Regulation of Oxytocin Receptor Expression in Cultured Human Myometrial Cells by Fetal Bovine Serum and Lysophospholipids

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Oxytocin receptor (OTR) expression in human myometrium increases over 150-fold from the beginning of pregnancy to the end. In the present studies, we examined potential mechanisms of OTR up-regulation, using myometrial cells in primary culture from women in late gestation. OTR ligand-binding sites and steady-state mRNA levels were down regulated by serum starvation, and up-regulated by restoration of fetal bovine serum (FBS). Transcriptional activity of the OTR gene was the same with or without FBS treatment, but FBS increased OTR mRNA half-life about 5-fold. Lysophospholipids (lysophosphatidic acid and sphingosine 1-phosphate), which are present in serum, had similar effects as FBS. Lysophospholipid receptor mRNAs of the endothelial differentiation gene (Edg) family (Edgs 1, 3, 4, and 5) were demonstrated in myometrial cells by RT-PCR. These G protein-coupled receptors have been shown to be coupled to Gi/o and to mediate activation of phosphoinositide-3-phosphate kinase. Indeed, the effects of the lysophospholipids and FBS were completely blocked by pertussis toxin, a G protein inhibitor. Likewise, inhibition of G protein signaling by elevation of intracellular cAMP or inhibition of phosphoinositide 3-phosphate kinase blocked FBS effects on OTR mRNA stability. We do not presently understand the mechanisms of OTR up-regulation in human myometrium in vivo, but the present studies might lead to the description of mRNA-stabilizing factors whose activity can be quantified in tissue samples during pregnancy to elucidate the process of OTR up-regulation. (Endocrinology 144: 61–68, 2003)

THE SENSITIVITY OF the myometrium to oxytocin (OT) increases strikingly during pregnancy. The classical work of Caldeyro-Barcia and Theobald (1) showed that infusion of less than 1 mU OT per minute is sufficient to induce labor contractions in women at the end of gestation, whereas more than 1000 times that dose is required to stimulate uterine contractions in nonpregnant women. Previous work indicated that the increase in sensitivity is due, at least in part, to the marked up-regulation of OT receptors (OTRs), as measured by the binding of [3H]OT (2, 3). This up-regulation of myometrial OTRs at the end of gestation is a general phenomenon that has been demonstrated in several species, including rat, rabbit, cow, pig, guinea pig, and human (2, 4–9). With the cloning of OTR genes, it was possible to demonstrate that the up-regulation in ligand-binding sites is associated with comparable increases in OTR mRNA concentrations in the myometrium (10–12).

Although we know which agents stimulate OTR up-regulation in the rat, virtually nothing is known regarding factors that up-regulate OTR concentrations in the human myometrium. In rats and rabbits, there is a sudden rise in myometrial OTR concentrations just before the initiation of labor (2, 4, 5). In contrast, OTR concentrations in the human myometrium increase steadily for most of pregnancy and are further elevated only about 2-fold at the onset of labor (3, 10). A fall in circulating progesterone in the presence of elevated estrogen levels plays a major role in up-regulating OTRs in the rat myometrium (2, 13), but in women progesterone levels do not decline at the end of gestation. Thus, it is possible that the mechanisms involved in up-regulating OTRs in the myometrium are different in the rat and human.

EMSAs of human OTR gene fragments indicated that there are two DNA regions in the 5′-flanking sequence and a region in the middle of the third intron that selectively interact with nuclear proteins from term myometrium, as opposed to nonpregnant myometrium or other tissues (14–16). However, in the absence of functional assays, it is not clear whether these protein/DNA interactions are relevant to transcriptional activity of the human OTR gene. There have been several reports on factors that down-regulate OTRs in human myometrial cells maintained in primary culture. These include IL-1β (17, 18) and interferon-α (19). A recent study showed that IL-6 treatment of cultured human myometrial cells for 4 h up-regulated OTR mRNA levels about 2.5-fold (20). However, studies from another laboratory using the same cell type showed that IL-6 down-regulated OTR mRNA levels (18).

