The Regulation of Oxytocin Receptor Expression in Human Myometrial Monolayer Culture

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Abstract

Clarification of the mechanism of oxytocin (OT)-induced contraction of the uterus seems to be essential for the elucidation of the mechanism of the initiation of labor. Although it has been suggested that estradiol (E) and progesterone (P) are involved in the expression of oxytocin receptors (OTRs), no consensus opinion has been formed on this topic. Thus we recently assessed the effects of E and P on OTR expression using cultures of human uterine cells and we examined the changes in the expressed OTRs following treatment of the cell with exogenous OT. The following results were obtained:

1. The total cellular concentration of OTRs as measured in the myometrial crude membrane fraction (total OTR concentration) showed an increase dependent on the E concentration and on the length of the incubation period in the presence of E. This increase following treatment with E (10^{-7} M) was suppressed by simultaneous treatment with P in a concentration-dependent manner; being suppressed by 50% when the concentration of P was 2.7 \times 10^{-6} M (E/P ratio = 0.037). When 1 nM OT was added to the culture, the total OTR concentration did not change within the first 30 minutes of incubation, but decreased by about 70% twenty-four hours after addition of OT.

2. The concentration of OTRs at the cell surface (surface OTR concentration), as measured in myometrial cells that adhered to the culture plate, did not change with time when the cells were cultured at 4°C in the presence of 1 nM OT. However, when cultured at 37°C, the surface OTR concentration showed decreases dependent on the concentration of OT added and the time after the addition of OT. This change was observed within 60 minutes after the addition of OT to the culture. This decrease in surface OTR concentration was suppressed by concanavalin A (ConA), an inhibition of the internalization of cell surface receptors.

These results indicate that in humans also, the expression of myometrial OTRs is regulated by change in the E/P ratio. The present study also revealed that OTRs, once expressed, soon disappear from the cell surface in the presence of exogenous OT (due to internalization of OTRs, i.e., dislocation of OTRs from the cell surface to the inside of the cells), and that prolonged exposure to OT even leads to the disappearance of intracellular OTRs. The present study thus suggests that the expression of human myometrial OTRs is
regulated by E and P, and that an agonist-induced desensitization mechanism at the receptor level, similar to that reported for β-adrenergic receptors, is also operating in this receptor.

Key words: oxytocin receptor, estrogen, progesterone, oxytocin, desensitization

Introduction

Oxytocin (OT), a powerful inducer of contraction of uterine muscles, is thought to play an important role in myometrial contraction at the beginning of labor. The concentration of oxytocin receptors (OTRs) in the myometrial cell membrane is the dominant factor determining the actions of OT in vivo. On the basis of this view, structural and functional studies of OTR-related intracellular signal transmission systems have been carried out by numerous investigators.

To date, however, investigators have reported different opinions on the relationship of blood OT levels and myometrial OTR concentrations in the third trimester of pregnancy to labor in human beings (Soloff et al., 1979; Den et al., 1981; Fuchs et al., 1982, 1983). The lack of a widely accepted view on this topic suggests that in vivo various factors are involved in determination of myometrial OTR concentrations.

It is known that progesterone (P) and estradiol (E) are produced in large amounts in fetuses, placentas and maternal bodies during the course of pregnancy. P and E are thought to be important determinants of myometrial OTR concentrations. However, to date no studies of the effects of P and E at the OT receptor level in humans have been published.

Some investigators have reported that treatment with OT for long periods of time reduced the OTR concentration in myometrial membranes (Engström et al., 1988a, 1988b). It therefore seems likely that the density of expressed OTRs changes depending on the presence of OT.

To elucidate the mechanism of the expression of human myometrial OTRs, we prepared a human myometrial cell culture system, taking advantage to avoid the influence of endogenous steroid hormones. Using this system, we measured: (1) the concentration of OTRs in an isolated crude membrane fraction from myometrial cells, and (2) the concentration of OTRs of myometrial cells in the cultured condition. The former concentration was regarded as the total cellular concentration of OTRs (total OTR concentration), and the latter as the concentration of OTRs at the cell surface (surface OTR concentration). These two concentrations were compared with each other. Furthermore, the effects of P and E on the total OTR concentration were assessed, and changes in total and surface OTR concentrations following the addition of OT to cultures were examined. On the basis of data from these analyses, desensitization mechanisms at the receptor level were also considered and discussed.

