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Expression of Membrane Prostaglandin E Synthase in Human Placenta and Fetal Membranes and Effect of Labor

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Initiation and maintenance of labor in humans is associated with an increase in prostaglandin synthesis by intrauterine tissues. The objective of the present study was to characterize the distribution of membrane-bound PGES (mPGES) protein and mPGES mRNA in human placenta, fetal membranes, and decidua at term and to determine whether any changes occurred with labor. Immunoreactive mPGES was found to be highly concentrated in amnion epithelial cells and the chorion laeve trophoblasts, with lower levels in the mesenchymal layers. The enzyme was at very low levels or undetectable in the decidual tissue. Much lower levels of mPGES protein and mRNA were found in placenta than in fetal membranes. mPGES was associated with the syncytiotrophoblast and in cells surrounding blood vessels. The expression of mPGES mRNA did not change with labor in full membranes or placenta, but Western analysis showed an increase in mPGES protein in chorion laeve and a decrease in mPGES protein in placenta during labor, with no change in the amnion. The differences in expression found among placenta, chorion, and amnion before and after labor would indicate that this enzyme is differentially regulated in these tissues at this time.

Key Words: Prostaglandins; placenta; fetal membranes; decidua; membrane prostaglandin E synthase; labor.

Introduction

Prostaglandins act as potent uterotonins at the time of labor, affecting myometrial contractility, uteroplacental blood flow, and cervical ripening *(1,2)*. The precursor of prostaglandins, arachidonic acid, is converted to prostaglandin H_2 (PGH_2) by the action of prostaglandin H synthase (PGHS) enzymes. PGH_2 is subsequently metabolized to various prostaglandins including PGE_2 and $PGF_{2\alpha}$ via prostaglandin isomerases. PGE_2 is a predominant prostaglandin in intrauterine tissues *(1–3)*, and its synthesis is dependent not only on the PGHS enzyme (Type 1 and 2), but also on the expression and activity of specific PGE synthases (PGESs), which convert PGH_2 to PGE_2 *(4,5)*. PGES is a member of a protein superfamily consisting of membrane-associated proteins that are involved in eicosanoid and glutathione metabolism (the MAPEG family) *(6)*. Two distinct forms of PGES have been identified requiring glutathione as a cofactor: cytosolic PGES (cPGES) and membrane bound PGES (mPGES). cPGES is identical to p23, which is reportedly the weakly bound component of the steroid hormone receptor/hsp90 complex and is constitutively expressed, and unaltered by proinflammatory stimuli in various cells and tissues *(7)*. Jakobsson et al. *(4)* identified and characterized the human microsomal mPGES enzyme and found that mPGES is inducible by interleukin-1β in lung carcinoma A549 cells. In addition, mPGES colocalized with both PGHS isozymes in the perinuclear envelope and is functionally coupled with PGHS-2 in marked preference to PGHS-1 *(8)*.

In sheep placenta, levels of mPGES increase progressively through pregnancy together with the levels of PGHS-2 *(9,10)*, corresponding to the increase in fetal levels of PGE_2. In the human, however, there are no published studies on the expression of cPGES or mPGES in intrauterine tissues. In late gestation, the fetal membranes have been shown to increase PGE_2 output during labor *(11)* and there are marked increases in the expression of PGHS-2 at this time *(12–14)*. Since cytokines increase the expression of the mPGES enzyme concomitantly with PGHS-2 in certain tissues *(4)*, and cytokines have been shown to stimulate prostaglandin production in the fetal membranes *(15)*, we focused our studies on the mPGES enzyme. The purpose of the present study was to examine the cellular distribution of mPGES at the protein and mRNA levels in human placenta, chorion, and amnion at term and to determine whether the expression of this enzyme changed with labor.

