0021-972X/03/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 88(11):5564–5571

doi: 10.1210/jc.2003-030875

Glucocorticoids Induce Cytosolic Phospholipase A_2 and Prostaglandin H Synthase Type 2 But Not Microsomal Prostaglandin E Synthase (PGES) and Cytosolic PGES Expression in Cultured Primary Human Amnion Cells

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This study examines the regulation of major enzymes in prostaglandin E_2 (PGE_2) synthesis by glucocorticoids in separate cultures of human amnion epithelial and fibroblast cells at term. Cytosolic phospholipase A_2 ($cPLA_2$), cytosolic PGES (cPGES), and microsomal PGES (mPGES) mRNA were expressed at similar levels in both cell types, whereas a greater prostaglandin H synthase type 2 (PGHS-2) mRNA expression was observed in amnion fibroblasts than in epithelial cells. Amnion fibroblasts produced 50-fold more PGE_2 per cell than epithelial cells. Dexamethasone (0.01–1 μM) increased PGE_2 production in amnion fibroblasts in a concentration-dependent manner but did not affect PGE_2 production in amnion epithelial cells. Both mRNA and protein expression of $cPLA_2$ and PGHS-2 but not cPGES and mPGES were increased in a dose-dependent manner by dexamethasone (0.01–1 μM) in amnion fibroblasts. Induction of $cPLA_2$ and PGHS-2 mRNA by dexamethasone was blocked by RU486. Dexamethasone did not affect PGHS-2, cPGES, and mPGES mRNA expression in amnion epithelial cells. In conclusion, amnion fibroblasts express a higher level of PGHS-2 mRNA and produced more PGE_2 per cell than amnion epithelial cells at term of human pregnancy. Glucocorticoids increase PGE_2 production only in the amnion fibroblasts mainly through induction of $cPLA_2$ and PGHS-2 expression. (*J Clin Endocrinol Metab* 88: 5564–5571, 2003)

PRETERM LABOR OCCURS in approximately 6–10% of all pregnancies and accounts for more than 75% of the perinatal mortality and morbidity rate (1). Despite progress in this field, the lack of identification of the mechanism of human parturition has limited the specific and effective diagnosis and treatment of preterm labor.

In most mammalian species, there is an increase in glucocorticoid concentration in maternal and fetal circulations as well as amniotic fluid toward the end of gestation and at the onset of labor (2, 3). This surge of glucocorticoid is believed to be crucial to maturation of fetal organs as well as to be integral to the cascade of events in the initiation and maintenance of labor (4). The concurrent increase in prostaglandin production, especially prostaglandin $(PG)E_2$ and PGF_{2a} with the rise of glucocorticoid concentration at term is one of the major events leading to labor (5). PGs have been identified as key factors inducing cervical ripening, myometrial contraction, and fetal membrane rupture at term (5).

Fetal membranes, in particular amnion, are believed to be the major PGE_2 source at term (6). The conversion of arachidonic acid into PGH_2 by prostaglandin H synthase (PGHS) is currently thought to be the rate-limiting step in prostaglandin synthesis (7). There are two isoforms of PGHS. PGHS-1 is constitutively expressed in many tissues, whereas PGHS-2 is the inducible isoform (7). The increase in prostaglandin synthesis at term and parturition is believed to be associated with increased expression of the enzyme PGHS-2 (5, 8). However, there are also other enzymes, cytosolic phospholipase A_2 ($cPLA_2$) and two isoforms of PGE synthase [cytosolic PGES (cPGES) and microsomal PGES (mPGES)] that are potential regulatory steps in PGE_2 synthesis in addition to PGHS. Cytosolic PLA_2 catalyzes the release of arachidonic acid, the initial and rate limiting substrate in prostaglandin synthesis (9), from phospholipids (10). Cytosolic PGES and mPGES are two terminal enzymes responsible for the specific synthesis of PGE_2 from PGH_2 (11, 12). It has been reported that cPGES is constitutively expressed (11), whereas mPGES is inducible by IL-1 in human alveolar A549 cells (12).

Amnion is believed to be a tissue of prime importance, anatomically and functionally, in the maintenance of pregnancy and during the initiation of parturition. It has been shown that PG synthesis increases in the amnion at term (5, 13), and glucocorticoids exert potent stimulation of PG output by inducing the expression of PGHS-2 in the amnion (8, 14). Previous work has demonstrated that all the four major enzymes ($cPLA_2$, PGHS-2, cPGHS, and mPGES) involved in PGE_2 synthesis were present in the amnion epithelium and fibroblast (15–17). However, few studies have addressed the regulation of $cPLA_2$, cPGHS, and mPGES by glucocorticoids in the amnion. Previous work also showed that the effect of

Abbreviations: cPGES, Cytosolic PGES; $cPLA_2$, cytosolic phospholipase A_2; FCS, fetal calf serum; GR, glucocorticoid receptor; GRE, glucocorticoid response element; 3β-HSD, 3β-hydroxysteroid dehydrogenase; mPGES, microsomal PGES; PG, prostaglandin; PGHS, PGH synthase; PR, progesterone receptor; QT-RT-PCR, quantitative real-time PCR.

glucocorticoids appears to be restricted to stimulation of PGHS-2 expression and PGE_2 production from amnion fibroblasts but not amnion epithelial cells (14, 18, 19). Thus, we performed studies in separate cultures of human amnion epithelial and fibroblast cells to investigate the regulation of $cPLA_2$, PGHS-2, cPGES, and mPGES expression and PGE_2 production by glucocorticoids in these separate cell types.

