Expression and regulation of prostaglandin E synthase isoforms in human myometrium with labour

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Since the controversies regarding the use of non-steroidal anti-inflammatory drugs (NSAIDs) and selective cyclo-oxygenase (COX)-2 antagonists for the treatment of preterm labour (PTL), more emphasis has been placed on investigating the terminal synthases involved in the production of prostaglandins (PGs) to allow more targeted therapy in PTL. Prostaglandin E₂ (PGE₂) is synthesized by one of three enzymes, cytosolic prostaglandin E synthase (cPGES), microsomal PGES-1 (mPGES-1) and microsomal PGES-2 (mPGES-2). We have determined (i) the immuno-localization of all three PGES enzymes in lower segment pregnant human myometrium, (ii) the expression of PGES and COX-2 mRNA expression at term and preterm gestation with and without labour and (iii) the effect of interleukin (IL)-1β on COX-2 and PGES mRNA and protein expression in human myometrial smooth muscle (HMSM) cell cultures. We show mPGES-1 protein located predominantly in myometrial and vascular smooth muscle cells (SMCs), whilst mPGES-2 protein is largely in stromal cells surrounding the SMC and cPGES is diffusely located throughout the myometrium. Expression of mPGES-2 mRNA increased with term labour and PTL and expression of COX-2 and mPGES-1 mRNA with term labour, whereas cPGES expression did not change. IL-1β stimulated release of PGE₂ by HMSM cells and increased COX-2 and mPGES-1 mRNA and protein expression. Thus, COX-2 expression and mPGES-1 expression are co-ordinately up-regulated in lower segment myometrium with term labour and with IL-1β treatment in HMSM cells.

Key words: prostaglandin E₂ synthase/myometrium/labour/myometrial smooth muscle

Introduction

Spontaneous preterm birth, defined as delivery before 37 weeks of gestation, complicates between 5 and 11% of all births and results in 70–80% of neonatal mortality and morbidity (Goldenberg and Rouse, 1998; Wen et al., 2004). The onset of labour, both at term and preterm, is multifarious, involving the up-regulation of numerous inflammatory mediators, including cytokines and prostaglandins (PGs), which culminate in a uterine transition from one of relative quiescence throughout gestation to that of active contractions during labour.

PGs are a class of lipid mediators that have long been implicated in the parturition process (Blaesealden and Johnston, 1985; Challis and Olson, 1988; Olson et al., 1993). In association with labour, PG output by fetal membranes (amnion and chorion-deciduala) and myometrium increases. The subsequent increased PG levels are in turn thought to be involved in both ripening of the cervix and stimulation of uterine contractions (Keirse et al., 1983; Skinner and Challis, 1985; Bennett and Elder, 1988; Keirse, 1993; Challis et al., 2000). Furthermore, drugs that inhibit PG synthesis, for example indomethacin, nimesulide and celecoxib, have been used, with some success, for the treatment of preterm labour (PTL) (Zuckerman et al., 1974; Sawdy et al., 1997; Berkman et al., 2003). However, the use of these drugs has been associated with many serious fetal side effects (Hendricks et al., 1990; Norton et al., 1993; Holmes and Stone, 2000). These compounds act by preventing the action of either one or both of the cyclo-oxygenase (COX) enzymes, COX-1 or COX-2. Indeed, it was the use of selective COX inhibitors, for the specific and differential inhibition of COX-2 over that of COX-1, that recently provided some initial optimism and a new research focus for the treatment of PTL (Mitchell and Olson, 2004). Despite this, recent studies including a randomized, double-blind, placebo-controlled trial of a COX-2-specific inhibitor (rofecoxib) have not only failed to show a decreased incidence of preterm delivery but demonstrated an increased risk of delivery before 37 weeks in high-risk women (Groom et al., 2005).

PG synthesis is complex with a number of potential rate-limiting steps. First, arachidonic acid is released by the action of one or more phospholipase A₂ (PLA₂) enzymes, and secondly, either COX-1 or COX-2 converts arachidonic acid to prostaglandin H₂ (PGH₂). Finally, PGH₂ is converted into biologically active PGs by specific synthase enzymes.

