Up-regulation of oxytocin receptors in non-pregnant rat myometrium by isoproterenol: effects of steroids

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Abstract

The objective of the present study was to further elucidate our previous observation that β_2 -adrenoceptor activation induces oxytocin receptor (OTR) expression in rat myometrium. We wanted to investigate whether the mechanism behind this effect was under the influence of gonadal steroids. Ovariectomized non-pregnant rats were treated with estrogen, progesterone or a combination of both for 3 days. Some rats were concomitantly treated with isoproterenol.

Estrogen treatment increased both OTR mRNA production and maximal binding of [³H]-oxytocin to isolated myometrial plasma membranes, but it did not affect contractility of isolated uterine strips challenged with oxytocin. When the estrogen regimen was combined with isoproterenol treatment, an augmented maximal contractile response ($E_{\max}$) to oxytocin was observed although no

further increase in OTR mRNA and binding was seen. Progesterone treatment did not in itself alter OTR mRNA, OTR binding or $E_{\max}$. However, OTRs were induced at the level of gene expression when progesterone was supplemented with isoproterenol infusion. Finally, progesterone suppressed the effect of estrogen on OTR mRNA production and binding when the two compounds were administered together. However, when isoproterenol treatment was added this effect was abolished and $E_{\max}$ was enhanced more than that seen following treatment with estrogen alone.

These data suggest that β_2 -adrenoceptor activation represents an important regulator of OTR expression/function in estrogen- and progesterone-dominated rat myometrium.

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Introduction

Oxytocin receptors (OTRs) increase in the endometrium (Sheldrick & Flint 1985) during luteolysis and in myometrial tissue at term (Fuchs *et al.* 1992). Since steroid hormones, in particular progesterone and estrogens, are thought to play pivotal roles in the process of luteolysis and in the initiation, maintenance and termination of pregnancy, it is not surprising that these substances should participate in the regulation of OTR concentrations. The precise mechanisms by which these gonadal steroids influence OTR formation and function are, however, not fully elucidated. The OTR gene comprises an estrogen response element, which imparts estrogen inducibility of OTR gene transcription (Bale & Dorsa 1997). In spite hereof, the results concerning the effects of estrogens on OTR have not been unequivocal. Thus, in some studies estrogens have been shown to increase the amount of uterine OTR mRNA (Quinones Jenab *et al.* 1997) and the number of OTR binding sites (Hixon & Flint 1987), while in others, estradiol has been reported to decrease uterine OTR concentrations (Silvia *et al.* 1991, Sheldrick

& Flick Smith 1993). On the other hand, the effects of progesterone on uterine OTR are, in general, agreed to be suppressive (Vallet *et al.* 1990, Lau *et al.* 1992). This effect may primarily be of a non-genomic character, due to the lack of a progesterone response element in the OTR gene. Thus, Grazzini *et al.* (1998) found progesterone to inhibit OTR function by direct binding to the OTR. In bovine endometrium a 200-fold rise in OTR was preceded by a decline in plasma progesterone (Fuchs *et al.* 1992). Indeed, the decline in plasma progesterone observed in some species at term has been suggested to be the stimulus that triggers the up-regulation of myometrial OTR and thereby initiates delivery (Hilliard *et al.* 1968).

Several observations indicate that hormones other than steroids also regulate OTR. These include oxytocin itself (Engstrøm *et al.* 1988a) and substances capable of stimulating cAMP production (Jeng *et al.* 1995). We recently showed that β_2 -adrenoceptor stimulation by isoproterenol increased rat myometrial OTR gene expression (Engstrøm *et al.* 1998). In accordance, Bale & Dorsa (1998) recently identified a novel region of the rat OTR promoter containing elements, which impart cAMP inducibility of

OTR gene transcription. In this context however, it is intriguing that some studies have shown that the cAMP analogue CPTcAMP inhibits the function of OTR (Anwer *et al.* 1989, 1990) and we speculate whether our previous observed effect of isoproterenol infusion on OTR dynamics is dependent also on gonadal steroids in the myometrial compartment. Thus, Jeng *et al.* (1995) have previously described a synergistic effect of cortisol and the cAMP elevating agent forskolin in elevating OTR concentrations in rabbit amnion cells.

The objective of the present study was to investigate the effect of isoproterenol infusion in ovariectomized rats on myometrial OTR binding, oxytocin induced uterine contractility and myometrial OTR gene expression, after pre-treatment with estrogen and progesterone alone or in combination.

Materials and Methods

Animals

Female Wistar rats (250–350 g) were maintained under controlled conditions in the Panum Institute Animal House. Food and water were freely available. All experiments conformed to the Guidelines on the Handling and Training of Laboratory Animals by Universities Federation for Animal Welfare.

Rats were anesthetized with a mixture of Dormicum (1.25 mg, Roche, Basel, Switzerland) and Hypnorm (0.4 ml, Janssen, Geel, Belgium). Through an incision in the lateral abdominal wall the rats were bilaterally ovariectomized. Thereafter, the incisions were sutured. Eight treatment groups were formed 7 days later. The groups received either no steroid injection (groups 1–2), a daily i.m. injection of 50 µg estradiol-benzoate (LEO, Copenhagen, Denmark, groups 3–4) (Stürmer 1968), a daily i.m. injection of 100 µg progesterone (Sigma Chemical Company, St Louis, MO, USA, groups 5–6) or both steroids (groups 7–8) for 3 consecutive days. The higher dosage of progesterone compared with estradiol-benzoate was chosen due to the high metabolic degradation rate of progesterone (Ganjam *et al.* 1975). During the steroid treatment period the rats were additionally given continuous infusions of isoproterenol (10 µg/h, Sigma Chemical Company, groups 1, 3, 5, 7) or saline (groups 2, 4, 6, 8) by means of osmotic mini-pumps (Engstrøm *et al.* 1998). The dosage regime of isoproterenol was selected from preliminary experiments in which 10 µg/h was found to be the minimal effective dose on OTR transcription. Following the treatment period the rats were anesthetized with CO₂ and decapitated. The abdomen was opened longitudinally and the uterine horns were removed. Some uterine horns were used for measurements of *in vitro* contractility. Others were freed from endometrium and parametrium and were subsequently used for isolation of mRNA or plasma membranes.

