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Labor and Inflammation Increase the Expression of Oxytocin Receptor in Human Amnion[1]

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ABSTRACT

The oxytocin/oxytocin receptor (OXT/OXTR) system plays an important role in the regulation of parturition. The amnion is a major source of prostaglandins and inflammatory cytokine synthesis, which increase both before and during labor. Amnion is a noncontractile tissue; therefore, the role played by OXT/OXTR in this tissue will be fundamentally different from the role played in myometrial contractions. In the present study, we demonstrate increased *OXTR* mRNA and protein concentrations in human amnion epithelial cells associated with the onset of labor. We show that incubation of primary human amnion epithelial cells with IL1B results in a rapid, transient up-regulation of *OXTR* mRNA expression, which peaks in prelabor samples after 6 h. Incubation of prelabor amnion epithelial cells with OXT results in a marked increase of prostaglandin E_2 synthesis, and we demonstrate that OXT activates the extracellular signal-regulated protein kinase signal transduction pathway to stimulate up-regulation of cyclo-oxygenase 2 in human amnion epithelial cells. The increased ability of human amnion to produce prostaglandins in response to OXT treatment suggests a complementary role for the OXT/OXTR system in the activation of human amnion and the onset of labor.

amnion, oxytocin, oxytocin receptor, parturition, prostaglandins

INTRODUCTION

The oxytocin receptor (OXTR) is a member of the G protein-coupled superfamily, and uterine sensitivity to oxytocin (OXT) markedly increases around the onset of labor. This is associated with both an up-regulation of *OXTR* mRNA levels and a strong increase in myometrial OXTR density, reaching a peak during early labor [1]. The physiological role of the OXT/OXTR system in myometrium is well understood, because OXT represents one of the best-known contraction mediators. The increase in OXTR before labor is not confined to the myometrium. In rabbit amnion, a 200-fold up-regulation of OXTR occurs at the end of pregnancy [2], and OXTR binding to OXT significantly increases in human fetal membranes with the onset of labor [3]. Studies in several species have shown OXT to stimulate uterine prostaglandin (PG) synthesis when administered near estrus and to stimulate PGE_2 production. This suggests a role for OXT/OXTR in the activation of the amnion that occurs at the time of labor.

The amnion plays an important role in the onset of human labor. It is a major source of PG and inflammatory cytokine synthesis, which increase both before and during labor. Increased PG synthesis in the amnion appears to occur first in the region overlying the cervix, where it is thought to mediate cervical ripening [4]. PGs, especially PGE_2 synthesized in the amnion, are thought to cross to the decidua and myometrium to mediate cervical ripening and lower segment remodeling and initiate contractions. Roseblade et al. [5] have suggested that when using physiological concentrations of PGE_2 in an in vitro model, only small amounts cross the membranes without being metabolized. However, Johnston et al. [6] have demonstrated marked transfer of PGE_2 across ovine fetal membranes in vivo after intra-amniotic PGE_2 injection. Amnion PG synthesis occurs principally via cyclo-oxygenase 2 (PTGS2), which itself is regulated by nuclear factor kappa B (NFKB) [7–10]. Labor, both term and preterm, is associated with increased expression and activity of PTGS2 in the amnion [11], and basal NFKB DNA binding and transcriptional activity increase in human amnion with labor onset. The NFKB changes persist in culture and represent a form of amnion differentiation, which we believe commits to labor and delivery [7]. Both basal and IL1B-induced NFKB DNA binding involves the p50-p65 heterodimer as well as the p65 and p50 homodimers [12]. Using whole-genome cDNA arrays, we recently studied the range of genes for which expression is increased in amnion with high levels of NFKB activity compared to low levels. OXTR expression showed the second-greatest difference, with a 24-fold increase in expression (after PTGS2, which was increased 40-fold), therefore strongly suggesting a role for NFKB in the regulation of OXTR in amnion [13].

Mitogen-activated protein kinases (MAPKs) are important signal pathway components and function as integrators of mitogenic and other signals originating from G protein-coupled receptors. Members from the three subgroups of the MAPK family—extracellular signal-regulated protein kinase (ERK), stress-activated protein kinase c-Jun NH_2-terminal kinase (MAPK8), and p38 MAPK (MAPK14)—mediate responses to proinflammatory stimuli and affect gene expression at the transcriptional and posttranscriptional levels. Each subtype of the MAPK family is activated by its own kinase system. The activation of MAPK3/1 (ERK1/2) involves activation of RAF followed by MAPK kinase (MAP2K) 1/2. When MAPK3/1

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gets activated, it exerts its effects via phosphorylation of proteins and/or transcription factors that can alter gene expression [14].

