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Oxytocin Receptor Regulation and Action in a Human Granulosa-Lutein Cell Line[1]

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ABSTRACT

Although oxytocin and its receptor have been identified in human ovary, its regulatory role in granulosa cell or corpus luteum function has not been clearly defined. To better understand oxytocin action in the human ovary, we have characterized the expression and function of oxytocin receptors in an immortalized human granulosa-lutein cell line, HGL5. Expression of oxytocin receptor mRNA was demonstrated by reverse transcriptase-polymerase chain reaction analysis, and by specific binding of an iodinated oxytocin antagonist (apparent dissociation constant of 131 ± 0.15 pM, and a B_{max} of 12 ± 0.5 fmol/μg DNA). Receptor levels were down-regulated by serum starvation, and rapidly up-regulated by serum restoration. Stimulation of protein kinase C activity increased oxytocin receptor levels in a concentration-dependent manner. Conversely, protein kinase C inhibition blocked up-regulation of oxytocin receptors. Treatment of cells with 10 nM oxytocin resulted in a rapid, transient increase in intracellular Ca^{2+}, and the response was blocked by an oxytocin antagonist. Because HGL5 cells secrete progesterone and estradiol in response to agents that elevate intracellular cAMP concentrations, we studied the effect of oxytocin on steroid production. Oxytocin enhanced the effects of forskolin on progesterone production. These results suggest that oxytocin augments the activity of luteotropins in vivo. Our studies are the first to show an ovarian cell line that expresses functional oxytocin receptors. These cells can serve as a useful model for studying oxytocin signal pathways and their cross-talk with respect to progesterone synthesis. These cells also will be useful in the analysis of mechanisms of oxytocin receptor regulation, including regulation of its gene.

calcium, corpus luteum, corpus luteum function, granulosa cells, mechanisms of hormone action, oxytocin, progesterone, signal transduction

INTRODUCTION

Oxytocin (OT) and oxytocin receptors (OTRs) are both expressed in human and subhuman primate ovaries [1–6], and it has been suggested that the local production of OT is important in regulating ovarian function [7, 8]. More specifically, OT may be a paracrine mediator of luteinization in primate ovary [5, 6]. Mayerhofer et al. [9], using primary cultures of human granulosa-lutein cells, found that OT augmented the effects of hCG on progesterone production in cells that were cultured for 5 days, but not 2 days. In view of our observations that fetal bovine serum (FBS) up-regulates OTR concentrations in cultured human breast tumor cells [10], it seems possible that the human granulosa cells acquired OTRs after 5 days in culture media containing FBS, but not by 2 days. Mayerhofer et al. [9] also found that OT elicited a rapid rise in intracellular Ca^{2+}.

To better understand the role of OT in the ovary, we examined the HGL5 human granulosa-lutein cell line developed by Rainey et al. [11]. These cells are insensitive to gonadotropins, but synthesize estradiol and progesterone in response to forskolin. Forskolin acts downstream of gonadotropin receptors by binding to the catalytic subunit of adenylyl cyclase and elevating intracellular cAMP concentrations. The increase in intracellular cAMP levels directly governs the activity of protein kinase A (PKA), which phosphorylates transcriptional factors (e.g., CREB) that induce the expression of steroidogenic enzymes such as steroidogenic acute regulatory protein (StAR), P450 side-chain cleavage enzyme, and 3β-hydroxysteroid dehydrogenase. These enzymes are the rate-limiting steps in progesterone synthesis [12–15]. The purposes of our studies were to determine whether HGL5 cells express functional OTRs, whether these receptors are regulated, and whether HGL5 cells may be autoregulated by the production of OT. More importantly, we sought to determine whether HGL5 cells respond to OT in the same manner as granulosa-lutein cells in primary culture so that the cell line can be used in future studies to elucidate the functions of OTRs in the human ovary.

MATERIALS AND METHODS

Materials

OT and OT antagonist (OTA), [d(CH_2)$_5$,Tyr(Me)^2,Thr^4,Tyr-NH_2^{9}]OVT were obtained from Peninsula Laboratories (Belmont, CA); 9 *cis*-retinoic acid, all-*trans*-retinoic acid, and forskolin were obtained from Sigma Chemical Company (St. Louis, MO); GF 109203X, PD-98059, U73122, and phorbol 12-myristate 13-acetate (PMA) were purchased from BIOMOL (Plymouth Meeting, PA); and FBS was purchased from Atlanta Biological (Atlanta, GA).

Cell Culture

Immortalized HGL5 cells of human luteinized granulosa cell origin [11], kindly donated by Dr. William Rainey, were cultured in F12/Dulbeccos modified Eagle medium (DMEM) supplemented with 2% NuSerum (Becton Dickinson, Fisher Scientific Co., Pittsburgh, PA), insulin-transferrin-selenium and penicillin/streptomycin (Gibco BRL Life Technologies, Rockville, MD) at 37°C in an atmosphere of 5% CO_2. To down-regulate OTR levels, NuSerum was replaced with 0.5% BSA for up to 48 h. The cells remained viable as verified by trypan blue staining of cells. To up-regulate OTR levels, serum-starved cells were placed in media containing either FBS or PMA at the concentrations indicated in the text.

