Prostaglandin F2α Potentiates Cortisol Production by Stimulating 11β-Hydroxysteroid Dehydrogenase 1: A Novel Feedback Loop That May Contribute to Human Labor

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In human pregnancy, cortisol and PGs are involved in the onset of labor and play an important role in the mechanisms leading to parturition. Recent studies have shown that at term, cortisol increases PG synthesis and decreases PG metabolism in chorion trophoblast (CT) cells. In CT, 11β-hydroxysteroid oxidase type 1 (11β-HSD1) converts biologically inactive cortisone to cortisol to regulate cortisol availability. In the present study, we have investigated whether 11β-HSD1 activity could be influenced by PGs. We have shown that in CT, PGF2α rapidly increased 11β-HSD1 reductase activity in a dose-dependent manner via the PGF2α, receptor, localized in the fetal membranes. PGF2α stimulated 11β-HSD1 activity through increased intracellular calcium mobilization, activation of PKC, and the phosphorylation of the 11β-HSD enzyme. We propose that within CT there is a novel feed forward loop by which PGF2α acts to promote cortisol production from cortisone through increases in 11β-HSD1, and this in turn leads to further net PG output for the onset of labor and birth. (J Clin Endocrinol Metab 86: 5585–5592, 2001)

LATE HUMAN GESTATION is associated with an increase in the synthesis of bioactive glucocorticoids by the fetus and PGs, in particular PGE2 and PGF2α, by intrauterine tissues. Both cortisol and PGs are required for the processes leading to birth. The increase in fetal glucocorticoids helps to promote lung maturation, required by the fetus for extraterine survival (1, 2). PGs have been shown to stimulate myometrial contractility, regulate cervical ripening, and precipitate membrane rupture at term. Alterations in synthesis of glucocorticoids and PGs may predispose to preterm birth, the major problem in obstetrical practice. Increased PG output results from elevated expression and activity of prostaglandin H2 synthase type 2 (PGHS-2) in amnion and chorion (3–6) and reduced mRNA and activity of the PG catabolizing enzyme, 15-hydroxyprostaglandin dehydrogenase (PGDH), particularly in preterm labor. Regulation of PG output is multifactorial, involving cytokines, growth factors, and steroids, including cortisol. An inhibitory effect of cortisol on PGDH activity in chorion and a stimulatory effect of cortisol on PGHS-2 in amnion has recently been reported (7, 8), suggesting an interaction between PGs and glucocorticoid. However, the source of that cortisol is unclear; potentially it could be derived from the mother or fetus by local formation in the fetal membranes (9).

Cortisol and its biologically inactive metabolite cortisone are interconverted through the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD). Two distinct isozymes of 11β-HSD, known as 11β-HSD1 and 11β-HSD2, have been characterized and cloned (10). 11β-HSD1 possesses both oxidase (cortisol to cortisone) and reductase (cortisone to cortisol) activities. This enzyme has a higher affinity for cortisone than for cortisol (11–13) and operates predominantly in a reductase direction. In contrast, 11β-HSD2, under physiological conditions exhibits only oxidase activity and has a much higher affinity for glucocorticoids. 11β-HSD1 is expressed in human placental intermediate trophoblast cells and vascular endothelium and is expressed abundantly in chorion trophoblast (CT) cells and decidua (14). 11β-HSD2 has been localized to placental syncytiotrophoblast (15). The high affinity of placental 11β-HSD2 for cortisol makes it more suited to regulate the amount of maternal glucocorticoid passing across the placenta into the fetal circulation. Glucocorticoids reaching the fetus from maternal circulation facilitate fetal organ maturation (16), but excessive amounts may result in fetal growth restriction and program adult-onset disease (17).

There is an increasing body of literature concerned with regulation of 11β-HSD2 activity and expression. The enzyme is up-regulated by cyclic AMP and its activators such as forskolin in the kidney and placenta (18, 19). The enzyme is down-regulated by progesterone and nitric oxide (20). Pre-