Materials and Methods

Amnion epithelial and fibroblast cell preparation

Fetal membranes were collected at term from elective cesarean section patients not in labor under a protocol approved by the University of Cincinnati Institutional Review Board. Patients treated with steroids or other antiinflammatory agents or with clinical indication of inflammation were excluded in this study. Amnion was peeled off the chorion and washed three times in cold PBS (pH 7.5). For amnion epithelial cell preparation, amnion tissue was digested with 0.125% trypsin (Sigma, St. Louis, MO) and 0.02% DNAase (Sigma) twice for 30 min at 37 C. The digestion media were collected, and the remaining amnion tissue was washed vigorously with PBS three times to wash residual epithelial cells off the amnion tissue. The wash solution was then combined with the previous trypsin digestion media. For the preparation of amnion fibroblasts, the remaining amnion tissue was further digested with 0.1% collagenase (Roche, Indianapolis, IN) at 37 C for 1 h. The digestion medium was then collected. Both trypsin (epithelium) and collagenase (fibroblast) digestion media were centrifuged at 2300 rpm for 15 min. Cell pellets were collected and resuspended in DMEM without phenol red (Sigma). Resuspended cells were loaded onto pre-prepared discontinuous Percoll (Sigma) gradients (5, 20, 40, and 60%, respectively), and the gradients were centrifuged at 2500 rpm for 20 min. A single band of cells around 20% Percoll concentration was collected and diluted with DMEM containing 10% fetal calf serum (FCS) (Atlas, Fort Collins, CO) and antibiotic-antimycotic (Life Technologies, Inc., Grand Island, NY) to a density of 10^6 cells/ml. Cells (3×10^6) were plated in each well of a 6-well plate. Cell culture was maintained at 37 C with a water saturated atmosphere of 5% CO_2 in air.

Immunocytochemical staining for vimentin and cytokeratin

To identify the cell types that were obtained after trypsin and collagenase digestion, immunocytochemical staining for cytokeratin (epithelial cell marker) and vimentin (mesenchymal cell marker) was carried out on cells cultured for 3 d on chamber slides using the avidin biotin peroxidase method (Vector ABC, Vector Laboratories, Burlingame, CA), as described previously (17). The cells were washed with PBS and fixed with 4% paraformaldehyde. Before applying primary antibodies, endogenous peroxidase activity was quenched in 0.3% H_2O_2, and then the cells were incubated with normal blocking serum. After removal of excess serum, the monoclonal vimentin antibody (Sigma) at 1:3000 dilution and cytokeratin antibodies (Sigma) at 1:1000 dilution were applied respectively as primary antibodies. After incubation with the primary antibodies for 30 min at 37 C, the cells were washed and appropriate secondary antibodies were then applied. Incubation was further carried out for 30 min at 37 C. After the cells were washed, cells were incubated with Vectastain ABC reagent (Vector) for 30 min. The color reactions were developed using 3-amino-9-ethyl carbazole (red color). Cells were counterstained with Carazzi's hematoxylin and examined by light microscopy. To test the specificity of immunocytochemical staining, cells were also stained with preimmune serum or PBS instead of primary antibodies, and then the same procedures as described above were followed.

Cell treatment, protein, and RNA extraction

On the third day of culture, amnion fibroblasts and epithelial cells were washed with PBS and culture medium was changed to FCS free DMEM and preincubated in the same medium for 1 h. Steroid hormones and trilostane were added into the FCS free medium to achieve final concentrations of 0.01–1.0 μM for dexamethasone (Sigma), 1 μM for both cortisol (Sigma) and cortisone (Sigma), and 2.5 μM for trilostane. Incubation with the above treatments was carried on for 24 h. This time point was chosen according to the preliminary study with 3, 8, and 24 h incubation. A maximal change was found at 24 h. The culture medium was then collected for PGE_2 RIA as described below. For protein extraction, cells were washed with PBS after removal of the culture media and then scraped off the plate into cell lysis buffer in the presence of protease inhibitors [leupeptin, pepstatin, 4-(2-aminoethyl) benzenesulfonyl fluoride, Na-p-tosyl-L-lysine-chloromethyl ketone, and sodium orthavanadate]. The cell lysate was then passed through a 20-gauge needle four times and centrifuged at 12,000 $\times$ *g* for 10 min at 4 C. The supernatant was collected and stored at −20 C for later protein analysis with Western blotting. For total RNA extraction, after removal of the culture medium, cells were washed with PBS and then scraped off the plate into cell lysis buffer (supplied with RNeasy kit, QIAGEN, Valencia, CA). Subsequent extraction and purification of total RNA from the cells was conducted using RNeasy kit (QIAGEN) according to the protocol provided by the manufacturer. The extracted RNA was then quantified spectrophotometrically at 260 nm. The integrity of the extracted RNA was assessed by agarose-formaldehyde gel electrophoresis.

