Prostaglandin endoperoxide H synthase mRNA expression in the human amnion and decidua during pregnancy and in the amnion at preterm labour

Jane E. Mijovic, Tamas Zakar¹, Jordanka Angelova and David M. Olson
Perinatal Research Centre, Departments of Physiology, Obstetrics and Gynaecology, and Pediatrics, University of Alberta, Edmonton, Alberta T6G 2S2, Canada
¹To whom correspondence should be addressed at: University of Alberta, Perinatal Research Centre, 227 Heritage Medical Research Centre, Edmonton, Alberta, T6G 2S2 Canada

We have examined the expression of prostaglandin endoperoxide H synthase (PGHS) isoenzymes in the amnion and the decidua during gestation, and the abundance of PGHS mRNA in the amnion at idiopathic preterm labour. PGHS-1 and -2 mRNA abundance in the amnion, determined with ribonuclease protection assays, was significantly (P < 0.05) higher at term than earlier during pregnancy. In contrast, neither PGHS-1 and -2 mRNA values, nor PGHS-specific activity, measured with a cell-free assay, was different in the decidua at term as compared to earlier gestational ages. In individual term patients, PGHS-2 mRNA values in the amnion were positively correlated with PGHS-2 mRNA values in the chorion laeve. PGHS-1 and -2 mRNA abundance was higher (P < 0.05) in the amnion after idiopathic preterm labour than in the absence of labour at the same gestational age (28–35 weeks). Thus, PGHS-1 and -2 are induced in the amnion at term. Furthermore, amniotic PGHS-2 changes in co-ordination with PGHS-2 concentrations in the chorion laeve. PGHS is not induced in the decidua at term. Increased amniotic PGHS expression may contribute to the enhanced intrauterine prostaglandin synthesis before term labour. Both PGHS isoenzymes may participate in the increase of PGHS activity in the amnion at preterm birth.

Key words: amnion/cyclo-oxygenase/decidua/pregnancy/preterm labour

Introduction

Intrauterine prostaglandins are implicated in the initiation of labour in various species, including humans. Amniotic fluid prostaglandin E2 (PGE2) and PGF2α concentrations rise at late gestation before the onset of labour (Dray and Frydman, 1976; Nieder and Augustin, 1983; Steinborn et al., 1995; Romero et al., 1996), and the local or systemic administration of prostaglandins leads to labour induction (Jacobs, 1986). The principal sources of prostaglandins in the pregnant uterus are the fetal membranes (amnion and chorion laeve) and the decidua (Olson and Zakar, 1993). The precursor of the prostanooids, arachidonic acid, is present in these tissues together with the enzymes that participate in its conversion to PGE2 and PGF2α. The irreversible, committing step of the biosynthetic pathway is catalysed by the prostaglandin endoperoxide H synthase isoenzymes, PGHS-1 and -2 (Herschman, 1994). These enzymes convert arachidonic acid to the prostaglandin endoperoxide PGH2, which is further metabolized to biologically active prostanoids including PGE2 and PGF2α. The importance of the PGHS enzymes in the process of human parturition is underlined by the observation that PGHS inhibitors, such as indomethacin and aspirin, prolong gestation and protract labour (Lewis and Sculman, 1973; Gamissans and Balasch, 1993).

It has been shown that both PGHS-1 and -2 are expressed in the gestational tissues (Slater et al., 1995; Trautman et al., 1996). Furthermore, the concentration of PGHS-1 and -2 mRNA was demonstrated to increase in the chorion laeve with advancing pregnancy, reaching significantly higher values at term than earlier during gestation (Mijovic et al., 1998). PGHS enzyme activity in the chorion laeve of term patients showed a close correlation with PGHS-2 mRNA abundance, but not with PGHS-1 mRNA abundance, suggesting that the PGHS-2 isoenzyme was predominantly responsible for the increased capacity of this tissue to produce prostaglandins preceding labour.

