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Differential Expression of the Enzymatic System Controlling Synthesis, Metabolism, and Transport of PGF2 Alpha in Human Fetal Membranes[1]

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ABSTRACT

The present study investigated the expression of genes and proteins associated with PGF2alpha biosynthesis, catabolism, and transport in matched amnion and choriodecidua of human term placenta. The concentration of PGF2alpha within fetal membranes depends on the balance between complex enzymatic systems responsible for, respectively, its synthesis—by prostaglandin-endoperoxide synthase 2 (PTGS2) and members of the aldo-keto reductase (AKR) family, AKR1C3 and AKR1B1—and its catabolic inactivation—through hydroxy-prostaglandin-dehydrogenase (HPGD). We observed that AKR1C3 shows equal basal expression (mRNA and protein) in choriodecidua and amnion but that AKR1B1 exhibits preferential expression in the choriodecidua. Expression of HPGD and solute carrier organic anion transporter family member 2A1 (SLCO2A1) was found primarily in the choriodecidua. We also evaluated whether an inflammatory environment induced by the gram-negative bacterial endotoxin lipopolysaccharide (LPS) affects expression of each candidate enzymes. The amnion responded to LPS with a small but significant decrease of *AKR1B1* mRNA expression. In contrast, we found a significant increase in *PTGS2* and *AKR1C3* mRNA expression in choriodecidua after LPS challenge, but such regulation was confirmed only at protein levels for PTGS2 and not for AKR1C3. Our results suggest that the choriodecidua appears to be the main tissue, which expresses maximally all the components (synthesis, degradation, and transport) controlling PGF2alpha levels.

AKR1B1, AKR1C3, aldo-keto reductases, fetal membranes, human parturition, parturition, placenta, pregnancy, prostaglandin-$F_{2\alpha}$, prostaglandin transporter, SLCO2A1

INTRODUCTION

Parturition encompasses composite physiological processes that require synchronization of uterine contractions, cervical dilatation, and rupture of fetal membranes. The control of onset of labor in women is as yet unknown because of the multiplicity of factors involved (hormonal, mechanical, genetic, and/or environmental). During the past decade, growing evidence has demonstrated a link between intrauterine infection/inflammation and spontaneous preterm labor/delivery [1–5]. Extensive data from the literature have reported that the cytokine-mediated inflammatory process is directly involved in the release of uterotonic agents such as prostaglandin (PG) leading to the rupture of fetal membranes. The fetal membranes (amnion and choriodecidua) are major sources of prostaglandins [6–9]. Numerous in vitro studies have shown that proinflammatory cytokines and bacterial endotoxin stimulate PG production from human term fetal membranes [10–13]. It is well established that the net production of PGs within the placenta and fetal membranes depends on the balance between complex enzymatic systems responsible, respectively, for their synthesis by prostaglandin-endoperoxide synthases, the constitutively expressed PTGS1, and the inducible PTGS2 (also known as cyclooxygenase, COX-1, and COX-2, respectively) [14] and by various terminal PG synthase (such as PGE and PGF synthase responsible for the selective production of PGE2 and PGF2α) on the one hand and for their metabolism/catabolism by the 15-hydroxy prostaglandin dehydrogenase (HPGD) enzyme on the other [15–19]. Previous studies have shown that human fetal membranes expressed various prostaglandin E synthases (PTGES1, also known as microsomal PGE synthase-1; PTGES2, also known as microsomal PGE synthase-2; and PTGES3, also known as cytosolic PGE synthase-3) [20, 21]. Labor has little if any effect on the expression of PTGES, but proinflammatory cytokines and bacterial endotoxin increase the activity of PTGS2 and reduce the activity of HPGD in the fetal membranes, thereby resulting in a net increase of PGE2 production [22]. But compelling evidence also supports a role for PGF2α in human labor. PGF2α concentration increases in amniotic fluid before the onset of spontaneous labor at term [23]. Moreover, a significant rise in PGF2α happens in the amniotic fluid during early labor [24] as well as in amniotic fluid of women with intra-amniotic infection associated to preterm labor [12]. This prostaglandin is known to increase its local concentration at the time of parturition and to induce myometrial contractions and cervical dilatation [6]. PGF2α can be mostly produced through reduction of PGH2 by 9,11-endoperoxide reductase. In humans, the aldo-keto reductase family 1, member C3 (AKR1C3), plays an important role in biosynthesis of PGs, catalyzing the formation of PGF2α and 9α-, 11β-PGF2α from PGH2, and PGD2, respectively [25]. More recently, a new enzyme responsible for PGF2α synthesis, the AKR1B5, has been described in bovine endometrium [26], and the AKR1B1 (the homolog of AKR1B5 in human) was found to be expressed and functional in the human endometrium [27, 28].

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TABLE 1. Primer pair sequences and Genbank accession numbers for real-time quantitative RT-PCR study.

Gene name	Genbank accession no.	Amplicon (size in base pair)	Oligonucleotide primer (5′ → 3′)
PTGS2	NM_000963	101	ATCATAAGCGAGGGCCAGCT
			AAGGCGCAGTTTACGCTGTC
AKR1B1	NM_001628	201	GATCGCAGCCAAGCACAATA
			CTCAACAAGGCACAGACCCTC
AKR1C3	NM_003739	500	GTAAAGCTTTGGAGGTCAC
			CACCCATCGTTTGTCTCG
SLCO2A1	NM_005630	101	CTTCCACCCGGTCTGTGGAG
			TCAGTTGCTTGGAGGTTGCA
HPGD	NM_000860	171	AAGCAAAATGGAGGTGAAGGC
			TGGCATTCAGTCTCACACCAC
GAPDH	NM_002046	380	GGAGAAGGCTGGGGC
			GATGGCATGGACTGTGG

According to these new findings, whether human fetal membranes express such enzyme family remains unknown.