PGE_2 RIA

To measure PGE_2 level in cultured media of amnion fibroblasts and epithelial cells, aliquots of collected media were incubated with 10,000 cpm ^{3}H-PGE_2 (Amersham Life Science, Arlington Heights, IL) and anti-PGE_2 antibody (1:500) (20) at 4 C overnight. Subsequently 0.2 ml 12% bovine γ-globulin and 0.5 ml 40% polyethylene glycol were added into the reaction mixture to precipitate antibody-bound PGE_2 in the reaction mixture. The antibody-bound and unbound ^{3}H-PGE_2 were separated by centrifugation at 2000 $\times$ *g* for 15 min. The supernatant containing the unbound ^{3}H-PGE_2 was aspirated and the pellet containing the bound ^{3}H-PGE_2 was dissolved in 0.1 M Tris buffer. Scintillation fluid was added into the resuspended pellet and the radioactivity of the bound ^{3}H-PGE_2 was counted using a liquid scintillation counter. The concentration of PGE_2 in the sample was calculated from a standard curve of known concentrations of PGE_2 standard (16–2000 pg/100 μl).

Quantitative real time-PCR

To measure $cPLA_2$, PGHS-2, cPGES, and mPGES mRNA levels in response to glucocorticoid treatment, quantitative real-time PCR (QT-RT-PCR) analysis was carried out using a Cepheid Smart Cycler (Cepheid, Sunnyvale, CA).

Ribonuclease-free DNase (Invitrogen, Carlsbad, CA) treatment of the extracted total RNA was performed before RT-PCR. Dnase-treated RNA (1.0 μg) was reverse transcribed with oligo$(dT)_{12–18}$ primer using Superscript II kit (Invitrogen). Some RNA samples with no reverse transcriptase enzyme were used as controls to further check the absence of genomic DNA contamination in the samples. Reverse transcription product (cDNA) was diluted three times for subsequent PCR and QT-RT-PCR. Paired oligonucleotide primers for amplification of human $cPLA_2$, PGHS-2, cPGES, and mPGES were designed using Primer Designer (Scientific and Educational Software, Durham, NC) against the sequences downloaded from GenBank. The primer sequences are listed in Table 1. To control sampling errors, QT-RT-PCR for the housekeeping gene β-actin was routinely performed on each sample. The primer sequences for human β-actin are also listed in Table 1.

TABLE 1. Primer sequences

		Primer sequence (5′-3′)
$cPLA_2$	Fwd	ATGGCCTTGGTGAGTGATTC
	Rev	TCAGGATCTGCTACAGCTGC
PGHS-2	Fwd	TGTGCAACACTTGAGTGGCT
	Rev	ACTTTCTGTACTGCGGGTGG
cPGES	Fwd	TCTTAGCCCCTTGGATTCCT
	Rev	ATTCTTAGCCCGGGATTCAG
mPGES	Fwd	GGAGAAAGCTCGCAACAACT
	Rev	TTCCCACCATACACTTGCTG
β-Actin	Fwd	TGTGTTGGCGTACAGGTCTTTG
	Rev	GGGAAATCGTGCGTGACATTAAG

Fwd, Forward; Rev, reverse.

QT-RT-PCR reaction solution consisted of 2.0 μl diluted RT-PCR product, 0.2 μM of each paired primer, 2.0 mM Mg^{2+}, 100 μM deoxynucleotide triphosphates, 2 U *Taq* DNA polymerase, and 1× PCR buffer. SYBR green (BMA, Rockland, ME) was used as detection dye. QT-RT-PCR conditions were optimized according to preliminary experiment. The annealing temperature was set at 61 C and amplification cycles were set at 45 cycles. The temperature range to detect the melting temperature of the PCR product was set from 60 C to 95 C. mRNA levels were measured by determining the cycle numbers at which the fluorescence threshold was reached. To control sampling errors, the ratio of cycle numbers for $cPLA_2$, PGHS-2, cPGES, and mPGES to β-actin was obtained respectively to quantify the relative mRNA expression level. The specificity of the primers was verified by examining the melting curve as well as subsequent sequencing of the QT-RT-PCR products.

For sequencing of the QT-RT-PCR products, PCR products was cloned using TOPO cloning kit (Invitrogen). The mixture of PCR product and pCR4-TOPO vector was transformed into TOP10 *Escherichia coli* cells and grown on a selective plate containing ampicillin overnight at 37 C. Positive colonies were picked and further cultured in LB medium containing ampicillin overnight at 37 C. Plasmid DNA was extracted from the harvested bacteria with QIAprep miniprep kit (QIAGEN) and quantified spectrophotometrically at 260 nm wavelength. One microgram of extracted plasmid DNA was sent for sequencing to the DNA Core of the University of Cincinnati.

Western blot

Western blotting was performed to measure $cPLA_2$, PGHS-2, cPGES, and mPGES protein levels in response to glucocorticoid treatment. The protein levels of the cell lysate were determined with protein assay reagent kit (Pierce, Rockford, IL). Samples containing the same amount of protein (20 μg) in loading buffer were electrophoresed on precast 8–16% Tris-glycine gel (Invitrogen). The protein bands were then transferred electrophoretically to a nitrocellulose membrane (Osmonics, Inc., Minnetonka, MN). The membrane was blocked for 1 h in Tris-buffered saline containing 5% nonfat milk powder and 0.1% Tween 20. The membrane was then incubated with 1:500 to 1:1000 dilution of anti-$cPLA_2$ (Santa Cruz Biotechnology, Santa Cruz, CA), anti-PGHS-2 (Cayman, Ann Arbor, MI), anti-cPGES (Cayman), and anti-mPGES (Cayman) antibodies overnight at 4 C. The membrane was then washed three times with Tris-buffered saline containing 0.1% Tween 20 and incubated for 1 h with horseradish peroxidase-conjugated donkey antirabbit IgG (for PGHS-2, mPGES) or donkey antimouse IgG (for $cPLA_2$, cPGES) at 1:10,000 dilution. After washing the membrane, the enhanced chemiluminescence detection system (Amersham, Piscataway, NJ) was used to detect the bands with peroxidase activity. The light-emitting bands were detected with x-ray film. The resulting band intensities were quantitated using an imager scanning densitometer (Alpha Innotech Corp., San Leandro, CA).