Two other components of the gestational tissues, the amnion and the decidua, are adherent to the chorion laeve on two sides, lining the amniotic cavity and the myometrium, respectively. The contribution of these tissues to the gestational age-dependent increase of intrauterine prostaglandin synthesis is unclear. Therefore, in the present investigation, we examined the expression of the PGHS isoenzymes in the amnion and the decidua capsularis during pregnancy. PGHS-1 and -2 mRNA values were determined in both tissues and PGHS activity was measured in the decidua of patients who delivered in the absence of labour at different times during gestation. In addition, the amniotic expression of PGHS-1 and -2 mRNA was determined at preterm labour in order to assess the possible involvement of changing PGHS concentrations in the control of prostaglandin production in this condition.

Materials and methods

Materials

[5,6,8,11,12,14,15-N³H]PGE2 (specific activity, 140 Ci/mmol) was purchased from Amersham Canada (Oakville, Ontario, Canada) and [α-32P]-cytidine 5‘-triphosphate was obtained from DuPont Canada
Prostaglandin synthases in the amnion and decidua
(Mississauga, Ontario, Canada). Protease K, ribonuclease-A and T<sub>1</sub>, RNase-free DNase and reduced glutathion were from Boehringer Mannheim Canada (Laval, Quebec, Canada). T<sub>7</sub> and T<sub>3</sub> RNA polymerases were supplied by BRL (Gaithersburg, MD, USA). Arachidonic acid was bought from NuChek Preparations (Ellysian, MN, USA). Sep-Pak C<sub>18</sub> cartridges were the products of Waters-Millipore (Milford, MA, USA). Phenylmethylsulphonyl fluoride (PMSF), leupeptin, diethylthiocarbamic acid, tryptophan and 1,4-piperazine-diethanesulfonic acid were purchased from Sigma (St Louis, MO, USA). The sources of the other chemicals are given below.

**Tissue collection**

Placentas with attached membranes were obtained from 43 patients who delivered without labour at different times of gestation. A total of 25 pregnancies were terminated by elective Caesarean section at term, defined as 37–41 weeks of gestational age (World Health Organization, 1977). Eight patients underwent Caesarean section at 21–36 weeks of pregnancy for medical reasons. The length of the pregnancies was calculated from the first day of the last menstrual period. The absence of labour was determined as &lt;2 cm cervical dilatation, intact membranes, and fewer than one uterine contraction per 10 min. These tissues were collected at the Women’s Centre, Royal Alexandra Hospital, Edmonton, Alberta, Canada. In all, 10 pregnancies were interrupted electively before 20 weeks of gestation. These tissues were supplied by the Central Laboratory for Human Embryology, University of Washington. Procedures for tissue collection, verification and storage were described previously in detail (Mijovic et al., 1998). Particular attention was paid to process the samples within 30 min after delivery, and to verify by histological examination the purity of the decidua, which was separated from the chorion laeve by sharp dissection. The amnion membrane was dissected bluntly from the chorio–decidua. Tissues were assigned to mRNA analysis and enzyme activity determination randomly according to availability and the design of the study.

For studying preterm labour, amnion membranes from five patients who gave birth after spontaneous labour at 28–35 weeks of pregnancy were collected. These patients presented with intact membranes and delivered within 72 h after admission. The control group was selected as patients who delivered by Caesarean section in the absence of labour at different times of gestation. As demonstrated in Table I, both PGHS-1 and -2 mRNA band intensities were divided by &gamma;-actin mRNA band intensities from the same RNA preparation, resulting in relative band intensity values that were used as the measure of mRNA abundance in the corresponding tissues. Abundance values were compared between the patient groups by the non-parametric Mann–Whitney U-test, since normal distribution of the data could not be ascertained. Enzyme activities in the decidua of patient groups who delivered term and preterm respectively, were also compared by Mann–Whitney U-test, again because the data were not normally distributed. Statistical correlations were analysed by simple linear regression. P ≤ 0.05 was considered significant.

**Results**

The expression of PGHS-1 and -2 mRNA in the amnion and the decidua during pregnancy was determined by measuring the steady-state value of these mRNA by specific ribonuclease protection assays. The tissues were collected after the termination of pregnancies in the absence of labour at different times of gestation. As demonstrated in Table I, both PGHS-1 and -2 mRNA abundance (i.e. the relative value of PGHS normalized against actin mRNA) were higher in the amnion at term than earlier during gestation. On the other hand, there was no significant difference between the PGHS-1 or the PGHS-2 mRNA values in the decidua of term patients as compared to patients who delivered before term.