Ligand Binding Assay for OTR

OTA was iodinated as described previously [16]. To determine B_{max} and dissociation constant (K_d), specific binding experiments were carried

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out with at least nine different concentrations of [^{125}I]OTA [17], and were repeated three times. The concentration range of [^{125}I]OTA was 1 to 500 nM. Whole cell assays were performed in 12-well culture plates. The cells were rinsed twice, and then incubated in 1 ml of PBS with a saturating concentration of [^{125}I]OTA (200 nM) at room temperature for 1 h. Nonspecific binding was determined by adding 1 μM unlabeled OTA along with [^{125}I]OTA. Cells were then rinsed three times with ice-cold PBS, suspended in 0.5 ml of 1 N NaOH, and the amount of radioactivity was determined in a gamma counter. DNA levels were determined by adding 1 ml of PBS containing 20 mM EDTA to companion wells, followed by freeze/thawing and fluorescence measurement using Hoechst 33258 dye [18]. The results are expressed as cpm bound specifically/μg DNA.

Determination of OTR, V_{1a} Vasopressin Receptor, and OT mRNA in Human Term Myometrium and HGL5 Cells by Reverse Transcriptase-Polymerase Chain Reaction

Total RNA was isolated [19], and reverse transcriptase-polymerase chain reaction (RT-PCR) was performed using the Perkin-Elmer GeneAmp RNA PCR kit and RNA PCR Core kit (Perkin-Elmer, Norwalk, CT). One microgram of total RNA was used for first-strand cDNA synthesis. The only modification of the manufacturer's recommended protocol was the use of 0.15 μM of antisense primer from each primer set for cDNA synthesis. For the PCR reaction, additional antisense primer was not added. Amplification products were analyzed by electrophoresis in 5% nondenaturing polyacrylamide (Fig. 1) and 2% agarose (Fig. 4B) gels, and the bands were visualized by ethidium bromide (1 μg/ml) staining. *Hae*III digested ϑX 174 RF DNA (New England Biolabs, Beverly, MA) was included for size estimation of the amplified DNA.

Primers used for RT-PCR of OTR and V_{1a} vasopressin receptors have been previously reported [10]. Multiplex analysis was carried out with primers encoding β-polymerase, which was used as a reaction control. The sequence of the sense primer for β-polymerase was 5′-AGT CCT GGT ACC TCC TTC AAG CTG-3′; and the antisense was 5′-GGG TAT TTT GCT ATA ACA GAT GCT GCT TTT-3′. These primers were designed to anneal to sites separated by one or more introns to prevent priming from genomic DNA. Correct amplification of primer pairs for the OTR, V_{1a}R, and β-polymerase resulted in products of 391, 534, and 266/208 base pairs (bp), respectively.

The sequences of the sense primer for OT was 5′-TGC TAC ATC CAG AAC TGC CC-3′, and the antisense primer was 5′-CGG AGC CAT CAA GTT TCA G-3′. These sequences correspond to bases 94–113 (sense) and 411–429 (antisense) of the OT gene (GenBank accession number NM000915). The expected size of the amplicon was 326 bp.

In experiments in which the effects of PMA on OTR expression were studied, RT-PCR was carried out using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal control. The sequences of the sense primer for GAPDH was 5′-CCA TGT TCG TCA TGG GTG TG-3′, and the antisense primer was 5′-TGC CCA CAG CCT TGG CAG CG-3′, corresponding to bases 461–480 (sense) and 711–730 (antisense) of the GAPDH gene (GenBank accession number NM002046). Correct amplification from primer pairs for GAPDH resulted in a product of 270 bp. The identities of OTR and OT PCR products were verified by cloning the amplified DNA fragments into *pCR*II (Invitrogen, Carlsbad, CA) followed by DNA sequence analysis. Each primer set was designed to cross at least one intron, to rule out priming from genomic DNA. Any contribution from genomic DNA was further eliminated by observing the lack of the appropriate PCR product in the absence of reverse transcription. The number of cycles used for amplification of PCR products was in the exponential range to ensure that the results were semiquantitative.