Abbreviations: 11β-HSD1, 11β-Hydroxysteroid oxidase type 1; Ca2+, calcium; CT, chorion trophoblast; FP receptor, PG F2α, receptor; ir-FP, immunoreactive FP receptor; PGDH, 15-hydroxyprostaglandin dehydrogenase; PGHS-2, prostaglandin H2 synthase type 2; TLC, thin-layer chromatography.
arious studies have failed to demonstrate regulation of 11 \( \beta \)-HSD1 activity or expression by these factors (18, 19). Recently it has been suggested that the products of arachidonic acid metabolism may alter 11 \( \beta \)-HSD2 activity (21). We have established that cortisol formed from cortisone within the fetal membranes can promote enzyme activities leading to increased formation of biologically active prostaglandins at the time of labor (22, 23). These PGs might then contribute to rupture of the fetal membranes and stimulation of uterine contractility. Therefore, we hypothesized that PGF\(_{2\alpha}\) generated within fetal membranes, might stimulate 11 \( \beta \)-HSD1 activity in CT cells in a manner that would increase the local formation of cortisol from cortisone. Our results demonstrate, for the first time, a novel interaction between PGF\(_{2\alpha}\) and 11 \( \beta \)-HSD1 in human fetal membranes that describes a positive feed-forward loop, which should result in increased output of glucocorticoid and prostaglandin at the time of labor.

**Materials and Methods**

**Chorionic trophoblast cell culture**

Trophoblast cells from choriodecidual tissue were isolated and cultured using a modification of the technique described by Kliman et al. (24). Briefly, human placentae were obtained from uncomplicated term pregnancies (38–40 wk, \( n = 27 \)) after elective cesarean section delivery. Patient consent and ethical approval was obtained before each placenta was collected, in agreement with the regulations of Mount Sinai Hospital and the University of Toronto. The chorion with adherent decidua was peeled off the amnion and digested three times for 60 min each with 0.125% trypsin (Sigma, St. Louis, MO) and 0.02% deoxyribonuclease I (Sigma), and 0.2% collagenase (Sigma) in DMEM (Life Technologies, Inc., Grand Island, NY) containing 10% FCS (Life Technologies, Inc.; Burlington, Ontario, Canada). The dispersed choriodecidual cells were filtered through a 200-\( \mu \)m nylon gauze and loaded onto a continuous Percoll (Sigma) gradient (5–70% in 5% steps of 3 ml each) and then centrifuged at 1,200 \( \times \) g for 20 min at room temperature to separate different cell types. Cytotrophoblast cells between the density markers of 1.049 and 1.062 g/ml were collected and plated in 24-well plates (Corning Costar Corp., Cambridge, MA) at a density of 10\(^6\) cells/ml. Cells were also plated in 6-well plates at 10\(^6\) cells/ml for immunoprecipitation, in 35-mm Petri dishes (10\(^6\) cells/ml) containing 1 glass coverslip for calcium imaging, or in 8-well chamber slides (Lab-Tek, Nalge Carrazzi) for immunostaining. The cells were cultured for 3 d at 37 \( ^\circ \)C in 5% CO\(_2\) and 95% air before experimental treatments. Under these conditions, the chorionic trophoblast cells formed small clumps or remained as single cells. Purity of the cell preparation was assessed after 3 d in culture by histochemical staining for cytokeratin, an epithelial cell lineage marker (DAKO Corp., Glostrup, Denmark) or vimentin, a mesenchymal cell lineage marker (DAKO Corp.) (25); cells were counterstained with Carrazzi’s hematoxylin.

**Immunohistochemistry for PGF\(_{2\alpha}\) receptor (FP receptor)**

**Trophoblast cells.** After 72 h, representative cultures were fixed with 4% paraformaldehyde and stored in 90% ethanol for further analysis. Cells were then rehydrated with serial increasing dilutions of ethanol ending with 100% ethanol before experimental treatments. Under these conditions, the choriodecidual cells were rehydrated with serial increasing dilutions of ethanol ending with 90% ethanol before embedding them in paraffin wax. The paraffin blocks were sectioned at 5 \( \mu \)m for immunohistochemistry. Tissue sections were deparaffinized in xylene, rehydrated, and washed in PBS. Staining was performed according to the same protocol described above except that the primary antibody used was 1:100 dilution and the immunizing peptide at 1:200.

**Preparation of \(^3\)H-cortisone**

We prepared \(^3\)H-cortisone by oxidizing \(^3\)H-cortisol (specific activity: 64 Ci/mol, Amersham Pharmacia Biotech, Buckinghamshire, UK) with chromium trioxide as described previously (26) and purifying it by two-layer chromatography (TLC: chloroform, ethanol, 95:3 vol/vol) before it was used in the enzyme activity experiments.