Materials and Methods

1. Materials

1) Myometrial tissue

Intact muscles were sampled from the anterior wall of the uterine body immediately after
the removal of the uterus from premenopausal women for the treatment of benign disease. Informed consent was obtained from each woman prior to myometrial sampling of the removed uterus.

2) Reagents and devices

Nunc 6-well culture plates (36 mm diam) were used to culture uterine muscle cells. Oxytocin, concanavalin A (ConA) and other reagents used in this experiment were purchased from Sigma. 125I-oxytocin (specific activity 2,200 Ci/mmol) was obtained from New England Nuclear (Boston, MA, U.S.A.).

2. Methods

1) Myometrial tissue preparation and culture

Human myometrial cells were cultured according to the method reported by Casey et al. (1987). Within 30 min after removal of the uterus, myometrial tissue was obtained from the anterior wall of fundus of the uterus. The myometrial tissue was placed in Hank’s balanced salt solution [HEPES (20 mM), pH 7.4]; the tissue was minced into fragments of approximately 1 mm³. The tissue fragments were transferred to Hank’s balanced salt solution that contained collagenase Type I (1 mg/ml), Type IA (1 mg/ml), DNase (0.2 mg/ml), penicillin (200 U/ml), streptomycin (200 µg/ml), and Fungizone (0.5 µg/ml). The mixture was incubated in a water bath at 37°C with agitation for 6 h. After the incubation the cells were separated from tissue debris by filtration through four layers of gauze cloth and collected by centrifugation at 600 x g for 10 min. The cells were then washed with Waymouth’s enriched medium that

Fig. 1. Phasecontrast photomicrograph of human myometrial smooth muscle cells in primary culture at nonconfluency (×300, left) and confluency (×60, right). The long, fusiform cells grew in parallel arrays.
contained fetal bovine serum (FBS), suspended in Waymouth Medium MB 752/1 [HEPES (25 mM), pH 7.4] with FBS (10%, vol/vol), 1% sodium pyruvate, MEM amino acid, MEM NE amino acid, MEM vitamin, penicillin (200 U/ml), streptomycin (200 µg/ml) and neomycin (400 µg/ml), plated at a density of 1 x 10^5 cells/well in 6-well plates and were maintained with 5 ml culture medium/well. Morphologically, the smooth muscle cells maintained in culture as above were characterized by a long, fusiform shape and a central nucleus; moreover the cells grow in parallel arrays (Fig. 1).

2) Treatment of myometrial cells with E, P or OT

At the point when the human myometrial cell culture became confluent, the medium was renewed with Waymouth Medium containing fatty acid free albumin (0.4%), and the cells were further incubated for 0–72 hours in the presence of E, P or OT.

At the end of incubation with OT, the supernatant was removed by aspiration and the cultured cells were immediately washed with ice-cooled 0.25 M glycine-HCl (pH 2.8).

3) Preparation of myometrial membranes

Cultured cells were harvested with a scraper and then incubated in ice-cooled hypotonic buffer (10 mM Tris HCl, 2.5 mM EDTA, pH 7.6) for 15 min. The culture was then homogenized with an Ultra-thrux homogenizer and centrifuged at 4°C and 10,000 rpm for 10 min. The sediment was used as a crude myometrial membrane fraction.

4) Binding assay for total OTR concentration

The myometrial membrane (50 µg) was incubated at 4°C for 60 min in 200 µl of binding buffer (50 mM Tris HCl, 10 mM MnCl₂, 1 mg/ml BSA, pH 7.4) containing ^125I-OT. The culture was then filtered through a Whatmann GF/F glass filter. Some samples (non-specifically bound samples) were subjected to the same binding assay in the presence of non-labeled OT (100 nM) along with the above-mentioned amounts of ^125I-OT. The radioactivity of each sample was measured using a gamma-counter.

5) Binding assay for surface OTR concentrations

Binding buffer (50 mM Tris HCl, 10 mM MnCl₂, 1 mg/ml BSA, pH 7.4), containing ^125I-OT, was added to the culture plate. After 60-minute incubation at 4°C, the supernatant was harvested as the free sample. A fraction, obtained by washing the culture in ice-cooled 0.25 M glycine-HCl (pH 2.8) three times, served as the bound sample. Samples subjected to binding assay in the presence of non-labeled OT (100 nM) plus the above mentioned amounts of ^125I-OT served as non-specifically bound samples. The radioactivity of each sample was measured using a gamma-counter.

