these are exhibited as well-defined intrinsic contractions in immersed, isolated tissue

Figure 2 Concentration–effect curves and typical traces for vehicle, (a) PGE₂, (b) PGF_{2α} and (c) U46619 in myometrium from term-pregnant, non-labouring donors (*n*=6). Agonists were added in a cumulative manner at 30-min intervals. Results are expressed as arithmetic means±S.E.M. and statistical significance was determined by two-way ANOVA with Bonferroni's *post hoc* test ***P*<0.01; ****P*<0.001 for PGE₂, PGF_{2α} and U46619 compared with vehicle.

Table 2 Mean EC₅₀ values (mol/l) and maximal excitatory response (E_m) for U46619 concentration-effect curves in the absence and presence of either SQ29,548 (10⁻⁶ mol/l) or GR32191B (10⁻⁶ mol/l) in myometrium from term pregnant, non-labouring (n=6) and donors in early (n=7) and late stages of labour (n=5). Maximal responses for excitation are expressed as percentage hypotonic shock. Data were analysed using two-way ANOVA mixed model with Bonferroni's *post hoc* test and results were expressed as arithmetic means ± S.E.M.

	U46619 alone		+ SQ29,548		+ GR32191B	
	EC ₅₀	E _m	EC ₅₀	E _m	EC ₅₀	E _m
Term pregnancy	1.3 × 10 ⁻⁷ ± 9.0 × 10 ⁻⁹	133.4 ^a ± 8.1	1.1 × 10 ⁻⁶ ± 9.0 × 10 ⁻⁶	67.4 ^c ± 4.6	5.5 × 10 ⁻⁶ ± 5.1 × 10 ⁻⁶	62.2 ^e ± 9.0
Early labour	3.7 × 10 ⁻⁷ ± 1.6 × 10 ⁻⁷	106.2 ^b ± 9.6	5.2 × 10 ⁻⁶ ± 4.8 × 10 ⁻⁶	28.7 ^b ± 9.9	4.7 × 10 ⁻⁶ ± 4.8 × 10 ⁻⁶	39.3 ^d ± 14.1
Late labour	3.5 × 10 ⁻⁷ ± 1.3 × 10 ⁻⁷	56.9 ± 9.7	7.7 × 10 ⁻⁷ ± 5.1 × 10 ⁻⁷	21.0 ± 7.6	5.4 × 10 ⁻⁶ ± 5.3 × 10 ⁻⁶	20.1 ± 0.7

Excitatory responses to U46619 were significantly different in myometrium taken at late labour compared to ^aterm gestation, no labour (P<0.001) and ^bearly labour (P<0.01). Responses to U46619 were also attenuated by action of SQ29,548 in ^clate pregnancy (P<0.001) and ^dearly labour (P<0.001) with similar antagonism by GR32191B at ^eterm and ^fearly labour.

(Crankshaw 2001, Popat & Crankshaw 2001, Hutchinson 2005). At parturition, regular and forceful uterine muscle contractions develop in a caudal direction from the fundus towards the cervix. However, in this *in vitro* study, the phasic contractions of lower segment myometrial strips declined by almost threefold in frequency and amplitude with progressive labour. This may correspond to extensive *in utero* collagen tissue remodelling, which facilitates cervical effacement and dilation for delivery of the fetus (Leppert 1995).

The attenuated myogenic force in lower segment tissues during labour was consistent with the topographical changes in contractile-associated protein expression and calcium transients (Astle *et al.* 2005, Riley *et al.* 2005). For the uterus to act in synchrony, specialised pacemaker cells transduce electrical signals via gap junctions between myocytes (Kilarski *et al.* 2000, Duquette *et al.* 2005). These gap junction transcripts become more pronounced in upper rather than lower segment myometrium during labour (Sparey *et al.* 1999), facilitating the vigorous spontaneous contractions of isolated fundus myometrium towards the cervix (Griffiths *et al.* 2006). At parturition, less sensitive isoforms of calcium-activated potassium channels are expressed in isolated lower segment tissues (Curley *et al.* 2004), whilst calcium-ATPase activity is reduced with uterine dystocia (Zyrianov *et al.* 2003). Although uterine constituents are similar, myometrial stiffness was shown to increase in women suffering from dysfunctional labour (Buhimschi *et al.* 2006). These factors may contribute to the diminished intrinsic myometrial activity with prolonged labour compared with tissues from non-labouring donors.