An additional level of regulation of PGs action at the cellular level involving PG transporters has recently been demonstrated. Because PGs predominate as charged anions, they cross cell membranes not by passive diffusion but rather through specialized carriers such as solute carrier organic anion transporter family member 2A1 (SLCO2A1) [29]. SLCO2A1 was initially postulated to mediate influx of PGs from the extracellular milieu for their cellular inactivation and more recently for transit of newly synthesized PGs to exert their biological actions in the bovine uterus and fetal membranes (amnion, chorion, and chorioallantois) [30, 31]. Although SLCO2A1 has emerged as a functional PG carrier in the human endometrium throughout the menstrual cycle and in decidualized endometrial stroma cells [30, 32, 33], its characterization in human fetal membranes and its precise role in parturition are still poorly understood.

The present study focused on the analysis of the complex enzymatic systems controlling PGF2α level in matched amnion and choriodecidua of human term placenta. The objectives were 1) to determine the cellular localization of PTGS2, AKR family members (AKR1C3, AKR1B1), HPGD, and SLCO2A1 protein expression in fetal membranes using immunohistochemistry (IHC); 2) to determine *PTGS2*, *AKR1B1*, *AKR1C3*, *HPGD*, and *SLCO2A1* mRNA expression in amnion and choriodecidua and to compare them with changes in protein levels; and 3) to evaluate whether an inflammatory environment induced by the Gram-negative bacterial endotoxin lipopolysaccharide (LPS) can affect the expression of each enzyme candidates.

MATERIALS AND METHODS

Materials

Monoclonal anti-AKR1C3 antibody was purchased from Sigma-Aldrich, polyclonal anti-HPGD antibody was from Cayman Chemical (Spibio, Montigny le Bretonneux, France), and polyclonal anti-PTGS2 and anti-GAPDH antibodies were purchased from Santa Cruz Biotechnology (TEBU-BIO S.A., Le Perray en Yvelines, France). Monoclonal anti-vimentin (VIM) and monoclonal anti-cytokeratin 7 (KRT7) were purchased from DAKO FRANCE (Trappes, France). Polyclonal anti-AKR1B1 and anti-SLCO2A1 antibodies were contributed by M.A. Fortier and characterized previously [32, 33].

Biological Samples

Placentas were collected from eight pregnant women delivered by elective caesarean section prior to the onset of labor (38th and 40th weeks of pregnancy) because of a diagnosed cephalo-pelvic disproportion in an apparently normal singleton pregnancy. This study was approved by the Comité Consultatif de Protection des Personnes pour la Recherche Biomédicale (Paris-Cochin, France), and informed consent was obtained from all donors. Fetal membranes were dissected free of the placenta under sterile conditions and washed with phosphate-buffered saline (PBS) in order to eliminate blood clots.

The amnion and chorion with adherent decidua were manually separated and cut into 1-cm^2 pieces. Explants were pooled and randomly distributed into 24-well plates (two explants per well, two wells per treatment) containing 2 ml of Dubelco modified Eagle medium supplemented with 10% fetal calf serum, 100 UI/ml of penicillin, and 100 μg/ml of streptomycin (Invitrogen SARL Life Technologies, Cergy-Pontoise, France). The explants were incubated under 5% CO_2 and 95% air at 37°C for 48 h to allow them to stabilize. The explants were incubated either without (basal) or with 10 μg/ml lipopolysaccharide from *Escherichia coli* 127:B8 (LPS) (Sigma-Aldrich, Saint Quentin Fallavier, France) for the indicated times in serum-free medium as previously described [34]. At the end of the incubation period, the explants of each well were pooled, weighed, and immediately frozen in liquid nitrogen before storing at −80°C for subsequent processing for either total RNA or protein extraction.

Immunohistochemistry

Immediately after the cesarean section, full-thickness fetal membranes from four term placentas were fixed in 4% formalin for 4 h at room temperature and then embedded in paraffin. Tissue sections of 5 μm were mounted on slides and dewaxed in xylene and rehydrated in ethanol/water. Immunostaining was performed with a universal streptavidin-peroxidase immunostaining kit (DAKO FRANCE). Nonspecific binding was blocked by incubation of tissue sections for 5 min in a blocking reagent containing 3% hydrogen peroxide and then in 3% serum albumin bovine in PBS buffer for 30 min. The sections were incubated with antibodies directed against AKR1B1 (1/100), AKR1C3 (1/100), PTGS2 (1/500), SLCO2A1 (1/500), HPGD (1/100), VIM (1/200), and KRT7 (1/200) for 30 min at room temperature. Sections were washed in PBS and incubated with a biotinylated secondary antibody for 15 min. They were then washed three times in PBS and incubated with streptavidin conjugated to horseradish peroxidase for 15 min. The sections were washed in PBS, and staining was detected by incubation for 2 min with the DAB (3, 3′-diaminobenzidine) chromogen. Controls were performed by incubating the sections with nonspecific rabbit or mouse purified IgG at the same concentration as the primary antibody. Preparations were counterstained with hematoxylin blue and mounted in an aqueous mounting medium (Ultramount Permanent Mounting Medium, Aqueous, DAKO FRANCE), then examined and photographed with an Olympus BX60 microscope.