All data are reported as mean ± SEM. A Student's *t* test or one-way ANOVA followed by the Student-Newman-Keuls test was used to assess significant differences between absolute values. Significance was set at $P < 0.05$. The values for n refer to the number of experiments performed with cell preparations from different patients.

Results

Characterization of cultured primary human amnion fibroblasts and epithelial cells

Morphological examination of cells isolated with trypsin showed round-shaped epithelial cells, which began to divide and form clusters around 24 h after plating. Immunocytochemical staining of the cells with the epithelial cell marker, cytokeratin, showed that more than 99% of the cells were positive for cytokeratin, suggesting an epithelial origin (Fig. 1). Morphological examination of cells dispersed using collagenase showed that these cells were irregular in shape at the time of plating and took on a spindle appearance and began branching after overnight incubation. Immunocytochemical staining of these cells with the mesenchymal cell marker, vimentin, showed that more than 90% of the cells were positive, indicating the mesenchymal origin (Fig. 1). To examine the specificity of the staining, a mixed cell preparation was also made. Immunocytochemical staining for vimentin showed that only spindle-shaped fibroblast but not round epithelial cell was stained positive (data not shown). In addition, cells stained with normal serum or PBS instead of specific primary antibody showed no obvious staining (data not shown).

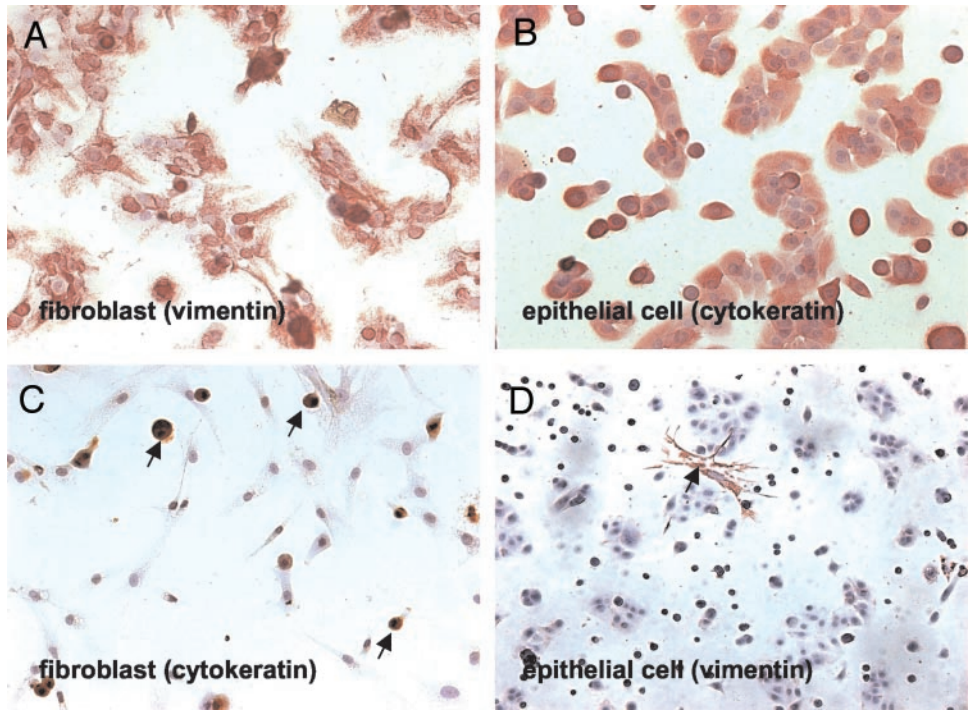

FIG. 1. Cultured primary human amnion fibroblasts (A and C) and epithelial cells (B and D) stained for mesenchymal cell marker, vimentin (A and D), and epithelial cell marker, cytokeratin (B and C). *Red color* indicates positive staining. *Arrows* indicate epithelial cells (C) and fibroblast (D), respectively. Magnification, ×200 (A and B), ×100 (C and D).

Effects of glucocorticoids on PGE$_2$ release in cultured primary amnion fibroblasts and epithelial cells

RIA showed that cultured primary human amnion fibroblasts produced about 50-fold more PGE$_2$ per cell than amnion epithelial cells (Fig. 2, A and B). Dexamethasone (0.01–1 μM) significantly increased PGE$_2$ production in a dose-dependent manner in amnion fibroblasts (Fig. 2A) but not in amnion epithelial cells (Fig. 2B). Cortisol (1 μM) was as effective as dexamethasone (1 μM) in the stimulation of PGE$_2$ output from the amnion fibroblasts, whereas cortisone (1 μM) caused modest but not significant increase of PGE$_2$ output from amnion fibroblasts (Fig. 3A). The increase of PGE$_2$ output on dexamethasone (1 μM) treatment is inhibited by co-treatment with RU486 (1 μM) as expected (Fig. 3B). However, treatment with trilostane (2.5 μM), a 3β-hydroxysteroid dehydrogenase (3β-HSD) inhibitor, had no influence on PGE$_2$ output in amnion fibroblasts (Fig. 3A).