In the case of prostaglandin E₂ (PGE₂), one of the predominant PGs produced within the uterus during pregnancy, the conversion of PGH₂ to PGE₂ is catalysed by PGE synthase (Jakobsson et al., 1999; Kudo and Murakami, 2005). Three distinct prostaglandin E synthase (PGES) enzymes have been identified: cytosolic PGES (cPGES) (Tanioka et al., 2000), microsomal PGES type-1 (mPGES-1) (Jakobsson et al., 1999) and microsomal PGES type-2 (mPGES-2) (Tanikawa et al., 2002; Hellwell et al., 2004; Kudo and Murakami, 2005). Expression of cPGES and mPGES-2 appears to be, for the most part, constitutive, whilst mPGES-1 expression may be induced in response to pro-inflammatory stimuli (Forsberg et al., 2000; Kudo and Murakami, 2005).
2005). In addition, these PGES enzymes may differentially couple with the upstream COX enzymes, for example mPGES-1 appears to have a greater preference for COX-2 (Murakami et al., 2000), cPGES for COX-1 (Tanioka et al., 2000) and mPGES-2 will couple equally with either COX-1 or COX-2 (Murakami et al., 2003). Thus, there is clearly the potential for intricate regulation of the synthesis of PGE₂ within the uterus during pregnancy.

To date, the cPGES and mPGES-1 enzymes have been identified in human fetal membranes and placenta (Alfaidy et al., 2003; Meadows et al., 2003, 2004) and mPGES-1 and mPGES-2 in human myometrium (Giannoulias et al., 2002a; Soorana et al., 2006).

In addition to the observed increases in PGs, the process of parturition also involves numerous other factors including the increased production of inflammatory cytokines such as interleukin (IL)-1β (Romero et al., 1992; Osman et al., 2003). Furthermore, IL-1β itself may also up-regulate genes involved in PG synthesis. For example, in human myometrial smooth muscle (HMSM) cells, IL-1β increases the expression of COX-2 (Belt et al., 1999; Erkinheimo et al., 2000; Soorana et al., 2005), and in other cell systems, IL-1β up-regulates both COX-2 and mPGES-1 mRNA and protein expression in a coordinate manner (Murakami et al., 2000; Catley et al., 2003).

Therefore, the aims of this study were to (i) determine the cellular localization of all three PGES proteins using immunohistochemistry and (ii) determine whether there were any changes in the mRNA expression of these isoforms in the pregnant human myometrium with labour at term or preterm. In addition, we have utilized primary cultured HMSM cells to (iii) examine the effects of IL-1β on PGES expression and assess the suitability of these cells as an in vitro model for the analysis of PGES regulation.

Materials and methods

Subjects

Human myometrial biopsies were collected from non-pregnant and pregnant women with local ethics committee approval (Walsgrave Hospital Trust, Coventry, UK, CRECO/09/09/08). Fully informed written consent was obtained from all patients in this study. Tissues for mRNA analysis were collected from the upper margin of the lower uterine segment following Caesarean section deliveries, at preterm before the onset of labour (PTNL, 28–36 weeks, n = 6), preterm following the onset of labour (PTL, 27–35 weeks n = 5), term not in labour (TNL, 37–41 weeks n = 7) and term in labour (TL, 39–42 weeks, n = 6). Labour was defined as regular contractions (>3 min apart) plus membrane rupture and cervical dilation (>3 cm) with no augmentation (oxytocin or PG administration). Samples were collected from women undergoing Caesarean section, without underlying disease, for fetal distress, breech presentation, previous section, placental praevia, maternal request or failure to progress. Non-pregnant myometrial samples (n = 3) were also obtained from pre-menopausal women undergoing hysterectomy for dysmenorrhoea. Tissues collected for RT–PCR analysis or culture of HMSM cells, were first separated from serosal or decidual components, were viewed under a dissection microscope and were then either snap-frozen in liquid nitrogen before RNA isolation or immediately cultured HMSM cells to (iii) examine the effects of IL-1β on PGES regulation.

