Myometrial prostaglandin E₂ synthetic enzyme mRNA expression: spatial and temporal variations with pregnancy and labour

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We have investigated the hypothesis that the expression of the enzymes involved in PGE₂ synthesis in the human uterus is co-ordinated. We have studied (i) the mRNA expression of the enzymes involved in PGE₂ synthesis [phospholipases (cPLA₂ and sPLA₂), prostaglandin H synthase (PGHS)-2 and PG E synthases (PGES-1 and -2)] and their relationship to the expression of inflammatory cytokines in samples of myometrium obtained from pregnant women undergoing caesarean section (LSCS) either before or after the onset of labour at or before term; and (ii) the effect of IL-1β, IL-6, TNF-α, PGE₂ and stretch on PGE₂ enzyme mRNA expression. We found that cPLA₂, sPLA₂ and PGHS-2 mRNA expression were greater in labour samples; cPLA₂, sPLA₂, PGHS-2, PGES-1 and -2 mRNA expression were greater in lower-than upper-segment samples; and there was no effect of gestational age. PGHS-2 mRNA levels correlated with those of PGES-1, cPLA₂, IL-1β and IL-8; PGES-1 mRNA levels correlated with those of IL-1β, IL-8 and cPLA₂. In primary cultures of uterine myocytes, cPLA₂ mRNA expression was increased by IL-1β and IL-6; PGHS-2 mRNA expression was increased by IL-1β, PGE₂ and stretch; and PGES-1 mRNA expression was increased by IL-1β only. These data show that labour is associated with increased expression of the enzymes involved in PGE₂ synthesis and their expression is greater in the lower uterine segment. The presence of associations between the levels of PGE₂ enzyme mRNA expression and the effects of IL-1β suggest that their expression is co-ordinated and that IL-1β is the responsible factor.

Key words: cytokines/labour/prostaglandin synthesis/stretch/uterine smooth muscle cells

Introduction

Many studies have confirmed the important role played by prostaglandins (PG) during human labour, which promote both cervical compliance and myometrial contractility. Most studies have suggested that in the process of PG synthesis at the time of labour, PG H synthase (PGHS) type 2 plays the most important role (Hirst et al., 1995b; Slater et al., 1995, 1999). However, several other enzymes are involved in PGE₂ synthesis and there are relatively little data available in terms of the spatial and temporal variations and the effect of labour on their expression and what factors may be important in their regulation.

PG synthesis involves the mobilization of the precursor arachidonic acid from membrane phospholipids, which is then converted to the PG intermediate PGH₂ by PG H synthase (PGHS). Specific PG synthase enzymes convert PGH₂ to the different families of PGs. The release of arachidonic acid from intracellular membrane phospholipids is catalysed by the action of phospholipase A₂ (PLA₂). These enzymes have been broadly divided into two classes, the secretory and cytosolic phospholipases (sPLA₂ and cPLA₂, respectively). sPLA₂ type-IIA and cPLA₂ type-IV have been identified within the human uterus (Skannal et al., 1997; Lappas et al., 2004) and the expression of sPLA₂, but not cPLA₂, has been found to increase in human myometrium with the onset of labour (Slater et al., 2004). Two isoforms of PGHS exist in most cells and are encoded for by two distinct genes—the constitutively expressed PGHS-1 and the inducible PGHS-2 (Hla and Neilson, 1992), but only the latter increases in association with labour in fetal membranes and myometrium (Hirst et al., 1995a; Slater et al., 1999; Challis et al., 2000; Lindstrom and Bennett, 2004). PG synthases (PGES), cytosolic PGES (cPGES or PGES-1) and membrane-bound PGES (mPGES or PGES-2) are responsible for PGE₂ synthesis (Jakobsson et al., 1999; Watanabe et al., 1999). Recent studies in humans have localized mPGES to fetal membranes (Meadows et al., 2003) and myometrial cells with no significant changes in the latter with gestational age or the onset of preterm or term labour (Giannoulias et al., 2002). Thus, several enzymes are involved in the synthesis of PGE₂, their expression may be co-ordinated and inflammatory cytokines are prime candidates. IL-1β is known to increase the expression and activity of PGHS-2 in human myometrial cells (Sooranna et al., 2005), but whether IL-1β or other inflammatory cytokines affect the expression of the other components of the PGE synthetic pathway in myometrium is not known.