Results

Localization of mPGES in Human Fetal Membranes and Placenta

The localization of mPGES in term human fetal membranes and placenta is shown in Figs. 1 and 2. In all mem-

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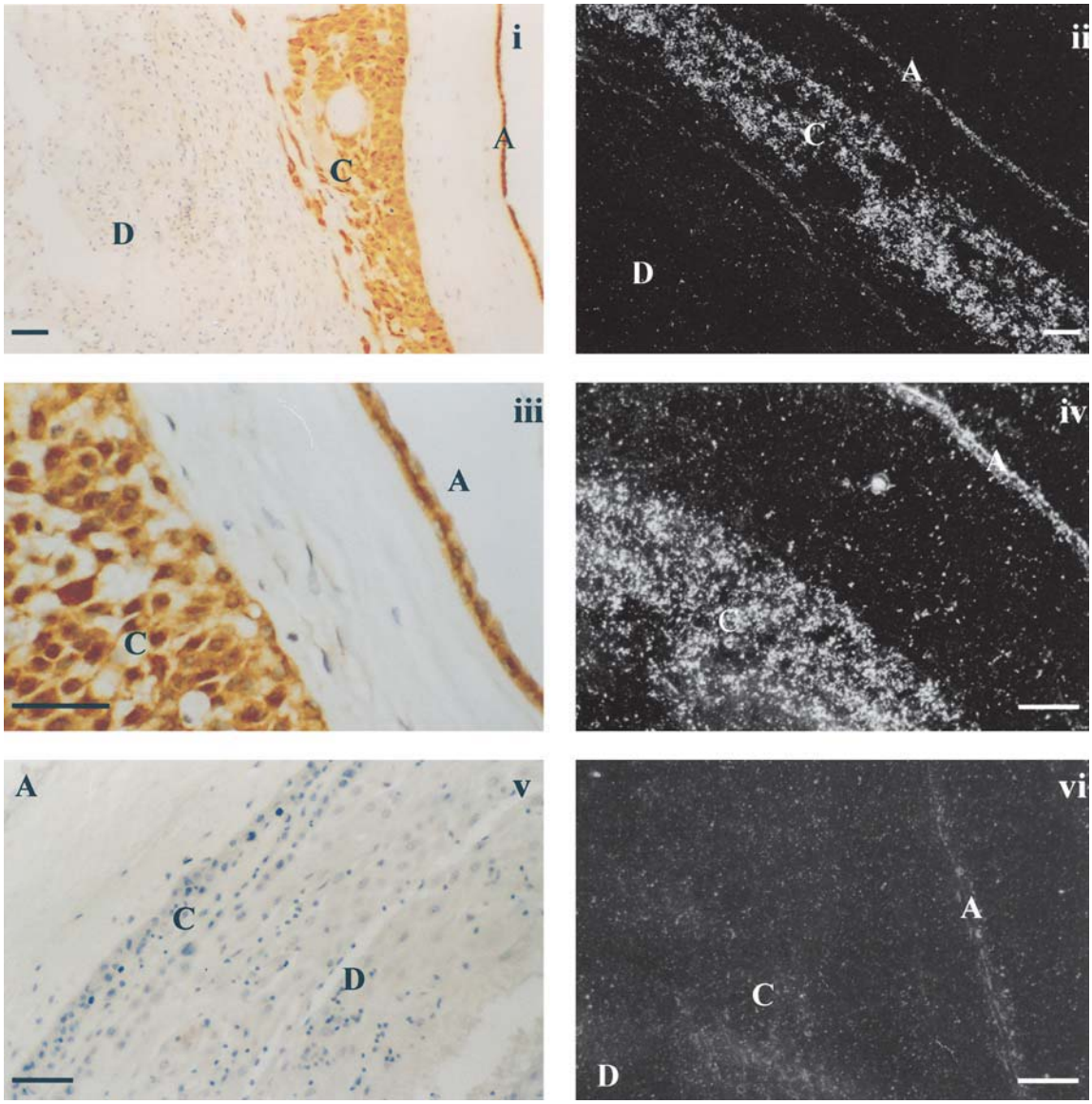