In rat myometrium during pregnancy, regulation of ERK activity is controversial. It has been reported to increase with advancing gestation, although some studies have documented a decline immediately before the onset of parturition [15], in contrast to others that have documented a rise with the onset of labor [16]. In nonpregnant women, expression of MAPK14 and MAPK3/1 was uniform throughout the uterus, whereas in pregnant women before and after labor onset, expression of MAPK14 and MAPK3 was significantly elevated in the upper compared to the lower uterine segment [17]. The role of OXT in MAPK activation is not well defined. Cytosolic phospholipase A_2 ($cPLA_2$) is an important enzyme providing substrate for cyclo-oxygenases and is activated by various agents that stimulate its phosphorylation on Ser505 by MAPKs [18, 19].

In the present study, we have investigated the labor-associated changes in OXTR expression and the effect of IL1B in human amnion epithelial cells. We provide evidence suggesting for a role of the OXT/OXTR system in PG production in human amnion, and we propose that an ERK-mediated PTGS2 up-regulation is involved in this process.

MATERIALS AND METHODS

Cell Preparation and Culture

Fetal membranes were obtained by elective cesarean section before labor at term (prelabor) or after spontaneous vaginal delivery at term (postlabor), where term was defined as 37–42 completed weeks of pregnancy. Institutional ethics committee approval was granted for the present study, and patients gave informed consent. Amnion epithelial cells were prepared from tissue as previously described [20]. In brief, amnion was separated from chorion and washed in PBS. Next, the membrane was cut into strips and incubated in 0.5 mmol/L of ethylenediaminetetra-acetic acid (EDTA; BDH Laboratory Supplies Ltd.) for 15 min at room temperature. The strips were then rinsed twice in PBS, followed by incubation in 2.5 g/L of dispase (Life Technologies) for 40 min at 37°C. Amnion epithelial cells were isolated by vigorous shaking for 3 min, after which strips were removed. Individual cells were collected by centrifugation at 400 × *g* for 10 min and grown in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum (Sigma), 2 mmol/L of L-glutamine, 100 U/ml of penicillin, and 100 μg/ml of streptomycin (Life Technologies) at 5% CO_2. All amnion epithelial cells used were primary cultures (no passages) and were usually cultured for 3–4 days before treatment.

Real-Time RT-PCR

Total RNA was extracted using RNA STAT-60 reagent (AMS Biotechnology) according to the manufacturer's specifications. After quantification, 1 μg of total RNA was digested with 0.5 μl of DNase I in 1× DNase I reaction buffer (Invitrogen) in a total volume of 5 μl at room temperature for 15 min. The reaction was terminated by the addition of 0.5 μl of 25 mM EDTA and incubation at 65°C for 15 min. The whole of this reaction was subsequently used for first-strand cDNA synthesis with SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen) according to the manufacturer's instructions. Gene expression was verified by real-time RT-PCR using the ABI PRISM 7700 Sequence Detection System (Applied Biosystems). TaqMan primers and probes to analyze the different transcripts were designed using the Primer Express software (Applied Biosystems). The data were analyzed using Sequence Detector version 1.7 software (Applied Biosystems), and the values obtained were normalized according to transcript levels of glyceraldehyde phosphate dehydrogenase (GAPDH) or ribosomal protein L19. RT-PCR expression data were assessed using the delta Ct method. The sequences used are shown in Table 1. Statistical significance was determined by ANOVA.

Protein Extraction, SDS-PAGE, Western Blot Analysis, and Immunodetection

Confluent amnion epithelial cells were rinsed in PBS and lysed in a buffer containing 10 mM Hepes, 10 mM KCl, 0.1 mM EDTA, 0.1 mM ethylene glycol tetraacetic acid, 2 mM dithiothreitol (DTT), 1% (v/v) Nonidet P-40 (NP-40), and Complete Protease Inhibitor Tablets (Roche Diagnostics). Cell lysates were incubated on ice for 10 min, and NP-40 was added to a final concentration of 1% (v/v). Lysates were vortexed and centrifuged for 30 sec at 4°C and 12 000 × *g*. The supernatants were retained as the cytosolic protein fraction. The pellets were resuspended in a buffer containing 10 mM Hepes, 10 mM KCl, 0.1 mM EDTA, 0.1 mM ethylene glycol tetraacetic acid, 2 mM DTT, 400 mM NaCl, 1% (v/v) NP-40, and Complete Protease Inhibitor Tablets. Samples were then shaken vigorously for 15 min on ice. Nuclear protein extracts were obtained in the supernatant after centrifugation for 5 min at 12 000 × *g* and 4°C. For whole-cell protein, cells were lysed on ice for 20 min in radioimmunoprecipitation assay buffer (1% NP-40, 1% sodium deoxycholate, 1% Triton X-100, 0.1% SDS, 150 mM NaCl, 10 mM Tris [pH 8.0], and 2 mM NaF). Protein concentrations were determined using BioRad assay (Bio-Rad Laboratories). Protein samples (40 μg) were denatured by boiling for 5 min and then separated by electrophoresis on an 8% SDS-PAGE gel for 80 min at 150 V. Transfer from gel to polyvinylidene fluoride membrane (Anachem) took place in a semidry chamber with three buffer systems as described by Pohnke et al. [21]. The membrane was activated in methanol, blocked in 5% milk protein solution (Marvel Lincs) for 1 h at room temperature, and then washed and hybridized with the primary antibodies (1:1000 dilution) overnight at 5°C in a fresh blocking buffer (1× PBS, 1% milk protein, and 0.1% Tween-20), followed by incubation with horseradish peroxidase (HRP)-conjugated secondary antibodies (1:2000 dilution). Signal detection was carried out using ECL Plus (Amersham). To confirm equal loading of each well, the blot membrane was treated with a stripping buffer (2% SDS, 62.5 mM Tris-HCl [pH 6.7], and 100 mM 2-mercaptoethanol) for 30 min at 50°C, washed in PBS-Tween-20, and then preblocked and reprobed with an antibody to human β-actin.