Previous work from this laboratory has shown that fetal bovine serum (FBS) up-regulates OTR ligand binding in human breast tumor (Hs578T) (21) and human ovarian granulosa-lutein (HGL5) (22) cell lines. The focus of the present work was to show that FBS elevates OTR expression in human myometrial cells in primary culture, examine the mechanisms, and establish the identity of active substances in serum. In the course of these studies, we found that lysophospholipids could effectively substitute for FBS in elevating OTR expression. These agents, which act through G pro-

Abbreviations: CHO, Chinese hamster ovary; Edg, endothelial differentiation gene; FBS, fetal bovine serum; LPA, lysophosphatidic acid; OT, oxytocin; OTA, oxytocin antagonist; OTR, oxytocin receptor; PI3K, phosphatidylinositol 3-kinase; PL, phospholipase; RPA, ribonuclease protection assay; S1P, sphingosine 1-phosphate.
tein-coupled receptors of the Edg (endothelial differentiation gene) family, are important in diverse biological processes, including cell proliferation, survival, cytoskeletal changes, migration, wound healing, angiogenesis, tumor invasion, and embryonic development (23). Edg 1, 3, and 5 are high-affinity sphingosine 1-phosphate (SIP) receptors, whereas Edg 2, 4, and 7 are lysophosphatic acid (LPA, 1-a-lysophosphatic acid, oleoyl) receptors (23). Our findings showed that both FBS and lysophospholipids (SIP and LPA) up-regulate OTR expression in human myometrial cells by stabilizing OTR mRNA concentrations rather than affecting transcriptional activity.

Materials and Methods

Reagents

Oxytocin and oxytocin antagonist [d(CH)2]Tyr(Me)2-Thr4-Tyr-NH2/Jornithine vasotocin were obtained from Peninsula Laboratories, Inc. (Belmont, CA). Pertussis toxin, genistein, wortmannin, arachidonylfluoromethyl ketone, aristolochic acid (8-methoxy-6-nitrophenanthro[3,4-d]-dioxole-5-carboxylic acid), and cytidine 5'-diphosphocholine were purchased from BIOMOL Research Laboratories, Inc. (Plymouth Meeting, PA). FBS was procured from Atlanta Biological (Atlanta, GA); MEM and cell culture reagents came from Life Technologies, Inc. (Grand Island, NY). Forskolin, cortisol, actinomycin D, uridine, 4-thiouridine, collagenase, and other chemicals were obtained from Sigma (St. Louis, MO); and the [5,6-3H]uridine came from NEN Life Science Products (Boston, MA).

Myometrial cell culture

The University of Texas Medical Branch Committee on Research Involving Human Subjects approved the use of human tissue. Myometrial samples were taken from women by cesarean section near term, not in labor, and cells were prepared as described previously and maintained in MEM containing 10% (vol/vol) FBS, 1 mm sodium pyruvate, 2 mm L-glutamine, penicillin G (100 IU/ml), streptomycin sulfate (100 μg/ml), and Amphotericin B (15 μg/ml) at 37 C (95% humidity) in the presence of 5% CO2 (24). The cells, which appeared to be homogenous morphologically, were used at confluence and were serum starved overnight (about 16 h) before treatment with FBS or other agents. Cells from each patient were examined separately between passage 2 and 10.

125I-OXA-binding assay

The concentration of OT-binding sites on intact cells was measured by using an iodinated OT antagonist (OTA), as described previously (5). Myometrial cells were incubated with a near-saturating concentration of [125I]OTA, and specific binding was determined by subtracting nonspecific binding (1000-fold excess of cold OT) from total binding.

RNA extraction

Total RNA from myometrial cells was isolated using the method of Chomczynski and Sacchi (25).