**Determination of 11 \( \beta \)-HSD1 activity**

Trophoblast cells from placenta or fetal membranes were cultured for 72 h. Then the media was replaced with FCS-free DMEM (pH 7.4) and incubated for 1 h. 11 \( \beta \)-HSD1 reductase activity (cortisone to cortisol) was measured using 1-\( \mu \)m cortisone containing 100,000 cpm \(^3\)H-cortisone as substrate and oxidase activity was measured using 1 \( \mu \)m cortisol containing 100,000 cpm \(^3\)H-cortisol. The activity assays were performed in the absence or presence of PGF\(_{2\alpha}\) (Oxford BioMedical Research, Cederlane, Hornby, Ontario, Canada). Fluprostanol, an FP receptor agonist, was also used to determine whether any PGF\(_{2\alpha}\) effect was mediated through the FP receptor. To investigate the role of calcium (Ca\(^{2+}\)) in this interaction, cells were incubated with A23187 (Ca\(^{2+}\) ionophore). To determine the source of mobilized calcium in CT cells for the PGF\(_{2\alpha}\) effect on 11 \( \beta \)-HSD1 activity, we incubated the cells in the presence of EGTA; extracellular calcium chelator; or BAPTA-am, an intracellular calcium chelator. To further examine the second messenger of the PGF\(_{2\alpha}\) we incubated cells with either PMA (10\(^{-5}\)–10\(^{-6}\) M), a PKC activator, or calphostin (10\(^{-7}\) M), a specific inhibitor for PKC. Each treatment was performed in triplicate for each preparation of cells (from three to six separate experiments; see Results). Media were collected after 30-min incubations at 37 \( ^\circ \)C, except in the experiments designed to examine the time course of the PGF\(_{2\alpha}\) effect on 11 \( \beta \)-HSD1 activity. Cell viability before and after drug treatment was examined by trypan blue exclusion.

To measure the conversion of \(^3\)H-cortisone to \(^3\)H-cortisol (reductase activity) or \(^3\)H-cortisol to \(^3\)H-cortisone (oxidase activity), a mixture of cortisol and cortisone (40 \( \mu \)g each) was added to the collected medium to allow the subsequent localization of the steroids during purification by TLC (27). Steroids in the media and cells were extracted with ethyl acetate (3 ml). The extract from the medium was dried under air, reconstituted with ethyl acetate (100 \( \mu \)l), and applied to a TLC plate (silica gel GF, Fisher Scientific, Pittsburgh, PA). Cortisol and cortisone were separated in the solvent system chloroform/ethanol (95:5, vol/vol). Steroids were visualized under UV light, scraped off the plate, and extracted with ethyl acetate. The solvent was dried, scintillation fluid added, and the radioactivity was counted in a liquid scintillation counter. The recovery rate of \(^3\)H-cortisone or \(^3\)H-cortisol was measured by adding \(^3\)H-cortisone or \(^3\)H-cortisol to culture media and processing the samples in an identical fashion. The mean recovery of \(^3\)H-cortisone was 88.3% ± 4.3% and 90% ± 3.8% for \(^3\)H-cortisol. In all cases, the background conversion, estimated from radioactivity in duplicate blank wells not containing cells, was subtracted from that in the experimental wells before analysis. Activity was expressed as femtomoles of cortisol
or cortisone produced per 10^6 cells per 30 min (fmoles/10^6 cells per 30 min).

Calcium imaging

Ratio imaging of intracellular free Ca^{2+} was performed using the acetoxymethyl ester of the fluorescent indicator Fura-2 (Molecular Probes, Inc., Eugene, OR) as described previously (28). Washed CT cells were incubated for 45 min at room temperature with Fura-2 (5 μM). The cells on the cover slips were rinsed, placed in a perfusion chamber on an inverted fluorescence microscope (Diophot 300, Nikon, Melville, NY), illuminated with a 75-W Xenon lamp, and viewed using a 40× fluorescence objective. For Fura-2 excitation, the shutter and filter wheel were controlled by Axon Imaging Workbench software (AIW 2.1, Axon Instruments, Foster City, CA) to provide sequential illumination at two alternating wavelengths, 340 and 380 nm. Fluorescence of Fura-2 was detected at an emission wavelength of 510 nm. Consecutive video frames were captured with an intensified charge coupled device camera (PTI IC-110, Monmouth, NJ). Digitized images were obtained by averaging four consecutive frames using an image-processing board also controlled by AIW 2.1 software. Fluorescence emission ratios following excitation at 340 and 380 nm were calculated by dividing averaged pixel values in circumscribed regions of individual responding cells in the field of vision. The values were corrected for background fluorescence obtained by imaging a field with no cells. Treatment with PGE_2 at the final concentration of 10^{-6} M was performed at room temperature.