6) Protein quantification

Protein was quantified by a Bio-Rad protein assay kit using BSA as standard.

Results

1) The effect of E and P on total OTR of the cultured myometrial cells.

In order to see how OT receptor expression in human myometrial cells were modified by E and P, the cells were cultured, subjected to various concentrations of E and/or P for varying length of time, crude membrane fraction containing total OTR was isolated from cells, and the
parameters of OTR of this fraction was analyzed by Scatchard plot using 100 pM $^{125}$I-OT. The analysis of the effect of addition of varying concentrations of E on total OTR revealed that the maximal binding ($B_{max}$) increased with the concentration of E, being 19.85, 28.12 or 36.20 fmol/mg protein at E concentrations of 0 (control), $10^{-8}$ M or $10^{-7}$ M, respectively. The dissociation constant (Kd) showed no significant changes by varying concentrations (Fig. 2).

When the cells were treated with $10^{-7}$ M E for varying duration of the time, the Scatchard analysis of total OTR of the cells revealed that $B_{max}$ increased with duration of the incubation time, being 17.85, 23.72 or 28.20 fmol/mg protein when incubated for 24, 48 or 72 hours, respectively. The Kd showed no time-related change (Fig. 3).

When the cultures were incubated for 72 hours in the presence of E ($10^{-7}$ M) and P ($10^{-9}$ M to $10^{-5}$ M), the binding assay was showed that the specific binding of OT was 2.78 fmol/mg protein in control samples (treated neither with E nor P) and 7.70 fmol/mg protein in samples

| $E_2$ concentration | Kd $\times 10^{-9}$ (M) | $B_{max}$ (fmol/mg protein) |
|---------------------|-------------------------|-----------------------------|
| control             | 1.16                    | 19.85                       |
| estradiol $10^{-8}$M| 1.24                    | 28.12                       |
| estradiol $10^{-7}$M| 1.23                    | 36.20                       |

Fig. 2. Representative Scatchard plots made with four or five points each of $^{125}$I-oxytocin specific binding to crude membrane fraction from myometrial culture cells. Myometrial cells were incubated without ($\triangle$) and with $10^{-8}$ M estradiol ($\circ$), $10^{-7}$ M estradiol ($\blacksquare$) for 72 h at 37°C. After incubation the cells were harvested, homogenized and their membrane fraction was obtained as noted in Materials and Methods. The membrane fraction was incubated in binding buffer containing $^{125}$I-OT (20, 40, 60, 80 or 100 pM). Using a method described in Material and Methods, a bound sample and a free sample were obtained for Scatchard plot analysis.

| Time course     | Kd $\times 10^{-9}$ (M) | $B_{max}$ (fmol/mg protein) |
|-----------------|-------------------------|-----------------------------|
| 24 hr culture   | 1.37                    | 17.85                       |
| 48 hr culture   | 1.32                    | 23.72                       |
| 72 hr culture   | 1.60                    | 28.20                       |

Fig. 3. Representative Scatchard plots made with four or five points each of $^{125}$I-oxytocin specific binding to crude membrane fraction from myometrial culture cells. Myometrial cells were incubated with estradiol $10^{-7}$ M for 24 h ($\blacksquare$), 48 h ($\circ$) and 72 h ($\triangle$) at 37°C. The cells were treated and analyzed as written in Fig. 2.
Fig. 4. Suppression of estradiol induced myometrial oxytocin receptor expression by progesterone. Myometrial cells were incubated in the presence of estradiol $10^{-7}$ M plus progesterone ($10^{-9}$ M to $10^{-5}$ M) for 72 h. After incubation the cells were harvested, homogenized and their membrane fraction was obtained as noted in the materials and methods. The membrane fraction was incubated in binding buffer containing 100 pM $^{125}$I-OT. Binding assay was performed also as described in Materials and Methods. When a semi-logarithmic plot analysis was carried out, using the OTR concentration in the presence of $10^{-7}$ M E as 100% and the control level as 0%, the P concentration causing 50% suppression of OTR expression was calculated at $2.7 \times 10^{-6}$ M. The E/P ratio at that time was 0.037.

These results suggest that the addition of E to the culture increase the cells' total OTR concentration in a time and concentration-dependent manner, while that of P inhibit these changes in the OTR concentration also in concentration-dependent manner.