Fig. 1. Localization of mPGES in human fetal membranes and decidua. mPGES was localized to amnion epithelial and chorion laeve trophoblast with little expression in the decidua. This distribution did not change with labor. Results show the use of an antibody dilution of 1:1000 (v/v). (i), (iii) PGES in term tissues; (v) control, preabsorbed with the PGES peptide; (ii), (iv) localization of PGES mRNA in human fetal membranes and decidua by *in situ* hybridization as shown in dark field; (vi) control, sense probe. A, amnion epithelial cells; C, chorion laeve; D, decidua. Bars = 20 μm. These data are representative of tissues obtained at term prior to labor (n = 10) and following labor (n = 10).

branes examined, there was high expression of mPGES protein in the amnion epithelial cells and the chorion trophoblast and much lower expression in the decidua (Fig. 1 [i] and [iii]). The strong staining was removed completely by preincubating the mPGES antibody with the synthetic peptide (amino acids 59–75) to which the antibody had been prepared (Fig. 1[v]). mPGES mRNA was also expressed in the amnion epithelial cells and chorion laeve trophoblast at very high levels and was essentially absent or at much lower levels in the decidua (Fig. 1[ii] and [iv]). No signal was obtained with the sense probe (Fig. 1[vi]). This localization is consistent with the production of PGE_2 by these tissues.

In the placenta, mPGES was localized to the syncytiotrophoblast (Fig. 2[ii]), and strong staining was also observed in cells surrounding the blood vessels (Fig. 2[i]). No staining was found when the primary antibody was preabsorbed with the peptide (Fig. 2[iii] and [iv]). mPGES mRNA levels in placenta were much lower than in fetal membranes; the signal was diffuse and could not be localized to individual cell types by emulsion autoradiography.