Measurement of Intracellular Ca^{2+}

Real-time recording of Ca^{2+} was performed in single cells, using methods that we have described previously [17]. In brief, cells grown on glass coverslips (Carolina Biological, Burlington, NC) for 48 h in 10% FBS, DMEM, and antibiotics were washed with a physiological medium (KRH) containing $NaCl_2$ 125 mmol/L, KCl 5 mmol/L, KH_2PO_4 1.2 mmol/L, $MgSO_4$ 1.2 mmol/L, $CaCl_2$ 2 mmol/L, glucose 6 mmol/L, and Hepes-NaOH buffer 25 mmol/L pH 7.4, then loaded with 2 μM fura-2 AM (Molecular Probes, Eugene, OR) for 50 min at 25°C to minimize dye compartmentalization. Loaded cells were washed 3 times with KRH and incubated for 60 min at 25°C in the dark with KRH 0.1% BSA. Loaded cells attached to coverslips were mounted on a Leiden Cover Slip Dish and placed in an Open Perfusion Micro-Incubator (Medical Systems Corp., New York, NY) covered with 3 ml KRH 0.1% BSA. Test peptides were dissolved in KRH 0.1% BSA and a volume of 3 ml was delivered to the Leiden dish after removal of KRH 0.1% BSA via vacuum pump. Cells were imaged using a Nikon Diaphot inverted microscope (Nikon, Garden City, NY), equipped with a Nikon phase contrast, oil immersion objective, and coupled to a dual monochrometer system via a fiber optic cable (Photon Technology International, South Brunswick, NJ). Fluorescence was detected using an intensified CCD camera (Dage-MTI, Inc., Michigan City, IN) and images were processed using ImageMaster software (Photon Technology). Molar concentration of Ca^{2+} was determined by the method of Grynkiewicz et al. [20], using 224 as the K_d for fura-2 and Ca^{2+}. Calcium saturation and zero Ca^{2+} were achieved by adding 0.3% Triton X-100 and 2.5 mM EGTA, respectively.

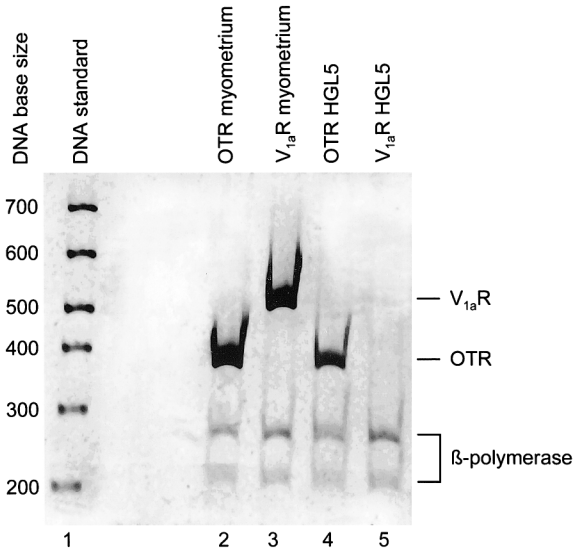

FIG. 1. RT-PCR analysis for the presence of OTR and V_{1a} vasopressin receptor mRNAs in HGL5 cells. The primer pairs and the tissue/cell source of RNA are indicated in each lane. Human β-polymerase primers were included in each RT-PCR reaction as a control. The expected sizes of the amplified DNA are as follows: hV_{1a}R, 534 bp; hOTR, 391 bp; and human β-polymerase, 266 and 208 bp. Human term myometrial RNA (1 μg) was used as the positive control for hOTR (lane 2) and hV_{1a}R (lane 3).

Progesterone Radioimmunoassay

A progesterone radioimmunoassay kit (Diagnostic Systems Laboratories, Inc., Webster, TX) was used according to the manufacturer's procedure to determine the amount of progesterone in the medium. The sensitivity of the assay was 0.1 ng/ml with an intraassay coefficient of variation of 1.7%, and an interassay coefficient of variation of 3.3%.

Prostaglandin Estrogen Measurements

PGE_2 levels in media were measured using an enzyme immunoassay system from Amersham Life Sciences (Arlington Heights, IL) as previously described [10]. The sensitivity of the assay was 2.5 pg/ml with intraassay coefficients of variation of 6.4% and the interassay coefficient of variation of 6.7%.

Statistics

All experiments were repeated in triplicate. ANOVA followed by the Newman-Keuls test were used to determine statistical differences between the means of treatment groups. Differences were considered to be significant at $P < 0.05$ level.

RESULTS

Demonstration of the Presence of OTR mRNA in HGL5 Cells by RT-PCR

RT-PCR was performed to initially determine whether OTR is expressed in HGL5 cells. Using amplimers specific for the human OTR and V_{1a} vasopressin receptors, we found that HGL5 cells expressed OTR mRNA (Fig. 1, lane

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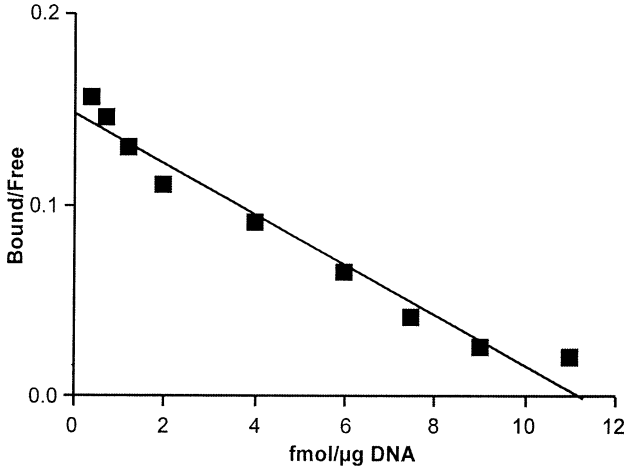