Effects of glucocorticoids on cPLA$_2$, PGHS-2, cPGES, and mPGES mRNA expression in cultured primary amnion fibroblasts and epithelial cells

The melting curve of QT-RT-PCR showed a single peak of melting temperature value for PCR products of cPLA$_2$, PGHS-2, cPGES, mPGES, and β-actin, respectively (data not shown). Sequence analysis of cPLA$_2$, PGHS-2, cPGES, mPGES, and β-actin PCR products showed complete alignment with the corresponding sequences of human cPLA$_2$, PGHS-2, cPGES, mPGES, and β-actin genes in the gene bank

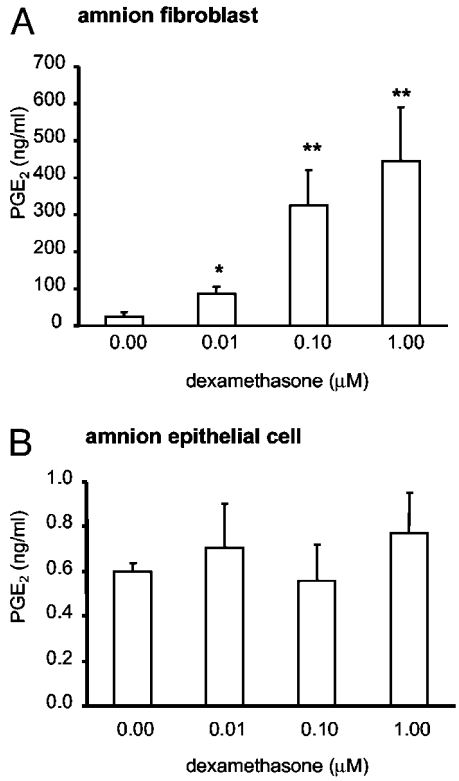

FIG. 2. Effect of dexamethasone treatment (0.01–1 μM) on PGE$_2$ production in cultured human amnion fibroblasts (A) and epithelial cells (B). *, $P < 0.05$; **, $P < 0.01$ *vs.* control (0 μM), n = 5 experiments (fibroblasts), n = 3 experiments (epithelial cells).

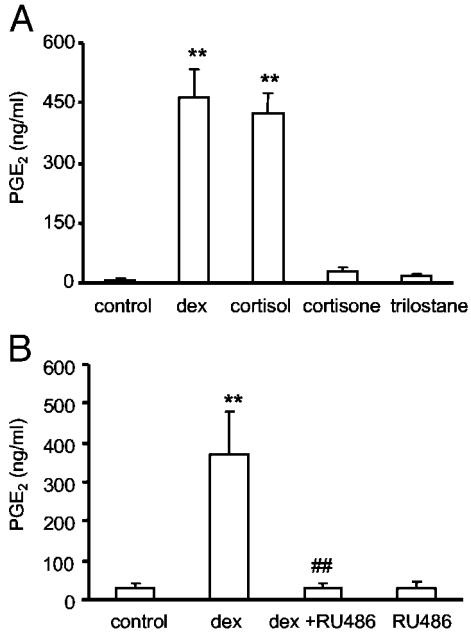

FIG. 3. Effect of different glucocorticoids (1 μM), trilostane (2.5 μM) (A), and RU486 (1 μM) (B) on PGE$_2$ production in cultured amnion fibroblasts. dex, Dexamethasone. **, $P < 0.01$ *vs.* control; ##, $P < 0.01$ *vs.* dex (n = 3–4 experiments).

(data not shown). Similar expression of cPLA$_2$ mRNA was observed between amnion fibroblasts and epithelial cells (Fig. 4), whereas amnion fibroblasts expressed a significantly higher level of PGHS-2 mRNA than amnion epithelial cells (Fig. 4). Similar levels of expression of either cPGES mRNA or mPGES mRNA were observed between amnion fibroblasts and epithelial cells (Fig. 4).

Dexamethasone (0.01–1 μM) treatment for 24 h significantly increased cPLA$_2$ and PGHS-2 mRNA levels in a dose dependent manner in the amnion fibroblasts (Fig. 5A) but did not affect cPGES and mPGES mRNA expression in the same cell type (Fig. 5A). In amnion epithelial cells, dexamethasone (0.01–1 μM) treatment for 24 h increased only cPLA$_2$ mRNA expression but not PGHS-2, cPGES, and mPGES mRNA expression (Fig. 5B). Cortisol (1 μM) was as effective as dexamethasone (1 μM) in the induction of cPLA$_2$ and PGHS-2 mRNA expression in amnion fibroblasts, whereas cortisone (1 μM), a metabolite of cortisol, did not affect cPLA$_2$ and PGHS-2 mRNA expression in amnion fibroblasts (Fig. 6A). RU486 (1 μM), a glucocorticoid and progesterone receptor antagonist, could partially but significantly inhibit the induction of cPLA$_2$ and PGHS-2 mRNA expression by dexamethasone (1 μM) (Fig. 6B). RU486 treatment alone also caused a significant increase in the expression of PGHS-2 mRNA in amnion fibroblasts but not to the same extent as dexamethasone (Fig. 6B). However, treatment with trilostane (2.5 μM) did not influence the expression of cPLA$_2$, PGHS-2, cPGES, and mPGES mRNA in amnion fibroblasts (Fig. 6A).