Ribonuclease protection assay (RPA)

Primers based on a portion of the human OTR cDNA sequence were used to amplify a 390-bp fragment that was reverse transcribed from human myometrial mRNA (22). The amplified DNA was cloned using pCRII (Invitrogen, Carlsbad, CA). The resulting plasmid was linearized at an internal site with HindIII, and the RNA polymerase was used to transcribe the probe from the linearized template, using a MAXscript kit (Ambion, Inc., Austin, TX). The probe, which is comprised of almost 400 bases of OTR and about 100 bases of vector sequence, was labeled with [32P]CTP (800 Ci/mmol), and purified by denaturing polyacrylamide gel electrophoresis (5% polyacrylamide, 8 m urea). Solution hybridization of the labeled RNA probe with 20 μg total RNA and subsequent RNase digestion were performed using an RPA II kit, according to the manufacturer’s instructions (Ambion, Inc.). Two protected fragments were isolated by denaturing electrophoresis (Fig. 1A). DNA markers were generated by Alul digestion of a pML plasmid and end labeled using γ32P-ATP and polynucleotide kinase after dephosphorylation with calf intestinal alkaline phosphatase. Gels were dried, quantified with a PhosphorImager (Molecular Dynamics, Inc., Sunnyvale, CA), and exposed to X-Omat AR films with intensifying screens (Kodak, Rochester, NY).

Riboprobes for cyclophilin mRNA and 18S RNA were generated from total template (Ambion, Inc.), and either one was used simultaneously with the OTR probe. The protected cyclophilin probe was used to normalize for variations in recovery of OTR-protected fragments after the various procedural steps. Completeness of digestion with RNase was verified by using yeast RNA instead of myometrial cell RNA. The results are expressed as the ratio of OTR mRNA to cyclophilin mRNA. To establish specificity of the human OTR probe, it was hybridized to RNA from several cell lines from different species that express OTRs (see Fig. 1A). These included Rin5 (rat insulinoma cells) (26), Chinese hamster ovary (CHO) cells transfected with the rat OTR (26), rabbit amnion cells (5), nonpregnant mouse myometrium, and LLC-PK1 (pig kidney epithelial) cells (27). Control cell lines not expressing OTRs included CHO and human liver (HepG2) cells.

Nuclear run-on assay

Cultured myometrial cells were rinsed twice in ice-cold PBS, disassociated from culture plates by scraping with a rubber policeman, and collected by centrifugation. The cells were resuspended in 4 ml ice-cold sucrose buffer (1.032 m sucrose, 3 mm CaCl2, 2 mm MgOAc, 0.1 m EDTA, 1 mm dithiothreitol, 0.5% Nonidet P-40, and 10 mm Tris-Cl, pH 8.0), and homogenized with 15 strokes of a Dounce homogenizer. Nuclei were isolated by sucrose gradient centrifugation according to the method of Greenberg and Bender (28). DNA plasmids for hybridization included the empty vector pGL2, pGL2 with 4.2 kb of 5′-flanking sequence from human OTR, full-length human 8-oxo cDNA as an internal control for the treatments, and full-length c-fos cDNA as positive control for the effects of the serum. The DNA samples were linearized with the appropriate restriction endonucleases, alkali denatured, and filtered through nitrocellulose membranes using a slot-blot apparatus (5 μg DNA per slot). Run-on transcription and RNA hybridization were carried out as described previously (28). Background labeling of the filters was reduced by treatment with DNase-inactivated RNase A (10 μg/ml) in 2× saline sodium citrate for 30 min at 37 C.

4-Thiouridine labeling and isolation of thiolated RNA

Transcription analysis by thiouridine labeling was carried out as described previously (29). Briefly, myometrial cells were incubated with either 100 μM 4-thiouridine or uridine and [5,6-3H]uridine (0.5 μCi/ml) for 1 h. Total RNA was extracted as described above, and the amount of tritium in each sample was determined by liquid scintillation counting. Equal amounts of tritiated samples were adsorbed to slurry of organonemural agarose (Affi-Gel 501, Bio-Rad Laboratories, Inc., Richmond, CA) for 2 h at 4 C. The gels were packed individually into sterile tuberculin syringes and rinsed, and then thiolated RNA was eluted and concentrated by ethanol precipitation. The amount of newly transcribed OTR in the eluted fraction was determined by RPA. A sample of RNA not subjected to affinity chromatography was used to measure steady-state OTR mRNA levels by RPA.