In this study, to test the hypothesis that the expression of the enzymes involved in PGE₂ synthesis is co-ordinated, we have investigated the
spatial and temporal variation in the mRNA expression of the enzymes involved in PGE\textsubscript{2} synthesis, cPLA\textsubscript{2}, sPLA\textsubscript{2}, PGHS-2 and PGES-1 and -2 in the human uterus; their inter-relationships and relationship to the expression of inflammatory cytokine (IL-1\textbeta and IL-8); and in primary cultures of human uterine smooth muscle cells, we have studied the effect of IL-1\textbeta, IL-6, TNF-\alpha, PGE\textsubscript{2} and stretch on their mRNA expression.

Materials and methods

Myometrial samples, collection and preparation

Paired upper and lower human myometrium were obtained from four groups of women (n = 6, in each group) at the time of caesarean section (LSCS) under the conditions of preterm with labour (PTL; 33.9 ± 1.5 weeks), preterm no labour (PTNL; 30.4 ± 1.5 weeks), term with labour (TL; 39.2 ± 0.5 weeks) or term no labour (TNL; 38.4 ± 0.5 weeks). Labour was defined as the presence of regular uterine contractions (every 3–4 min) resulting in cervical effacement and dilatation. Myometrial samples were removed from the upper margin of the incision made in the lower uterine segment (lower), while the upper segment was removed from just below the fundus. The indications for LSCS included previous LSCS, failure to progress and breech presentation. All women were invited to continue with the protocol that is in compliance with the Institution Review Board of the University of Cincinnati (Cincinnati, OH, USA).

Primary myocyte cultures

Biopsies (0.5 x 0.5 cm\textsuperscript{3}) of term human myometrium were collected from the time of LSCS from women not in labour (n = 6) into Dulbecco’s modified Eagle’s medium (DMEM) containing 100 munits/ml penicillin and 100 μg/ml streptomycin. Samples were stored at 4°C for no ≥3 h prior to cell preparation for culture. Tissue from LSCS was removed from the upper margin of the incision made in the lower segment of the uterus; mean gestational age was 39 weeks (38 + 3 – 39 + 2). The indications for LSCS included fetal distress, previous LSCS, failure to progress and breech presentation. All specimens were obtained after patient consent and according to the guidelines set forth in the protocol that is in compliance with the Institution Review Board of the University of Cincinnati (Cincinnati, OH, USA).

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Forward primer sequence</th>
<th>Reverse primer sequence</th>
<th>Genbank/EMBL Accession number</th>
<th>Nucleotide number</th>
<th>R\textsuperscript{2}</th>
<th>Efficiency</th>
</tr>
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<tr>
<td>GAPDH</td>
<td>5′-TGTGACATCAAGAAGTTGGTGTAAG-3′</td>
<td>5′-TCCTTGGAGGCCCATGAGCCAC-3′</td>
<td>BC014085</td>
<td>1644–1883</td>
<td>0.999999</td>
<td>1.12</td>
</tr>
<tr>
<td>Beta actin</td>
<td>5′-GGGAAATCTGGCAGGACATTA-3′</td>
<td>5′-TGTGTTGGCGTACAGGTCTTT-3′</td>
<td>BT019932</td>
<td>617–891</td>
<td>0.999999</td>
<td>0.64</td>
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<td>cPLA2</td>
<td>5′-ATGCTTACAGCTGTTGATG-3′</td>
<td>5′-TGACATGCTAGTACAGCTG-3′</td>
<td>NM024420</td>
<td>1546–1724</td>
<td>0.999444</td>
<td>0.96</td>
</tr>
<tr>
<td>SPLA2-IA</td>
<td>5′-AAGGAAAGCAGCTAGTCT-3′</td>
<td>5′-TGACAGGTAGTGCTGCT-3′</td>
<td>NM000300</td>
<td>375–567</td>
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<td>PGES-1</td>
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<td>5′-TTCCCACTACTTTGCTG-3′</td>
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<td>5′-TCTGAGATTCTGAGCTGTTG-3′</td>
<td>BC008729</td>
<td>675–898</td>
<td>0.999011</td>
<td>0.81</td>
</tr>
</tbody>
</table>

The r\textsuperscript{2} values obtained and the efficiencies during quantitative real-time PCR using the primers pairs are also listed.