Experiments were initially performed to determine which PGES isozymes were expressed in myometrial tissue and HMSM cells. Each PCR was run with 2.5μl of cDNA (or negative control) and 1μl BioTag DNA Polymerase (Bioline, London, UK) in a final amplification volume of 25μl. PCR primers used were (5′–3′): cPGES (GenBank NM 006601, official name PTGES3) ATGCAGCCGTCTTCTGCCA (sense), TTACTCCAGATCTGGCAT (antisense); mPGES-1 (GenBank NM 004878, official name PTGES) TGCCCTGCCACAGCTTGTTAGA (sense), CCACCAATCTGGAAGGACATCT (antisense); mPGES-2 (GenBank NM 0275072, official name PTGES2) GCCAGACGCGAGGATGAA (sense), TCACCGGACCAAGCATAT (antisense); COX-2 (Genbank NM000963, official name PTGS2) TTCATAATGAGATTTGGAAGAATGCT (sense), AGATCATCTTGGCTTGAATCTT (antisense). The PCR was performed for 32 cycles of 30 s of denaturation at 94°C, 30 s of annealing at 55°C (cPGES), 58°C (COX-2) or 60°C (mPGES-1 and mPGES-2) and 30 s of elongation at 72°C. The PCR was followed by a 10-min extension at 72°C. Amplification products (cPGES: 482 bp, mPGES-1: 550 bp and mPGES-2: 351 bp) were analysed using agarose gel electrophoresis, purified using a Qiagen gel extraction kit and then either snap-frozen in liquid nitrogen before RNA isolation or immediately cultured HMSM cells to (iii) examine the effects of IL-1β on PGES regulation.

Results

RNA isolation and generation of cDNA

Total ribonucleic acid (RNA) was isolated using a Qiagen RNeasy RNA Isolation System (Qiagen Inc., Ontario, Canada) and digested with DNase I (Qiagen). For cDNA synthesis, total RNA (500 ng) was first denatured at 70°C for 5 min and incubated at 37°C for 60 min in a total volume of 20 μl, with 200 ng random hexanucleotide primers (Promega) and 200 U Superscript II as recommended by the manufacturer (Invitrogen). The reaction volume was then brought up to 100 μl, and the resultant cDNA was utilized for subsequent standard and real-time PCR amplification. For each sample analysed a negative RT control reaction (RT-ve) was also performed as a control, in which the reverse transcriptase enzyme was absent.

RT–PCR and real-time RT–PCR

Experiments were initially performed to determine which PGES isoforms were expressed in myometrial tissue and HMSM cells. Each PCR was run with 2.5μl of cDNA (or negative control) and 1μl BioTag DNA Polymerase (Bioline, London, UK) in a final amplification volume of 25μl. PCR primers used were (5′–3′): cPGES (GenBank NM 006601, official name PTGES3) ATGCAGCCGTCTTCTGCCA (sense), TTACTCCAGATCTGGCAT (antisense); mPGES-1 (GenBank NM 004878, official name PTGES) TGCCCTGCCACAGCTTGTTAGA (sense), CCACCAATCTGGAAGGACATCT (antisense); mPGES-2 (GenBank NM 0275072, official name PTGES2) GCCAGACGCGAGGATGAA (sense), TCACCGGACCAAGCATAT (antisense); COX-2 (Genbank NM000963, official name PTGS2) TTCATAATGAGATTTGGAAGAATGCT (sense), AGATCATCTTGGCTTGAATCTT (antisense). The PCR was performed for 32 cycles of 30 s of denaturation at 94°C, 30 s of annealing at 55°C (cPGES), 58°C (COX-2) or 60°C (mPGES-1 and mPGES-2) and 30 s of elongation at 72°C. The PCR was followed by a 10-min extension at 72°C. Amplification products (cPGES: 482 bp, mPGES-1: 550 bp and mPGES-2: 351 bp) were analysed using agarose gel electrophoresis, purified using a Qiagen gel extraction kit and subsequently verified by sequencing. Subsequently, to determine whether there were any labour-associated changes in lower segment myometrium or any cytokine induced changes in HMSM cells in the expression of the PGES or the COX-2 enzymes, we utilized real-time RT–PCR using an ABI PRISM 7000 Sequence detection system and Taqman expression assays from Applied Biosystems (Foster City, CA, USA). The real-time PCR reactions were carried out in triplicate in a final volume of 25 μl with 2.5 μl cDNA. The results were calculated using the comparative 2–ΔΔCₚ method according to the manufacturer’s instructions (ABI PRISM 7000 sequence detector) previously described (Livak and Schmittgen, 2001). Gene expression was normalized to expression of the 18s rRNA.