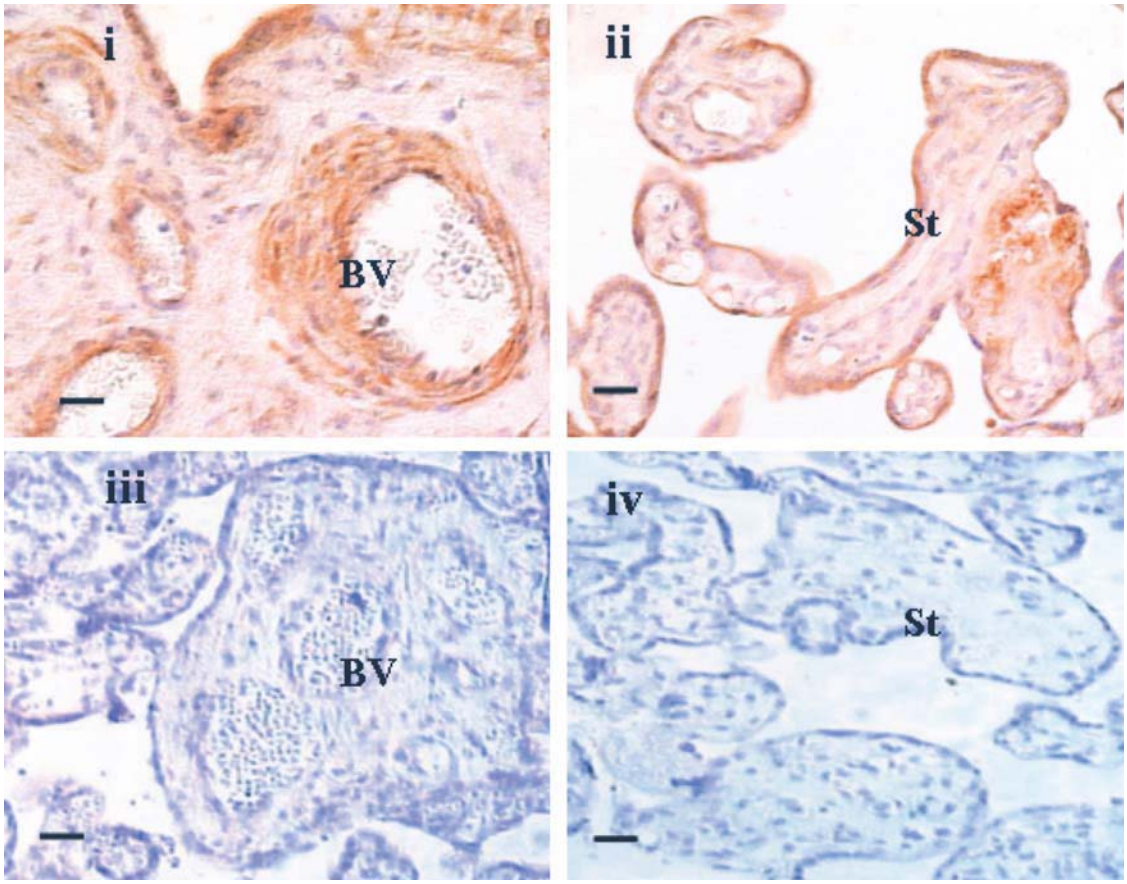

Fig. 2. Localization of mPGES in placental villous tissues from patients at term. There was high expression in the blood vessels (BV) (i), and syncytiotrophoblast layer (St) (ii). Plates (iii) and (iv) represent controls, preabsorbed with PGES peptide. Bars = 50 μm for (i) and (ii) and 25 μm for (iii) and (iv).

Protein extracts from samples of placenta, chorion, and amnion of six nonlaboring patients were compared for the expression of mPGES. Figure 3 shows a representative blot of a band of 16 kDa corresponding to mPGES. The amnion and chorion have similar relatively high expression of mPGES, whereas the placenta expresses much lower levels of the enzyme. The difference was highly significant ($p < 0.001$) when mPGES levels in chorion or amnion were compared with those in placenta.

Effect of Labor on mPGES Expression in Human Placenta, Chorion, and Amnion

The effect of labor on mPGES levels in placenta, chorion, and amnion tissue was examined from six nonlaboring patients and seven patients in labor (Fig. 4). There was no significant change in the level of mPGES protein in the amnion; however, there was a significant decrease in the level of placental mPGES protein with labor and a significant increase in the chorion mPGES protein. Before and after labor no differences were found in mRNA levels of the mPGES in placenta and fetal membranes using film autoradiography (Fig. 5), although the levels in placenta were much lower than in the membranes.

Discussion

The present study has revealed that the mPGES enzyme was expressed in human placenta and fetal membranes although the level of expression in the amnion and chorion was much higher than in the placenta. In the placenta, mPGES was localized to the syncytiotrophoblast and to the cells surrounding the blood vessels. In the fetal membranes, mPGES was highly expressed in the trophoblast layer of the chorion and amnion epithelial cells with low expression in the decidua. In the placenta, the mPGES protein levels declined during labor, whereas in the chorion the levels increased and in the amnion there was no change. mRNA levels, as estimated by film autoradiography, did not change during labor in the whole membranes or in placenta. In the latter case, it is possible that the low signal-to-noise ratio presented a problem for quantification.

The human amnion and chorion are thought to be an important source of PGE_2 involved in labor *(11–14)*, and the high levels of mPGES found in these tissues would support this view. In both placenta and fetal membranes, mPGES has the same localization as that reported for PGHS-2 *(16, 17)*. Previous studies have also shown that mPGES is co-