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unpaired samples and Wilcoxon matched pairs test for paired samples, as appropriate, using InStat 3 for Macintosh, GraphPad, San Diego, CA, USA. Differences were considered statistically significant at $P < 0.05$. Pearson correlation coefficients were calculated using Prism 4 for Macintosh, GraphPad.

**Results**

**Expression of PGE synthetic enzyme mRNA in myometrium**

For cPLA$_2$, overall, levels were higher in labour (L) versus non-labour (NL, $P < 0.01$) and in lower segment (LS) versus upper segment (US, $P < 0.05$) samples and not different between preterm (PT) versus term (T) samples; differences between NL and L samples defined by region and gestational group are shown in Figure 1a. For sPLA$_2$, overall, levels were higher in L versus NL ($P < 0.001$) and in LS versus US ($P < 0.0001$) samples and not different between PT versus T samples; differences between NL and L samples defined by region and gestational group are shown in Figure 1b. For PGHS-2, overall, levels were higher in L versus NL ($P < 0.0001$) and in LS versus US ($P < 0.01$) samples and not different between PT versus T samples; differences between NL and L samples defined by region and gestational group are shown in Figure 1c. For PGES-1, overall, levels were higher in LS versus US ($P < 0.0001$), and not different between L versus NL and PT versus T samples; differences between NL and L samples defined by region and gestational group are shown in Figure 1d. For PGES-2, overall, levels were higher in LS versus US ($P < 0.05$) samples and not different between L versus NL and PT versus T samples; differences between NL and L samples defined by region and gestational group are shown in Figure 1e. The cytokine (IL-1$\beta$) and IL-8 data are not shown.

**Correlations with myometrial PGE synthetic enzyme mRNA**

Correlations were found between cPLA$_2$ and both PGHS-2 ($r = 0.65$, $P < 0.0001$) and PGES-1 ($r = 0.55$, $P = 0.0003$, Table II); PGHS-2 and PGES-1 ($r = 0.86$, $P < 0.0001$, Figure 2a), IL-1$\beta$ ($r = 0.48$, $P < 0.0005$) and IL-8 ($r = 0.31$, $P < 0.03$, Table II); PGES-1 and IL-1$\beta$ ($r = 0.82$, $P < 0.0001$, Figure 2b) and IL-8 ($r = 0.6$, $P < 0.0001$, Table II).

**Effect of IL-1$\beta$, IL-6, TNF-α, PGE$_2$ and stretch on PGE synthetic enzyme mRNA expression**

cPLA$_2$ mRNA expression was increased by IL-1$\beta$ ($P < 0.01$) and IL-6 ($P < 0.05$, Figure 3a); sPLA$_2$ mRNA expression was unaffected by any treatment (Figure 3b); PGHS-2 mRNA expression was increased by IL-1$\beta$ ($P < 0.01$), PGE$_2$ ($P < 0.05$) and stretch ($P < 0.05$, Figure 3c); PGES-1 mRNA expression was increased by IL-1$\beta$ only ($P < 0.01$, Figure 3d); and PGES-2 mRNA expression was unaffected by any treatment (Figure 3e).

**Discussion**

These data show that labour is associated with increased expression of cPLA$_2$, sPLA$_2$ and PGHS-2 mRNA and that the lower segment expresses higher mRNA levels of key enzymes involved in PGE$_2$ synthesis. Further, we found associations between PGE$_2$ synthetic enzyme mRNA levels and those of inflammatory cytokines and increments in the key PGE$_2$ synthetic enzyme mRNA expression in response to IL-1$\beta$. Overall, these data suggest that the expression of the enzymes involved in PGE$_2$ synthesis is co-ordinated and that the prime candidate for this effect is IL-1$\beta$.