PGHS mRNA abundance in the amnion and decidua of individual patients is presented in Figure 1, as a function of gestational age. The diagrams in Panels A and B clearly suggest a
Table I. Prostaglandin endoperoxide H synthase (PGHS)-1 and -2 mRNA abundance (relative value of PGHS normalized against γ-actin mRNA) in the amnion and the decidua during pregnancy (relative band intensity)

<table>
<thead>
<tr>
<th>Time of pregnancy termination</th>
<th>Before term</th>
<th>At term&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amnion PGHS-1 mRNA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients</td>
<td>12</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Gestational age, weeks; mean (range)</td>
<td>17.8 (7.5–35)</td>
<td>38.6 (37.1–40.1)</td>
<td></td>
</tr>
<tr>
<td>Relative mRNA abundance, median (range)</td>
<td>0.11 (ND–0.33)</td>
<td>0.30 (0.01–0.83)</td>
<td>$P &lt; 0.01$&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Amnion PGHS-2 mRNA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients</td>
<td>13</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Gestational age, weeks; mean (range)</td>
<td>19.1 (7.5–35)</td>
<td>38.5 (37.1–40.1)</td>
<td></td>
</tr>
<tr>
<td>Relative mRNA abundance, median (range)</td>
<td>0.00 (ND–0.97)</td>
<td>0.96 (0.06–2.8)</td>
<td>$P &lt; 0.01$&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Decidua PGHS-1 mRNA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients</td>
<td>12</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Gestational age, weeks; mean (range)</td>
<td>17.9 (7.5–35)</td>
<td>38.6 (37.1–40.1)</td>
<td></td>
</tr>
<tr>
<td>Relative mRNA abundance, median (range)</td>
<td>0.55 (ND–1.78)</td>
<td>0.23 (0.04–1.2)</td>
<td>$P &lt; 0.01$&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Decidua PGHS-2 mRNA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients</td>
<td>15</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Gestational age, weeks; mean (range)</td>
<td>17.6 (7.5–35)</td>
<td>38.5 (37.1–40.1)</td>
<td></td>
</tr>
<tr>
<td>Relative mRNA abundance, median (range)</td>
<td>0.00 (ND–4.7)</td>
<td>0.21 (0.01–3.44)</td>
<td>NS&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Tissues were collected from patients whose pregnancies were terminated in the absence of labour. PGHS-1 and -2 mRNA values and, for reference, γ-actin mRNA values were determined by ribonuclease protection assays. The autoradiographic signals were quantified by densitometry.

<sup>a</sup>Delivery between 37–41 completed weeks of gestation.

<sup>b</sup> ND = not detected, entered as zero in the statistical calculations.

<sup>c</sup> Significantly different by the Mann–Whitney U-test.

<sup>d</sup> NS = not significant.

Figure 1. Prostaglandin endoperoxide H synthase (PGHS)-1 and-2 mRNA abundance in the amnion and decidua during pregnancy. RNA was isolated from the amnion (Panels A and B) and decidua (Panels C and D) of patients who delivered in the absence of labour at different times of gestation, and PGHS-1 (Panels A and C) and PGHS-2 mRNA levels were determined by specific ribonuclease protection assays. Each point represents an individual patient.
rise in PGHS-1 and -2 mRNA values in the amnion at term, while no such trend is apparent in the decidua as demonstrated in Panels C and D. Rather, decidual PGHS-1 and -2 mRNA expression varied widely among patients throughout gestation.

To determine the activity of PGHS in the decidua during gestation, particulate fractions were prepared from homogenates of decidua samples collected at different times of pregnancy, and PGHS activity was measured. Figure 2 shows the specific activity values according to gestational age. Varying amounts of enzyme activity were detected in the decidua during gestation. There was no apparent tendency of PGHS activity to change at term. Statistical comparison of PGHS specific activities between the two groups of patients who delivered at term and before term respectively, indicated no significant difference (at term: 41.1 ± 29.9 pg PGE₂/μg protein/min, mean ± SD; 30 and 3–82, median and range; n = 8, before term: 28.7 ± 19.8; 25 and 2.5–55; n = 10; Mann–Whitney U-test).