Western Blot Analysis

Whole homogenates were prepared from amnion and choriodecidua explants treated as indicated. Tissues were homogenized (400 mg/ml) with an Ultra-Turrax apparatus (IKA-WERKE, VWR, Strasbourg, France) in ice-cold homogenization buffer containing 100 mM Tris HCl, pH 7.4, 2 mM $MgSO_4$, 2 mM EDTA, 10% glycerol, and the protease inhibitor cocktail (P-2714) (Sigma-Aldrich). Protein concentration was determined by the method of Bradford, using bovine serum albumin as standard. Equal amounts of proteins were loaded and separated by SDS-PAGE on 10% or 12% gels and transferred onto a Hybond-P membrane. Nonspecific binding sites were blocked by incubating the membranes with 5% low-fat dried milk in Tris Buffer Saline Tween (TBST) (10 mM Tris HCl, pH 7.5, 0.15 M NaCl, 0.1% Tween 20). The membranes were then incubated overnight at 4°C in TBST/1% milk with the indicated antibodies at the appropriate concentrations: AKR1B1 (1/3000),

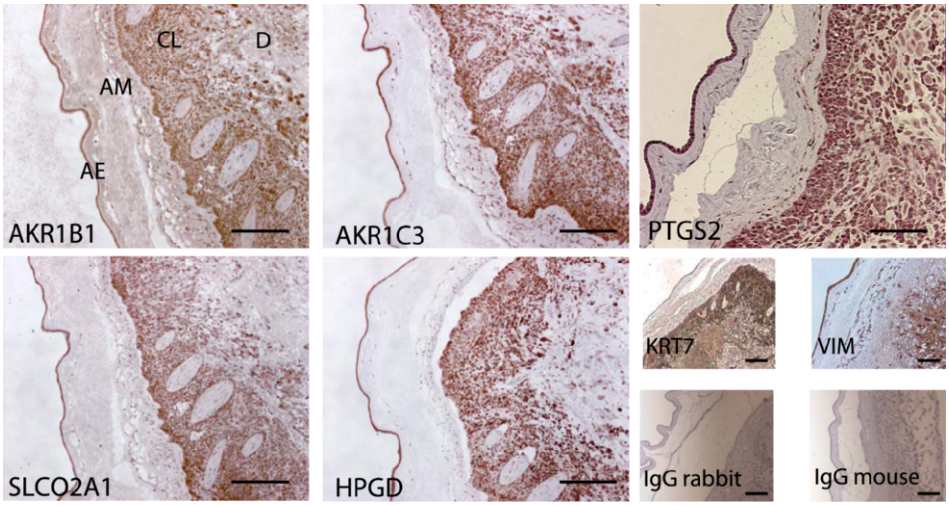

FIG. 1. Immunolocalization of PTGS2, AKR1B1, AKR1C3, SLCO2A1, and HPGD in human fetal membranes. Immunohistochemistry was performed on serial sections of fetal membranes. Tissue-positive controls were conducted with cytokeratin 7 (KRT7) and vimentin (VIM) antibodies, and negative controls were performed with nonimmune rabbit or mouse IgG. AE, amniotic epithelium; AM, amniotic mesenchymal layer; CL, chorionic layer; D, decidual cells. Bar = 100 μm.

AKR1C3 (1/500), PTGS2 (1/1000), SLCO2A1 (1/5000), or HPGD (1/200). Membranes were washed with TBST and incubated with either donkey anti-rabbit or anti-mouse secondary antibody conjugated with horseradish peroxidase. The blots were developed with ECL+ reagents and visualized on Kodak X-ray films (GE Healthcare Bio-sciences, Orsay, France). Molecular-weight markers were run in parallel.

For standardization, the membranes were stripped with a buffer containing 62.5 mM Tris (pH 6.2), 2% sodium dodecyl sulfate, and 100 mM β-mercaptoethanol at 50°C for 30 min and reprobed with a polyclonal antibody raised against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1/1000). Densitometry analysis was performed using a FUJIFILM LAS-1000 Camera System and analyzed by Advanced Image Data Analysis software (Raytest, Courbevoie, France).

RNA preparation. Total RNA was extracted from amnion and choriodecidua explants using the TRIZOL reagent method (Invitrogen SARL) as previously described [35]. Briefly, explants were homogenized in TRIZOL reagent, and total RNA was recovered by phenol/chloroform extraction, isopropanol precipitation and ethanol washes, according to the manufacturer's instructions. Total RNA was quantified by spectrophotometry, and RNA integrity was verified by nondenaturing agarose gel electrophoresis.

Synthesis of human cDNA for AKR1B1, AKR1C3, SLCO2A1, HPGD, PTGS2, and GAPDH. Reverse transcription was performed on 1 μg of total RNA extracted from placental membranes at 37°C with oligo(dT) primer and M-MLV reverse transcriptase (Invitrogen). Reaction mixture for polymerase chain reaction (PCR), including Taq polymerase (QBiogene, MP BIOMEDICALS, Illkirch, France), was prepared as suggested by the manufacturer. Primers were designed using the Primer Selection program from GCG (Genetics Computer Group). The identity of all PCR products was confirmed by DNA sequencing (COGENICS, Meylan, France), and sequence homology analysis was verified using the Basic Local Alignment Search Tool [36]. These fragments were used to design real-time PCR primers and to quantify templates for the real-time PCR standard curves.