FIG. 2. Scatchard analysis of [125I]OTA binding by HGL5 cells. Cells were plated in 12-well plates in the presence of 30% FBS, DMEM, and antibiotics to maximize OTR levels. Whole cell binding assay and DNA content were performed as described in *Materials and Methods.*

4), but not the closely related V_{1a} vasopressin receptor (Fig. 1, lane 5) mRNA. In contrast, both OTR and V_{1a} vasopressin receptor mRNAs were expressed in human myometrium (Fig. 1, lanes 2 and 3). The identities of OTR and V_{1a} vasopressin receptor PCR products were verified by cloning the amplified DNA fragments into *pCR*II, followed by DNA sequence analysis (data not shown).

Scatchard Analysis of [125I]OTA Binding by HGL5 Cells

Binding studies were further carried out using [125I]OTA as the ligand. Scatchard analysis showed a single class of high-affinity binding site (K_d = 0.13 ± 0.015 nM) and B_{max} of 12 ± 0.5 fmol/μg DNA (Fig. 2). Using an estimate of 7 pg of DNA/cell, 12 fmol/μg DNA is equal to about 50 000 receptors per cell.

Up-Regulation of [125I]OTA Binding by HGL5 Cells with Increasing FBS Concentrations and with Phorbol Ester

Incubation of serum-starved cells with increasing amounts of FBS resulted in a concentration-dependent increase in the amount of [125I]OTA bound to whole cells (Fig. 3A). Maximal stimulation, about 15-fold, occurred with 30% and 40% FBS. Stimulation by 30% FBS was lost upon heating the FBS at 95°C for 10 min (Fig. 3B, heat treat, lane 2). Pretreatment of cells with the selective protein kinase C (PKC) inhibitor GF 109203X (10 μM) for 1 h blocked the ability of 10% FBS to up-regulate OTR levels. The phospholipase C inhibitor, U73122 (1 μM), also blocked FBS-stimulation of OTR up-regulation (Fig. 3B, lane 4), whereas pretreatment with the MEK inhibitor, PD-98059 (10 μM), increased OTR levels to those obtained

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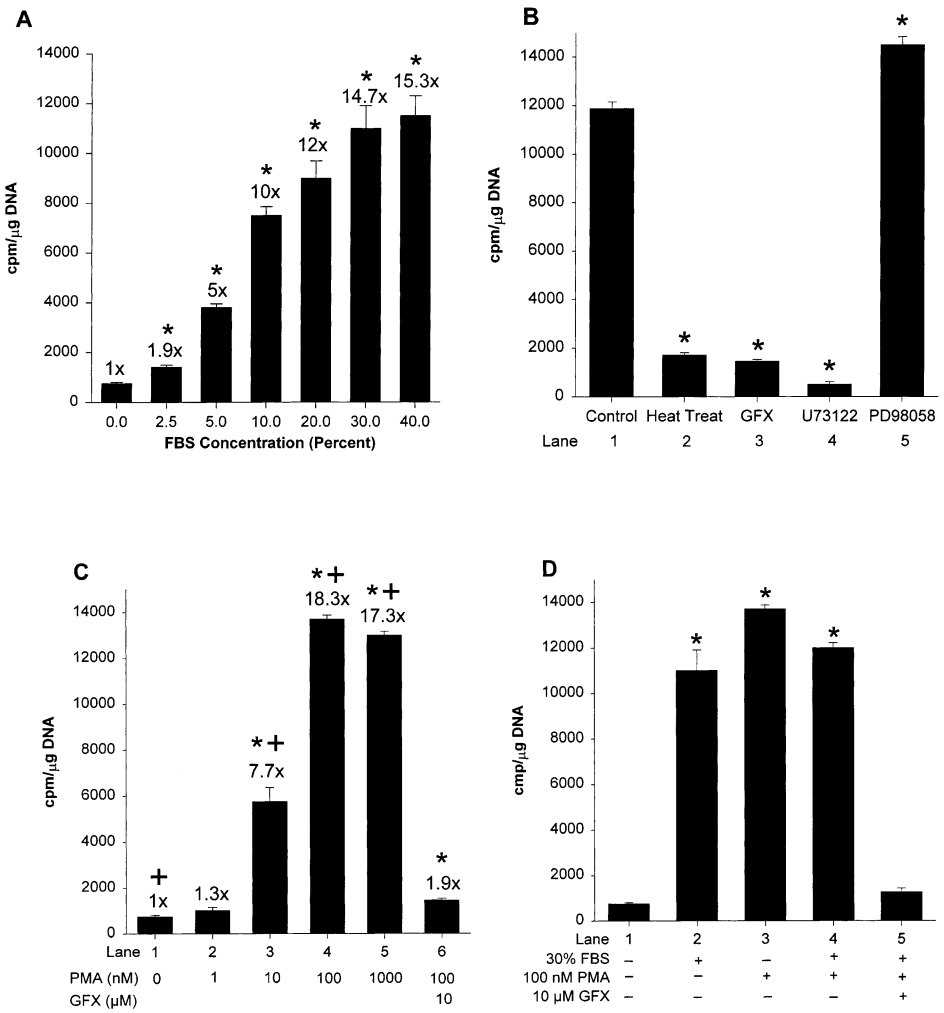