Determination of Edg mRNAs in human term myometrial cells by RT-PCR

First-strand cDNA was synthesized using the first-strand cDNA synthesis kit (Promega Corp., Madison, WI), and PCR was carried out using specific primer sets for each Edg mRNA and the GeneAmp RNA PCR kit (Perkin-Elmer, Foster City, CA). The primer pairs used were: Edg 1: 5′-ATTTCCGCCCCTTCTCTGCTACTA-3′ and 5′-ATAAATGTCCTGGGT TGCC-3′; Edg 2: 5′-AACCACATACTTACTCTTACTA-3′ and 5′-GGCT CCAAATCTCTATCTTCAG-3′; Edg 3: 5′-AACTGGTCTGTCGACC TCGT-G- and 5′-TTCACCCGACGTTTACCTCAC-3′; Edg 4: 5′-CTTCTAC ACCGGCCATCTCA-3′ and 5′-CTTCAAGCTTACCACCCATCCAC-3′; Edg 5: 5′-TGGGAAAACGCGAGACCAC-3′ and 5′-TGGACCGGA-
PCR was performed for 35 cycles of 30 sec at 95°C, 1 min at 58°C, and 1 min at 72°C. The amplified DNAs were cloned using the TOPO TA pCRII cloning kit (Invitrogen). Dideoxynucleotide reactions were performed using a cycle-sequencing protocol and AmpliTaq DNA polymerase (Perkin-Elmer), and DNA sequence analysis was performed using PE Applied Biosystems model 373 A analyzer (Perkin-Elmer).

**Statistical methods**

Assays were carried out in triplicate, and the results are expressed as the mean ± se. Cells from at least two different patients, usually three, were used. A t test was used to compare treated groups with control groups. All tests were made at the 0.05 level of statistical significance.

**Results**

**Treatment of serum-starved human myometrial cells with FBS up-regulates OTR ligand-binding site and mRNA concentrations**

Treatment of serum-starved human myometrial cells with 5% FBS for 8 h caused about an average 5-fold increase in steady-state OTR mRNA concentrations in cells from three patients (Fig. 1B). The maximal increase in OTR mRNA occurred with about 2.5% FBS, and significant differences were seen with as little as 0.1% FBS (Fig. 1B). In contrast, 5% FBS had no effect on the mRNA levels of either of the G protein-coupled receptors Edg1 or Edg2 after 8 h of treatment (data not shown). These findings show that the effects of FBS on OTR mRNA are specific.

Deprivation of human myometrial cells in primary culture of FBS for 24 h resulted in a major reduction in 125I-labeled OTA-binding sites (data not shown). Replenishment of FBS for 24 h restored antagonist binding (Fig. 2). The increase in labeled antagonist binding was about 10-fold in low-passage cells, passages 2 to 4 (P-2 to P-4), from three separate patients (Fig. 2). Maximal effects occurred with about 5% FBS (Fig. 2). Vasopressin V1a receptor transcripts, which are present in myometrial tissue samples (30), were not detectable in the myometrial cells using RT-PCR (data not shown). Thus, the potential binding of [125I]OTA to V1a receptors can be ignored.

**Effects of FBS on transcription of OTR mRNA**

Although OTR mRNA levels were significantly up-regulated by FBS treatment, transcriptional activity of the OTR gene was essentially unchanged following the addition of FBS, as measured by nuclear run-on assays (Fig. 3). Transcriptional activity of the human β-actin gene was very low because hybridization of nascent transcripts to the β-actin cDNA probe was not greater than that to control plasmid DNA (pGL2, Fig. 3). FBS treatment caused a marked induction of c-fos expression by 15 min, as previously established (31), thereby validating the method (Fig. 3). In addition to nuclear run-on assays, transcription rates were determined in cells that were pulse labeled with 4-thiouridine (100 μM) for 1 h at different time points after addition of FBS. Newly synthesized thiolated RNA was isolated by affinity chromatography and quantified by RPA. In this set of experiments, addition of FBS resulted in about a 3-fold increase in steady-state OTR mRNA levels after 3 h and about a 4-fold increase at 6 h (Fig. 4). However, there were no changes in thiolated OTR transcripts at either time point (Fig. 4). These results indicated that the increase in steady-state OTR mRNA levels after FBS treatment does not occur as the result of increased...
transcriptional activity of the OTR gene but instead must be due entirely to increased OTR mRNA stability.