Antibodies and Materials

The following antibodies were obtained from Santa Cruz Biotechnologies: goat anti-PTGS2 (COX-2) (C20), goat anti-OXTR (C-terminus), and HRP-conjugated secondary antibodies raised against goat, rabbit, and mouse immunoglobulin Gs. Rabbit monoclonal antibodies to phospho-$cPLA_2$, phospho-MAPK14 (p38 MAPK), phospho-MAPK3/1 (ERK1/2 p44/42 MAPK), and mouse monoclonal MAPK8 (SAPK/JNK) were from Cell Signaling Technology. The monoclonal anti-smooth muscle-actin antibody was from Sigma. PGE_2 enzyme immunoassays were from Amersham (GE Healthcare UK Ltd.). Cells before treatment with OXT (Alliance Pharmaceuticals) or IL1B (R&D Systems) were serum-deprived for 16 h and stimulated for the required times.

Statistical Analysis

Multiple comparisons were analyzed using one-way ANOVA. The effects of OXT and IL1B treatments with nonstimulated effects were compared using *t*-test. Differences of $P < 0.05$ were considered to be statistically significant.

RESULTS

OXTR *Is Expressed in Humans, and Both mRNA and Protein Expression Significantly Increase in Association with Labor*

Primary human amnion epithelial cell cultures were established from patients before and after the onset of labor. Using real-time RT-PCR, we found that the expression of *OXTR* mRNA in postlabor primary amnion epithelial cells was 16-fold higher than in prelabor cells ($P < 0.001$; n = 4 sets of amnion epithelial cells isolated from 4 patients each group, duplicate samples) (Fig. 1A). Whole-cell protein was extracted, and Western blot analysis demonstrated that OXTR protein expression also significantly increased in association with labor (Fig. 1, B and C).

IL1B Increases OXTR *Expression in Prelabor Amnion to Postlabor Levels But Does Not Further Increase Expression in Postlabor Cells*

Time-course experiments were performed to assess *OXTR* mRNA expression after treatment with IL1B (1 ng/ml) for 30 min as well as 1, 2, 4, 6, and 24 h before and after the onset of labor. Treatment with IL1B resulted in a significant up-

TABLE 1. The primer pairs, probes, and GenBank accession numbers for the primer pairs used.

mRNA	Sequences*	GenBank/EMBL Accession no.
	Primers	
OXTR	F: GATGGGAAGGGTGGT	NM_000916
	R: CAAGGACCCCAGCATTTGTT	
PTGS2	F: GCTCAAACATGATGTTTGCATTC	AY151286
	R: GCTGGCCCTCGCTTATGA	
PTGES	F: TCTTAGCCCCTTGGATTCCT	BC008280
	R: ATTCTTAGCCCGGGATTCAG	
GAPDH	F: GAAGGTGAAGGTCGGAGT	XM_068376
	R: GAAGATGGTGATGGGATTTC	
RPL19	F: GCGGAAGGGTACAGCCAAT	NM_000981
	R: GCAGCCGGCGCAAA	
	Probes	
OXTR	CCTCCTGACCTCAAAGTGTATTTGCCTTTAAGC	NM_000916
GAPDH	ATTTGGTCGTATTGGGCGCCTGGTCACC	XM_068376