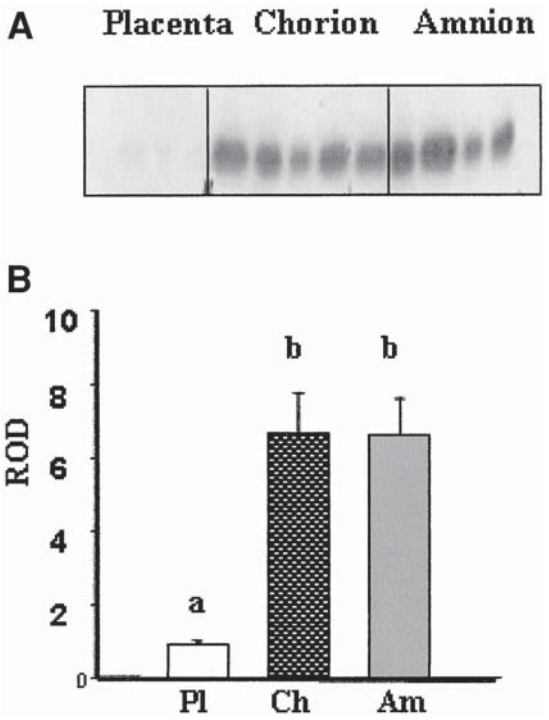

Fig. 3. Differential expression of mPGES in human placenta, chorion, and amnion. (**A**) Representative Western blot of mPGES protein (16 kDa) in placenta and amnion from four nonlaboring patients and chorion from five nonlaboring patients; (**B**) histogram representing relative optical density (OD) of mPGES protein, for six nonlaboring patients for each tissue, normalized to the heavy IgG chain to control for protein loading. Data were analyzed by one-way analysis of variance (ANOVA) ($\alpha = 0.05$). All values are mean ± SEM. Values with different letters are significantly different from each other ($p < 0.05$).

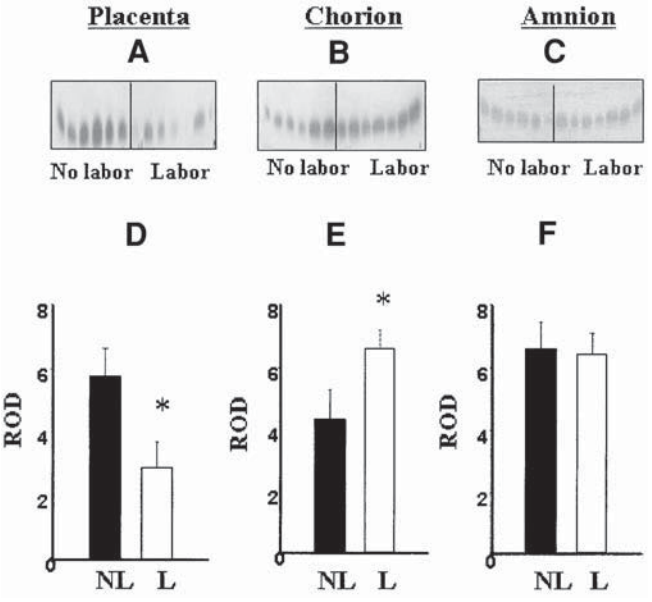

Fig. 4. Effect of labor on mPGES expression in human placenta, chorion, and amnion. Protein from patients in labor (L, $n = 7$) and not in labor (NL, $n = 6$) were examined. (**A–C**) Representative Western blots of mPGES protein (16 kDa) changes with labor in placenta, chorion and amnion respectively; (**D–F**) histograms corresponding to blots in (A), (B), and (C), respectively. The relative OD of mPGES protein was normalized to the heavy IgG chain to control for protein loading. Data were analyzed by student's *t*-test. All values are mean ± SEM.