2) Changes in total OTR concentrations of the cultured cells following the treatment with OT

Total cellular content of OTRs as determined by the specific binding using $^{125}$I-OT (100 pM), was $25.78 \pm 2.07$ fmol/mg protein in non-OT-treated control samples. After 30 minutes of the cells incubation in the presence of 1 nM OT, the specific binding was $23.66 \pm 1.05$ fmol/mg protein; a value which did not differ significantly from that for control samples. After 24 hours of the incubation in the presence of 1 nM OT, however, the specific binding decreased to $7.88 \pm 2.05$ fmol/mg protein, which was significantly (about 70%) lower than the control level (Fig. 5).

3) Changes in surface OTR concentrations following OT treatment

When the cells were incubated at 4°C in the presence of 1 nM OT, the OT specific binding to OTRs on the myometrial cell surface remained almost unchanged during the first 60 minutes of incubation. When the cells were incubated at 37°C, the specific binding decreased inversely as the concentration of OT or the length of time after addition of OT increased. The OT-specific binding after 60 minutes incubation in the presence of 10 pM, 100 pM, 1 nM or 10 nM of OT was 5.53, 5.89, 2.57 and 1.72 fmol/mg protein, respectively (Fig. 6). These results indicate that while the total OTR concentration was relatively constant during the 30-min period after the addition of OT, the surface OTR concentration decrease markedly.
Fig. 5. Oxytocin induced loss of total cellular content of oxytocin receptors. Myometrial cells were incubated with 1 nM oxytocin for 30 min and 24 h at 37°C. After incubation the cells were treated as described in Fig. 4 and binding assay was performed as described in Materials and Methods. Data shown are mean±SD of results obtained in six separate experiments.

|          | average | SD    |
|----------|---------|-------|
| OT (−)   | 25.78   | 2.07  |
| 30 min   | 23.66   | 1.05  |
| 24 hr.   | 7.88    | 2.05  |

Fig. 6. Oxytocin induced loss of oxytocin receptors at the cell surface. Myometrial cells were incubated with 1 nM oxytocin at 4°C (■) or incubated with 10 pM (○), 100 pM (△), 1 nM (□) and 10 nM (■) at 37°C for 60 min. After cells were washed in ice-cold 0.25 M glycine–HCl (pH 2.8), they were incubated in the binding buffer containing 90 pM [125I]-OT. Binding assay was performed as described in Materials and Methods.

Fig. 7. Representative Scatchard plots made with six points each of [125I]-oxytocin specific binding to myometrial culture cells (oxytocin receptors at cell surface). Myometrial cells were incubated without (■) and with 1 nM oxytocin (●) for 30 min at 37°C. After they were washed in ice-cold 0.25 M glycine–HCl (pH 2.8), Binding buffer containing [125I]-OT (22.5, 45, 67.5, 90, 112.5 or 130 pM) was added to the culture plate and the cells were further incubated for 60 minutes at 4°C (binding assay). Using a method that described in Material and Methods, a bound sample and a free sample were obtained for Scatchard plot analysis.
Fig. 8. Effect of ConA on oxytocin induced loss of oxytocin receptors at the cell surface. Myometrial cells were preincubated for 60 min at 37°C with or without 250 μg/ml ConA. Oxytocin (1 nM) was then added to the cultures, and the incubation was continued for another 30 min. The cells were treated as described in Fig. 6. Data shown are mean±SD of results obtained in six separate experiments.

|          | average | SD   |
|----------|---------|------|
| control  | 13.36   | 0.62 |
| OT       | 9.44    | 0.35 |
| OT + ConA| 13.26   | 0.25 |

When changes in the surface OTR concentrations following treatment with 1 nM OT were analyzed using Scatchard plots, the Bmax, i.e., the surface OTR concentration, in samples treated with 1 nM OT (12.11 fmol/mg protein) was smaller by about 54% than that in non-OT-treated samples (25.78 fmol/mg protein). The Kd was not affected by treatment with OT, being $4.47 \times 10^{-8}$ M in non-OT-treated samples and $4.66 \times 10^{-8}$ M in samples treated with 1 nM OT (Fig. 7). These results indicate that OT induced decrease of the specific binding to OTRs is derived from decrease of the number of OTRs on the myometrial cell surface.