FIG. 3. Specific [125I]OTA binding by HGL5 cells. **A**) The effect of increasing FBS concentrations. Specific binding after addition of a fixed amount of [125I]OTA is expressed as cpm bound per μg DNA. The fold-stimulation in binding relative to serum-deprived cells (1×) is shown at the top of each bar. *, Different from lane 1 control ($P < 0.05$). **B**) Serum-deprived cells (48 h in 0.5% BSA, DMEM, and antibiotics) were incubated with 30% FBS (control) or heat-treated FBS (95°C for 15 min) for 24 h, or pretreated with either 10 μM GF 109203X, 1 μM U73122, or 20 μM PD-98059 for 1 h before treatment with 30% FBS. *, Different from lane 1 control ($P < 0.05$). **C**) Serum-deprived cells were incubated with 0–1000 nM PMA for 24 h. The fold-stimulation in binding relative to serum-deprived cells (1×) is shown at the top of each bar. *, Different from control ($P < 0.05$); +, different from lane 6, 100 nM PMA/10 μM GF109203X ($P < 0.05$). **D**) Serum-deprived cells were treated with 30% FBS, 100 nM PMA alone, or together for 24 h. Groups of cells were also pretreated for 1 h with 10 μM GF 109203X before FBS and PMA stimulation. Specific [125I]OTA binding was then determined. *, Different from lane 1 control ($P < 0.05$). Each value is the mean ± SEM of triplicate determinations for **A**–**D**.

with FBS treatment (Fig. 3B, lane 5). Treatment of cells with phorbol 12-myristate 13-acetate (PMA) for 24 h caused a concentration-dependent increase in OTA binding. A maximal increase of 18.3-fold was obtained with 100 nM PMA (Fig. 3C, lane 4). The phorbol ester, PMA, has been used extensively to study the involvement of PKC in cellular function. PMA binds directly to PKC and replaces the requirement for agonist-induced diacylglycerol (DAG) for activation. Both the classical and nonclassical PKC isoforms are activated by PMA (reviewed in [21]). Pretreatment of HGL5 cells with 10 μM GF 109203X blocked the PMA-induced (100 nM) rise in OTR levels (Fig. 3C, lane 6). Combined treatment of cells with maximally effective concentrations of FBS and PMA gave no further up-regulation of OTRs (Fig. 3D, lane 4). The effects of the combined treatment were blocked by GF 109203X, suggesting that PMA and FBS work through a common, PKC-mediated pathway (Fig. 3D, lane 5).

Because retinoids such as retinoic acid (*cis*-RA) and all-*trans*-retinoic acid antagonize PKC-mediated processes by the interaction of liganded retinoic acid receptors with PKC [22], and with the PKC-activated transcriptional factors, c-Jun/c-Fos [23–26], we investigated whether these compounds blocked FBS-induced and PMA-induced up-regulation of OTR levels. Cells were treated with 1 μM 9-*cis* retinoic acid either 1 h before or 4 h after addition of FBS or PMA. Pretreatment blocked both the FBS-stimulated and PMA-stimulated up-regulation of OTR ligand binding sites (Fig. 4A, lanes 3 and 6) and mRNA levels (Fig. 4B, lane 7). However, there was no effect of 9-*cis* retinoic acid when added 4 h after FBS or PMA (Fig. 4A, lanes 4 and 7; Fig. 4B, lane 8). All-*trans*-retinoic acid gave the same results as 9-*cis* retinoic acid (data not shown). Pretreating cells with actinomycin D (Fig. 4B, lane 6) blocked the effects of PMA (Fig. 4B, lane 4) on OTR mRNA up-regulation, suggesting that mRNA expression is transcriptionally regulated (Fig. 4B, lane 7).

