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Activation of Functional Oxytocin Receptors Stimulates Cell Proliferation in Human Trophoblast and Choriocarcinoma Cell Lines*

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ABSTRACT

Despite oxytocin receptors (OTR) being present in human chorio-decidual tissues, their expression and role in placental trophoblast cells in the context of tumor growth or physiological functions related to cell proliferation have never been examined. In the present study we demonstrate the presence and functionality of OTR in normal human trophoblast cell lines (ED77 and ED27) and a choriocarcinoma cell line (BeWo). RT-PCR and immunofluorescence analysis revealed the presence of OTR messenger RNA and protein in these cells. Binding studies using [^{125}I]oxytocin ([^{125}I]OT) antagonist confirmed the presence of specific binding sites in ED27, ED77, and BeWo cells. OTR functionality was demonstrated by measuring the OT-induced increase in the intracellular calcium concentrations. This effect was dose dependent and was blocked by the selective OT antagonist d(CH_2)$_5$[Tyr(Me)^2,Thr^4, Tyr-NH_2^{9}]OVT (OT antagonist). Furthermore, two proteins with apparent molecular masses of 125 and 60 kDa became tyrosine phosphorylated in all of the cell lines after OT stimulation (and an additional protein of 45 kDa in BeWo choriocarcinoma cells), suggesting that this peptide can stimulate tyrosine kinase activity. Finally, we observed a dose-dependent OT stimulation of cell proliferation associated with OTR activation that was completely abolished by the selective OT antagonist. These findings provide the first evidence of the presence of functional OTR in normal trophoblast cell lines as well as in choriocarcinoma cells and show that a specific effect of OT on normal and neoplastic trophoblast is to promote cellular proliferation. (*Endocrinology* **142:** 1130–1136, 2001)

OXYTOCIN (OT) IS a nonapeptide hormone that acts via specific G-coupled receptors (OTR) (1) and is involved in various reproductive functions as a neuroregulator or in a paracrine manner in ovary or uterus (2, 3). The uterine OTR in myometrial and endometrial cells mediate the OT-promoting effect on PG production involving mitogen-activated protein kinase activity (4, 5). The expression of OTR in the myometrium increases during pregnancy until the onset of labor, and this increase in OTR is paralleled by the increasing near-term myometrial responsiveness to OT (2, 3). At the feto-maternal interface, OTR are up-regulated at parturition and seem to be involved in the amplification of labor (3, 6, 7). As in the uterus, OT also promotes PG production in the decidua and amnion (7). In addition, chorio-decidual tissues have been reported to synthesize OT themselves (8), and this synthesis is estrogen stimulated and increases at parturition (9). Taken together these data provide clear evidence that OT and OTR are part of an autocrine and paracrine network involved in regulation of the timing of labor onset. Despite these extensive studies, to the best of our knowledge the role of OT as a placental regulator or growth factor and in particular as a promoter of trophoblast proliferation in a physiological or neoplastic context has never been investigated.

In previous studies we observed that OT acts as a negative regulator of cell proliferation in different neoplastic tissues, including human breast carcinomas (10), rodent mammary tumors (11), human nervous tumors (12), and human osteosarcoma cell lines (13), all of which express OTR. Human endometrial carcinomas also express OTR, and OT inhibits the proliferation of endometrial cancer cells (14). This OT-induced antiproliferative effect is coupled to nonconventional OT signaling, activated via the cAMP-protein kinase A pathway (12, 14, 15). Other researchers also suggested that OT could activate this unusual pathway in breast cancer cells (16). The evidence of a growth-regulating effect of OT has also been demonstrated in human endothelial cells (17) and human uterine smooth muscle cells (18). In these cases, however, OT exerts a positive, stimulating effect on cell proliferation that is calcium (17, 18) and protein kinase C dependent (17).

As the expression of OTR at the feto-maternal interface has never been correlated to the growth-regulating properties of their natural ligand, we decided to investigate the possible role of OT as a trophoblast growth factor under both phys-

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iological and neoplastic conditions. In this study we demonstrate the expression and functionality of OTR in human normal and neoplastic trophoblast-derived cell lines, and we present experimental evidence that the OTR-OT system may play an important role in regulating the proliferation of human trophoblast and choriocarcinoma cells.