In order to explain the observed decline of cells' surface OTRs, the cells were pretreatment of concanavalin A (ConA), a known inhibition of the internalization of cells' surface receptors, and then incubated with 1 nM OT. The specific binding after 30 minutes incubation in the presence of 1 nM OT was $9.44 \pm 0.35$ fmol/mg protein, which was significantly lower than the control values ($13.36 \pm 0.62$ fmol/mg protein). However, in the samples treated with 250 μg/ml ConA prior to the addition of 1 nM OT, the specific binding after 30 minutes incubation was $13.26 \pm 0.25$ fmol/mg protein, which was almost equal to the control level, indicating the complete arrest of the decrease of the surface OTR concentration by ConA (Fig. 8).

**Discussion**

The regulation of the action of OT, a strong inducer of contraction of the uterine muscles, seems to depend on systemic OT levels and myometrial OTR concentrations.

OTR concentrations in human uterine muscles are known to increase gradually with time during pregnancy. Fuchs et al. (1984) reported that the OTR concentration per unit DNA in the third trimester of pregnancy was 80-100 times higher than in non-pregnant women. Martine, T., et al. (1991) and Sakamoto et al. (1981) reported a 5- to 10-fold increase in the OTR concentration per unit protein in the third trimester of pregnancy.

E and P, which are abundant during pregnancy, seem to be involved in the sensitivity of
uterine muscles to OT. Follett et al. (1964) and Kuriyama et al. (1976) reported that treatment of rats with P reduced their sensitivity to OT, while treatment of rats with E enhanced it. Studies at the level of receptors were carried out by Soloff (1975) using rats and by Nissenson et al. using rabbits. Both studies revealed that the OTR concentrations increased when E was in excess over P, and that P suppressed the E-induced expression of OTRs.

In human, however, no reports have been published concerning the effects of steroid hormones on the expression of myometrial OTRs. Since the myometrial OTR concentration varies greatly among individuals, it is difficult to carry out this kind of in vivo study because of the restricted availability of the uterine samples.

In the present study we, therefore, used cultured human myometrial cells. We assumed that the OTR concentration in the crude membrane fraction of the myometrial cells obtained from the culture in the presence of E and/or P represents the total cellular concentration of OTRs (total OTR concentration), and examined the changes in this concentration. A possible disadvantage of using cultured cells lies in that the type of expression of OTRs is changing during the cultivation.

We believe however that the signal transmission system of the myometrial cells were preserved well after incubation, because:

1. the pattern of the release of calcium following stimulation with OT was normal in the primary cultures (Fujii et al., 1993); and

2. the production of diacylglycerol (DG), fatty acids and prostaglandin (PG) in response to phsphatidylinositol (PI) did not differ between cultured myometrial cells and previously studied myometrial strips obtained in the third trimester of pregnancy (Morimoto et al., 1993).

As found in this study OTR concentration in the myometrial culture, when treated with E ($10^{-7}$ M), increased with time between 24th to 72nd hour after the addition of E. Furthermore, this increase was found to depend on increasing E concentrations. That is, the total OTR concentration rose to 140% of the control level (untreated samples) following treatment with $10^{-8}$ M of E and to 180% following treatment with $10^{-7}$ M of E. Regarding the actual concentration of E which is known to increase with time during the course of pregnancy, we have previously shown in vivo that the E concentration in the third trimester of pregnancy was as high as $10^{-8}$ M to $10^{-7}$ M although the variation of this parameter between individuals was great (Oku et al., 1988). The findings combined with previous findings indicate that the increased E concentrations are the cause of increase in OTR concentrations during the course of pregnancy. The increase in the OTR concentration in the presence of $10^{-7}$ M E was suppressed by treatment with P. This suppression was dependent on the concentration of P; that is, it was greater at a P concentration of $10^{-6}$ M than at the concentration of $10^{-7}$ M. The concentration of P which could suppress the E-induced increase in the total OTR concentration by 50% was $2.7 \times 10^{-6}$ M (E/P ratio=$0.037$). The average concentration of P in the third trimester of pregnancy is of the order of $10^{-6}$ M (Oku et al., 1988). Then the decrease in P concentration found immediately before the beginning of labor seems to be explained by the increase in OTR concentration.