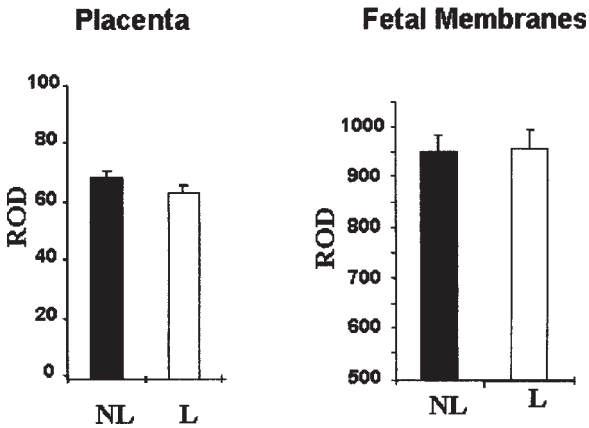

Fig. 5. Effect of labor on mPGES mRNA expression in human placenta and fetal membranes. mRNA expression was quantified by film autoradiography in placental tissues at term not in labor (NL, $n = 6$), and following labor (L, $n = 6$), and fetal membranes not in labor ($n = 12$) and following labor ($n = 12$). The relative ODs for both tissues are standardized to the same control. Data were analyzed by student's *t*-test. All values are mean ± SEM. There was no significant difference in expression between tissues obtained before and after labor.

localized with PGHS enzymes in the perinuclear envelope *(8,18)*. Although the present study did not examine this possibility, such colocalization within the tissues is certainly a possibility.

High levels of mPGES were found in the amnion and no change was seen in the level of expression during labor. This contrasts with the reported increase in PGHS-2 levels at labor in this tissue *(12)* and would suggest that mPGES is not rate limiting in the production of PGE_2 in the amnion. However, the level of mPGES increased with labor in the chorion, indicating that the chorionic enzyme is regulated at this time. Thus, it is clear that there is differential regulation of prostaglandin formation in the amnion and chorion during labor. In vitro studies have shown that cytokines can simultaneously increase expression of PGHS-2 and mPGES in A549 cells *(19)*. It is possible that the effect in chorion seen at labor is owing to cytokine action in this tissue, since the membranes have been suggested to contain a cytokine network *(15)* and cytokine action may be involved in the increase in prostaglandin production that occurs at labor *(3)*.

Recent data from our laboratory have shown that mPGES is also present in human myometrium *(20)*. However, in this tissue enzyme levels are much lower than those found in the placenta. Western blot analysis of placenta, chorion, and amnion protein extracts revealed a unique band at 16 kDa. By contrast, in myometrium the major band has a molec-

ular weight of 180 kDa with a minor band at 16 kDa *(20)*. Kinetic studies have shown that the smaller weight, 16 kDa protein is the active form *(4,21)*. If this distribution reflects active enzyme, then production of PGE_2 would appear to be more important in amnion, chorion, and placenta than myometrium.

In placenta, as in the chorion, mPGES was localized to trophoblast cells, but in the former it was also highly expressed in the cells surrounding blood vessels. The high expression surrounding blood vessels is consistent with the previously reported distribution in human vascular smooth muscle cells and endothelial cells *(22)*, in the myometrium *(20)*, and in the ovine placenta *(10)*. This localization is consistent with the important role that PGE_2 has in the regulation of uterine and placental blood flow *(23)*. The decrease in mPGES levels in the placenta at labor may then result in reduced PGE_2 production during labor and alterations in placental blood flow at this time.

Prostaglandins have numerous autocrine/paracrine effects in fetal membranes and placenta at labor including stimulation of the expression of matrix metalloproteinases *(24)*. We have recently shown that PGE_2 and $PGF_{2\alpha}$ act locally to increase cortisol concentration via regulation of the enzymes 11βHSD 1 and 2 in chorion trophoblast cells and placenta, respectively *(25)*. In the present study, mPGES protein levels exhibited differential changes in the placenta and chorion trophoblast. A significant increase and a significant decrease in protein expression were found in chorion and placental extracts, respectively. These opposite changes in mPGES protein at the time of labor may have physiologic significance with respect to the type of prostaglandin produced in each tissue at the time of labor. However, note that the levels of mPGES protein might not necessarily reflect enzyme activity. Therefore, mPGES activity measurements at the time of labor and investigation of the changes in other synthases involved in the production of different prostaglandins will enable us to better understand not only the production of prostaglandins at labor but also the physiologic role that locally produced prostaglandins have in the placenta and fetal membranes.