Materials and Methods

Cell lines and reagents

Spontaneously transformed first trimester human trophoblast cell lines (ED27) and (ED77) were provided by Dr. D. Kniss, Ohio State University (Columbus, OH). The human choriocarcinoma cell line BeWo was obtained from the European Collection of Animal Cell Cultures (Salisbury, UK). MCF7 (breast carcinoma cell line) and HT29 (colon carcinoma cell line) were obtained from American Type Culture Collection (Manassas, VA). Cells were routinely cultured in RPMI 1640 medium (Life Technologies, Inc., Burlington, Canada) supplemented with 10% FCS (Life Technologies, Inc.). Native OT and vasopressin (AVP) were provided by Dr. Per Melin (Ferring Pharmaceuticals Ltd., Malmo, Sweden). The OTR antagonist d(CH_2)$_5$[Tyr(Me)^2,Thr^4,Tyr-NH_2^{9}]OVT (OT antagonist) (19) and the selective OT agonist [Thr^4])oxytocin ([Thr^4]OT) (20) were gifts from Dr. Maurice Manning (Toledo, OH). Epidermal growth factor (EGF) was purchased from Life Technologies, Inc.

RT-PCR

Total RNA was extracted according to the guanidine thiocyanate-cesium method (21) from ED27, ED77, and BeWo cells. The concentration of RNA was estimated by spectrophotometry, and RNA degradation was monitored by agarose gel electrophoresis. Oligonucleotide primers were designed to amplify a 391-bp fragment of human OTR complementary DNA according to the method of Takemura *et al.* (6).

Five micrograms of RNA obtained from each cell line were reverse transcribed in 20 μl reaction mixture containing 200 U Superscript reverse transcriptase (Life Technologies, Inc.) and 84 pmol antisense primer for OTR. Each reaction product was then amplified by PCR buffer containing 2.5 U Polytaq *Taq* DNA polymerase (Polymed, Sambuca, Firenze, Italy) and 12.5 pmol each of the specific primer pair for OTR.

After 20 cycles of amplification, 25 μl of each reaction product were electrophoresed, stained with ethidium bromide on 1% agarose gels, and transferred onto nylon membranes by vacuum blotting. MCF7 breast carcinoma cells and HT29 colon carcinoma cells were used as positive and negative controls, respectively.

Southern blot analysis was performed to further test the specificity of the RT-PCR product. Membranes were hybridized at 42 C overnight with 25 pmol digoxigenin-labeled OTR oligonucleotide probe. The membranes were then washed with 2 $\times$ SSC (standard saline citrate)-0.1% SDS for 10 min at room temperature and with 0.5 $\times$ SSC-0.1% SDS for 30 min at 42 C. Digoxigenin-labeled specific hybrids were visualized using an immunological detection system (Roche Molecular Biochemicals, Mannheim, Germany) employing antidigoxigenin antibodies conjugated with alkaline phosphatase. Detection was performed using chemiluminescent substrate disodium 3-(4-methxyspiro;1,2-dioxetane-3,2-(5-chloro)tricyclo(3.3.1.^{3,7})decan;-4-yl)phenylphosphate CSPD (Roche Molecular Biochemicals) according to the manufacturer's instructions. All blots were exposed to x-ray films with intensifying screens at room temperature for 3 h.

Immunofluorescence and flow cytometry

In a standard immunofluorescence procedure, ED27, ED77, and BeWo cells were grown on glass coverslips for 5 days. After washing in PBS, cells, either unfixed or fixed in methanol for 5 min at $-$20 C, were incubated at room temperature for 30 min with a primary monoclonal antibody (MAb) to human OTR (clone IF3) (22) diluted 1:20 in PBS. Cells were then rinsed in PBS and finally incubated for 30 min at room temperature with the appropriate fluorescein-labeled secondary antiserum (Sera-Lab Ltd., Sussex, UK) diluted 1:20 in PBS. The reaction was evaluated with a Leitz Orthoplan fluorescence microscope equipped with Xenon lamp and epifluorescence apparatus (Leitz, Rockleigh, NJ). An unrelated primary MAb (Common Leukocytic Antigen, DAKO Corp., Glostrup, Denmark) as well as omission of primary antibody were used as negative controls.

The percentage of positive cells was evaluated by flow cytometry on both unfixed and fixed cells. Cells (5×10^6/ml) were suspended in PBS and incubated for 30 min with anti-OTR IF3 MAb and with the secondary fluorescein isothiocyanate-labeled antibody, as described for the immunofluorescence procedure. The percentage of fluorescent cells was then analyzed using a FACSort (Becton Dickinson, San Jose, CA).