Real-Time PCR Analysis

Four independent fetal membranes were analyzed in duplicate. Before doing real-time PCR, the samples were checked for any DNA contamination by performing PCR reactions done without reverse transcription of the RNA extracts. For each transcript to study, real-time PCR was carried out using the real time Applied 7000 PCR system (Applera France SA, Courtaboeuf, France) with 5% of the retrotranscribed product (equivalent to 50 ng of RNA) and 300 nM of gene-specific oligonucleotide pairs, in a total volume of 25 μl of reagent SYBR Green PCR Mastermix (Applera France SA). Primers (Table 1) were designed using Primer Express software (Applera France SA). Real-time PCR

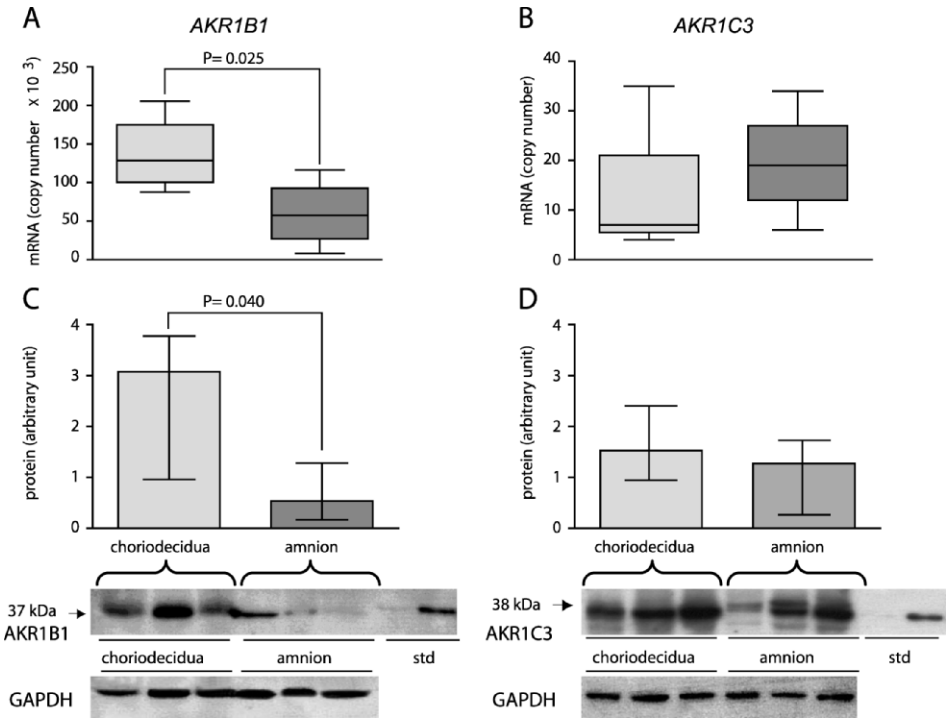

FIG. 2. Expression of AKR1B1 and AKR1C3 in matched amnion and choriodecidua. Quantification of *AKR1B1* (**A**) and *AKR1C3* (**B**) mRNA was performed by real-time PCR. Data, expressed as the copy number, are represented as box plot showing median and 25th and 75th percentiles, the minimum and the maximum data values of four samples measured in duplicate. Protein expression was analyzed by Western blotting (**C** and **D**) on homogenates of three matched amnion and choriodecidua explants and with recombinant AKR1B1 (**C**) or AKR1C3 (**D**) proteins used as positive control (std). The detection of GAPDH in each sample served as a loading control. Representative experiments are shown with three different matched amnion and choriodecidua membranes. Densitometric quantification of bands represents median (top of columns), and the highest and lowest values (bars) of three samples were analyzed four times. There is a statistically significant difference between choriodecidua and amnion both at mRNA and at protein levels for AKR1B1 but not for AKR1C3.