Immunoprecipitation

Phosphorylation and immunoprecipitation of 11β-HSD1. Chorionic trophoblast cells (3 × 10^6 cells/well, 6-well plate) were washed twice with PBS and incubated for 90 min at 37°C in phosphate-free DMEM containing 0.4 mM/μl [32P]orthophosphate. Cells were then washed with phosphate-free media and stimulated for 10 min with PGE_2 or the PMA, as described in the figure legend. After rapidly rinsing twice with cold PBS, the cells were lysed with cold RIPA lysate buffer (100 mM NaCl, 50 mM NaF, 0.1% SDS, 1% sodium deoxycholate, 1% Triton X-100, 1 mM EDTA, 1 mM EGTA, 0.1 mM PMSF, 0.1 mM aprotinin, 1 mM orthovanadate, and 50 mM Tris HCl, pH 7.5) for 20 min at 4°C. The total cell lysates were collected with a cell scrapper, vortexed vigorously, and centrifuged (1,500× g for 20 min at 4°C). The precollected cell extracts were transferred to new tubes. The protein content of the supernatant was measured by the Bradford method using a protein assay kit (Bio-Rad Laboratories, Inc., Mississauga, Ontario, Canada) with BSA as a standard. Immunoprecipitation was then performed as described below:

11β-HSD1 immunoprecipitation. Cell extracts (200 μg protein) were incubated with an 11β-HSD1 antibody (5 μg/ml), a generous gift from Dr. K. Yang (29), for 2 h with continuous rotation at 4°C. Antibody-A-conjugated agarose beads (30 μl/μl) were then added, and the samples were incubated for an additional 2 h with continuous rotation. The immunocomplexed beads were collected by centrifugation and washed three times with RIPA lysis buffer. 11β-HSD1 was eluted from the beads with Laemmli buffer (0.5 M Tris HCL, 10% SDS, 20% glycerol, 0.2% bromophenol blue, and 10% mercaptoethanol) and finally resuspended in 40 μl Laemmli buffer containing 10% mercaptoethanol for subsequent gel electrophoresis using a 12% polyacrylamide gel. The gel was dried and autoradiography performed. Immunoblots with multiple exposures were quantified using Duo Scan transparency Scanner and NIH Image 6.1 software to determine the relative abundance of phosphorylated 11β-HSD1. Immunoprecipitation was conducted in four independent experiments.

Statistical analysis

All data are shown as mean ± SEM for the number of experiments with different chorion specimens from different patients. A t test or one-way ANOVA test was used to assess statistical differences. The effects of treatment on 11β-HSD1 activity were determined by one-way ANOVA, followed by post hoc multiple comparisons (t, Newman-Keuls, or Tukey's HSD) method. Relative optical density determinations were analyzed by t test. Statistical significance was set at P < 0.05. Calculations were performed using Sigma Stat (Jandel Scientific Software, San Rafael, CA).

Results

Characterization of cell type

After 72 h of culture, chorionic trophoblasts appeared as rounded cells that either remained as single cells or formed small clumps. The cultured cells were 85% ± 3% (n = 24) cytokeratin positive and vimentin negative, suggesting the presence of mainly trophoblast cells and few fibroblast or decidual cells. Cell viability, assessed by trypan staining, was 95% before and after treatment.

FP receptor expression

Immunoreactive FP (ir-FP) receptor protein was identified in both fetal membranes and CT cells (Fig. 1). Ir-FP receptor was localized to the amnion epithelial layer, the CT cells, and extensively within the decidual stromal cell layer of the fetal membranes (Fig. 1A). Purified CT cells after 72 h in culture continued to exhibit intense ir-FP receptor expression (Fig. 1C).

Preabsorption of the FP receptor antibody with excess immunizing peptide completely eliminated staining from both chorion layer (Fig. 1B) and CT cells (Fig. 1D), thus confirming to the specificity of the immunostaining response.