OT binding

Binding assays were performed on crude membrane preparations; briefly, cells were homogenized in a glass potter, washed twice, and resuspended in the binding buffer (50 mM Tris-HCl and 5 mM MgCl, pH 7.4). Membrane proteins (10 mg) were incubated with increasing concentrations of [^{125}I]OT antagonist (10–800 pM) for 60 min at 30 C. Nonspecific binding was determined in the presence of 1 mM OT for each [^{125}I]OT antagonist concentration point. Bound and free radioactivities were separated by filtration over Whatman GF/C filters (Clifton, NJ) presoaked in 10 mg/ml bovine serum. Data were fitted with a binding isotherm curve (PRISM version 3.0, GraphPad Software, Inc., San Diego, CA).

Calcium measurements

ED 27, ED77, and BeWo cells grown on glass coverslips were treated alternatively with OT at different concentrations (1 nM to 10 μM), AVP (1 μM), selective OT agonist [Thr^4]OT (1 μM), and OT antagonist (1 μM). In selected experiments EGTA was used to chelate extracellular calcium. The intracellular calcium levels were evaluated as follows.

The cells were loaded with the acetoxymethyl ester form of indo-1 (Molecular Probes, Inc., Eugene, OR; incubation for 45 min with 2.5 μM at 37 C). The medium was then replaced with standard Tyrode solution, and the coverslips were placed on an inverted IM-35 Carl Zeiss microscope (New York, NY) with a $\times$100 fluorescence objective. Diaphragms were used to observe single cells. Fluorescence signals were taken at an excitation wavelength of 380 nm and emissions of 400 and 480 nm using a spectrophotometer from Cairn Ltd. (Newnham, UK). Intracellular calcium ($[Ca^{2+}]_i$) values were calculated according to the formula: $[Ca^{2+}]_i = K_d\ \beta\ (R - R_{min}/R_{max} - R)$ (23), where R is the ratio of the cellular fluorescence signals ($R = F_{400}/F_{480}$), K_d is assumed to be 820 nM, β is defined as $F_{480,\ zero\ Ca}/F_{480,\ saturating\ Ca}$, and R_{max}, R_{min}, and β were obtained by calibrations using the calcium ionophore ionomycin (Sigma, St. Louis, MO). Background and autofluorescence corrections were performed before each experiment. All experiments were carried out at 22–24 C.

Tyrosine phosphorylation

ED27, ED77, and BeWo cells were serum-starved overnight and then treated with 100 nM OT for 20 min. Control and OT-treated cells were lysed in Laemmli solubilization buffer as previously described (24). For each sample, 50 μg solubilized proteins were separated on an 8% SDS-PAGE, transferred to polyvinylidene difluoride paper (Amersham Pharmacia Biotech, Aylesbury, UK), and then decorated with antiphosphotyrosine antibodies (Upstate Biotechnology, Inc., Lake Placid, NY) as previously described (24). The changes in tyrosine phosphorylation were quantified by the Chemi Doc System using Quantity One software (Bio-Rad Laboratories, Inc., Hercules, CA).

Cell proliferation

ED27, ED77, and BeWo cells were seeded in triplicate in 24-multiwell plates at a density ranging from 5000–8000 cells/ml. To evaluate the effect of OT on cell proliferation, 24 h after plating OT was added to culture medium at concentrations ranging from 10 nM to 1 μM. The medium was changed every 48 h. At 48 and 96 h of culture, cells were counted in double blind by two independent investigators using a hemocytometer. Each experiment was repeated three times. EGF (20 ng/ml) was added to culture medium of ED27 cells and used as a positive control for its known promoting effect on trophoblast cell growth. HT29 colon carcinoma cells were used as a negative control.

Statistical analysis was carried out using ANOVA followed by Bonferroni correction. The cut-off for significance was 0.05.

Results

Detection of OTR messenger RNA (mRNA) by RT-PCR

Using the RT-PCR procedure, ED27, ED77, and BeWo cells (Fig. 1, lanes 1, 3, and 4) as well as MCF7 cells (positive control, lane 2), showed a 391-bp signal corresponding to OTR mRNA. Colon carcinoma cells (HT29) were used as a negative control (Fig. 1A, lane 5).

Immunofluorescence and flow cytometry

The reactivity of the IF3 anti-OTR MAb was analyzed on ED27, ED77, and BeWo cells by means of immunofluorescence and flow cytometry. By immunofluorescence, all of the cell lines showed both bright spots outlining the cellular membrane and intracytoplasmic fluorescent granules (Fig. 1B, choriocarcinoma BeWo cells; normal trophoblast ED27 and ED77 cells not shown).

By flow cytometry, OTR positivity was evident in 93% of ED77 cells, 92% of ED27 cells, and 78% of BeWo cells. No signal was detected when the primary MAb was omitted or the unrelated MAb was used (Fig. 1C).