The results of the present study show a change in the contractile activity of isolated lower segment human myometrium in response to EP, FP and TP receptor stimulation prior to and following labour onset. Most diverse was the action of PGE₂ on myogenic activity, corresponding to the heterogeneous EP₁₋₄ receptor subtypes within plasma and nuclear membranes of myometrial cells (Coleman *et al.* 1994, Bhattacharya *et al.* 1999, Leonhardt *et al.* 2003). In myometrium taken from term pregnant, non-labouring donors, PGE₂-attenuated contractions in a concentration-dependent manner followed by relative tissue excitation. This biphasic response to PGE₂ corroborates results from superfusion assays (Senior *et al.* 1993) and indicates that inhibitory receptors supersede contractile EP receptor function at term pregnancy. Stimulation of EP₃/EP₁ receptors by sulprostone confirms the existence of contractile-mediated EP receptor pathways in term pregnant myometrium (Senior *et al.* 1993), perhaps via the EP₃-VI receptor isoform (Wing *et al.* 2003). Even so, a tenfold reduction in the potency of sulprostone is a likely result of parallel decreases in the majority of EP₃-splice variants in gravid compared with non-gravid human myometrium (Matsumoto *et al.* 1997, Wing *et al.* 2003, Astle *et al.* 2005). This may be a mechanism required for pregnancy maintenance. In addition, potent EP₂ agonist responses (Senior *et al.* 1993) and PGE₂-induced cAMP

Figure 3 Concentration–effect curves for vehicle, PGE₂, PGF_{2α} and U46619 in myometrium taken from donors in (a) early and (b) late labour. Early and late labours were defined as 3–8.5 and 9 cm to full dilation of the cervix. Results are expressed as means $\pm$ S.E.M. and statistical analysis was performed using two-way ANOVA with *post hoc* Bonferroni's adjustment * $P < 0.05$; *** $P < 0.001$ for PGE₂ or U46619 compared with vehicle. Traces show typical responses to U46619 in myometrium excised from donors in (a) early and (b) late stages of labour.

formation via elevated G α s-coupled adenylyl cyclase activity may further contribute to uteroquiescence during pregnancy (Yardley 1992, Europe-Finner *et al.* 1994). Thereby a shift in EP receptor dynamics and signal transduction pathways may mediate the onset of parturition.

During early and late stages of labour, PGE₂ caused uterorelaxation in myometrial strips until full cessation of contractility. The mean potency of PGE₂ function in labouring samples was analogous to myometrium taken prior to labour onset ($EC_{50} = 6.8 \times 10^{-8} \pm 4.1 \times 10^{-8}$ mol/l), even though no contractile responses were evoked. These results substantiate a previous *in vitro* study using myometrium

obtained during active labour (Wikland *et al.* 1984). Excitatory responses to PGE₂ were solely at the fundus whilst lower myometrial activity was suppressed; this indicates a regional change in the complement of functional EP receptor subtypes.

EP₂ receptors have particularly been implicated in labour-associated events due to altered temporal and regional myometrial expression. In relation to changes in the hormonal milieu, EP₂ receptor expression has been reported to decline towards term gestation (Brodt-Eppley & Myatt 1999, Leonhardt *et al.* 2003); although, it remains unaltered (Brodt-Eppley & Myatt 1999, Astle *et al.* 2005, Sooranna *et al.*

2005) or increases during parturition (Grigsby *et al.* 2006). Opposite to EP₃ receptor expression, total EP₂ mRNA and nuclear EP receptors were greater in lower myometrial segments compared with upper myometrial segments (Astle *et al.* 2005, Grigsby *et al.* 2006). This reflected the maintained dose-related inhibitory effect of butaprost, a selective EP₂ agonist, at term pregnancy (Senior *et al.* 1993, Duckworth *et al.* 2002) and following labour onset (unpublished observations). By contrast, high myometrial EP₄ and EP₁ expression was consistent regardless of gestational age, labour and regional location (Leonhardt *et al.* 2003, Astle *et al.* 2005, Grigsby *et al.* 2006). This suggests that EP-mediated uterorelaxation at labour may operate via EP₂ predominance or due to a paucity of functional contractile receptors.