In conclusion, the presence of mPGES in placenta and fetal membranes and the changes in its expression that occur during labor indicate that it is differentially regulated within each of the tissues and may have a different role to play in the provision of prostaglandins within each tissue.

Materials and Methods

Tissue Preparation

Placenta with attached fetal membranes and decidua was obtained immediately after spontaneous vaginal delivery or elective cesarean section at term (38–41 wk of gestation) in accordance with ethical standards at the Ottawa Hospital–General Campus and Mt. Sinai Hospital, Toronto. Separate pieces of placenta, chorion, and amnion were directly fast frozen in dry ice–cooled isopentane and stored at –80ºC for Western blot analysis. For immunohistochemistry, placental tissue was fixed in 4% paraformaldehyde, paraffin embedded, sectioned at 5 μm on a microtome (Histocut; Reichert-Jung, Cambridge Instruments, West Germany), placed on Super-frost Plus slides (Fisher, Fair Lawn, NJ), and processed as described in the next section. For *in situ* hybridization, fetal membranes were rolled and frozen in dry ice–cooled isopentane, sectioned on a cryostat, and thaw mounted onto cooled Superfrost/Plus slides. The sections were post-fixed by immersion in 10% buffered formalin phosphate and washed in phosphate-buffered saline (PBS).

Immunohistochemical Analysis

Briefly, slides were deparaffinized and then rehydrated in a graded series of ethanol dilutions. Slides were then incubated with rabbit antihuman polyclonal antibody raised against a peptide corresponding to amino acids 59–75 (CRS DPDVERSLRAHRND) of human membrane-bound PGES (Cayman, Ann Arbor, MI) at a dilution of 1:200 in antibody dilution buffer. The avidin-biotin-peroxidase technique (Vectastain ABC Kit; Vector, Burlingame, CA) for immunostaining was utilized with diaminobenzidine (Sigma, St. Louis, MO) as the substrate *(26)*. Slides were counterstained with Carazzi's hematoxylin followed by dehydration in a graded series of ethanol dilutions, cleared in xylene substitute, and mounted with Permount (Fisher). To test the specificity of the antibody, tissue sections were also stained with primary antibody preabsorbed with excess immunizing peptide.

Western Blot Analysis

At the time of tissue collection, fetal membranes destined for Western blot analysis were separated into chorion and amnion for every patient. Samples of frozen placenta, chorion, and amnion were homogenized on ice for 1 min in RIPA lysis buffer (50 n*M* Tris-HCl [pH 7.5], 150 m*M* NaCl, 1% (w/v) sodium deoxycholate, 0.1% sodium dodecyl sulfate, 100 m*M* sodium orthovanadate (Sigma), 1% (v/v) Triton X-100 [Fisher], and Complete MiniEDTA-free protease inhibitors [Roche; Dorval, Canada]). Homogenates were centrifuged at 4ºC and 15,000*g* for 15 min, and supernatants were collected. Protein concentrations were determined by the Bradford *(27)* assay. Polyacrylamide gels were prepared (stacking gel: 4%; separating gel: 14% for mPGES). Proteins (50 μg/well) were separated by polyacrylamide gel electrophoresis and then transferred electrophoretically to a 0.45-μm pore nitrocellulose membrane (Bio-Rad, Hercules, CA). Transfer was confirmed by protein visualization with Ponceau S (Sigma). Blots were washed with PBS-T, pH 7.5 (150 m*M* NaCl, 10 m*M* Na_2HPO_4, 1.5 m*M* NaH_2PO_4, and 0.1% Tween-20 [Sigma]) and incubated overnight with blocking solution (5% skim milk powder in PBS-T). Subsequently, blots were incubated with primary antibody for rabbit anti-human mPGES (1:1000 dilution in blocking solution)