Western blot analysis

HMSM cells were harvested in 100 μl of 10 mM Tris/HCl (pH 7.5), 0.15 M NaCl, 1.5 mM MgCl₂, 0.65% (v/v) NP-40, 0.5 mM phenylmethylsulphonyl fluoride (PMSF) and 0.5 mM dithiothreitol (DTT). HMSM cellular proteins (10 μg) were denatured at 95°C for 10 min in Laemmli sample buffer (Sigma) and protein samples run on 10% sodium dodecyl sulphate (SDS) polyacrylamide gels and transferred to Hybond-enhanced chemiluminescence nitrocellulose paper (Amersham Pharmacia, Bucks, UK) using standard techniques. Transfer efficiency and equal loading of protein samples were assessed by incubating membranes with Ponczeau red solution (Sigma). Membranes were probed with rabbit polyclonal anti-human PGES (no. 160150), mPGES-1 (no. 160140) or mPGES-2 (no. 160145) (Cayman Chemical, Ann Arbor, MI, USA).
or goat polyclonal COX-2 (SC-1745) (Santa Cruz) followed by incubation with a conjugated secondary antibody at a dilution of 1:2000 (Dako, UK). In all cases, proteins were visualized using chemiluminescence (ECL) (Amersham Pharmacia Biotech) according to the manufacturer’s instructions.

**Immunohistochemistry**

Myometrial tissue sections (5 μm) were deparaffinized in xylene and rehydrated by passing through a graded alcohol series. Antigen retrieval was performed using 1% antigen-unmasking solution (Vector laboratories, Burlingame, CA, USA) incubated at 96°C for 60 min. To localize the PGES isozymes, we used the Vectastain Elite ABC detection kit (Vector Labs) following the manufacturer’s protocol. Briefly, endogenous peroxidase activity was quenched by incubation in 3% hydrogen peroxide. The tissue sections were then blocked in 1% antibody host serum and incubated overnight with the desired polyclonal primary antibody at 4°C. Primary antibodies, cPGES (1:250) and mPGES-1 (1:250) and mPGES-2 (1:400) were diluted in 1% serum/phosphate-buffered saline (PBS) or as a control incubated with the respective blocking peptides as recommended by the manufacturer (Cayman Chemical). Incubation of the sections with pre-absorbed antibody or omitting of the primary antibody gave no staining. The colour reaction was developed with a biotinylated secondary antibody, addition of avidin/biotinylated complex followed by incubation with 3,3-diaminobenzidine-tetra-hydrochloride (DAB) solution with metal enhancer. Sections were counterstained using Harris haematoxylin, dehydrated in an increasing ethanol series, cleared in xylene and the coverslips mounted in DPX mounting media (all Sigma). For all PGES enzymes, serial sections were analysed from two patients in each group.

**Analysis of PGE₂ release**

PGE₂ released in culture media was measured by radioimmunoassay (RIA) using an anti-PGE₂ antibody (Sigma) according to the manufacturer’s instructions.

**Statistics**

Data are presented as the mean ± SEM. Comparison of two means was made using a Mann–Whitney two-tailed t-test. Comparison of more than two means was made using analysis of variance (ANOVA) Kruskal–Wallis test followed by Dunn’s multiple comparison test using PRISM statistics software (GraphPad software Inc., San Diego, CA, USA). A value of P ≤ 0.05 was considered significant.

**Results**

**Expression of cPGES, mPGES-1 and mPGES-2 in human lower segment myometrium**

Expression of the PGES isozymes (cPGES, mPGES-1 and mPGES-2 mRNA) was observed in all non-pregnant and pregnant myometrial samples examined as determined using standard RT–PCR and sequence analysis (data not shown).