OT binding

Specific binding was detected in ED27, ED77, and BeWo cells tested with the specific radiolabeled [^{125}I]OT antagonist (not shown). Saturation studies performed on ED77 cells showed a K_d of 247 ± 77 pM (n = 3) for [^{125}I]OT antagonist, a value comparable to those shown by other OTR-expressing cells (25, 26), and a calculated binding capacity of approximately 25 ± 12 fmol/mg protein (three independent experiments). This level of expression is similar to that recently measured in breast cancer cells in culture (26).

Intracellular Ca^{2+}

Application of OT in ED27, ED77, and BeWo cell lines induced a slow, persistent, and dose-dependent increase in cytosolic free $[Ca^{2+}]_i$ (total of 60 cells; Fig. 2, A–C). The addition of 10 mM EGTA in the bath during the response induced a rapid and complete decline of $[Ca^{2+}]_i$ to the resting levels in all of the cell lines, showing that a calcium entry was involved in the response (Fig. 2D). EGTA application failed to induce any effect on $[Ca^{2+}]_i$ in resting conditions. Only in 2 of 60 cells tested could a release from intracellular stores be detected (not shown). In ED27 cells the application of 1 μM [Thr^4]OT, an OTR-selective agonist (20), caused a similar OT response (Fig. 2E). Cell perfusion with 1 μM AVP did not trigger any $[Ca^{2+}]_i$ increase (Fig. 2F). In contrast, the selective antagonist of OTR (1 μM) inhibited the response triggered by 1 μM OT in ED27 and BeWo cells (Fig. 2, G and H).

Tyrosine phosphorylation

Upon OT stimulation, three major proteins of about 125, 60, and 45 kDa became tyrosine phosphorylated in BeWo choriocarcinoma cells, indicating that OT indeed may stimulate tyrosine kinase activity (Fig. 3). The increased band intensity was quantified by the Chemi Doc system and Quantity One software (+19%, +13%, and +20% after OT treat-