Effects of glucocorticoids on cPLA$_2$, PGHS-2, cPGES, and mPGES protein expression in cultured amnion fibroblast

The level of cPLA$_2$, PGHS-2, cPGES, and mPGES protein expression was analyzed with Western blotting. Results

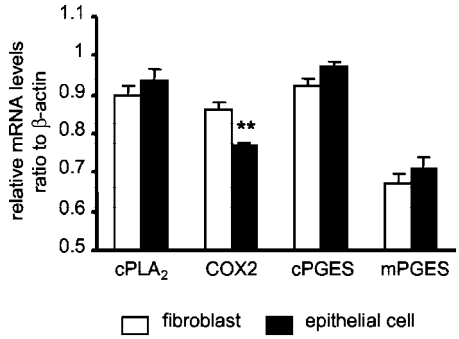

FIG. 4. Relative cPLA$_2$, PGHS-2, cPGES, and mPGES mRNA expression levels in cultured human amnion fibroblasts (n = 11 experiments) and epithelial cells (n = 6 experiments). **, $P < 0.01$ *vs.* PGHS-2 mRNA of fibroblast.

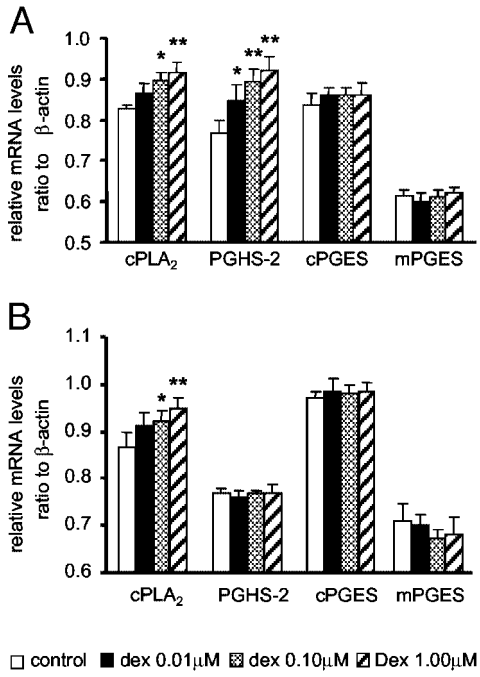

FIG. 5. Effect of dexamethasone (dex) on cPLA$_2$, PGHS-2, cPGES, and mPGES mRNA expression in cultured amnion fibroblasts (A), epithelial cells (B). *, $P < 0.05$; **, $P < 0.01$ *vs.* control (0 μM), n = 4–5 experiments.

showed that dexamethasone (0.01–1 μM) treatment for 24 h increased cPLA$_2$ and PGHS-2 protein expression dose-dependently in amnion fibroblasts, whereas cPGES and mPGES protein expression was not affected (Fig. 7). This is in support of the mRNA changes observed with QT-RT-PCR.

Discussion

In this study, separate amnion epithelial and fibroblast cell cultures were examined to evaluate the regulation of PGE$_2$ production as well as cPLA$_2$, PGHS-2, cPGES, and mPGES expression by glucocorticoids in each cell type. Using these relatively pure amnion epithelial and fibroblast cell preparations, we found amnion fibroblasts produced higher levels of PGE$_2$ than amnion epithelial cells per cell as reported in an earlier study (21). We also found cPLA$_2$, cPGES, and

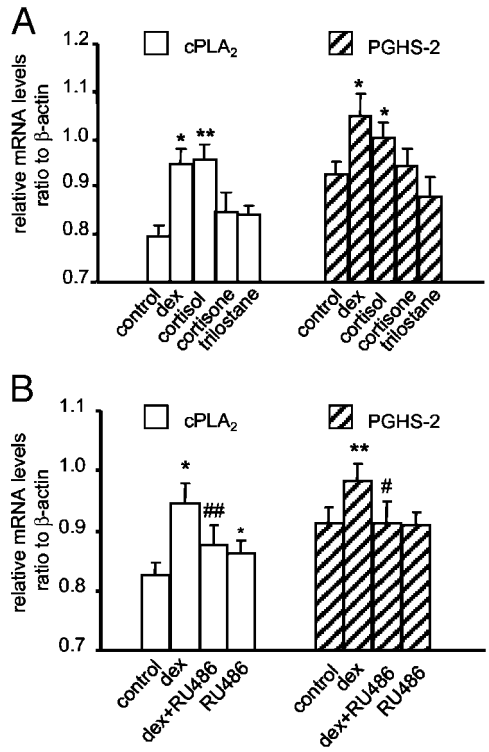

FIG. 6. Effect of different glucocorticoids (1 μM), trilostane (2.5 μM) (A), and RU486 (1 μM) (B) on cPLA$_2$ and PGHS-2 mRNA expression in cultured human amnion fibroblasts. dex, Dexamethasone. *, $P < 0.05$; **, $P < 0.01$ *vs.* control; #, $P < 0.05$; ##, $P < 0.01$ *vs.* dex (n = 4–6 experiments).

mPGES mRNA were expressed at similar levels between these two cell types. However, a significantly higher level of PGHS-2 mRNA expression was observed in the amnion fibroblast when compared with the amnion epithelial cell. This could explain, at least in part, the low output that the amnion epithelial cells have in PGE$_2$ production as well as the crucial role of PGHS-2 in prostaglandin synthesis in the amnion.