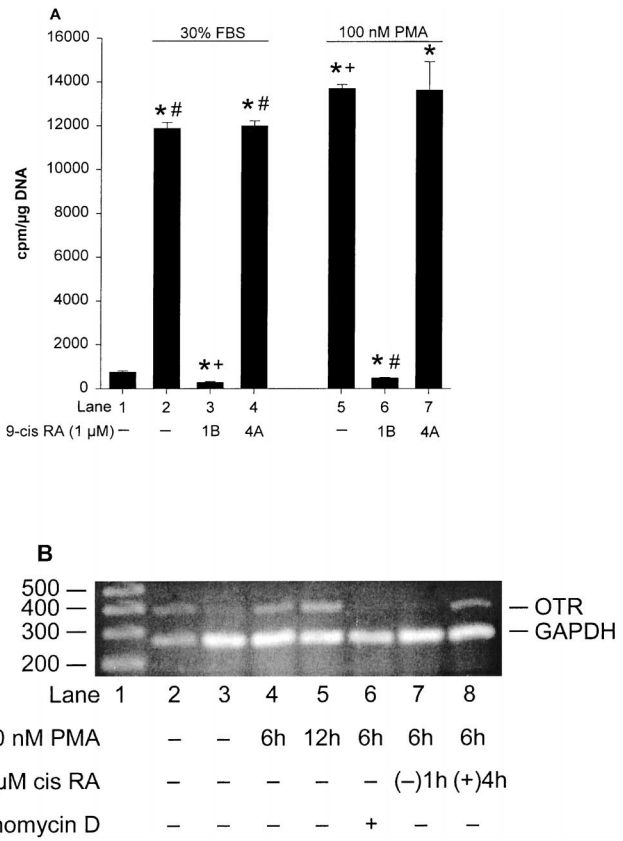

FIG. 4. Regulation of OTR protein and mRNA levels. **A**) Time-dependent inhibition of OTR up-regulation by retinoic acid in HGL5 cells. Cells were treated with 1 μM 9-*cis* retinoic acid 1 h before (1B, lanes 3 and 6) either 30% FBS or 100 nM PMA treatment, or 4 h after 30% FBS or 100 nM PMA treatment (4A, lanes 4 and 7). Specific [^{125}I]OTA binding was then determined. Each bar is the mean ± SEM of triplicates. *, Different from lane 1, untreated control ($P < 0.05$); +, different from lane 2, 30% FBS treatment ($P < 0.05$); #, different from lane 5, 100 nM PMA treatment ($P < 0.05$). **B**) RT-PCR analysis of OTR mRNA regulation by PMA and 9-*cis* retinoic acid in serum-starved HGL5 cells. GAPDH primers were included in each RT-PCR reaction as an internal loading control. The expected sizes of amplified DNA are 391 bp for hOTR, and 270 bp for GAPDH. Lane 1 depicts DNA standard markers of 200, 300, 400, and 500 bp. Description of treatment groups are as follows: lane 2, human term myometrial RNA as a positive control; lanes 3, 4, and 5, serum-starved HGL5 cells treated for 0, 6, and 12 h, respectively, with 100 nM PMA; lane 6, serum-starved HGL5 cells treated with 50 ng/ml actinomycin D 30 min before 6-h 100 nM PMA treatment; lanes 7 and 8, serum starved HGL5 cells treated with 1 μM 9-*cis* retinoic acid 1 h before (lane 7) and 4 h after (lane 8) the 6-h 100 nM PMA treatment. The data in **B** are representative of three experiments.

OT-Stimulated Intracellular Ca^{2+} Transients in Individual HGL5 Cells

OTR in HGL5 are functionally coupled to signal generation, as the addition of OT (10 nM) caused a rapid and transient increase in intracellular Ca^{2+} (Fig. 5A). Treatment of cells with 1 μM oxytocin antagonist had no effect on intracellular Ca^{2+}, but it blocked the effects of OT (Fig. 5B). The effects of the antagonist were specific for OT, as there was no effect on bombesin-stimulated Ca^{2+} transients (Fig. 5B), which are mediated by another G protein-coupled receptor.

Effect of OT on Progesterone Synthesis

Treatment of HGL5 cells with 100 nM OT for 48 h had no effect on the amount of progesterone secreted into the medium (Fig. 6, lane 2). However, addition of 10 μM forskolin caused a significant, 10-fold increase in progesterone production at the end of 48 h (Fig. 6, lane 3; $P < 0.05$). Cotreatment of cells with forskolin and OT (Fig. 6, lane 4) resulted in a significant doubling of the level of progesterone secreted, as compared to the effects of forskolin alone (Fig. 6; $P < 0.05$). All totaled, there was a 20-fold increase in progesterone production over basal levels.

DISCUSSION

Up-regulation of OTRs in vivo have been shown to be critical for the increased sensitivity of target sites to OT action [27, 28]. Previous studies from this laboratory and others have shown that OTR expression is regulated differently in different cell types. Whereas up-regulation of OTRs in a human breast tumor cell line occurs through activation of PKC and AP-1 transcriptional factors [10, 29], OTR levels in the rat uterus are up-regulated by estrogen [27, 30]; in rabbit amnion, cAMP and glucocorticoids are up-regulators [28]. Our present results demonstrate that HGL5 cells express OTRs that can be up-regulated in culture by heat-sensitive factors present in serum. The identity of these factors remains to be determined, but evidence for PKC mediation arises from the findings that the effects of serum can be mimicked by administration of the PKC activator PMA, and blocked by the PKC selective inhibitor, GF 109203X. This was confirmed by blocking serum-induced OTR up-regulation by inhibition of phospholipase C