* F, Forward; R, Reverse

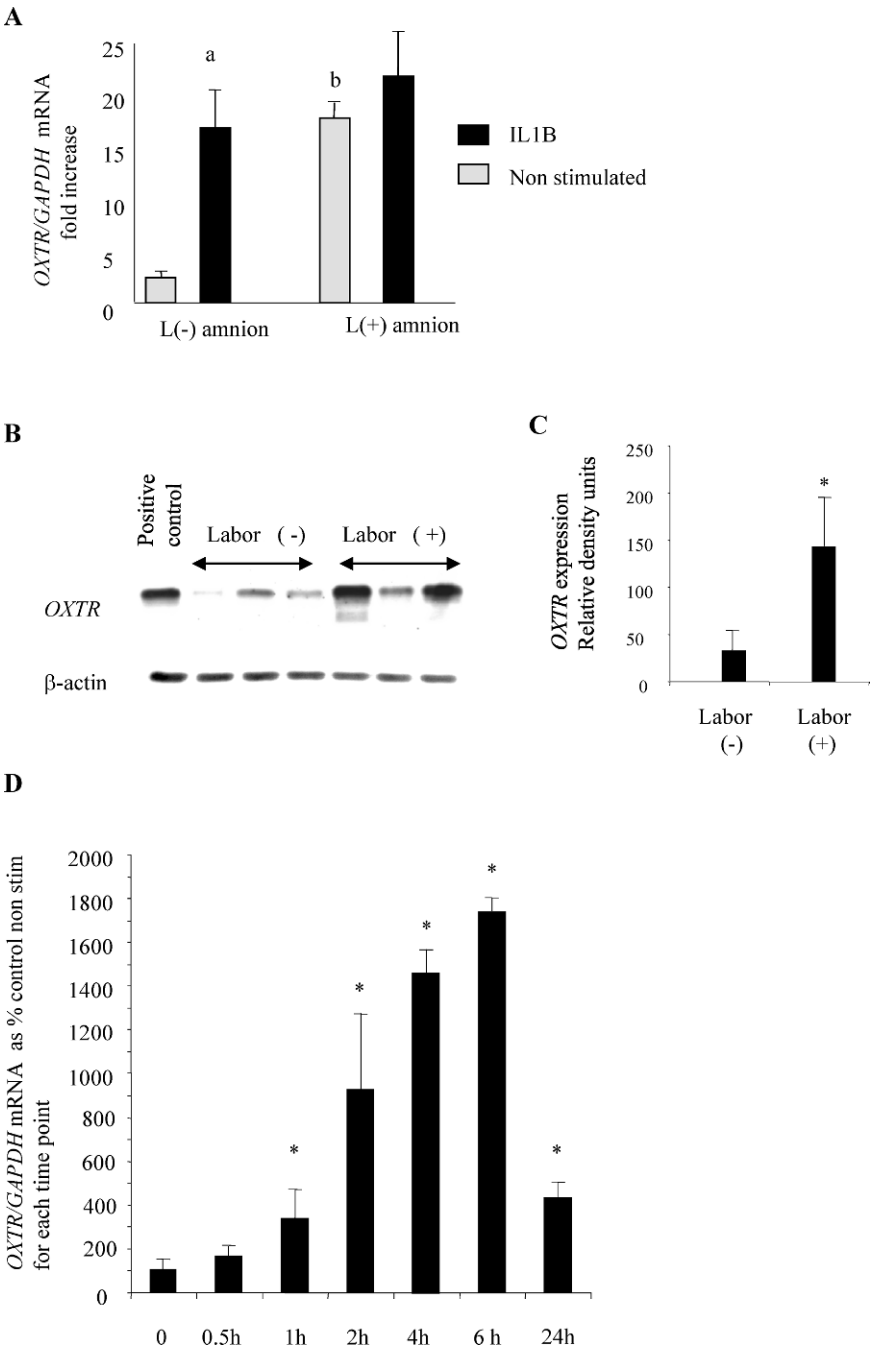

FIG. 1. Expression of *OXTR* in human amnion epithelial cells. **A**) Expression of *OXTR* mRNA measured by real-time RT-PCR in prelabor (L-) and postlabor (L+) amnion epithelial cells. Both L- and L+ cells were treated with IL1B (1 ng/ml) for 6 h. Results are corrected for *GAPDH* expression and given as the fold-increase in *OXTR/GAPDH* mRNA expression (n = 4 sets of amnion epithelial cells isolated from 4 patients each group, duplicate samples; ANOVA: $^{a}P < 0.05$, L- a IL1B-stimulated vs. nonstimulated cells; $^{b}P < 0.05$, L- vs. L+ cells. **B**) Western blot analysis for OXTR in amnion epithelial cells taken before (-) and after (+) the onset of labor. Controls with β-actin confirmed equal protein loading. **C**) Densitometric quantification of OXTR protein expression normalized against actin protein levels (n = 3 sets of amnion epithelial cells isolated from 3 patients; ANOVA: $*P < 0.05$). **D**) Time course of *OXTR* mRNA measured by real-time RT-PCR in prelabor (-) amnion epithelial cells after treatment with IL1B (1 ng/ml). Results are corrected for *GAPDH* expression and given as the percentage of untreated cells for each time point (n = 4 sets of amnion epithelial cells isolated from 4 patients in each group, duplicate samples; ANOVA: $*P < 0.05$ vs. nonstimulated cells). Treatment with IL1B resulted in a significant up-regulation of *OXTR* expression that peaked 6 h after treatment.