It has been reported that there are 7–10 times more epithelial cells than mesenchymal fibroblast cells in the amnion at term (22). In this study, we found that cultured primary amnion fibroblasts produced about 50-fold more PGE$_2$ than amnion epithelial cells per cell. Taking the cell number of each cell type into consideration, amnion fibroblasts may still produce about 5 times more PGE$_2$ than amnion epithelial cells at term *in vivo*. Therefore, we suggest amnion fibroblasts rather than amnion epithelial cells might be the major source of PGE$_2$ at term or during parturition.

Glucocorticoids are commonly used in the treatment of immune and inflammatory disorders. One of the major reported mechanisms of glucocorticoid modulation of the inflammatory response is inhibition of the release of arachidonic acid from phospholipids and inhibition of PGHS-2 expression induced by proinflammatory cytokines in a number of cell lines (23, 24). In certain cells, however, glucocorticoids act paradoxically by stimulating rather than inhibiting PG production, such as in rat gastric mucosa, murine fibroblast, and fetal rat lungs (25–27). PG production by human amnion has been suggested to be involved in the onset and progression of labor. Interestingly, glucocorticoids

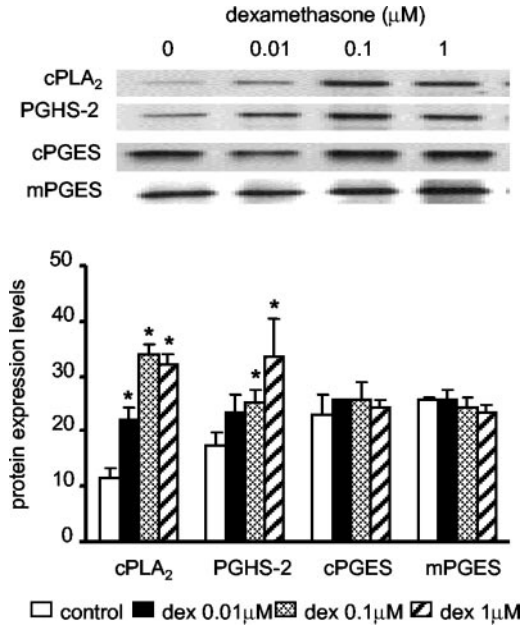

FIG. 7. Effect of dexamethasone (dex) on cPLA2, PGHS-2, cPGES, and mPGES protein expression in cultured human amnion fibroblasts. *Upper panels* are representative Western blots; *bottom panel* is the average data of three different experiments. dex, Dexamethasone. *, $P < 0.05$, *vs.* respective control.

have been shown to stimulate PG production in the amnion (8, 14, 18, 19). This is in marked contrast to its reported inhibitory action on the induction of PG production by proinflammatory cytokine (23, 24). If true *in vivo*, this would imply very different effects of glucocorticoids on basal *vs.* cytokine-stimulated PG production. Our preliminary data (not shown) did not show a glucocorticoid inhibition of IL-1β-induced cPLA2 and PGHS-2 mRNA expression in primary amnion cell cultures. In a recent paper, dexamethasone has been shown to block interleukin-1β-induced uterine contractions in pregnant rhesus monkey; however, the site of the glucocorticoid action was not determined (28).

In a mixed amnion cell culture, glucocorticoid exposure up-regulated PGHS-2 mRNA and immunoreactive protein in the amnion mesenchymal cells but not in the amnion epithelial cells (14). Using purified amnion epithelial and fibroblast cells, Blumenstein *et al.* (19) found that glucocorticoids up-regulated PGE_2 production and PGHS-2 expression in amnion fibroblast but decreased PGE_2 production in epithelial cells, whereas Whittle *et al.* (21) found that amnion epithelial cells responded to glucocorticoid with increased PGE_2 output. The causes for the different responses of amnion epithelial cells to glucocorticoids in terms of PG synthesis are not very well understood. Earlier work by Gibb and Lavoie (18) demonstrated that glucocorticoids inhibited PG production by freshly isolated amnion cells but stimulated PG production by amnion cells maintained in cultured for a few days, suggesting culture conditions of amnion epithelial cells may determine their response to glucocorticoids in PG production. In this study, we found that dexamethasone dramatically increased PGE_2 output in amnion fibroblasts maintained in culture for 3 d but not in epithelial cells maintained in culture for the same period, suggesting the incubation time of cultured cells is unlikely the cause for the different responses of amnion epithelial cells and fibroblasts to glucocorticoids in this study. In consideration of the facts of much lower PGHS-2 mRNA expression level and less PGE_2 production in amnion epithelial cells, we assume these facts might contribute to the relative unresponsiveness of amnion epithelial cells to glucocorticoids.