Serum starvation of cultured human myometrial cells markedly reduces the half-life of OTR mRNA

Myometrial cells maintained in 5% FBS were treated with actinomycin D (1 μg/ml) at t = 0 to inhibit transcription of the OTR gene, and incubation was continued in either the presence or absence of 5% FBS. OTR mRNA levels were quantified by RPA, and the data were normalized to the concentration of cyclophilin mRNA in each sample. The half-life of OTR mRNA in cells maintained in 5% FBS was about 60 h (Fig. 5). In the absence of FBS, the half-life of OTR mRNA was reduced to about 12 h (Fig. 5).

Factors in FBS causing up-regulation of OTRs

FBS contains numerous growth factors and activators that might potentially up-regulate OTR concentrations. Work in the rat showed that 17β-estradiol up-regulated OTR concentrations in vitro when added to uterine explants, and the effects of estrogen were antagonized by progesterone (32). However, neither 17β-estradiol (1 nm) nor progesterone (100 nm), alone or in combination, had any effect on OTR ligand-binding sites in human myometrial cells after 24 h treatment (data not shown). Cortisol and forskolin both elevate OTR expression by rabbit amnion cells in primary culture (33). Again, neither agent (100 nm cortisol or 25 μM forskolin) was effective in up-regulating OTR ligand-binding sites in human myometrial cells after 24 h of treatment (data not shown). Using a series of pathway inhibitors, we found that treating the cells with the cytosolic phospholipase (PL)A2 inhibitor, arachidonyl trifluoromethyl ketone (20 μM), completely blocked the effects of 5% FBS (data not shown). Other PLA2 blockers such as aristolochic acid (100 μM) and cytidine 5'-diphosphocholine (100 μM), which are selective for ionophore-stimulated PLA2 and brain PLA2, respectively, had no effect.

Because lysophospholipids are formed through the actions of cellular PLA2 and exert complex effects on target cells through actions on cognate G protein-coupled receptors, we examined their effects on regulating OTR expression. Addition of increasing concentrations of LPA and S1P increased OTR mRNA concentrations after 8 h of treatment (Fig. 6). LPA and S1P were about equipotent. At the highest concentrations of LPA and S1P examined, 100 μM (which is in the high end of the concentration range found in the blood), OTR mRNA expression was increased to a level almost comparable with that achieved with 10% FBS stimulation (Fig. 1B).

Lack of effect of LPA and S1P on OTR transcription

As with FBS stimulation (Fig. 4), treatment of myometrial cells with LPA or S1P (10 μM) increased steady-state levels of OTR mRNA but had no effect on transcriptional activity of the gene, as measured by the thiolated RNA method (Fig. 7).

Presence of lysophospholipid receptors in human myometrial cells

There are eight known lysophospholipid receptors (Edg), all of which are coupled to G proteins. The expression of Edg family members by myometrial cells was determined by RT-PCR. Specific primers for seven of the eight Edg family members were designed based on the DNA sequences in the GenBank database and the Oligo Version 4.0 software (National Biosciences, Plymouth, MN) to minimize secondary structure formation and homodimerization. We also performed RT-PCR on RNA from a human bladder cell line (T24). Amplicons corresponding in size to Edgs 1, 2, 3, and 5 were obtained using myometrial cell RNA (Fig. 8A), and Edgs 1, 2, 3, 4, and 5 amplification products were found in T24 cells (Fig. 8B). A major 750-bp amplicon was obtained with the Edg 8 primers and myometrial RNA, but it was not the correct size. No amplification was seen with primers for Edg 7 in either type of cell. We cloned Edgs 1, 2, 3, 4, and 5 cDNAs in pCRRII (Invitrogen) and confirmed their identities by DNA sequencing.
Preliminary analysis of signal pathways involved in FBS/LPA/S1P-mediated OTR mRNA stability

Edg 2, or the more recently designated LPA1, is a multifunctional receptor that is coupled to Gi/o, Gq/11, and G12/13 proteins (23). Accordingly, multiple signaling pathways are activated by LPA, including inhibition of adenylyl cyclase, stimulation of PLC and PLD, increased inositol phosphate and intracellular calcium concentrations; increased protein kinase C activity; arachidonic acid release; activation of phosphatidylinositol 3-kinase (PI3K) and MAPK; and Rho-dependent phosphorylation of cytoskeletal proteins (23). To determine whether the effects of the lysophospholipids on OTR mRNA expression were mediated by Gi/o, myometrial cells were concomitantly serum starved and pretreated with pertussis toxin (100 ng/ml) overnight before treatment with the lysophospholipids. In the absence of pertussis toxin, both