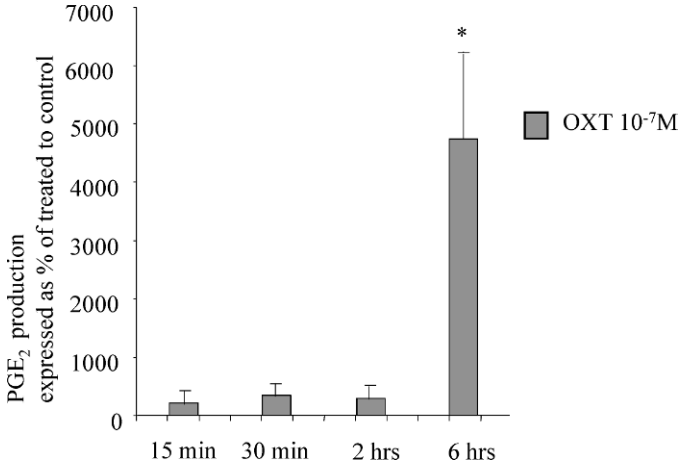

FIG. 2. OXT-stimulated PGE_2 production in human amnion epithelial cells. PGE_2 production by prelabor cells after treatment with OXT (10^{-7} M) for 15 min, 30 min, 2 h, and 6 h is shown. Results are expressed as the percentage of control (nonstimulated cells) production for each time point (n = 3 sets of amnion epithelial cells isolated from 3 patients, triplicate samples; ANOVA: *$P < 0.05$).

regulation of *OXTR* mRNA expression. This peaked in the prelabor cells at 6 h after treatment and resulted in a 17-fold induction of *OXTR* expression ($P < 0.001$; n = 4 sets of amnion epithelial cells isolated from 4 patients, duplicate samples) (Fig. 1D). The level of expression that was observed in the prelabor cells was significantly less than that in the postlabor cells. Treatment with IL1B in prelabor cells brought the level of *OXTR* expression to that observed in postlabor cells. In postlabor cells, IL1B caused a further, but nonsignificant, increase in *OXTR* expression (Fig. 1A).

PGE_2 Release by Primary Human Amnion Significantly Increases after OXT Stimulation

Time-course experiments were performed with prelabor cells to assess the effect of OXT treatment in PGE_2 production. OXT (10^{-7} M) stimulation for 6 h resulted in a 40-fold increase of PGE_2 production ($P < 0.05$; n = 3 sets of amnion epithelial cells isolated from 3 patients, triplicate samples) (Fig. 2).

Changes in PTGS2 and Phospho-$cPLA_2$ in Amnion after OXT Stimulation

Amnion epithelial cells from patients before the onset of labor were treated for 6 h with either OXT (10^{-7} M) or IL1B (1 ng/ml). Treatment with IL1B resulted in a 3-fold increase in *PTGS2* mRNA expression ($P < 0.001$; n = 7 sets of amnion epithelial cells isolated from 7 patients, duplicate samples) and OXT induced a 2-fold increase in *PTGS2* mRNA expression ($P < 0.005$; n = 7, as described above) (Fig. 3A), whereas the levels of PGE synthase (*PTGES*) mRNA were not affected (Fig. 3B). Western blot analysis confirmed this increase in protein level (Fig. 3, C and F). Phospho-$cPLA_2$ also increased after OXT treatment (Fig. 3, D and G). Western blot analysis for β-actin confirmed equal loading (Fig. 3E).

OXT Activates the MAPK Pathway in Amnion Epithelial Cells, Specifically via ERK

To determine the identity of the MAPK potentially involved in OXT-stimulated PTGS2 up-regulation, immunoblotting of amnion cell lysates was performed using antibodies to dually