Unlike PGE₂, PGF_{2α}-elicited monophasic excitation in isolated lower myometrium taken at term pregnancy. Uterotonic responses were quantitatively similar to previous superfusion and immersion studies (Word *et al.* 1992, Senior *et al.* 1993, Crankshaw & Dyal 1994, Friel *et al.* 2005b). This was associated with a transient rise in intracellular calcium release in both intact myometrium and myocytes (Carrasco *et al.* 1996, Shlykov & Sanborn 2004) and preceded a decline in responsiveness to oxytocin (unpublished observations). To support pregnancy, myometrial FP mRNA was shown to decline compared with the non-pregnant state (Matsumoto *et al.* 1997, Sooranna *et al.* 2005); this correlated with a decrease in the potency of PGF_{2α}-induced contractions (Senior *et al.* 1992, 1993). At term parturition, human FP receptor expression significantly increased indicating hormonal and physiological influences on PG receptors (Brodt- Eppley & Myatt 1999). Even so, this study showed that the response to PGF_{2α} was attenuated during both stages of labour in lower segment myometrial strips. Weak responses were also observed in superfusion, with marked stimulation by PGF_{2α} evoked only in paired fundal-end specimens taken during active labour (Wikland *et al.* 1984). This topographical difference in FP receptor activity parallels the decline in smooth muscle content of cervical tissue compared with the fundus (Adelantado *et al.* 1988). Moreover, instead of modulating activity, it is plausible that FP receptor populations mediate PGF_{2α}-stimulated glycosaminoglycan activity for uterine compliance in the lower segment during labour (Weiss 2000). Combined doses of PGE₂ and PGF_{2α} suppressed contractile activity in lower-isolated myometrium after labour onset (Wikland *et al.* 1984). Whilst reflecting a high PGE₂ binding affinity, this also indicates the predisposition of the lower uterus to relax in order to subserve the birth process.

The TX mimetic U46619 produced dynamic contractions in isolated human myometrium taken at term pregnancy. Significant attenuation with selective TP antagonists SQ29,548 and GR32191B confirmed TP-mediated responses in this study. In uterine muscle from non-pregnant donors, *in vitro* responses to U46619 were irrespective of menstrual status and excision site (Senchyna & Crankshaw 1999) and functional potencies were analogous to myometrium from term pregnant, non-labouring donors (Senior

et al. 1992, 1993). In addition, no regional or labour-related changes in the expression of TP receptor genes were detected in isolated baboon myometrium (Smith *et al.* 2001). This suggests that the effector-coupling affinity and density of myometrial TP receptors were not substantially influenced by the hormonal milieu. Although TPα- and TPβ-splice variants have been identified in human myocytes and vasculature from both non-pregnant and term pregnant donors (Moore *et al.* 2002, Moran *et al.* 2002), little is known about labour-associated changes in human TP receptor function and expression.

The results of this study demonstrated concentration-dependent spasmogenic effects of U46619 in isolated lower myometrium after labour onset. Despite a twofold reduction in contractile activity between early and late stages of labour, tissue excitation was significantly augmented compared with initial low myogenic contractions. Moreover, urinary TX excretion was increased at late gestation and heightened during labour, which corresponded to uterine activity (Noort & Keirse 1990). This implicates a maintained function for TP receptors during parturition. Two target mediators in the TP signalling cascade, rho-associated coiled coil-forming protein kinase (ROCKI) and its isoform ROCKII sensitise the uterus to calcium (Kureishi *et al.* 1997) and may account for the potentiated contractile responses in this study. Aberrant ROCKI expression has been associated with uterine contractile dysfunctions such as preterm labour and prolonged labour at term (Moore & Lopez Bernal 2003). Moreover, an increase in RhoA mRNA at parturition implies TX involvement in the preparatory and stimulatory phases of labour (Noort & Keirse 1990). As a result, cognate TP receptors may control uterine tone required for foetal descent during labour and possibly uterine involution *post partum*. Future research should examine the activated TX signal cascade to fully elucidate the mechanisms of uterine contraction.

In conclusion, this study showed that myometrial EP, TP and FP receptors are dynamic in nature at term pregnancy and during parturition. It seems likely that a change in the balance of these receptors and signal transduction pathways would mediate the transition from uterine quiescence to activation. Despite ethical constraints limiting research to the lower uterus in the present study, TP receptor function seemed to predominate. Therefore, targeting TP receptors or their downstream regulatory pathways in the parturient uterus may help to improve tocolytic therapy for labour-associated disorders.

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