Skannal *et al.* (29) demonstrated that cPLA$_2$ activity in human amnion increased with gestational age and was highest at term in the absence of labor, suggesting a role of cPLA$_2$ in mediation of arachidonic acid mobilization and PG synthesis at labor. Using the homologous recombination method to generate mice deficient in cPLA$_2$, Uozumi *et al.* (30) found female cPLA$_2$ null mice failed to deliver offspring, which further indicates a crucial role of cPLA$_2$ in parturition. In spite of the recognition of the role of cPLA$_2$ in parturition, few studies have addressed the regulation of cPLA$_2$ expression in the amnion. Using amnion-derived WISH cells, Xue *et al.* (32) and Hansen *et al.* (31) showed that IL-1β and TNFα provoked a time-dependent increase in the expression of the cPLA$_2$ mRNA and protein. As is the case with PGHS-2, glucocorticoids alone also inhibit cPLA$_2$ expression or inhibit the induction of cPLA$_2$ expression by proinflammatory cytokines in most nonintrauterine tissues (32–34). However, we found glucocorticoids up-regulate cPLA$_2$ mRNA expression both in amnion epithelial cells and amnion fibroblasts, which is in obvious contrast to the inhibitory effect of glucocorticoids in most of nonintrauterine tissues. In spite of up-regulation of cPLA$_2$ mRNA expression with glucocorticoid treatment in amnion epithelial cells, we found there was no corresponding increase of PGE_2 output. This could be due to the lower and unresponsive expression of rate-limiting enzyme PGHS-2 to glucocorticoids in this cell type.

Recent studies by Meadows *et al.* (17) demonstrated that both cPGES and mPGES were immunolocalized in the amnion. Moreover, they found there was no differences in amounts of either cPGES and mPGES mRNA or protein in amnion at term or preterm, with or without labor (17). Although it has been reported that mPGES expression could be induced by IL-1β in alveolar A549 cells and rheumatoid synovial cells (12, 35), we found no changes of cPGES and mPGES expression with glucocorticoid treatment in amnion epithelial cells and fibroblasts. Martin *et al.* (36) also found that cortisol infusion did not affect mPGES protein expression in ovine placentome.

The effects of glucocorticoids are normally mediated through intracellular glucocorticoid receptor (GR). Two isoforms of GR (GRα and GRβ), which originate from the same gene by alternative splicing of the GR primary transcript (37), have been identified. GRα is the predominant isoform that possesses steroid binding activity. Upon binding glucocorticoids, GRα translocates from cytoplasm to nucleus in which it acts as a transcription factor to regulate target gene expressions. Due to a lack of a steroid binding domain in the carboxyl terminus, GRβ does not bind glucocorticoids (37). Studies showed that GRβ could inhibit the gene transactivating effect of GRα by forming impaired heterodimers with GRα (38). However, there were also studies challenging this concept (39). By using immunocytochemistry, Sun *et al.* (40) demonstrated that nuclear GR was found in amnion epithe-

lium, mesenchyme, and the chorion leave. Our recent study (41) also found that both GRα and GRβ mRNAs were expressed at similar levels in the amnion fibroblast and epithelial cell. These findings provide a molecular basis for the actions of glucocorticoids in the fetal membranes.

We found in this study that the induction of both $cPLA_2$ and PGHS-2 expression as well as PGE_2 release by glucocorticoid in the amnion fibroblast was blocked by cotreatment with RU486. Although it has been very well recognized that RU486 is capable of blocking both GR and progesterone receptor (PR) (42), it has also been reported that term human amnion, chorion, and placenta have no detectable PR (43–45) and exogenous progesterone did not affect the PGE_2 output in cultured human amnion cells (46). Earlier work showed the presence of 3β-HSD and the production of progesterone in the amnion, although much less than in placenta and chorion tissues (47, 48). In this study, we found trilostane, a potent inhibitor of 3β-HSD and endogenous progesterone synthesis, did not affect $cPLA_2$, PGHS-2 expression, or PGE_2 output in the amnion fibroblast. Therefore, the blocking effect of RU486 on glucocorticoid's induction of $cPLA_2$, PGHS-2 expression, and PGE_2 output is very likely through GR rather than PR. With regard to the induction of PGHS-2 expression by RU486 treatment alone, we assume this effect is possibly due to the partial glucocorticoid agonist effect of RU486 (49).

Classically, glucocorticoids, upon binding to GR, stimulate gene expression through glucocorticoid response element (GRE) within the promoter region of the respective gene, whereas the inhibitory effect of glucocorticoids on gene expression is usually mediated through a negative GRE or interference with other transcription factors (50). Sequence analysis revealed the presence of one or more GREs but no negative GRE in the promoter region of $cPLA_2$ and PGHS-2 genes (51, 52). We speculate that the paradoxical stimulating effect of glucocorticoid on $cPLA_2$ and PGHS-2 expression in the amnion fibroblast might be the result of direct interaction of glucocorticoid/GR complex with GRE within the promoter region of the respective gene. How this interaction happens in the amnion fibroblast but not in the inflammatory tissues remains to be elucidated.

In conclusion, this study suggests that amnion fibroblasts expressed higher PGHS-2 mRNA and produce more PGE_2 per cell than amnion epithelial cells at term of pregnancy. Glucocorticoids increase PG production only in the amnion fibroblast through induction of $cPLA_2$ as well as PGHS-2 expression rather than cPGES or mPGES expression.

Acknowledgments

Received May 20, 2003. Accepted July 30, 2003.

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This work was supported by National Institutes of Health RO1 Grant HD31514 and the National Basic Research Program of China (Grant G1999054000).

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