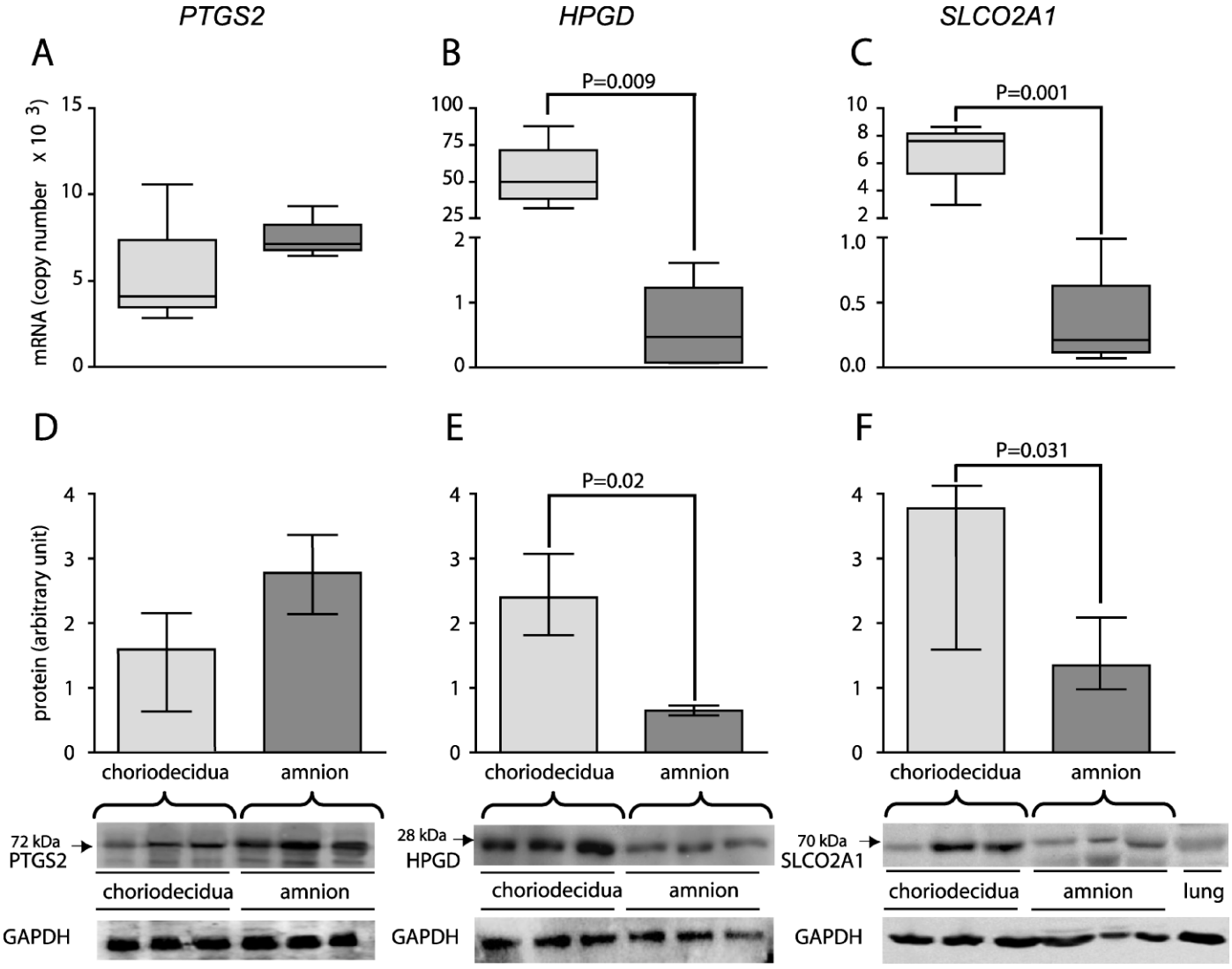

FIG. 3. Expression of PTGS2, HPGD, and SLCO2A1 in matched amnion and choriodecidua. Quantification of *PTGS2*, *HPGD*, and *SLCO2A1* mRNA was performed by real-time PCR (**A**–**C**). Data, expressed as the copy number, are represented as box plot showing median and 25th and 75th percentiles, the minimum and the maximum data values of four samples measured in duplicate. Protein expression was also analyzed by Western blotting (**D**–**F**) on homogenates of three matched amnion and choriodecidua explants and with a homogenate of rat lung used as positive control. The detection of GAPDH in each sample served as a loading control. Representative experiments are shown with three different matched amnion and choriodecidua membranes. Densitometric quantification of bands represents median (top of columns), and the highest and lowest values (bars) of three samples were analyzed four times. There is a statistically significant difference between choriodecidua and amnion both at mRNA and protein levels for HPGD and SLCO2A1.

conditions were 2 min at 50°C and 15 min at 95°C, followed by 45 cycles of 15 sec at 95°C and 1 min at 60°C. Standard curves were generated in parallel using serial dilutions of the purified PCR product as a polymerase template. The presence of a specific and unique PCR product was verified by an ABI prism-generated melting curve profile. Quantification of targets was calculated from their respective standard curves and was expressed as number of RNA copies per 50 ng total retrotranscribed RNA. No significant difference of expression for GAPDH was detected (analysis of variance [ANOVA], $P < 0.05$) between the different tissues (amnion, choriodecidua) and in the presence or absence of LPS.

Statistical Analysis

For transcript analysis, statistical significance was determined using log transform data and two-way ANOVA. ANOVA was followed by post hoc tests using the SYSTAT software (GMBH, Erkrath, Germany). Values at $P < 0.05$ were considered statistically significant. Data are represented as box-and-whiskers graphs. The boxes extend from the 25th percentile to the 75th percentile with a line at the median. The whiskers extend above and below the box to show the highest and lowest values. For Western blot data regarding protein expression in amnion and choriodecidua membranes, there were only three replicates so that values are graphed as the median (top of columns) and the highest and lowest values (bars). The effect of LPS on protein expression in explants of fetal membranes was assessed by ANOVA, and the bar graphs represent the mean ± SEM.

RESULTS

Immunolocalization of AKR1C3, AKR1B1, SLCO2A1, PTGS2, and HPGD in the Human Fetal Membranes

Chorionic trophoblast and amniotic epithelium were evidenced using cytokeratin-7 (KRT7), whereas vimentin (VIM) was used to identify the mesenchymal layer of the amnion and decidual stromal cells (Fig. 1). Positive immunostaining for AKR1B1 and AKR1C3 was found mainly in trophoblast cell layer of the chorion leave and in the decidual stromal cells and faintly in the amniotic epithelium, while a poor staining was detectable in cells of the mesenchymal layer