Next, we explored the relationship of PGHS message induction in the amnion and the chorion laeve. Tissues were collected from a group of term patients following elective Caesarean delivery, and PGHS-1 and -2 mRNA abundance in the amnion and chorion laeve of individual patients were correlated. As demonstrated in Figure 3, lower panel, PGHS-2 mRNA values in the two tissues of the same patients showed significant positive correlation. PGHS-1 mRNA values, however, did not correlate with each other (Figure 3, upper panel).

We assessed the change of PGHS mRNA values in the amnion during preterm labour by comparing PGHS mRNA abundance in a group of patients who gave birth spontaneously before term with a group who delivered at a similar gestational age without labour. Figure 4 shows the results of ribonuclease protection assays where PGHS-1 and -2 mRNA values were determined in the amnion of five patients following spontaneous preterm birth and preterm Caesarean delivery in the absence of labour respectively. As indicated by the autoradiographic images and the densitometric evaluation of signal intensities (Table II), both PGHS-1 and -2 mRNA values were significantly higher after spontaneous preterm delivery than following elective Caesarean section at the same mean gestational age.

Discussion
It is well documented that PGE₂ and PGF₂α accumulate in the amniotic fluid at term before the onset of labour (Dray and Frydman, 1976; Nieder and Augustin, 1983; Steinborn et al., 1995; Romero et al., 1996). The predominant cause of the increased amniotic fluid prostaglandin concentrations is the enhanced production of prostanoids by the gestational tissues, although a decreased rate of metabolic inactivation cannot be excluded in certain cases (van Meir et al., 1997). The increased intrauterine prostaglandin concentrations may be instrumental to the initiation of labour.

The two principal stages of prostaglandin biosynthesis are the phospholipase-evoked release of arachidonic acid from cellular phospholipids and the conversion of arachidonic acid to prostaglandins. Free arachidonic acid is present in the amniotic fluid and in the amnion, chorion laeve and the decidua at concentrations of 0.1–2.5 μM around the time of labour onset (MacDonald et al., 1974; Filshie and Anstey, 1978). Phospholipase C and A₂ are expressed in the gestational tissues, however, their specific activity was reported to stay constant at late pregnancy (Olson and Zakar, 1993; Skannal et al., 1997). This suggests that a continuous supply of arachidonic acid is present for further metabolism in the pregnant uterus at term. Therefore, the capacity of the gestational tissues to synthesize prostaglandins from arachidonic
amnion tissue at term, and correlation analysis indicated that the elevation of enzyme activity was due to the induction of the PGHS-2 isoenzyme. It has also been demonstrated that PGHS activity increases at late gestation in the amnion membrane (Teixeira et al., 1994), which is adjacent to the chorion laeve and lines the amniotic cavity. In the present study we have obtained evidence showing that, similar to the chorion laeve, PGHS-1 and -2 mRNA abundance is elevated in the amnion at term prior to labour, PGHS specific activity values in the amnion tissues analysed for mRNA values were not available, thus correlations between PGHS-1 or -2 mRNA abundance and enzyme activity were not determined. However, in previous studies we have found that, in amnion membranes before and after term labour, PGHS activity correlated positively and significantly with PGHS-2 mRNA values, but not with PGHS-1 mRNA values (Hirst et al., 1995). It is therefore reasonable to conclude that in the amnion as well as in the chorion laeve PGHS activity at late gestation is dependent predominantly on the expression of PGHS-2.

Our data also suggest that PGHS-2 expression is up-regulated in the amnion and the chorion laeve in a co-ordinated manner at term, since PGHS-2 mRNA abundance in the two fetal membranes showed significant positive correlation in term patients (Figure 3). At the same time, PGHS-1 mRNA abundance was not correlated in the two tissues, indicating independent regulation. These observations are in general agreement with the disparate control of the two PGHS genes in many other cells and tissues, where PGHS-1 is expressed in a developmentally regulated fashion, while PGHS-2 is induced by agonists (Herschman, 1994).