Immunohistochemical analysis demonstrated the presence of protein for all three PGES enzymes with distinct cellular localization patterns for each being observed (Figure 1, Table I). The mPGES-1 protein was most highly localized throughout the cytoplasm of myometrial and vascular smooth muscle cells (SMCs), while little or no staining was observed in the stromal cells (Figure 1C, G and K). For cPGES, staining was weak and scattered in myometrial smooth muscle (MSM), vascular smooth muscle and vascular endothelial cells, with no staining observed in the stromal cells (Figure 1B, F and J). In contrast, mPGES-2 expression was most abundant within stromal cells surrounding the MSM cells and to a lesser extent in vascular endothelium, and staining was diffuse in vascular and myometrial SMCs (Figure 1D, H and L). In addition, cPGES, mPGES-1 and mPGES-2 were all abundantly expressed within the adherent decidual component in the samples collected. Exclusion of primary antibody or pre-absorption with the antigen peptide eliminated positive staining (Figure 1A, E and I).

**Labour-associated changes in the expression of PGES and COX-2 mRNA**

To determine any labour-associated changes in the expression of the PGES mRNAs, we utilized real-time RT–PCR. No significant changes in the expression of cPGES with gestation or labour at term or preterm were observed in the lower segment myometrial samples (Figure 2B). Expression of mPGES-1 mRNA was higher in the TL group compared with PTNL (P < 0.05) and TNL (P = 0.06) groups; no significant changes were observed in samples from preterm deliveries (Figure 2C). Expression of mPGES-2 mRNA was significantly higher in the TL group compared with TNL (P < 0.05) and PTNL (P < 0.05) groups and in PTL group compared with PTNL group (P < 0.05) (Figure 2D). Expression of COX-2 mRNA was highest in the TL group compared with all other groups (P < 0.05); no significant changes were observed with labour at preterm (Figure 2A).

**Effect of IL-1β on COX-2 and PGES expression in HSM cells**

Treatment of HSM cells with IL-1β increased expression of mPGES-1, mRNA (***P < 0.001) and protein, as determined using real-time RT–PCR and western blot analysis respectively (Figure 3A and B). Release of PGE₂ was also increased by IL-1β-treated HSM cells (***P < 0.001) (Figure 3C). In contrast, no significant effects of IL-1β treatment on either cPGES or mPGES-2 expression were observed. Treatment with IL-1β also resulted in a concomitant increase in the expression of COX-2 mRNA (**P < 0.001) and protein in HSM cells (Figure 3A and B).

**Discussion**

PGs have long been associated with the onset of human labour, and their synthetic enzymes have been put forward as members of a group of ‘contraction-associated proteins’ (CAPs) postulated to be fundamental for uterine activation during labour (Challis and Lye, 1994). In this context, increased type-IIA secretory PLA₂ expression is observed in amniotic fluid and serum (Koyama et al., 2000) and myometrium with labour both at term and preterm (Slater et al., 2004). Similarly, whilst both COX-1 and COX-2 are expressed in human myometrial tissue, there are, perhaps surprisingly, no consistent findings with regard to changes in expression in association with labour. Thus, studies have variously reported no labour-associated changes in COX-1 or COX-2 (Moore et al., 1999; Sparey et al., 1999; Giannoulis et al., 2002b), a labour-associated decrease in COX-2 (Zuo et al., 1994) and increased COX–2 expression in association with term labour (Slater et al., 1999; Erkinheimo et al., 2000; Havelock et al., 2005; Soorana et al., 2006). Such differences may be explained by differences in sample site, small sample size, proximity to labour of the TNL group as well as variations in cervical dilation and drug regimens. Given the potential, and controversial, use of COX-2-selective inhibitors for PTL treatment/tocolysis, COX-2 mRNA expression was re-examined using real-time PCR as a more robust method of quantification. We now confirm significant increases in the myometrial expression of COX–2 mRNA in association with term labour. Despite this, no significant changes were detected with PTL, suggesting that COX-2 may not play a key role in the development of PTL in these cases. Whilst this result was initially surprising, the use of COX-2-selective inhibitors for PTL treatment has recently been questioned as, in addition to potentially serious fetal side effects (Holmes and Stone, 2000), rofecoxib may actually increase the incidence of preterm delivery in high-risk women (Groom et al., 2005).