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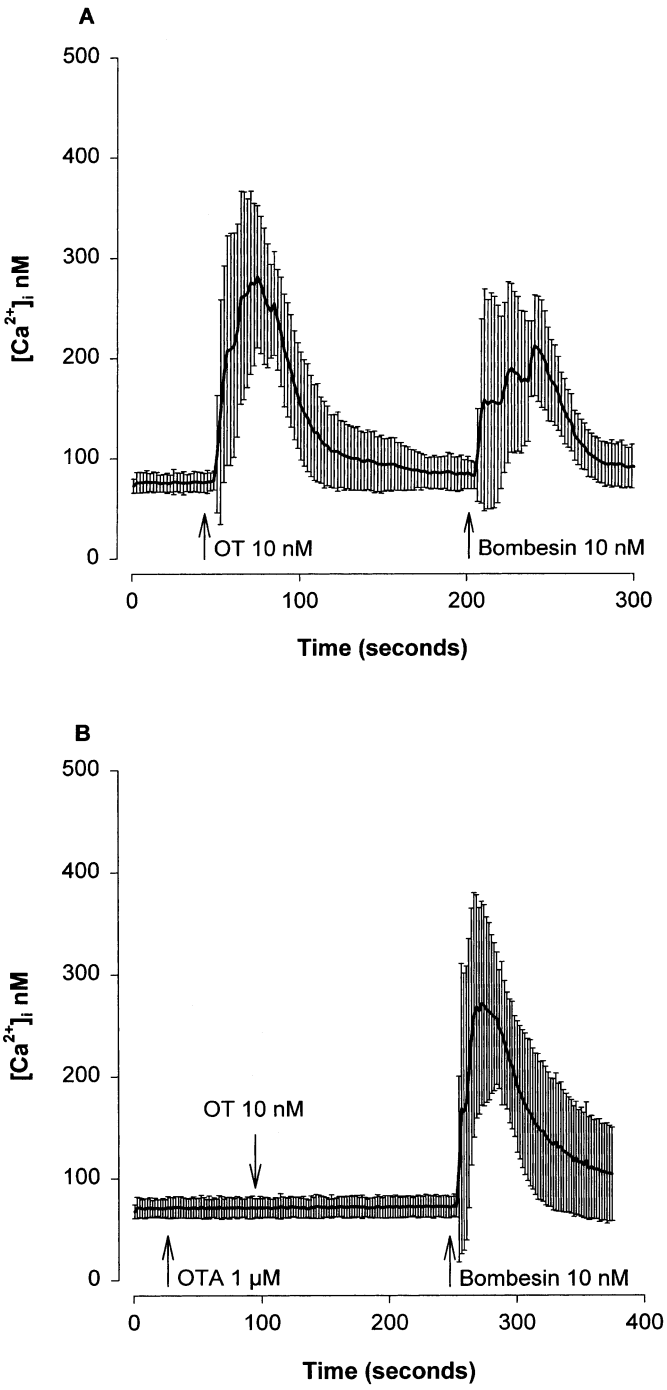

FIG. 5. OT-stimulated Ca^{2+} transients in HGL5 cells. **A**) Real-time intracellular Ca^{2+} concentration in individual cells in response to 10 nM OT. **B**) Inhibition of OT effects by OT antagonist. Cells were preincubated with 1 μM OTA for 100 sec before addition of 10 nM OT. Ten nanomolar bombesin was used as a control to demonstrate calcium responsiveness. Each experiment was performed in triplicate. Each time point represents the mean ± SEM from 40 individual cells.

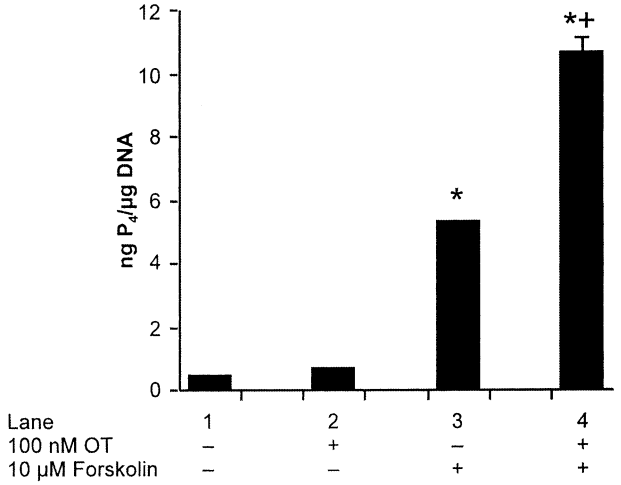

FIG. 6. Effect of OT and OT plus forskolin on progesterone production by HGL5 cells. Each bar is the mean ± SEM of triplicates. *, Different from control ($P < 0.05$); +, different from forskolin treatment ($P < 0.005$).