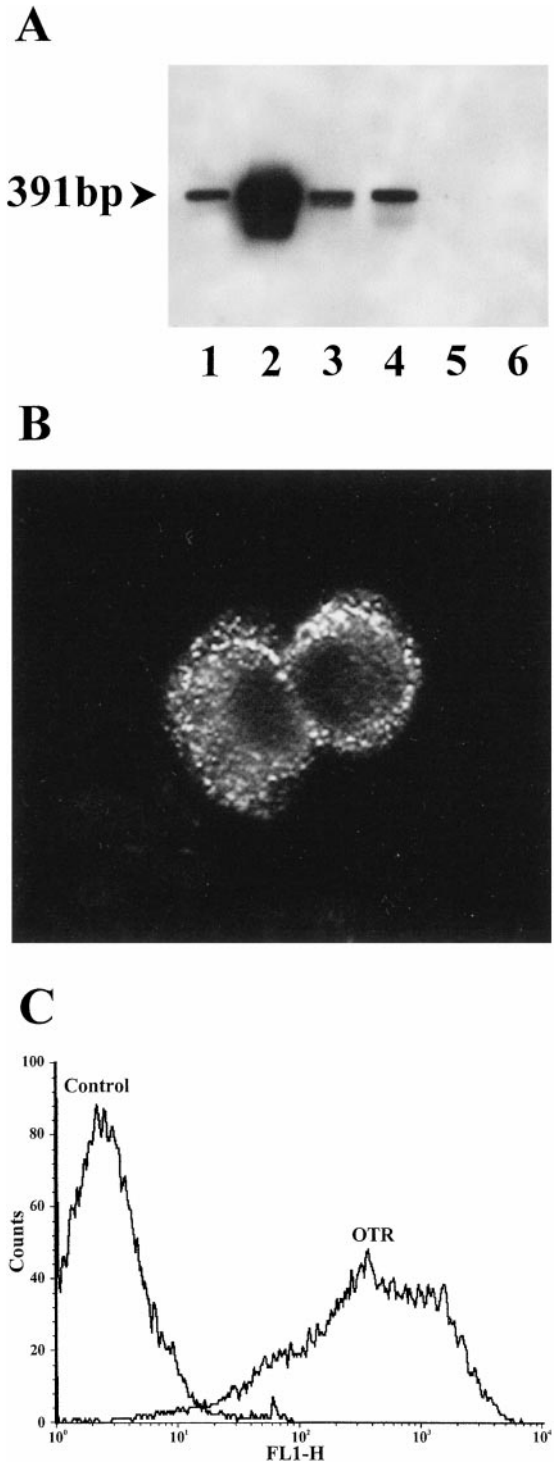

FIG. 1. A, RT-PCR for OTR mRNA detection. Using the RT-PCR procedure, a signal corresponding to OTR mRNA (391 bp) was observed in all cell lines tested (lane 1, BeWo; lane 3, ED27; lane 4, ED77). Breast cancer cells MCF7 were used as a positive control (lane 2). HT 29 colon carcinoma cells were used as a negative control (lane 5). Lane 6, Water. B, Immunofluorescence for OTR on BeWo choriocarcinoma cells. The immunofluorescent reaction using IF3 MAb revealed numerous fluorescent spots lining the cell membrane. C, Flow cytometric analysis of the IF3 anti-OTR MAb with the BeWo choriocarcinoma cells. The anti-OTR MAb shows a strong reaction with 80% of cells. *x*-axis, fluorescence intensity/cells (FL1-H); *y*-axis, number of cells registered per channel. There were 30,000 cells analyzed.