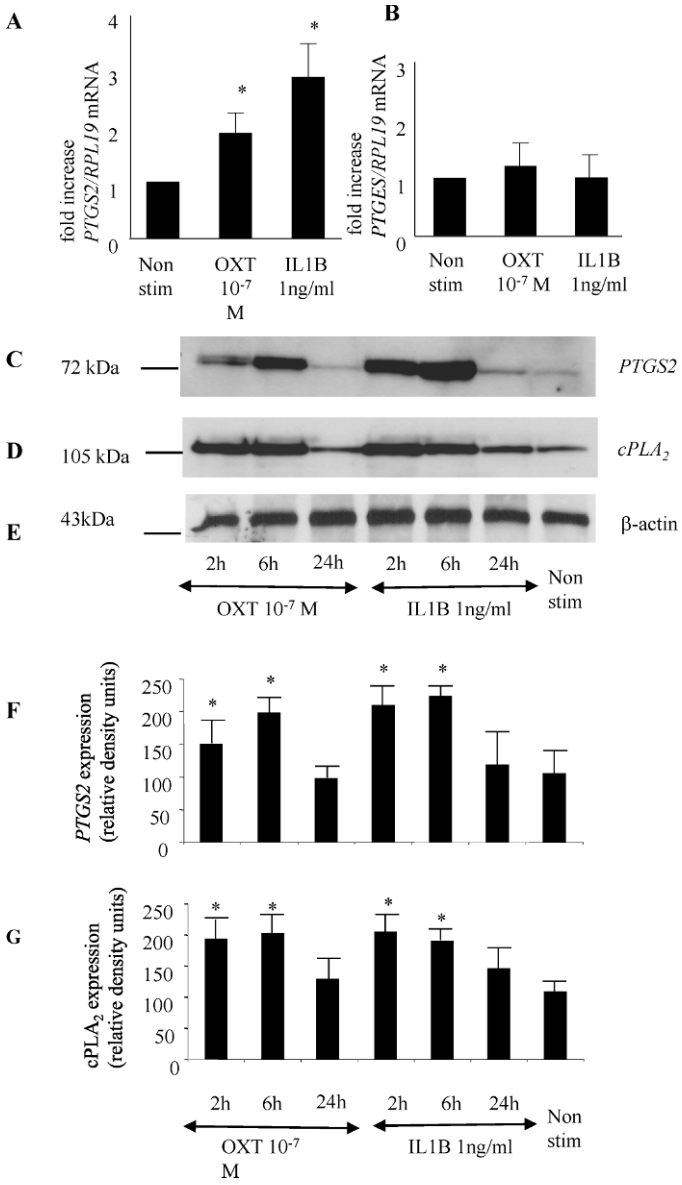

FIG. 3. OXT stimulation effects in the PG biosynthetic pathway in human amnion epithelial cells. Prelabor cells were treated with OXT (10^{-7} M) or IL1B (1 ng/ml) for 6 h, after which mRNA was extracted and real-time RT-PCR was performed. **A**) Real-time RT-PCR for *PTGS2*. Results are corrected for ribosomal protein L19; treatment with IL1B resulted in a 3-fold increase in *PTGS2* mRNA expression (n = 7 sets of amnion epithelial cells isolated from 7 patients/duplicate samples; ANOVA: *$P < 0.001$ vs. stimulated cells), and OXT induced a 2-fold increase in *PTGS2* mRNA expression (n = 7, as above; ANOVA: *$P < 0.005$ vs. stimulated cells). **B**) Real-time RT-PCR for *PTGES*. Results are corrected for L19. **C**–**E**) Prelabor amnion epithelial cells were treated with OXT (10^{-7} M) or IL1B (1 ng/ml) for 6 h, after which cytoplasmic protein was extracted. Western blot analysis for PTGS2 (**C**), phospho-$cPLA_2$ (**D**), and β-actin (**E**; control) is shown. OXT stimulation up-regulates both enzymes, as does treatment with IL1B. PTGS2 is degraded when it catalyses PG synthesis, which explains the down-regulation observed after 24 h of treatment. Controls with β-actin confirmed protein equal loading. **F** and **G**) Densitometric quantification of PTGS2 and cPLA2 protein expression normalized against actin protein levels (n = 3 sets of amnion epithelial cells isolated from 3 patients; ANOVA: *$P < 0.02$ vs. nonstimulated cells).

phosphorylated MAPK3/1, phospho-MAPK14, and phospho-MAPK8. We found that OXT stimulation increases phospho-MAPK3/1, whereas phospho-MAPK14 and phospho-MAPK8 were unaffected (Fig. 4, A–C). Pretreatment of amnion

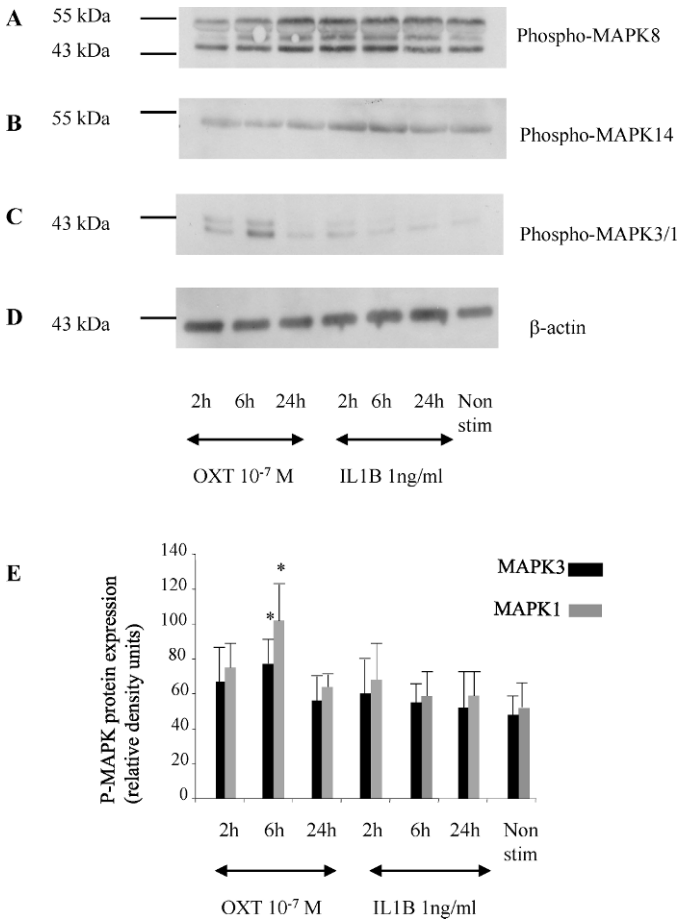

FIG. 4. OXT stimulation effects in the MAPK pathway in human amnion epithelial cells. Prelabor cells were treated with OXT (10^{-7} M) or IL1B (1 ng/ml), after which cytoplasmic protein was extracted. **A**–**D**) Western blot analysis for phospho-MAPK8 (**A**), MAPK14 (**B**), phospho-MAPK3/1 (**C**), and β-actin (**D**; control). Controls with β-actin confirmed equal protein loading. **E**) Densitometric quantification of phospho-MAPK3 and phospho-MAPK1 expression normalized against actin protein levels (n = 3 sets of amnion epithelial cells isolated from 3 patients; ANOVA: $^{*}P < 0.05$ vs. nonstimulated cells).