(U73122), which precedes activation of PKC. Similar results were shown with an established breast tumor cell line Hs578T [10, 29]. These previous studies indicated that PKC activation resulted in the interaction of AP-1 transcription factors with a binding site located in the proximal promoter region of the human OTR gene [29]. The present studies, showing retinoic acid inhibition of both FBS-stimulated and PMA-stimulated up-regulation of OTRs is consistent with the involvement of AP-1 proteins. Retinoids antagonize AP-1 activity by interfering with c-Jun and c-Fos interaction with AP-1 binding sites on target genes [23–26]. Parenthetically, because granulosa and luteal cells express retinoic acid receptors [31] as well as synthesize retinoic acid [32], this system may antagonize the up-regulation of ovarian OTRs in vivo. Thus, alterations in PKC activity and retinoic acid levels may play a role in the timed appearance and disappearance of ovarian OTRs in vivo.

There is substantial experimental evidence suggesting that OT has a role in luteolysis in ruminants. $PGF_{2\alpha}$ produced by the endometrium in response to OT causes the demise of the corpus luteum. In humans, in whom the luteal life span is independent of the uterus, OT stimulated $PGF_{2\alpha}$ synthesis appears to take place within the ovary itself [33]. Thus, OT injected into the human corpus luteum causes a fall in serum progesterone and shortens the luteal phase [33]. Oxytocin injection also causes a rise in 15-keto-dihydro-$PGF_{2\alpha}$, a $PGF_{2\alpha}$ metabolite [33]. This prostaglandin likely mediates the effects of OT, because changes in serum progesterone caused by OT were prevented by pretreatment with a prostaglandin synthetase inhibitor [33]. Treatment of HGL5 cells with 100 nM OT for 24 h did not affect PGE_2 synthesis over control levels (data not shown). Addition of 1 μM arachidonic acid for 24 h resulted in increased prostaglandin synthesis by 3-fold to 4-fold, but OT had no additional effect on prostaglandin levels (data not shown).

Rather than being luteolytic in HGL5 cells, OT evoked responses that are similar to those seen in human granulosa-lutein cells in primary culture [9]. Both HGL5 cells and human granulosa-lutein cells in primary culture respond to OT with an increase in intracellular Ca^{2+} concentration. Oxytocin alone was incapable of stimulating progesterone synthesis in either cell type. However, OT significantly augmented the effects of agents that stimulate the hCG-cAMP pathway. Thus, OT may be involved in fine-tuning progesterone release. The reason why OT alone does not stimulate progesterone synthesis is not presently known. Oxytocin may potentiate the effects of cAMP by enhancing the uptake of cholesterol or by increasing the accumulation of progesterone in the medium as the result of inhibition of progesterone metabolism. An understanding of the effects of OT is important because it can elucidate distinct mechanisms that affect circulating levels of progesterone.

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Ovarian granulosa cells undergo profound changes in growth and differentiation during the processes of ovulation and luteinization (reviewed in [5, 6, 13–15, 34]). These changes are determined by specific, highly regulated responses to gonadotropins, steroids, and growth factors. One of the most dramatic changes in granulosa cell function is the switch from proliferating, preovulatory phenotype, to the nonproliferating, terminally differentiated luteal cells. It is not surprising, therefore, that OT may have different activities (and functions) in granulosa cells at different stages of development. Thus, in earlier stages of development, OT may coactivate the production of progesterone, whereas at more terminal stages of development OT could be luteolytic, causing a reduction in progesterone production through a prostaglandin-mediated mechanism. In support of this conclusion, analysis of mice deficient in both OT and cyclooxygenase-1 has shown that OT exerts significant effects on both the corpus luteum and the uterus during pregnancy [35]. At low concentrations OT can delay labor by delaying the normal decrease in plasma progesterone. This happens when ovarian OTRs are high relative to that of the uterus. When uterine OTRs are elevated, prostaglandins are produced, and this causes luteolysis. The authors propose that the down-regulation of OT receptors in the corpus luteum and induction of OT receptors in the myometrium serve to shift the predominant consequence of OT action during murine pregnancy from labor inhibition (luteotropic) to labor promotion (luteolytic) [35].

OT is expressed in the corpora lutea of cows, primates, and pigs, indicating that it acts in a paracrine/autocrine fashion (reviewed in [15]). Einspanier et al. [5, 6] have demonstrated that cultured preovulatory follicles but not small follicles from subhuman primates respond to hCG by secreting OT, and that OT then enhances progesterone synthesis. However, we were unable to demonstrate the presence of OT precursor mRNA in HGL5 cells utilizing RT-PCR (data not shown). Parenthetically, an advantage of using HGL5 cells is that one can examine the mechanisms of cross-talk between OT, forskolin, and other signaling pathways regulating steroidogenesis without concern for the contribution of endogenous OT to confound the results.

In summary, a human granulosa cell line has been shown to express functional OTRs. OTR levels are regulated by a PKC-mediated signaling pathway that can be antagonized by retinoic acid. HGL5 cells represent the first established cell line to allow examination of the cross-talk between oxytocin and the cAMP signaling pathways in the regulation of a key steroid hormone involved in reproduction and the menstrual cycle. The switch in roles for OT from a luteotrophic to luteolytic agent during the menstrual cycle is an intriguing phenomenon, and may be best studied using the HGL5 system in the future.

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