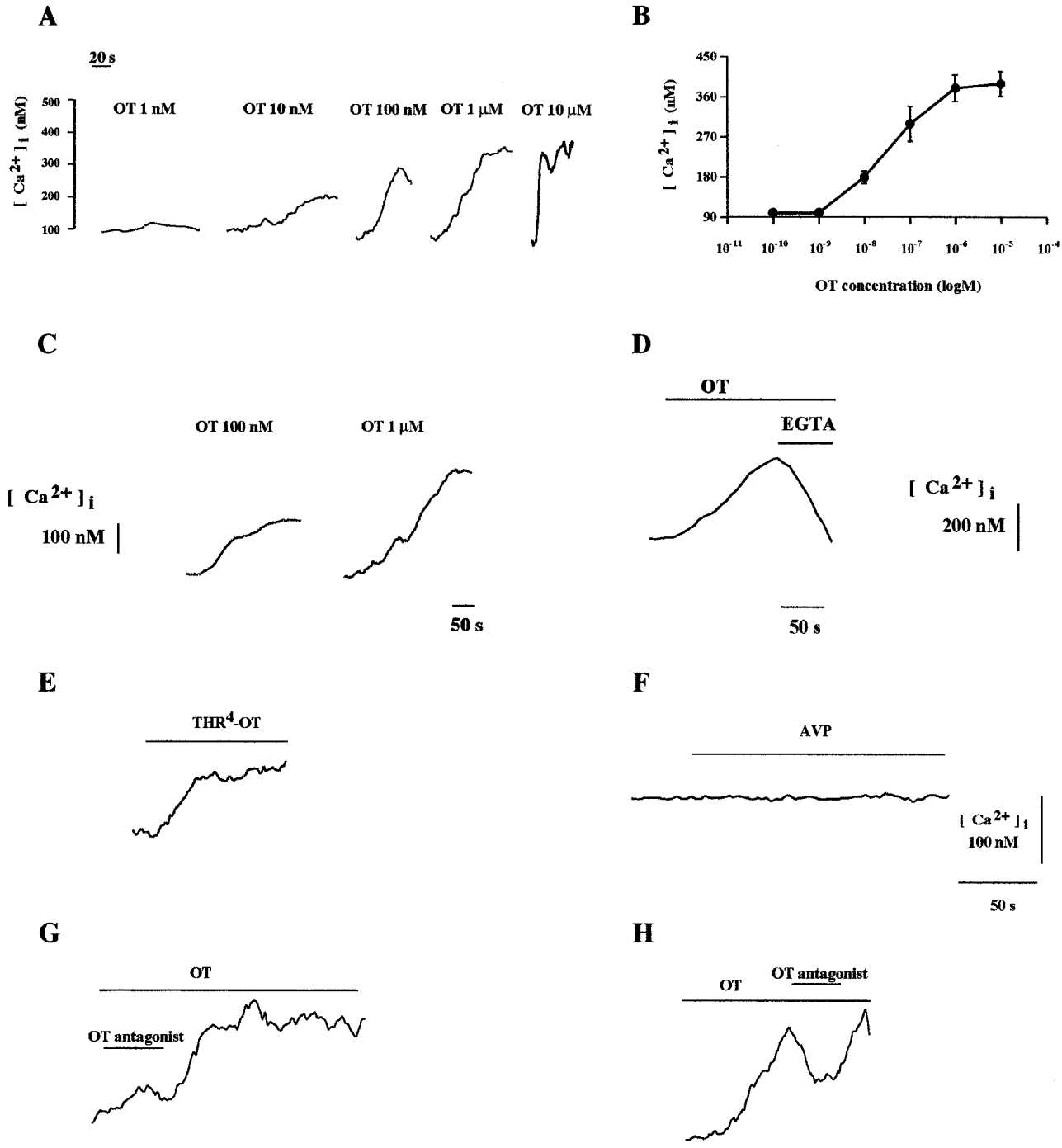

FIG. 2. A, Cytosolic calcium increase induced by application of OT at different concentrations (1 nM to 10 μM) in ED 27 cells (indo-1 measurement on single cells). OT application induced a dose-dependent increase in cytosolic calcium concentrations. B, Dose-response curve on ED 27 cells. The dose-response curve showed that OT concentrations required to determine an increase in cytosolic calcium range from 10 nM to 1 μM. C, Cytosolic calcium increase induced by OT application in BeWo choriocarcinoma cells. OT (100 nM and 1 μM) produced a significant increase in cytosolic calcium. The effect was abolished by the addition of 10 mM EGTA to the external bath (D). E and F, Effect of the OT agonist [Thr^4]OT and AVP on cytosolic calcium levels. In BeWo cells the OT agonist [Thr^4]OT (1 μM) triggered a calcium increase similar to that induced by OT. On the contrary, no effect was produced by AVP (1 μM). G and H, Effect of OTA on the cellular response to OT. Addition of OT antagonist (1 μM) during OT application caused a significant decrease in $[Ca^{2+}]_i$ in ED27 (G) and BeWo cells (H).

ment for the 125-, 60-, and 45-kDa bands, respectively). In both ED27 and ED77 normal trophoblast cells, two proteins of 125 and 60 kDa became tyrosine phosphorylated after OT treatment (+31% and +19% for the 125- and 60-kDa bands, respectively; −2% for the 45-kDa band; Fig. 3).

OT effect on cell proliferation

All of the cell lines responded to OT treatment with a significant increase in cell proliferation, which was evident at the first time point (48 h). OT (1 μM) effected 77% and 65%

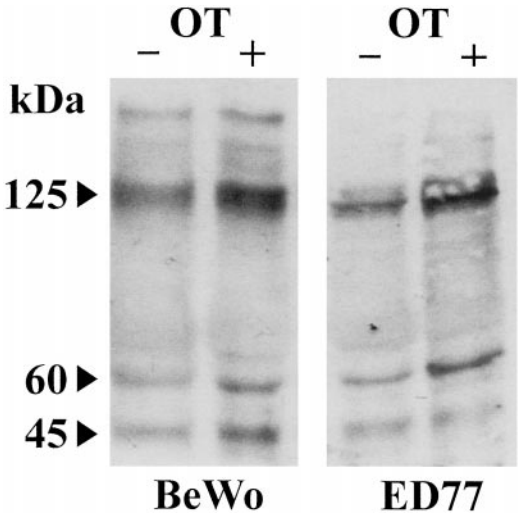

FIG. 3. Tyrosine phosphorylation in BeWo and ED77 cells after OT treatment. Twenty-minute treatment with 100 nM OT increased tyrosine phosphorylation of three major protein of about 125, 60, and 45 kDa in BeWo cells and two major proteins of 125 and 60 kDa in ED77 cells, suggesting that OT may stimulate tyrosine kinase activity. The pattern of phosphorylation of ED27 cells was identical to that of ED77 cells (not shown).

increases in BeWo choriocarcinoma cell number, respectively, at 48 and 96 h of treatment. The OT effect was dose dependent (all OT concentrations *vs.* control: **, $P > 0.001$; *, $P < 0.05$; Fig. 4C) and fully abolished by the incubation with the selective OTR antagonist, which inhibited cell proliferation when used alone (OT antagonist *vs.* control at 48 h: *, $P < 0.05$; Fig. 3C).

In ED27 and ED77 human normal trophoblast cells the mitogenic effect of OT was also dose dependent and time persistent. At 48 h only 10 μM OT significantly enhanced cell proliferation (up to a 71% increase in ED27 cells; $P < 0.001$). At 96 h all OT concentrations produced a significant (**, $P < 0.001$; Fig. 4, A and B) increase in cell number. Treatment with 1 μM OT produced 200% and 270% increases in ED77 and ED27 cell number, respectively. Addition of the OTR antagonist completely abolished the OT effect. The use of OT antagonist alone reduced cell growth at all time points (*, $P < 0.05$).

The addition of EGF to culture medium brought about a significant increase in ED27 cell number compared with that under basal conditions. The increase, however, was only 15% more than that caused by 1 μM OT (not significant; data not shown).