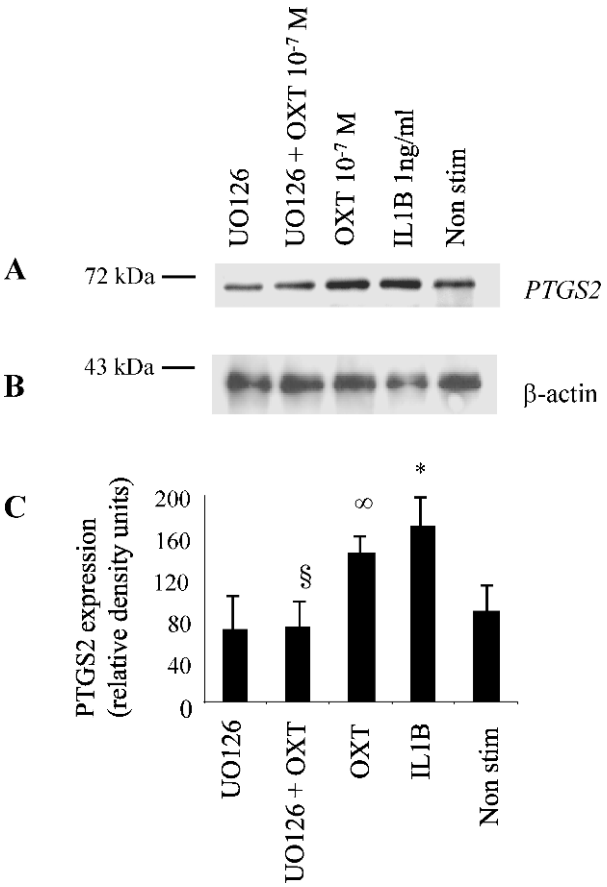

FIG. 5. MAP2K1/2 inhibition of the OXT-induced PTGS2 up-regulation in human amnion epithelial cells. Prelabor cells were pretreated with the MAP2K1/2 inhibitor UO126 (Sigma) for 2 h before OXT stimulation (10^{-7} M), after which cytoplasmic protein was extracted. **A** and **B**) Western blot analysis for PTGS2 (**A**) and β-actin (**B**; control). Controls with β-actin confirmed protein equal loading. **C**) Densitometric quantification of PTGS2 expression normalized against actin protein levels (n = 3 sets of amnion epithelial cells isolated from 3 patients; ANOVA: $^{*}P < 0.04$, IL1B-treated vs. nonstimulated cells; $^{\infty}P < 0.05$, OXT-treated vs. nonstimulated cells; $^{\S}P < 0.05$, UO126+OXT-treated vs. OXT-treated cells).

epithelial cells with the MAP2K1/2 inhibitor UO126 (10 mM; Sigma) for 2 h before OXT stimulation resulted in a significant decrease in the OXT-stimulated increase of PTGS2 (Fig. 5, A and B).

DISCUSSION

We have demonstrated an increase in *OXTR* mRNA and protein concentrations in human amnion epithelial cells associated with the onset of labor, as has been previously shown in human choriodecidua and fundal myometrium [1, 22, 23].

In the present study, incubation of primary human amnion epithelial cells with IL1B results in a rapid, transient up-regulation of *OXTR* mRNA expression. This peaked in prelabor amnion epithelial cells at 6 h after treatment and resulted in a 17-fold induction of *OXTR* mRNA expression. This response of *OXTR* expression to IL1B is similar to that which we have previously reported in myometrium [15], and further supports the concept that OXTR is regulated in a paracrine as well as endocrine fashion. Rauk et al. [24] as well as Mitchell and Schmid [25] have reported that IL1B down-regulates *OXTR* mRNA in myometrial cells. Schmid et al. [26] used an immortalized cell line derived from nonpregnant human myometrium, whereas Rauk et al. [24], using pregnant primary myocytes and measuring *OXTR* mRNA by Southern blot analysis, showed an early, transient increase in *OXTR* expression, followed by a significant decrease after 25 h. To us, this seems to be counterintuitive. It is well established that IL1B concentrations within the uterus increase at the time of both term and preterm labor [27], as does the expression of several ''labor-associated proteins,'' such as PTGS2 and IL8, each of which is up-regulated by IL1B [8, 10, 28–30]. *OXTR* expression is also up-regulated at term, at a time when IL1B concentrations are high and PTGS2 and IL8 expression increases. Our data suggest that IL1B increases *OXTR* expression in primary human amnion epithelial cells. The time course of the effect is similar to that seen with stimulation of both PTGS2 and IL8 by IL1B [28, 31]. The level of expression that was observed in the prelabor amnion epithelial cells was significantly less than that in the postlabor cells. Incubation with IL1B in prelabor cells brought the level of *OXTR* expression to that observed in postlabor cells. In postlabor cells, IL1B caused only a small, nonsignificant further increase in *OXTR* expression. Loudon et al. [28] have previously shown that PGE_2 production is lower in prelabor than in postlabor