Effects of PGE_2 on 11β-HSD1 reductase activity

To examine the time course of PGE_2 action, 11β-HSD1 reductase activity was measured after different incubation times (15–60 min) in the absence or presence of PGE_2 (10^{-6} M). Results are shown in Fig. 2. At each time point, PGE_2 significantly increased 11β-HSD1 reductase activity (Fig. 2A); the difference was statistically significant after 15 min. Therefore, all further determinations were performed after 30-min incubation.

We examined the dose-dependent stimulation of 11β-HSD1 activity by PGE_2. There was a dose-dependent stimulation of cortisol to cortisol conversion, in the presence of increasing concentrations of PGE_2 from 10^{-10} M to the maximal effect at 10^{-6} M (Fig. 2B). We also investigated the effect of PGE_2 on 11β-HSD1 activity under the same conditions. PGE_2 (1 μM) significantly increased cortisol production by 45 ± 4% (n = 5, data not shown).

Effects of PGE_2 on 11β-HSD1 oxidase activity

11β-HSD1 has both reductase and oxidase activity with the former predominant in the CT cells (14). To examine whether PGE_2 has the same effect on both reactions, we incubated CT cells in the presence of 1 μM cortisol or cortisol in the absence or presence of PGE_2 1 μM for 30 min. Results are shown in Fig. 3. PGE_2 stimulated reductase activity with no enhancement of the low level of oxidase activity present in CT cells.

Fluprostanol effect on 11β-HSD1 activity

Although PGE_2 is a potent FP agonist, it is not very selective, having appreciable agonist activity at PGE_2 and
However, fluprostenol, a PGF2α analog, has been demonstrated to be equipotent with PGF2α at the FP receptor but with much reduced agonist activity at other prostanoid receptors (30, 31). To determine whether the PGF2α effect on 11β-HSD1 activity is mediated through the FP expressed in chorion trophoblast cells, we examined the effect of fluprostenol (10⁻¹²–10⁻⁶ M) on 11β-HSD1 activity (Fig. 4). Fluprostenol stimulated 11β-HSD1 activity in a dose-dependent fashion indicating that the effect of PGF2α on 11β-HSD1 depends on interaction of the hormone with FP receptor.

**Effect of PGF2α on calcium release in chorion trophoblast cells**

It has been well documented in many other systems that PGF2α increases intracellular calcium on activation of FP receptor (32–34). PGF2α (1 μM) increased the intracellular

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**Fig. 1.** Cellular localization of FP receptor immunoreactivity in fetal membranes and purified CT. A, Representative staining of three independent patients at term pregnancy. C, Representative staining of three independent purified CT cells after 72 h in culture. Preabsorption abolished the positive staining in both chorion and amnion of the fetal membranes (B) and in purified CT cells (D). a, Amnion; c, chorion; d, decidua.

**Fig. 2.** Time course (2A) and dose response (2B) of PGF2α effect on 11β-HSD1 reductase activity in CT cells. A, Cells were incubated 15–60 min in the presence of [³H]cortisone, in the presence (solid line) or absence (dotted line) of PGF2α (10⁻⁶ M). Data are mean ± SE; each point is the mean of replicate determinations from four to six chorions. *, P < 0.05 (unpaired t test). B, Cells were incubated for 30 min in the presence of [³H]cortisone in the presence of PGF2α (10⁻¹²–10⁻⁴ M). Data are mean ± SE; each point is the mean of replicate determinations from four to six chorions. *, P < 0.05; **, P < 0.001.
calcium concentration of CT cells in less than 2 min. The ratio 340/380 was increased from 0.44 to 0.56 following application of PGF$_{2\alpha}$/H9251 (Fig. 5). Because the effect of PGF$_{2\alpha}$/H9251 via FP receptor is mediated by an increase in intracellular calcium, we examined whether A23187, a calcium ionophore, was able to reproduce the effect of PGF$_{2\alpha}$/H9251. Incubation (30 min) with A23187 (10$^{-12}$–10$^{-6}$ M) resulted in a significant (100%) increase of 11-HSD1 activity (Fig. 6). Activation of FP receptor is followed by an increase in intracellular concentration of Ca$^{2+}$. To determine whether intra- or extracellular calcium was mobilized to activate 11-HSD1, we measured the enzyme activity in the absence or presence of EGTA (2 mm), an extracellular calcium chelator, or BAPTA-am (1 mm), an intracellular calcium chelator. The two chelators have been used separately in two independent sets of experiments. Results are given in (Fig. 7, A and B). Neither EGTA nor BAPTA alone affected the basal activity of 11β-HSD1. BAPTA (10$^{-6}$ M) but not EGTA in the presence of A23187 (10$^{-6}$–10$^{-12}$ M) reproduced the effect of PGF$_{2\alpha}$/H9251.
PGF$_{2a}$ significantly decreased 11β-HSD1 activity, compared with the effect of PGF$_{2a}$ alone. These results strongly suggested that PGF$_{2a}$ increased 11β-HSD1 activity in CT cells by mobilizing intracellular calcium stores.