for 1 h. Blots were then rinsed six times for 5 min each with PBS-T and incubated with secondary rabbit anti-serum conjugated with horseradish peroxidase (1:3000 dilution in blocking solution; Amersham Pharmacia Biotech) for 1 h. Blots were washed six times, 5 min each, and the antibody-antigen complex was detected using the Amersham Pharmacia Biotech ECL detection system. The membranes were then exposed to X-OMAT blue film (Kodak, Rochester, NY). A major band of 16 kDa corresponding to the known molecular weight of mPGES was clearly visible in all specimens tested.

The intensities of immunoreactive bands were measured by scanning (6200C scanner, Hewlett Packard (Canada), Mississauga, Ontario) and analyzing the image on a desktop computer using Scion Image software (v.4.0.2; Scion, Frederick, MD). Protein bands were digitized, and the mean pixel density for each band was analyzed to obtain relative OD units for each protein.

In Situ *Hybridization*

The 50-mer antisense oligonucleotide probe for PGES (complementary to bases 727–776 of the human PGES gene *[28]*) was synthesized by the molecular biology facility at University of Ottawa Biotechnology Research Institute using an Oligo 1000 DNA synthesizer (Beckman, Palo Alto, CA). The uniqueness of the sequence for PGES was verified with a nucleotide sequence database using Blastn 1.4.6 MP *(29)*. Hybridization with the corresponding sense probe, prepared in a similar fashion, served as a control.

Hybridization procedures have been described previously *(9)*. The probes were labeled using terminal deoxynucleotidyl transferase (Gibco-BRL) and ^{35}S-labeled deoxyadenosine 5'a-thio triphosphate (1300 Ci/mmol; NEN, Dupont Canada, Mississauga, Ontario, Canada). The labeled probe was purified using a Nensorb 20 column (NEN; Dupont Canada). Slides were allowed to air-dry at room temperature and then incubated overnight in a moist chamber at 45ºC with 5000 cpm/µL of radiolabeled probe in hybridization buffer. Hybridization buffer contained 4X saline sodium citrate (SSC) (1X SSC is 150 m*M* sodium chloride, 15 m*M* sodium citrate), 50 m*M* sodium phosphate (pH 7.0), 1 m*M* sodium pyrophosphate (pH 7.0), 50% deionized formamide, 0.02% polyvinylpyrrolidone, 10% dextran sulfate, and 40 m*M* dithiothreitol (DTT). After washing for 20 min at room temperature and for 45 min at 55ºC in 1X SSC containing 10 m*M* DTT, slides were rinsed in 1X SSC containing 10 m*M* DTT and in 0.1X SSC (10 s each). Slides were then dehydrated in graded ethanol, air-dried, and exposed to X-ray film (BIOMAX MR; Kodak). Emulsion autoradiography was carried out using standard procedures with Ilford K5 liquid emulsion (Ilford, Mobberly, England). The slides were developed after 14 d using standard procedures and were counterstained with 0.1% cresyl violet to permit identification of nuclei.

Quantification and Autoradiography

Quantification was carried out as we have done previously *(9)*. In each experiment, all control and experimental sections were processed simultaneously to allow direct comparison between groups. For *in situ* hybridization, the sections were exposed together with ^{14}C standards (Amersham, Little Chalfont, UK) to ensure analysis in the linear region of the autoradiographic film. Each tissue was examined in duplicate, and three areas corresponding to 3 mm^2 of the original tissue were chosen at random. The relative OD of the signal on the autoradiographic film was quantified, after subtraction of background values, through the use of a computerized image analysis system (Imaging Research, St. Catherines, Ontario, Canada). The values obtained represent an average density over the areas measured.

Statistical Analyses

Results are expressed as the mean ± SEM for each patient group analyzed. Statistical comparisons were made using one-way ANOVA or student's *t* test (SigmaStat; Jandel, San Rafael, CA).

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