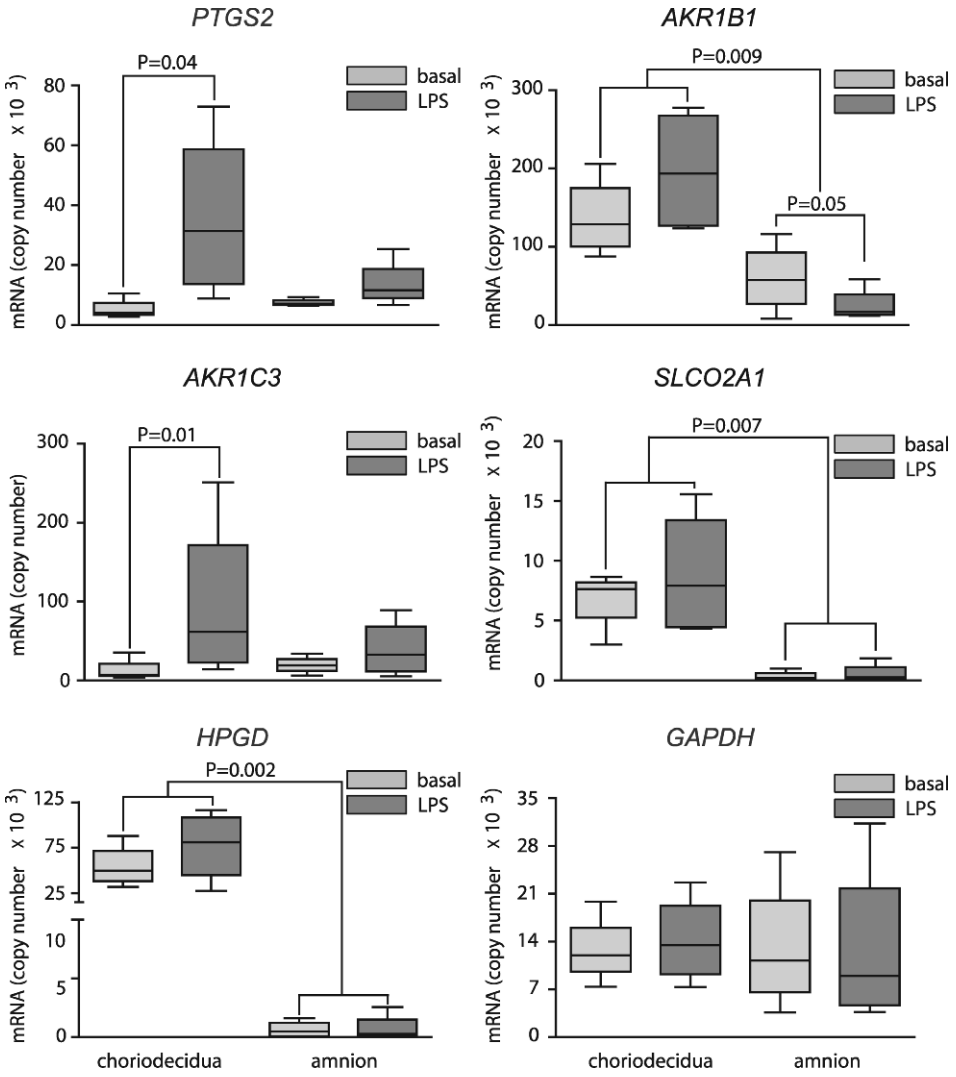

FIG. 4. Effect of LPS on *PTGS2*, *AKR1B1*, *AKR1C3*, *SLCO2A1*, and *HPGD* expression in amnion and choriodecidua. The effect of 4 h of LPS treatment (10 μg/ml) was determined on *PTGS2*, *AKR1B1*, *AKR1C3*, *SLCO2A1*, *HPGD*, and *GAPDH* mRNA expression by real-time PCR. Data are expressed as copy number mRNA and are represented as box plot showing median and 25th and 75th percentiles, the minimum and the maximum data values of four independent samples measured in duplicate. In choriodecidua, there is a statistically significant difference between basal and LPS-stimulated treatment for *PTGS2* and *AKR1C3* but not for *AKR1B1*, *SLCO2A1*, *HPGD*, and *GAPDH*. In amnion, there is a significant effect of LPS only on *AKR1B1*. Significant differences between choriodecidua and amnion are evidenced for *HPGD*, *AKR1B1*, and *SLCO2A1*.

of the amnion. A similar pattern of staining was observed for immunoreactive SLCO2A1 and HPGD. We observed an intense staining for PTGS2 mainly in amnion epithelial cells as well as in chorionic trophoblastic and decidual cells. By contrast, a moderate immunostaining of PTGS2 was present in amnion mesenchymal cells. The immunostaining was abolished when nonimmune serum was substituted for the primary antibody.

Expression of AKR1B1 *and* AKR1C3 *in Amnion and Choriodecidua*

Expression of AKR1B1 and AKR1C3 was analyzed by quantitative RT-PCR and Western blotting in matched amnion and choriodecidua samples (Fig. 2). In both components of fetal membranes, we detected a high mRNA copy number of *AKR1B1* instead of a low mRNA copy number of *AKR1C3*. Contrasting patterns for AKR1B1 expression were observed between amnion and choriodecidua. The choriodecidua exhibited significantly more AKR1B1 expression than the amnion (Fig. 2A). This difference was confirmed at the protein level as indicated after integration of the immunoreactive band for AKR1B1 (Fig. 2C). Messenger RNA expression and protein levels of AKR1C3 in amnion and choriodecidua were equivalent (Fig. 2, B and D).

Expression of PTGS2, HPGD, *and* SLCO2A1 *in Amnion and Choriodecidua*

As shown in Figure 3, A and D, comparable levels of expression of PTGS2 (mRNA and protein) were observed in amnion and choriodecidua. The levels of expression of *HPGD* mRNA (Fig. 3B) and *SLCO2A1* mRNA (Fig. 3C) were significantly higher in choriodecidua than in amnion. These differential expressions were confirmed at the protein level by Western blotting, where the integrated signal for the immunoreactive band at 70 kDa corresponding to SLCO2A1 protein (Fig. 3F) and the immunoreactive band at 28 kDa corresponding to HPGD (Fig. 3E) were significantly more intense in the homogenates of choriodecidua than in amnion samples.