Myometrial cPLA$_2$ mRNA expression and/or enzyme activity were unchanged with the onset of labour in the sheep and mouse (Zhang et al., 1996; Wu et al., 1998; Winchester et al., 2002), but increased in the guinea pig cervix (Rajabi and Cybulsky, 1995). Studies in human myometrium report a non-significant increase in cPLA$_2$ protein levels with advancing gestation (Korita et al., 2002) and an increase in myometrial sPLA$_2$, but not cPLA$_2$, mRNA expression with the onset of labour (Slater et al., 2004). These results are consistent with our sPLA$_2$, but not our cPLA$_2$, data. However, given that we found relationships between cPLA$_2$ and both PGHS-2 and PGES-1 and none for sPLA$_2$ and that cPLA$_2$, but again not sPLA$_2$, mRNA expression was increased by IL-1$\beta$ and IL-6, it seems that it is likely that cPLA$_2$ is a part of the co-ordinated up-regulation of the PGE$_2$ synthetic enzymes associated with labour. However, although IL-1$\beta$ increased cPLA$_2$ mRNA expression, there was no association between myometrial levels of IL-1$\beta$ mRNA and those of cPLA$_2$, suggesting in this case that the effect of IL-1$\beta$ may not be direct.

The expression of PGHS-2 mRNA in human myometrium is reported to increase with the onset of labour in humans (Hirst et al., 1995a; Slater et al., 1999; Challis et al., 2000; Lindstrom and Bennett, 2004). Our data are consistent with this except that the increase in PGHS-2 mRNA in the upper segment with the onset of preterm labour did not reach statistical significance. What is responsible for the increase in PGHS-2 expression with the onset of labour is not clear, but IL-1$\beta$ may play an important role. This would be consistent with previous observations (Bartlett et al., 1999; Sooranna et al., 2005) and the results of this study showing associations between IL-1$\beta$ and PGHS-2 ($r = 0.48$) in myometrial samples and an increase in PGHS-2 mRNA expression in response to IL-1$\beta$. Interestingly, the association between IL-1$\beta$ and PGHS-2 (0.48) was weaker than that observed between PGES-1 and PGHS-2 ($r = 0.86$) and we saw an increase in PGHS-2 mRNA in response to PGE$_2$, the product of PGES-1. This may account for the strong relationship between PGHS-2 and PGES-1. Indeed, PGE$_2$ has been reported to increase PGHS-2 mRNA stability in other tissues (Faour et al., 2001; Tamura et al., 2002) and the administration of nimesulide, a PGHS-2 inhibitor, to labouring sheep reduced myometrial PGHS-2 mRNA expression (Wu et al., 1998).

Sheep myometrial expression of PGES mRNA showed a tendency to reduce around the time of labour (Pulliser et al., 2004). In humans, PGES expression was similar in preterm and term samples obtained from the lower uterine segment before and after the onset of labour studied by immunohistochemistry, western analysis and in situ hybridization (Giannoulias et al., 2002). Neither study differentiated between the types of PGES and therefore may not have identified more subtle changes in expression. In our study, we found that PGES-1 mRNA expression was greater in the lower than in the upper segment and that in the lower segment, PGES-1 mRNA expression was increased with the onset of labour. Further, we found that PGES-1 mRNA levels related closely to those of IL-1$\beta$ ($r = 0.82$) and that IL-1$\beta$ increased PGES-1 mRNA expression in primary cultures of human uterine smooth muscle cells. These data suggest that IL-1$\beta$ increases PGES mRNA expression, leading to an increase in PGE$_2$ levels, which in turn increases PGHS-2 mRNA levels perhaps via an effect on PGHS-2 mRNA stability. What proportion of the IL-1$\beta$-induced increase in PGHS-2 mRNA levels in uterine smooth muscle cells is mediated via its increase of PGE$_2$ is uncertain, but in other tissues this has been given as a potential explanation for the relatively modest effects of IL-1$\beta$ on a PGHS-2 promoter construct (Tamura et al., 2002).
Figure 1. (a) Log cPLA2, (b) sPLA2, (c) log PGHS-2, (d) log PGES-1 and (e) PGES-2 : beta actin mRNA ratio (median, interquartile range and range) in paired upper and lower human myometrial samples obtained from four groups of women \( (n = 6, \) in each group), at the time of caesarean section (LSCS) under the conditions of preterm with labour (PTL; 33.9 ± 1.5 weeks), preterm no labour (PTNL; 30.4 ± 1.5 weeks), term with labour (TL; 39.2 ± 0.5 weeks) or term no labour (TNL; 38.4 ± 0.4 weeks). *\( P < 0.05\), **\( P < 0.01\) and ***\( P < 0.001\). NS, not significant.
Myometrial PGE₂ enzyme expression and labour