When OT is administered continuously to enhance uterine contraction at the end of pregnancy (at a stage where the sensitivity of uterine muscles to OT is augmented), a decrease
in the sensitivity of the uterine muscles to OT during this treatment is often seen even though
the dose level of OT is kept constant. In connection with this phenomenon, Fuchs et al. (1984)
reported that the OTR concentration in uterine muscles, removed during cesarean section, was
lower in women who had received OT to induce uterine contraction. Engstrom et al. (1988a; 1988b)
reported that the treatment of rats with OT for long periods of time reduced not only
the OTR concentration of the myometrial membrane fraction but also the contractility of
uterine muscles.

Mechanisms for such a desensitizing phenomenon at the level of receptors have recently
been reported to be similar to that of \( \beta \)-adrenergic receptors (\( \beta \)-ARs) (Mahan et al., 1985; Dayes et al., 1990; Hausdorff et al., 1990). When the \( \beta \)-ARs are stimulated with an agonist
for short periods of time, the extent of phosphorylation of their C-terminal tail begins to
decrease, and this change probably leads to there desensitization (Bouvier et al., 1988; Haus-
dorff et al., 1989). When the stimulation is prolonged quickly the number of \( \beta \)-ARs may be
anticipated to decrease, leading to the complete desensitization of receptors. Two mecha-
nisms have been proposed to explain decreases in the number of \( \beta \)-ARs. One is called
sequestration (i.e., internalization of receptors together with G protein-free components
attached to the cell membrane into the cell interior), occurring within about half a hour of the
start of stimulation with an agonist (Strader et al., 1987; Cheung et al., 1989). The other
mechanism is called down-regulation (i.e., disappearance of the \( \beta \)-ARs themselves when they
are stimulated with an agonist for more than several hours) (Bouvier et al., 1989; Valiquette
et al., 1990).

A recent study by Kimura et al. (1992) revealed that the OTR is a G protein-coupled
receptor which contains seven transmembrane helices, and that it belongs to the same super-
family of receptors as the \( \beta \)-AR. To date, however, very few studies have been published
concerning the mechanism of OTR desensitization at the receptor level. The only suggestion
made to date about this mechanism is the possibility of down-regulation.

When we examined the changes in total OTR concentrations following treatment with OT,
the concentration decreased significantly (by about 70%) when the samples were incubated in
1 nM OT for long periods (24 hours). This result is similar to the above-mentioned findings
reported by Fuchs et al. (1984) and Engström et al. (1988a; 1988b). If this findings are
combined with the results reported for \( \beta \)-ARs, it seems likely that this significant decrease of
total OTR concentration after OT treatment of the myometrial cells reflects down-regulation
which involves the degradation of OTRs the execution of which may need rather prolonged
period of times.

A reported experiment using uterine muscle strips revealed that OT-induced uterine
contraction tended to become weaker 30–60 minutes after OT treatment (Eiler et al., 1989). In
the present study, however, the total OTR concentration showed no change during the 30-
minute period after the addition of OT. Therefore, OTR desensitization which may be
occurring relatively soon after the treatment with OT, as observed in the above-mentioned
experiment with uterine muscle strips, may be attributable to some unknown mechanism other
than the down-regulation.

To explore this unknown mechanism, we examined changes in the surface OTR concentra-
tion (the concentration of OTRs at the cell surface) as measured in myometrial cells that adhered to the culture plate. The surface OTR concentration showed a marked tendency to decrease, dependent on the concentration of OT and the length of time of incubation with OT. This tendency was seen within 30–60 minutes of the addition of OT, although the total OTR concentration had shown no change by that time. This phenomenon was not seen when myometrial cells were cultured at 4°C or when the internalization of OTR was inhibited by ConA (Waldo et al., 1983). This phenomenon can therefore be interpreted as reflecting internalization of OTRs into the cell, i.e., sequestration.

Although the OT concentration in living tissue varies greatly among individuals, Chard et al. (1970), Kumaresan et al. (1974), and Dawood et al. (1977) all reported that the OT concentration increased gradually with time in the latter half of pregnancy and reached 10–100 pM. Sequestration, observed in the present study, seems to work as a mechanism of desensitization in vivo.

Additional studies are needed to clarify these mechanisms in more detail and to analyze factors involved in the regulation of these mechanisms. In any event, the present study has revealed that down-regulation and sequestration, triggered by stimulation with OT, regulate the expression of OTRs on the myometrial cell surface. This finding also adds a new knowledge to previous one obtained in animal model that E and P regulate the total number of OTRs expressed.

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