Effect of LPS on PTGS2, AKR1B1, AKR1C3, SLCO2A1, *and* HPGD *Expression*

The effect of LPS on *PTGS2*, *AKR1B1*, *AKR1C3*, *SLCO2A1*, and *HPGD* mRNA expression is presented in Figure 4 with LPS for 4 h. In choriodecidua explants, we found that treatment with LPS increased significantly *PTGS2* and *AKR1C3* mRNA expression. LPS treatment had no significant effect on *AKR1B1*, *SLCO2A1*, or *HPGD* mRNA expression. It has to be noted that AKR1B1, SLCO2A1, and HPGD are significantly expressed at a higher level in choriodecidua than

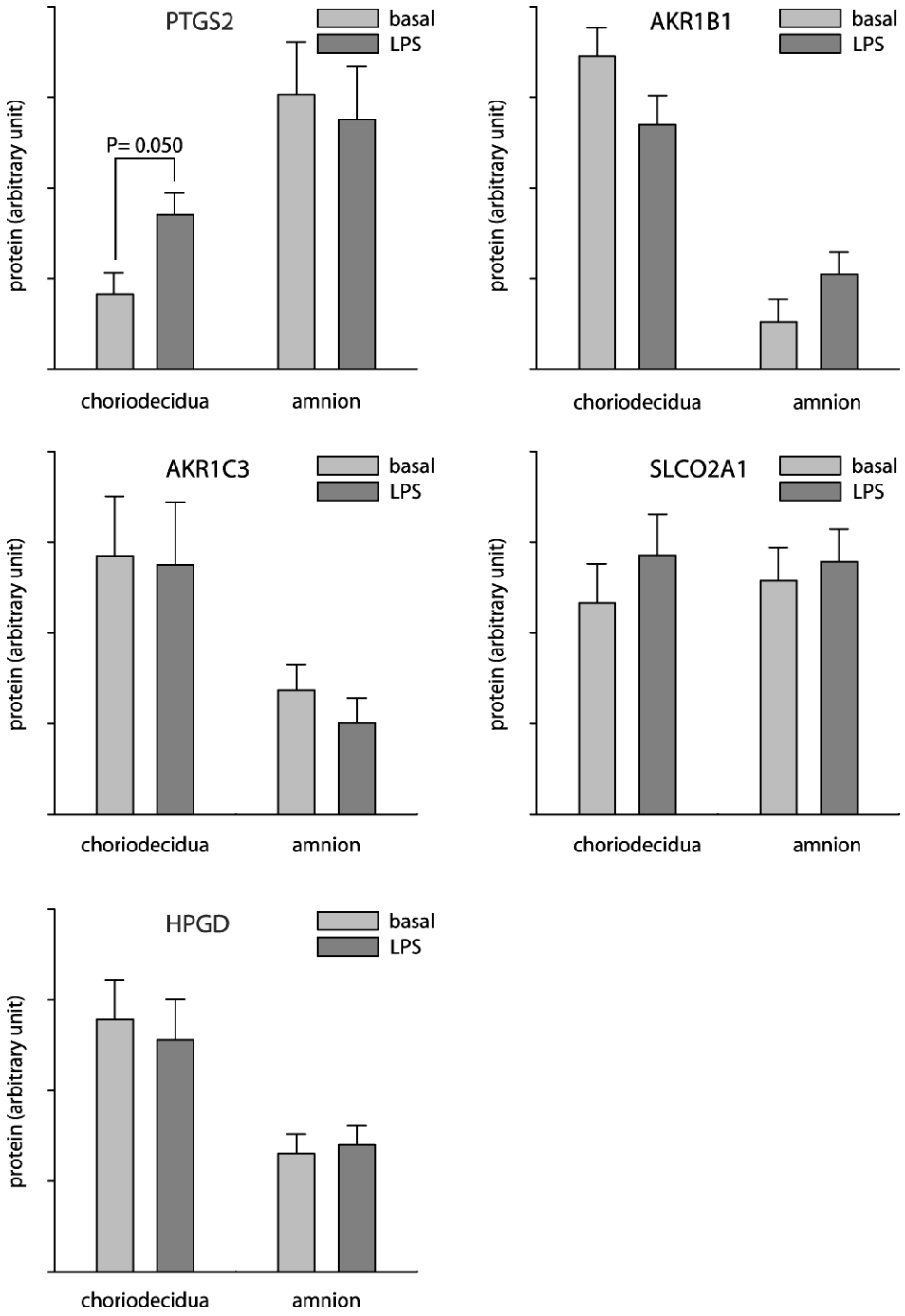

FIG. 5. Effect of LPS on PTGS2, AKR1B1, AKR1C3, SLCO2A1, and HPGD expression in amnion and choriodecidua explants. The effect of LPS treatment (10 μg/ml) was determined on PTGS2, AKR1B1, AKR1C3, SLCO2A1, and HPGD proteins analyzed by Western blot. Data are expressed as mean ± SEM of the quantified densitometry signals. LPS treatment increases PTGS2 protein in choriodecidua ($P = 0.050$) but not in amnion ($P = 0.754$). LPS had no effect on other protein in both choriodecidua and amnion membranes (AKR1C3 [$P = 0.376$; $P = 0.917$], AKR1B1 [$P = 0.148$; $P = 0.108$], SLCO2A1 [$P = 0.662$; $P = 0.405$], HPGD [$P = 0.755$; $P = 0.712$]).