Table II. The associations of the PGE₂ synthetic enzyme mRNA levels with each other and with those of the cytokines IL-1β and IL-8, correlation coefficient (r, 95% confidence limits, P value)

<table>
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<tr>
<th></th>
<th>PGHS-2</th>
<th>PGES-1</th>
<th>IL-1β</th>
<th>IL-8</th>
</tr>
</thead>
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<tr>
<td>cPLA₂</td>
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<td>0.55</td>
<td>0.24</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>0.45–0.78 (&lt;0.0001)</td>
<td>0.33–0.72 (&lt;0.0001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sPLA₂</td>
<td>−0.01</td>
<td>0.2</td>
<td>0.19</td>
<td>0.004</td>
</tr>
<tr>
<td>PGHS-2</td>
<td>−</td>
<td>0.86</td>
<td>0.48</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.77–0.92 (&lt;0.0001)</td>
<td>0.23–0.67 (&lt;0.0005)</td>
<td>0.037–0.54 (&lt;0.05)</td>
</tr>
<tr>
<td>PGES-1</td>
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<td>−</td>
<td>0.7–0.89 (&lt;0.0001)</td>
<td>0.39–0.75 (&lt;0.0001)</td>
</tr>
<tr>
<td></td>
<td>0.77–0.92 (&lt;0.0001)</td>
<td>0.09</td>
<td>0.1</td>
<td>0.02</td>
</tr>
<tr>
<td>PGES-2</td>
<td>−0.01</td>
<td>−</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 2. The relationship (a) between log PGES-1 and log PGHS-2 \( r = 0.86 \, (0.77–0.92) \), \( P < 0.0001 \) and (b) between log IL-1β and log PGES-1 \( r = 0.82 \, (0.7–0.89) \), \( P < 0.0001 \) in myometrial samples obtained from women before and after the onset of labour before and at term as shown by the symbols, closed circles, term labour; open circles, term no labour; closed triangles, preterm labour; open triangles, preterm no labour.
Figure 3. Percentage change in (a) cPLA2, (b) log sPLA2, (c) log PGHS-2, (d) log PGES-1 and (e) log PGES-2 : GAPDH mRNA ratio (median, interquartile range and range) in primary cultures of uterine smooth muscle cells exposed to TNF-α (1 ng/ml), IL-1β (1 ng/ml), IL-6 (1 ng/ml), PGE2 (1 nmol/l) all for 24 h and 11% stretch for 6 h. *P < 0.05 and **P < 0.01.
2002), but our data are consistent with those of Korita et al. (2002) who found that cyclical stretch of primary cultures of human uterine myocytes did not increase cPLA₂. Perhaps the sequence of events is that stretch of the lower segment increases the expression of IL-8 (Loudon et al., 2004), resulting in the previously described inflammatory cell infiltrate (Osman et al., 2003). The activated neutrophils release inflammatory cytokines, and these in turn promote the expression of the enzymes involved in PGE₂ synthesis. This is consistent with the increases in uterine smooth muscle cell expression of cPLA₂, PGHS-2 and PGES-1 mRNA in response to IL-1β described in this study. However, while each of the key enzymes in the PGE₂ synthetic pathway relate to each other, only PGHS-2 and PGES-1 are associated with IL-1β, suggesting the effect of IL-1β on cPLA₂ mRNA expression may be indirect.

These data support the hypothesis that the PGE₂ synthetic enzymes are regulated in a co-ordinated fashion with the onset of labour and suggest that IL-1β is the key factor. Further, they have shown that the lower segment of the uterus is the main site of PGE₂ synthetic enzyme mRNA expression.

Acknowledgements

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References


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