Discussion

In this study we provide the first evidence of OTR expression in human trophoblast-derived cell lines, both normal (ED27 and ED77 cells) and neoplastic (BeWo cells). More importantly, we show that in these cells OTR receptors are functionally active and that, through OTR, OT stimulates cell proliferation. This effect of OT is dose dependent and is similar in both choriocarcinoma and normal trophoblast cells.

In our experiments OT stimulated cell proliferation through a selective activation of OTR that led to an increase

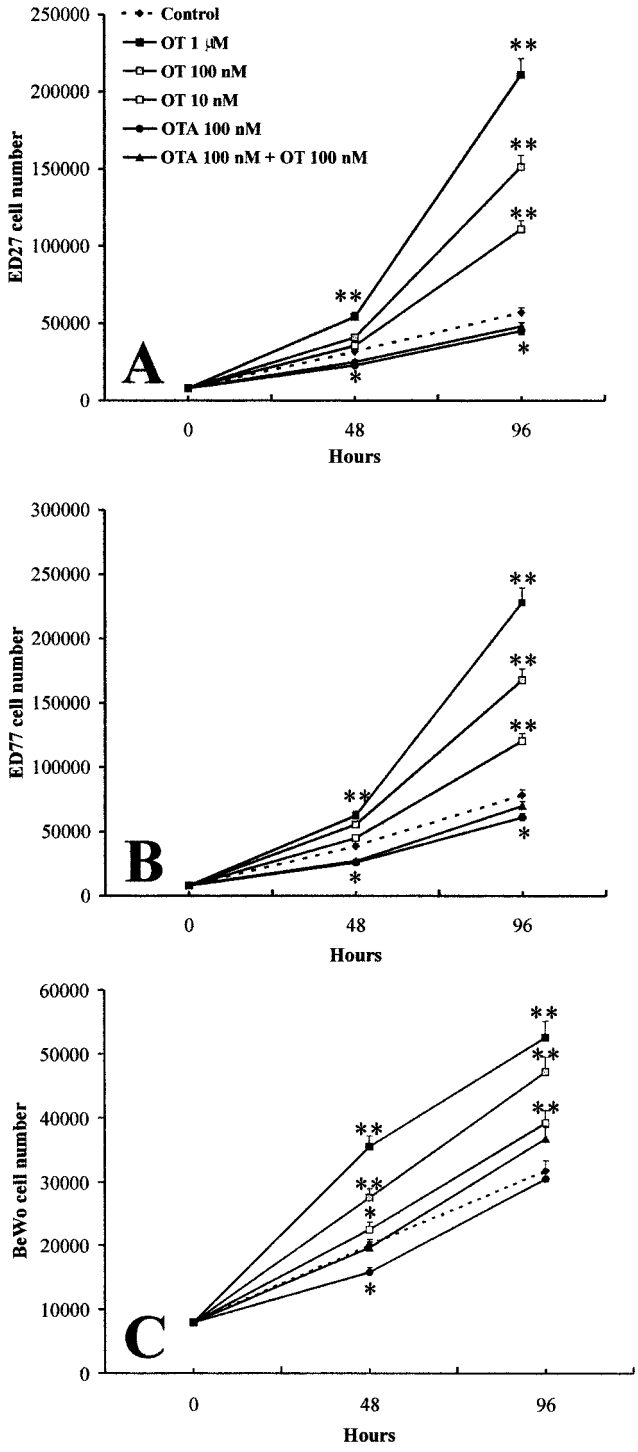

FIG. 4. Effect of OT and OT antagonist treatment on cell proliferation of ED27, ED77, and BeWo cells. OT significantly increased cell proliferation in ED27 (A), ED77 (B), and BeWo cells (C). In all graphs: *, $P < 0.05$; **, $P < 0.001$ (OT-treated cells *vs.* control). The addition of the selective OT antagonist (OTA) reverted the effect of OT. Moreover, when the 100 nM OT antagonist was used alone, cell proliferation was significantly reduced at any time point (*, $P < 0.05$, OTA *vs.* control). All experiments were performed in triplicate. Statistical analysis was carried out by ANOVA followed by Bonferroni's correction. The cut-off for significance was 0.05.

in intracellular $[Ca^{2+}]_i$ and tyrosine phosphorylation. The OT effects on calcium uptake were dose dependent and were completely abolished by simultaneous incubation with the selective OT antagonist, which inhibited cell proliferation when used alone. Moreover, the treatment with [Thr^4]OT, a selective OTR agonist, evoked a response similar to OT, whereas no effect on the $[Ca^{2+}]_i$ increase was observed after AVP perfusion. The lack of effect on the $[Ca^{2+}]_i$ increase after AVP perfusion ruled out the possibility of a contribution of other receptors of the AVP/OT receptor family, namely the V1a, V1b, and V2 subtypes (27), through which OT may exert a pharmacological effect, although with a lower efficacy with respect to AVP.