Fig. 4. Relative concentration of thiolated OTR transcripts in human myometrial cells after 5% FBS treatment. Cells were pulse labeled with 4-thiouridine for 1 h at 1, 3, or 6 h after addition of FBS, and the thiolated transcripts were isolated by affinity chromatography. The concentrations of transcript and steady-state (total) OTR mRNA in the same samples were assessed by ribonuclease protection assay. *, P < 0.05, compared with untreated controls (B, basal).

Fig. 5. Reduction in OTR mRNA stability following removal of FBS (5%). Cells were treated with actinomycin D (1 μg/ml) at t = 0 to inhibit transcription, and OTR mRNA decay was assessed by ribonuclease protection assays. Each point is the mean ± SEM of at least three determinations. One-phase exponential decay plots were calculated using GraphPad software (GraphPad Software, Inc., San Diego, CA). The goodness-of-fit R² values for +FBS and −FBS were 0.77 and 0.96, respectively. OTR mRNA half-life was estimated to be 60 h in myometrial cells maintained in 5% FBS and 12 h in the absence of FBS.

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Fig. 6. Dose-dependent effects of LPA and S1P on OTR mRNA steady-state concentrations. Each point is the mean of three determinations ± SEM. *, P < 0.05, compared with untreated controls (basal).

Fig. 7. Effects of LPA or S1P (10 μM) on transcription of the OTR gene, as measured by pulse labeling with 4-thiouridine. *, P < 0.05, compared with untreated controls (B, basal).
LPA and S1P (10 μM) treatments for 8 h increased the concentration of OTR mRNA relative to that of cyclophilin mRNA, as determined by ribonuclease protection assays (Fig. 9). Pretreatment with pertussis toxin almost totally blocked the effects of both lysophospholipids on OTR mRNA levels. By comparison, pretreatment of the cells with the general tyrosine kinase inhibitor, genistein (25 μM), was ineffective in reducing OTR mRNA expression (Fig. 9). Pretreatment with pertussis toxin also obliterated the effects of FBS on OTR mRNA levels (Fig. 9). These findings indicated that the active components of FBS work through Gi/o stimulation, which is consistent with the activators in FBS being lysophospholipids.

Edg-mediated pathways result in a decrease in intracellular cAMP concentration and an increase in PI3K activity. As further evidence that lysophospholipids mediate the effects of FBS on myometrial cell OTR expression, the reversal of these signals resulted in a marked reduction in the half-life of OTR mRNA. Thus, treatment of myometrial cells with forskolin (25 μM) to elevate intracellular cAMP concentrations or wortmannin (10 μM) to inhibit PI3K activity resulted in half-lives of OTR mRNA of approximately 8 and 11 h, respectively, compared with about 60 h for untreated cells (Fig. 10).

Discussion

Cloning of the human OTR gene was reported in 1994 (34), but little progress has been made in subsequent years toward understanding regulation of OTR gene expression in the human myometrium. It has been assumed that the marked up-regulation occurring during pregnancy is the result of increased transcriptional activity. However, there have been no functional transcriptional studies carried out on the human OTR gene in the human myometrium to date. No doubt part of the problem has been the extremely poor efficiency of DNA transfer using human myometrial cells in primary culture (Jeng, Y.-J., S. L. Soloff, and M. S. Soloff, unpublished observations). The present studies are actually the first to examine OTR transcriptional activity in human myometrium. The findings show that expression of the OTR gene in cultured human myometrial cells from pregnant patients near term is constitutive, with regulation occurring at the level of mRNA stability. Our previous work (35) using transfected human OTR promoter-driven reporter constructs in a human breast tumor cell line showed that both FBS-induced
and basal transcriptional activity require a region between bases –85 and –65 in the 5′-flanking sequence. These findings are consistent with the results obtained from functional assays of constitutively expressed housekeeping genes. In contrast, OTR gene expression in rabbit amnion cells is transcriptionally activated by cortisol and forskolin treatments (29). Thus, the expression of the OTR gene in human myometrium appears to be regulated differently from that in rabbit amnion.