**Mediation of PGF$_{2a}$ effects by PKC**

In many other systems, it has been shown that PGF$_{2a}$ stimulates PKC (35–37). To determine whether 11β-HSD1 activation by PGF$_{2a}$ involved the activation of PKC, the effect of the PKC activator, PMA, was examined (Fig. 8A). PMA (10$^{-8}$–10$^{-6}$ M) for 30 min stimulated 11β-HSD1 activity by 40%, thus reproducing the effect observed with PGF$_{2a}$. Conversely, calphostin C, a PKC inhibitor, prevented the PGF$_{2a}$-induced increase in 11β-HSD1 activity (Fig. 8B). Calphostin C (10$^{-7}$ M) was preincubated with the cells beginning 15 min before the incubation with $^3$H-cortisone (1 μM) in the absence or presence of PGF$_{2a}$ (10$^{-8}$ M). There was no effect of calphostin C alone on the basal activity of 11β-HSD1; however, coincubation of calphostin C with PGF$_{2a}$ significantly decreased the effect of PGF$_{2a}$.

**In vitro 11β-HSD1 phosphorylation**

To examine further PGF$_{2a}$-dependent 11β-HSD1 activation, in vitro phosphorylation was assayed. A representative autoradiogram of phosphorylated 11β-HSD1 that was immunoprecipitated and then resolved by gel electrophoresis is shown in Fig. 9A. The amount of phosphorylated 11β-HSD1 was determined by densitometry of the band at 34 kDa. 11β-HSD1 phosphorylation was increased 2.5-fold with PGF$_{2a}$ (1 μM), and 2-fold with PMA (200 nM) (Fig. 9B).

**Discussion**

The present study demonstrates the potential for local production of cortisol from cortisone in human chorionic tissue at term and shows that PGF$_{2a}$ increases 11β-HSD1 reductase activity in the CT cells. This action was associated with and dependent on increased free calcium availability and PKC activation. The PGF$_{2a}$ effect occurred within a few minutes. A similar rapid effect of PGF$_{2a}$ has been reported for other effects of the hormone in other systems, including the effect of PGF$_{2a}$ on the contraction and proliferation of glo-
merular mesangial cells and the stimulatory effect on protein kinase signaling in bovine luteal cells (32, 38). The dose dependence of the PGF2α-induced increase in 11 β-HSD1 (10^{-10}–10^{-7} \text{m}) is consistent with physiological levels of PGF2α, in human placenta at term (39). Therefore, the present data support the hypothesis that under physiological conditions, locally produced PGF2α may activate 11 β-HSD1 reductase activity in the fetal membranes, specifically the CT cells, in which 11 β-HSD1 and the FP receptor are colocalized. Our results demonstrate for the first time a novel interaction between PGs and glucocorticoid metabolism in CT cells that should create and then accelerate a local feed-forward loop with the progression of labor.

In human intrauterine tissues, 11 β-HSD1 is expressed in placental intermediate trophoblast cells, the vascular endothelium, the chorionic trophoblast cells, and the decidua (14). Our CT cell preparation was predominantly cytokeratin positive, confirming their origin from cells of epithelial lineage. The effect of PGF2α to stimulate 11 β-HSD1 is likely a specific effect on CT cells, although we cannot exclude the possibility that a portion of this effect may be mediated via decidual cells in our preparation. Decidual cells have both 11 β-HSD1 oxidase and reductase activity, with predominantly oxidative activity (14, 40), and express the FP receptor. However, a major contamination of the CT preparation by decidual cells is unlikely because there was very little oxidase activity in our chorionic preparations that was not enhanced on exposure to PGF2α.