OTR are coupled to both $G_{\alpha q}$ and $G_{\alpha i}$ subunits, which mediate the triggering of a number of intracellular signaling pathways, including protein kinase C activation (28). Protein tyrosine phosphorylation is an early event of the proliferative signaling triggered by growth factors. Recent reports suggested an involvement of protein tyrosine phosphorylation in OT signal transduction, as a tyrosine kinase inhibitor partially inhibits the OT-induced calcium increase (28). The mechanism by which OT stimulates tyrosine phosphorylation has not been elucidated, but may be mediated by $G_{\beta\gamma}$ dissociating from $G_{\alpha i}$ (28). Tyrosine phosphorylation in OT signaling contributes to activate both p38 mitogen-activated protein kinase and extracellular signal-regulated kinase 2 or to participate in the intracellular calcium increase (5, 28). Here we investigated protein tyrosine phosphorylation induced by OT in both normal trophoblast and choriocarcinoma cell lines. Interestingly, in both cell types we observed a significant increase in tyrosine phosphorylation of two proteins of 125 and 60 kDa. In BeWo choriocarcinoma cells OT treatment also determined the phosphorylation of a 45-kDa protein. Although we have not identified these proteins, we can suggest from their mol wt that the first one is FAK or its related protein Pyk2. Tyrosine phosphorylation of FAK has indeed been reported to occur upon OT stimulation of $CD8^+$ T lymphocytes (29). It is possible to speculate that stimulation of FAK and Src tyrosine kinase, which is common to several growth factors, may contribute to the proliferative activity of OT reported here.

Very few observations have been previously reported on the possible role of OT as a factor regulating cell proliferation, and they were controversial. In previous studies on breast (10, 11, 15), nervous (12), and endometrial (14) tumor cell lines, we observed that OT inhibited cell proliferation and that this inhibitory effect was mediated via the cAMP-protein kinase A pathway, whereas $[Ca^{2+}]_i$ and inositol phosphate were not involved. Identical demonstration of lack of an OT-dependent increase in intracellular calcium in breast cancer cells was reported by others (16).

Beside this evidence in neoplastic cells, in a recent paper Thibonnier *et al.* (17) documented that OTR are present in human vascular endothelial cells and that OT stimulates endothelial cell proliferation *in vitro*. This trophic effect is coupled to the cytosolic calcium increase and protein kinase C response, the conventional OT signaling (17). Similarly, it has been reported that OT induces the hyperplasia of human uterine smooth muscle cells *in vitro* through the increase in $[Ca^{2+}]_i$ (18).

The discrepancies observed on the biological effects of OT on cell proliferation (inhibition or stimulation) and the specific coupling between signaling and effect on cell growth (Ca^{2+} together with increased proliferation; cAMP together with decreased proliferation) indicate that OT may activate two intracellular distinct pathways. The choice of one or the other pathway may be explained by either a cell type specificity or the activation of different OTR subtypes or even the AVP receptors, including V2 receptor subtype and its intracellular signaling, involving cAMP (27).

The data presented here are consistent with the observations reported in endothelial (17) and smooth muscle uterine cells (18), as the cellular proliferation of normal and neoplastic trophoblast cells lines is stimulated by OT through calcium and tyrosine phosphorylation. The relatively high doses of OT required to stimulate cell proliferation may depend on the presence of OT-degrading enzyme produced by trophoblast cells (30).

In conclusion, our data demonstrate that functional OTR are present in both human normal trophoblast and choriocarcinoma cell lines and that through these binding sites OT stimulates cell proliferation. The future validation of these observations on fresh human placental tissue could definitely indicate whether OT should be considered a true growth factor for trophoblast-related tissues. Even though previous studies elucidated the role of OT at the feto- maternal unit in relation to the timing of labor and parturition (3, 6–8), no data were provided before on other biological effects of OT in either normal or neoplastic trophoblast-derived cells. Interestingly, as it has been reported that OT is produced at that site through a paracrine/autocrine loop (8), it may act locally to promote cell proliferation. These data, therefore, suggest new implications for the pathogenesis of chorion-derived tumors and open new perspectives on a possible therapeutic role for OT agonists and antagonists.

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