Contrary to the amnion, we detected no gestational age-dependent differences of PGHS expression in the decidua capsularis. In addition, PGHS-1 and -2 mRNA and activity values varied widely among patients throughout pregnancy. This suggests that PGHS expression in the decidua may be controlled by mechanisms that are independent of gestational age. Ascending intrauterine infection and inflammation may increase prostaglandin production in the gestational tissues and is believed to trigger preterm and augment term labour (Cox et al., 1993; Romero et al., 1993). However, patients involved in the present investigation were not in labour, had undilated cervix, and were devoid of infection and inflammation according to the usual clinical and histological criteria. Thus, the reasons for the high decidua values of PGHS expression in some pregnancies are unclear. Considering that correlation

<table>
<thead>
<tr>
<th>Mode of delivery</th>
<th>Without labour</th>
<th>Spontaneous</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PGHS-1 mRNA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients</td>
<td>4</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Gestational age (weeks; mean ± SD)</td>
<td>31.2 ± 3.1</td>
<td>30.5 ± 2.2</td>
<td>NS</td>
</tr>
<tr>
<td>Relative mRNA abundance, median (range)</td>
<td>0.18 (0.11–0.33)</td>
<td>0.66 (0.43–1.34)</td>
<td>P &lt; 0.02b</td>
</tr>
<tr>
<td><strong>PGHS-2 mRNA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Gestational age, weeks; mean ± SD</td>
<td>32.0 ± 2.2</td>
<td>30.6 ± 3.2</td>
<td>NS</td>
</tr>
<tr>
<td>Relative mRNA abundance, median (range)</td>
<td>0.33 (0.05–0.97)</td>
<td>1.64 (0.74–1.94)</td>
<td>P &lt; 0.04b</td>
</tr>
</tbody>
</table>

Amnion samples were obtained after spontaneous preterm delivery or following Caesarean section in the absence of labour. mRNA values were determined by ribonuclease protection assays, and normalized against actin RNA values.

a NS = not significantly different.
b Significantly different by the Mann–Whitney U-test. Statistical power reached 0.8 at α = 0.05.
analysis of PGHS activity and values indicated (Hirst et al., 1998; and this study, results not shown) that both PGHS-1 and -2 contribute to the capacity of the decidua to produce prostaglandins, the impact of high decidual PGHS expression on certain pregnancies may be significant, though yet undefined.

PGHS-1 and -2 mRNA values were elevated in the amnion after spontaneous preterm labour as compared with preterm delivery in the absence of labour. This is in agreement with the previously described increase of PGHS activity in the amnion following preterm birth (Teixeira et al., 1994). The patients included in these studies did not show signs of intrauterine infection or inflammation, and the preterm labour cases were classified as idiopathic. PGHS-1 and -2 expression was also found elevated in the chorion laeve at preterm labour, as reported in an earlier study (Mijovic et al., 1998). In situ hybridization with full thickness membrane preparations indicated that PGHS-1 and -2 mRNA were expressed in the mesenchymal, but not in the epithelial, cells of the fetal membranes delivered spontaneously before term. The present data thus support the notion that idiopathic preterm labour is associated with the enhanced expression of both PGHS isoenzymes in the mesenchymal components of the fetal membranes. Term labour, on the other hand, is accompanied by the selective increase of PGHS-2 expression in all cell types of the amnion and the chorion laeve (Hirst et al., 1995; Mijovic et al., 1997).

In conclusion, our data suggest that the amnion matures before the onset of term labour, as reflected by increased PGHS-1 and -2 expression. PGHS-2 mRNA abundance is enhanced in the amnion and the chorion laeve in a coordinated fashion, contributing to an increase of PGHS activity, prostaglandin synthesis and intrauterine prostaglandin accumulation prior to labour. PGHS induction in the decidua does not contribute to the increased prostanoid biosynthetic capacity of the gestational tissues at term. Idiopathic preterm labour is associated with an increase of the amniotic values of PGHS-1 and -2 mRNA, which is similar to preterm labour-related changes detected in the chorion laeve, but different from PGHS regulation accompanying normal term labour.

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References


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