Moving down the synthetic pathway to PGE₂, the finding of at least three distinct PGES genes adds considerably to the potential for the
Figure 1. Immuno-localization of cPGES, mPGES-1 and mPGES-2 protein in human lower segment myometrium samples from pregnant women. Representative sections show (B) cPGES, (C) mPGES-1, (D) mPGES-2 at term non-labour (1 × 100), (F) cPGES, (G) mPGES-1, (H) mPGES-2 in vascular smooth muscle (1 × 200) and (J) cPGES, (K) mPGES-1 and (L) mPGES-2 in myometrial smooth muscle at 30-week gestation non-labour (1 × 200). Negative control slides (A, E and I) showed no staining. The brown colour indicates positive staining. cPGES, cytosolic prostaglandin E synthase; mPGES, microsomal PGES-type-1; MSM, myometrial smooth muscle; De, decidual cells; VSM, vascular smooth muscle; VE, vascular endothelium; S, stromal cells.
intricate regulation of PGE$_2$ synthesis. In this context, this study clearly demonstrates the expression of all three PGES genes in human myometrial tissue and furthermore suggests distinct patterns of protein localization and mRNA expression. Thus, cPGES protein was diffusely distributed in most cell types, mPGES-1 protein was generally localized to both myometrial and vascular SMCs, and mPGES-2 protein was most abundantly found in stromal cells. In terms of changes in gene expression, cPGES mRNA was unaffected by labour, whereas mPGES-2 mRNA was significantly up-regulated with both term labour and PTL. Likewise, an upward trend in mPGES-1 mRNA expression was observed with term labour. Whilst mPGES-2 mRNA expression has been described as constitutive (Kudo and Murakami, 2005), mice treated with inflammatory mediators show increased mPGES-2 expression, and this is consistent with the current findings (Murakami et al., 2003). With regard to mPGES-1, these data are in agreement with the localization of mPGES-1 in HMSM cells (Giannoulis et al., 2002a; however, we believe our study to be the first to document the localization of cPGES and mPGES-2 protein in pregnant human myometrium. A recent paper by Soorana et al. (2006) has also examined the mRNA expression of COX-2, mPGES-1 and mPGES-2 in human myometrial tissue and MSC cell cultures. In keeping with our present data, they show increased expression of COX-2 mRNA with term labour in lower segment myometrium (Slater et al., 1999; Soorana et al., 2006). However, in contrast, they found COX-2 mRNA expression significantly increased in PTL compared with preterm non-labour myometrial samples, whereas in our sample set the observed increase was not statistically significant. With regard to mPGES-1, the pattern of mRNA expression shows similar trends in both studies, with higher expression in laboured groups compared with non-laboured groups. However, although Soorana et al. do not report these differences to be significant, we found mPGES-1 mRNA expression was significantly higher at term labour compared with preterm non-labour and $P = 0.06$ compared with term non-labour. With regard to mPGES-2, we report a small but significant increase in the expression of mPGES-2 mRNA with labour both at term and preterm, which was not found by Soorana et al. It is not clear how best to explain these differences of COX-2 and mPGES mRNA expression reported here with that of Soorana et al. (2006). However, this may prove to be down to subtle differences in methods used, and numbers and areas of sample collection.

Nevertheless, one suggestion raised by our data is that mPGES-2, and possibly mPGES-1, could, like COX-2, be included as one of the CAPs involved in the onset of labour. Furthermore, if, and this by no means certain, the inhibition of PGE$_2$ synthesis is beneficial as a tocolytic, then the inhibition of mPGES-2, or mPGES-1, rather than COX-2 could provide a safer target for treatment of PTL. However, in considering this option, it should be noted that compensatory regulatory mechanisms may exist between mPGES-1 and mPGES-2 (Kubota et al., 2005). Thus, in a mouse model of infection-induced PTL, lipopolysaccharide (LPS) up-regulated expression of mPGES-1, but not of cPGES or mPGES-2 in wild-type mice, whereas in mPGES-1 knockout mice, LPS treatment increased the myometrial expression of mPGES-2 (Kubota et al., 2005). Likewise, IL-1β stimulates COX-2 expression in HMSC cells (Bartlett et al., 1999), and we now show that along with increased PGE$_2$ release, the expression of both COX-2 and mPGES-1, but not cPGES and mPGES-2, is increased by IL-1β. This is also in keeping with the recent data reported by Soorana et al. (2006), who also show increased expression of COX-2 and mPGES-1 mRNA in HMSC cells in response to IL-1β (Soorana et al., 2006). These data, in common with other cell types (Murakami et al., 2000; Kudo and Murakami, 2005), suggest that COX-2 and mPGES-1 are co-ordinately regulated in HMSC cells, and this may provide a system for the possible study of the reciprocal regulation of PGES isoforms.