The increase in cortisol concentrations at term may result partly from an autocrine/paracrine regulation of 11 β-HSD1 and 11 β-HSD2 activities in the fetal membranes and placenta, respectively. In human choriocarcinoma JEG3 cells, it has recently been shown that PGs and leukotrienes decrease 11 β-HSD2 activity by 40% (21). We found the same inhibitory effect of PGE2 on 11 β-HSD2 activity in primary human placental trophoblast culture (N. Alfaidy et al.). A decrease in 11 β-HSD2 activity was earlier reported in both human and guinea pig placenta near term (41–43). The inhibitory effect of PGs on 11 β-HSD2 dehydrogenase activity in the placenta leads to an increase in the rate of transplacental transfer of cortisol between the mother and fetus. In addition, the stimulatory effect of PGs on 11 β-HSD1 reductase activity in fetal membranes also leads to an increase in locally formed cortisol derived from either maternal or fetal cortisone. This coordinated regulation of the 11 β-HSD enzyme complex by PGs suggests a local positive feed-forward loop between cortisol and PG production at term in the human intrauterine tissue.

Primary PGs (PGE2 and PGF2α) act through specific receptors. There are at least four subtypes of PGE2 receptors (EP1, EP2, EP3, and EP4) and two subtypes of PGF2α receptors (FPα and FPβ). In this study we showed for the first time that FP receptor is expressed in both fetal membranes and purified CT cells. The specific subtype expression of FPα and FPβ and the expression of PGE2 receptor subtypes have yet to be investigated in both human placenta and fetal membranes. Although this report is focused on effects of PGF2α, we do not wish to imply that PGs of other series are unimportant in this response. In preliminary studies we found that PGE2 also increased chorionic 11 β-HSD1 activity, but the mechanism of this effect remains to be explored.

Several convergent arguments suggest that the FP receptor mediates the observed PGF2α effect. Fluprostrenol, which acts exclusively at the FP receptor, reproduces the PGF2α-induced increase in 11 β-HSD1 activity. In the present study, we showed that FP receptor-mediated 11 β-HSD1 activation was associated with a rapid elevation (within 1 min) of free intracellular calcium concentration in CTs. The increase in free calcium appears to be of intracellular origin because BAPTA but not EGTA significantly attenuated the PGF2α effect. Calcium, the effector of the FP pathway, mimics PGF2α action, as demonstrated by the increase in 11 β-HSD1 activity in the presence of A23187. Our studies also demonstrate that in vitro, PGF2α phosphorylates and stimulates 11 β-HSD1 activity. A similar level of 11 β-HSD1 phosphorylation was also obtained after PMA treatment, suggesting PKC activation. Moreover, calphostin C significantly reduced the PGF2α effect.

11 β-HSD1 belongs to the short-chain alcohol dehydrogenase superfamily (46, 47). The active site consists of an invariant YXXK motif and usually contains a serine and/or threonine residue at Y+1 and Y+3, potential sites of phosphorylation. A recent study (48) has shown that serines at the 11 β-HSD1 active site play an important role in determining the rate of catalysis. The amino acid sequence of human 11 β-HSD1 deduced from its cDNA sequence contains a potential PKC phosphorylation site TAR at position [64.66] and SIR at the position [196.198]. The data reported in the present study suggest that 11 β-HSD1 is phosphorylated on PKC activation and that its activity may be regulated by phosphorylation-dephosphorylation. Further studies on the phosphorylation mechanism of 11 β-HSD1 may enhance our understanding of the regulation of this enzyme.

Earlier studies have reported that the fetal membranes, in particular the chorion layer, may provide an extraadrenal source of cortisol at term in human pregnancy (49). In the present study, we have described a mechanism for the stimulation of chorionic cortisol production from circulating inactive precursors through activation of 11 β-HSD1. We suggest that in the fetal membranes, 11 β-HSD1 may be pivotal to the integrated cellular regulation between glucocorticoid and PGs. 11 β-HSD1 is discretely colocalized with PGHS-2 and PGDH, consistent with paracrine/autocrine regulation between glucocorticoids and PGs. We speculate that this novel pathway, through which PGF2α stimulates 11 β-HSD1 activity in human chorion trophoblasts to generate biologically active cortisol, could have more general applicability in these tissues. In turn, cortisol stimulates PG synthesis and decreases PG metabolism. This feed-forward loop in the human fetal membranes may contribute to the mechanisms of human parturition and potentially to the major clinical condition of preterm birth.

Acknowledgments

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