in amnion. In amnion explants, the only significant change after LPS treatment was a decrease in *AKR1B1* mRNA expression. Amnion and choriodecidua explants were then incubated with or without 10 μg/ml LPS for 24 h, and the protein expression of PTGS2, AKR1B1, AKR1C3, SLCO2A1, and HPGD was evaluated (Fig. 5). LPS treatment had no significant effect on AKR1B1, AKR1C3, SLCO2A1, and HPGD protein expression. LPS increased PTGS2 protein ($P = 0.050$) only in choriodecidua. As indicated in Figure 4, this result correlates to the increase of *PTGS2* mRNA. By contrast, the LPS-induced increase of *AKR1C3* mRNA did not parallel an increase of the AKR1C3 protein in the choriodecidua explants.

DISCUSSION

In the present study, we examined in amnion and choriodecidua, the expression of genes and proteins associated with PGF2α biosynthesis, catabolism, and transport in an integrated manner, as a first attempt to understand the response of fetal membranes following LPS exposure to mimic inflammation. We have confirmed that the human fetal membranes possess all the committed steps necessary for autoregulation of PGF2α action. Thus, both PTGS2 and HPGD are localized in human fetal membranes as previously reported [10, 15–19]. Our analysis of the relative expression of these enzymes demonstrates that amnion epithelial cells and chorionic trophoblastic cells displayed similar PTGS2 expres-

sion. In contrast, the chorion expressed predominantly HPGD. This regional distribution within the fetal membranes has previously been reported [15, 16]. Our present data focus attention on the role of the AKR family members [37]. PGF2α biosynthesis involves AKR1C3 and now AKR1B1, the homolog of AKR1B5, which has been previously shown to be most likely the relevant enzyme for the synthesis of luteolytic PGF2α in the bovine endometrium [26–28]. The present report indicates that AKR1B1 was expressed at higher levels than AKR1C3 in both fetal membranes. AKR1C3 showed equal expression (mRNA and protein levels) in choriodecidua and amnion, but the transcripts for *AKR1B1* were more abundant in choriodecidua than in amnion. This is consistent with the AKR1B1 protein expression and with the IHC localization of AKR1B1, which shows choriodecidual preference. AKR1B5, the bovine homolog of AKR1B1, was shown to have complementary actions in the bovine endometrium: generation of PGF2α and inactivation of progesterone [26]. As PGF2α and progesterone are produced by the chorion [38], we cannot exclude that AKR1B1 acts as a multifunctional enzyme in the fetal membranes.

The PG transporter (SLCO2A1) has been identified in human reproductive tissues [32, 33, 39]. It has been proposed that SLCO2A1 mediates both the efflux of newly synthesized PGs to exert their biological action through their cell surface G-protein coupled receptors and the influx of PGs from the extracellular milieu for their cellular inactivation. In the present study, SLCO2A1 is preferentially expressed in choriodecidua compared to amnion. SLCO2A1 protein expression follows that of mRNA, and cellular localization shows that SLCO2A1 protein is highly expressed in the choriodecidua. This is a logical site of expression given the documented role of chorion for PG inactivation during pregnancy [24]. Interestingly, the choriodecidua appears to be the main tissue, which expresses maximally all the components (synthesis, degradation, and transport) controlling PGF2α levels.

Growing evidence has demonstrated an association between intrauterine infection/inflammation and preterm birth [4, 5, 40]. Intra-amniotic or intrauterine injection of dead bacteria or bacterial products, including LPS, is sufficient to promote preterm labor in mouse, rabbit, and sheep [41]. Because PG production by fetal membranes is believed to be an important initiator of term and preterm labor, we expected changes in the expression of synthesis, metabolism, and transport of PGF2α in human choriodecidua and amnion following LPS-induced inflammatory environment.

A classical concept in humans is that choriodecidua appears as the first tissue colonized by microbial pathogens during an ascending intrauterine infection. Experimental evidence indicates that choriodecidua produces 10-fold-higher levels of proinflammatory cytokines than amnion [34, 42, 43]. The present study demonstrates that in amnion, inflammatory conditions have almost no effect on the gene expression of *PTGS2*, *AKR1C3*, *HPGD*, and *SLCO2A1*. In contast, in choriodecidua, we demonstrated that LPS induced expression of *PTGS2* at mRNA and protein levels without altering expression of AKR1B1. An increase of *AKR1C3* mRNA levels was observed on LPS addition, but such regulation was not confirmed at the protein levels. The underlying cause of differential gene expression remains to be determined. It is also of note that expression level of HPGD is much more elevated in the choriodecidua compared to amnion, although we have not observed an inhibitory effect of LPS on this enzyme. The LPS has been previously reported to inhibit the activity of HPGD in the fetal membrane [11] and stimulated PGF2α release from the decidua and the intact fetal membrane [44, 45]. Interestingly, when chorion and decidua are separated, the chorionic production of prostaglandins always exceeds that of decidua, suggesting a potential paracrine inhibitory pathway between the two tissues [46]. Therefore, we cannot exclude that a similar mechanism may operate in vivo between choriodecidua and amnion. As quoted by others [15–17], the choriodecidua may act as a protective barrier for regulating the paracrine and the autocrine action of bioactive PGF2α until the time of spontaneous labor. Our data also support the idea that the choriodecidua is a major site for local control of enzymes of PGF2α biosynthesis and is more responsive than amnion to an inflammatory insult.

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