However, whilst increases in mRNA expression may not necessarily equate to increased protein and PGE$_2$ production, it is important to note that such events could also be a consequence, rather than a cause, of the labour process. In this respect, the precise role of uterine PGE$_2$ has yet to be fully defined. Certainly, all four PGE$_2$ receptor (EP) receptor subtypes through which PGE$_2$ acts are expressed in pregnant human myometrial tissue, and these also demonstrate distinct spatial localization (Leonhardt et al., 2003; Astle et al., 2005; Grigsby et al., 2006). Activation of EP$_1$ and/or EP$_3$ will elicit contractile responses, whilst EP$_2$ and/or EP$_4$ are coupled to cyclic adenosine monophosphate (cAMP) and may explain the anti-inflammatory and relaxatory effects on MSM (Word et al., 1992; Slater et al., 2006). Indeed, the effects of elevated PGE$_2$, which occur following increased expression of COX-2 and mPGES-1 or mPGES-2, may not only facilitate cervical ripening or membrane rupture but also relax, rather than stimulate, myometrial contraction (Slater et al., 2006). Therefore, the exact temporal and spatial relationship between sites of COX-2, mPGES-1 and mPGES-2 expression, i.e. where PGE$_2$ is produced, and the localization of each EP receptor type will have profound consequences for the physiological roles, whether paracrine or autocrine, of the PGE$_2$ produced. For example, mPGES-1 is clearly localized to MSM cells of the

### Table 1. Localization summary of prostaglandin E synthase (PGES) proteins in pregnant myometrium as determined by immunohistochemistry

<table>
<thead>
<tr>
<th>PGES</th>
<th>Myometrial smooth muscle</th>
<th>Vascular smooth muscle</th>
<th>Vascular endothelium</th>
<th>Stroma</th>
<th>Decidua</th>
</tr>
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<tbody>
<tr>
<td>cPGES</td>
<td>(+)</td>
<td>(+)</td>
<td>+</td>
<td>–</td>
<td>++</td>
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<tr>
<td>mPGES-1</td>
<td>+++</td>
<td>+++</td>
<td>–</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>mPGES-2</td>
<td>(+)</td>
<td>(+)</td>
<td>+</td>
<td>++</td>
<td>+++</td>
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</tbody>
</table>

cPGES, cytosolic prostaglandin E synthase; mPGES-1, microsomal PGES-1; –, no cells stained; +, few cells stained; ++, moderate number of cells stained; ++++, most cells stained; (+), scattered/diffuse staining. Staining intensity refers to the relative numbers of cells stained.
myometrial tissue, which also express both contractile EPs and relaxatory EP3 and EP4 receptors. Furthermore, both EP1 and EP3 are up-regulated in association with term labour in lower segment myometrium (Astle et al., 2005). By contrast, whilst mPGES-2 is also expressed in MSM cells, this enzyme is preferentially localized to the surrounding stromal cells and therefore suggests a role distinct from that of mPGES-1. Whilst it is postulated that EP3 is also involved in post-partum involution of the uterus or that EP2 may facilitate relaxation of the lower segment to facilitate delivery (Astle et al., 2005), it is equally clear that many fundamental questions remain unanswered. For example: What governs which EP receptor signal will predominate in a given cell type? Are there functional switches from pro-pregnancy to pro-labour types of response? Do autocrine PGE2 and paracrine PGE2 actually ‘see’ the same EP receptors on a target cell? In addition, the presence of PGES enzymes and EP receptors in vascular endothelial and vascular smooth muscle also requires further illumination.

In conclusion, these data demonstrate the presence of three distinct PGES enzymes in pregnant human myometrium and therefore support a role for locally produced PGE2, possibly produced via COX-2 and mPGES-1 and mPGES-2, in the regulation of myometrial activity. Furthermore, the spatial and labour-associated changes of these enzymes imply distinct physiological functions within the different cell types of the pregnant uterus. Therefore, the further elucidation of the role and regulation of the PGES enzymes, the involvement of the cytokine network and the subsequent signalling via the various EPs is still necessary to understand the mechanisms of parturition.

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