Several different human cell types have been shown to express functional OTRs in culture and respond to OT treatment with a rise in intracellular Ca2+ concentrations. These cell types include breast tumor cells (21), osteoblasts and osteosarcoma cells (30), an established endometrial cell line (36), immortalized ovarian granulosa cells (22), bladder epithelial cells (T24 cells, which were originally thought to be human umbilical vein cells, ECV304) (37), and myometrial cells (19, 38). It is noteworthy that OTRs are up-regulated by FBS in virtually all of these human cell types, but myometrial cells in primary culture from rats and rabbits do not express OTRs after exposure to FBS (Jeng, Y.-J., and S. L. Soloff, unpublished observations). Human myometrial cells in primary culture, prepared from nonpregnant patients having relatively low myometrial OTR concentrations, acquire OTRs in culture (39–41). In view of the results of the present studies, it is possible that this up-regulation is due to the presence of FBS in the culture medium. Thus, OTR expression can be up-regulated in a number of human cell types in culture by FBS, and this up-regulation may be unique to human cells.

The effects of FBS on myometrial OTR mRNA expression could be mimicked by lysophospholipids in micromolar concentrations, consistent with circulating levels of LPA (23). The actions of FBS, LPA, and S1P occurred at the OTR mRNA stability level because the addition of either FBS, LPA, or S1P to serum-starved myometrial cells did not increase nascent OTR transcripts but caused an increase in steady-state levels of OTR mRNA. LPA and S1P are produced during activation of most cell types by enzymatic degradation of phospholipids (23). LPA synthesis is initiated by the release of membranous microvesicles enriched in phosphatidic acid formed by sequential actions of inositol lipid-specific phospholipase C and diacylglycerol kinase or by phosphatidyl choline-specific phospholipase D. PLA2 catalyzes the final step in this LPA synthesis pathway. LPA occurs naturally as an albumin-bound serum factor generated during blood clotting from bound serum factor generated during blood clotting from activated platelets (23).

The presence of Edgs 1, 2, 3, and 5 in human myometrial cells is consistent with the effectiveness of both LPA (Edg 2) and S1P (Edg 1, 3, 5) on OTR mRNA levels. Nilsson et al. (42) showed that LPA induced significant increases in [3H]thymidine incorporation into quiescent human myometrial cells and the proliferative effects could be blocked by pertussis toxin, consistent with numerous observations that LPA receptors are coupled to Gαi/o proteins. LPA also induced a rapid rise in cytosolic free Ca2+ concentration. A number of studies have shown that PI3K mediates the effects of LPA, but this signaling pathway is not universal (23). Our findings showing that pertussis toxin blocked FBS, LPA, and S1P stimulation of OTR mRNA steady-state levels and forskolin and wortmannin reduced OTR mRNA half-life are consistent with the known Edg-mediated signal pathways.

It is not clear at the present time whether the same mechanisms of OTR regulation seen in these studies occur in vivo. Anecdotal evidence suggesting that OTR mRNA stability may increase during pregnancy is based on the observation that the rise in concentration is gradual rather that abrupt, as seen in rats and rabbits. Agents such as corticotrophin-releasing hormone and β-adrenergic mimetics, which have been shown to stimulate uterine quiescence (43, 44), stimulate the production of intracellular cAMP. These agents might act in part via cAMP-stimulated reduction in myometrial OTR mRNA stability. Although there were clear differences in OTR mRNA half-lives between the forskolin- or wortmannin-treated cells and cells in FBS alone, the absolute half-life values should be interpreted with caution because they involve the use of actinomycin D, which can result in extended half-life estimates.

It is not presently possible to directly determine OTR mRNA stability in myometrial tissue from patients. However, as more is learned about factors involved in increasing OTR mRNA stability, it may be possible to examine changes in the concentration or activity of these agents in the human myometrium during pregnancy to determine the importance of this process in up-regulating OTRs in vivo.

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