cells and that PGE_2 production in prelabor cells can be stimulated by IL1B to levels similar to that found in postlabor cells. This shows that the pathways for PG synthesis are activated in postlabor cells, and our present data suggest that up-regulation of *OXTR* is a feature of amnion activation and is sensitive to inflammatory cytokines.

Amnion is not a contractile tissue; therefore, the physiological role of the OXT/OXTR system in amnion must be in some respect different from its role in myometrium. In rabbit amnion [2], up-regulation of *OXTR* occurs at the end of pregnancy, and OXT has been shown to stimulate PGE_2 production. Similarly, we have found that release of PGE_2 by human amnion epithelial cells is significantly increased after OXT stimulation. This supports a role for OXT/OXTR in the activation of the amnion that occurs at the time of labor. The present study shows that OXT causes increased PG synthesis through up-regulation of PTGS2. It is established that the PTGS2 enzyme, which mediates the committing and rate-limited step of PG biosynthesis, generating a PGH_2 intermediate that is converted to the terminal PGs, is central to increased PGs synthesis in the human amnion at the time of labor [10, 32, 33].

In cultured bovine endometrial cells, OXT has been shown to stimulate $PGF_{2\alpha}$ via activation of the phospholipase C second-messenger cascade, activation of phospholipase A_2 [34], and induction of PTGS2 [35]. In ovariectomized ewes, injection of OXT induced an increase in serum PGFM (stable metabolite of $PGF_{2\alpha}$), which was associated with an increase in endometrial concentrations of PTGS2 [36]. In rabbit [37], the OXT-stimulated increase in PGE_2 is virtually immediate, with concentrations in the medium reaching near-maximal values by 15 min, which is too rapid to involve new synthesis of PTGS2. This suggests that in rabbit, OXT leads to increased availability of PG precursors, which are synthesized into PGs via pre-existing PTGS2. Our studies in human amnion show that PG synthesis occurred between 2 and 6 h following OXT treatment and was associated with increased PTGS2 expression but no changes in PTGES expression. This shows that in human amnion, unlike rabbit amnion but similar to bovine endometrium, transcriptional regulation of PTGS2 is required for the OXT-stimulated induction of PG synthesis.

We previously demonstrated that OXT can be extracted and measured from the amnion in samples taken both before and after the onset of spontaneous labor [23]. We demonstrated that labor was associated with a significant increase in OXT peptide in amnion, chorion, and decidua. The source of the measured OXT, which acts as ligand to the OXTR in amnion, is unclear, but three possibilities exist. First, neurohypophysial OXT may diffuse from the maternal circulation across the amnion to the epithelium. Although the amnion is avascular, we have found large amounts of OXT to be present in decidua, chorion, and amnion where the placenta was delivered shortly after administration of a bolus of exogenous Syntocinon (Novartis Pharmaceuticals UK Ltd) to the mother, suggesting that OXT (and OXT analogues) can diffuse from the maternal circulation to the amnion (unpublished data). Second, it has been proposed that the fetus may secrete OXT into the amniotic fluid [38]. Third, we have demonstrated that choriodecidua is capable of synthesizing full neurophysin-OXT peptide directly [23]. Studies in rat uterine epithelial cells have demonstrated a clear polarization in OXT and OXTR expression, whereby OXT is secreted from the apical membrane to act on apical OXTR via an autocrine loop [39]. This is particularly pertinent, because in rodents, the uterine epithelium is the primary source of PGs and produces the $PGF_{2\alpha}$ surge required to initiate luteolysis, progesterone withdrawal, and the onset of labor.

In CHO cells transfected with the rat OXTR, stimulation with OXT resulted in the specific phosphorylation and activation of MAPK1 [40]. We have found that OXT activated the ERK signal transduction pathway to stimulate up-regulation of PTGS2 in human amnion epithelial cells. We also have found both MAPK3 and MAPK1 to be activated in human amnion after 2 h of OXT treatment, which does not apply to the other principal MAPKs, MAPK14 and MAPK8.

In summary, we have found that human *OXTR* expression increases in postlabor amnion epithelial cells and that treatment with IL1B stimulates *OXTR* expression before the onset of labor. The increased ability of human amnion to produce PGE_2 in response to OXT treatment suggests a complementary role of the OXT/OXTR system in the activation of human amnion and in the onset of labor. In addition to its role in mediating contractions, the role of OXT in biochemical processes that lead to the onset of labor suggests that the potential clinical use of OXT antagonists requires re-evaluation.

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