Primers and construction of internal mRNA standard

The oligonucleotide primers (DNA Technology, Aarhus, Denmark) used for detection of OTR mRNA were selected from a sequence published by Rozen *et al.* (1995) using a computer program (Oligo Primer Analysis, National Biosciences, Plymouth, MN, USA):

Sense primer: 5' GGG ACG TCA ATG CGC CCA AGG AA 3' (nucleotides 2816–2838)

Antisense primer: 5' ACC AAT AGA CAC CTA ATG CA 3' (nucleotides 3921–3940)

Basic local alignment search tool (Blast) (Altschul *et al.* 1990) was used to search all non-redundant databases (GenBank+EMBL+DDBJ+PDB) for sequence homology. No homology with any known products other than the rat OTR was found. The amplified OTR DNA fragments consisted of 375 bp. The exact identity of the PCR product was confirmed by sequencing (Engstrøm *et al.* 1998). Using a PCR-MIMIC construction kit (Clontech, Palo Alto, CA, USA) an internal 240 bp DNA standard was constructed (Engstrøm *et al.* 1997). The internal standard RNA was constructed mainly as described by Faure *et al.* (1995). A composite primer, comprising 37 nucleotides of bacteriophage T7 RNA-polymerase promoter followed by the sequences of our usual sense primers, was used for amplification of the 240 bp DNA sequence by PCR. The resulting 277 bp product was re-amplified using our antisense primers and a primer consisting of the initial 23 nucleotides of the T7 RNA-polymerase promoter region. Following HPLC purification the re-amplified products were used for production of RNA by *in vitro* transcription (Riboprobe, Promega, Madison, WI, USA). The resulting RNA standards were quantitated by UV detection at 260 nm (Gene-quant, Pharmacia, Stockholm, Sweden). Subsequently the RNA standards underwent reverse transcription (RT) in order to verify that the resulting products were indistinguishable from the internal DNA standard.

Isolation of myometrial mRNA

The isolation of poly(A⁺) mRNA was performed using a MicroPoly(A)Pure kit (Ambion Inc., Austin, TX, USA). Approximately 30–300 mg myometrium free from parametrium and endometrium were homogenized in 800 µl lysis solution. An aliquot was mixed with two volumes dilution buffer. Tissue debris was removed by centrifugation for 10 min at 12 000 g and 4 °C. A total of 1 ml supernatant was combined with 20 mg oligo dT resin and agitated for 60 min at room temperature. The oligo dT resin was washed thrice with 1 ml binding buffer followed by three washings with 1 ml wash buffer. It was subsequently transferred to a spin column and centrifuged at 5000 g at room temperature. A total of 500 µl wash buffer was added to the column and the oligo dT resin re-centrifuged. To

ensure complete removal of ribosomal RNA this step was repeated until the absorbance of the flow-through wash at 260 nm was below 0.05. Poly(A⁺) mRNA was thereafter eluted with 200 µl 65 °C elution buffer followed by overnight precipitation in 20 µl 5 M NH₄OAC, 1 µl glycogen (5 mg/ml) and 500 µl 96% ethanol. The solution was centrifuged at 15 000 *g* for 20 min at 4 °C, the pellet washed with 70% ethanol to remove remaining salts and finally the pellet was resuspended in DEPC-H₂O and stored frozen at -80 °C.

RT-PCR

RT was performed in a mixture consisting of 250 µM dNTP, 40 U MMLV-RT (Promega), 31.2 U RNA-guard, 200 pmol antisense primer and 5 µl internal RNA standard in Promega RT buffer. Incubations were carried out for 60 min at 37 °C and the resulting cDNA used immediately or stored at -80 °C.

PCR was carried out with 5 µl cDNA, 37.5 µM of each dNTP, 1.0 U Taq polymerase (Pharmacia, Stockholm, Sweden), 40 pmol sense primer and antisense primer in PCR buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl₂, pH 9.0). Amplification took place in a Perkin Elmer Model 460 thermocycler. Cycling parameters were: 95 °C for 2 min followed by 27 cycles consisting of 1.5 min at 94 °C, 56 °C for 45 s and 70 °C for 2 min. After the last cycle the incubations continued for 5 min at 72 °C followed by lowering of the temperature to 4 °C. PCR products were used immediately or stored at -80 °C.

Quantitation of PCR products was carried out by means of HPLC using a TSK DEAE-NPR column (Engstrøm *et al.* 1997). Following chromatography PCR products were detected by UV at 254 nm (Fig. 1). The area of the 240 bp PCR products of the internal standard represented 0.050 amol. Hence, the amounts of the 375 bp PCR products could be quantitated relative to this standard and it was finally related to tissue wet weight. The average value of the uterine horns of each animal was calculated to represent the amount of specific mRNA.

Binding of [³H]-oxytocin to isolated myometrial plasma membranes

Preparation of rat myometrial plasma membranes was performed by subcellular fractionation (Engstrøm *et al.* 1988b). Subsequent binding of ³H-oxytocin was carried out as previously described (Engstrøm *et al.* 1988a). Plasma membranes were incubated for 60 min with concentrations of ³H-oxytocin varying from 0.42 to 24.20 nM (New England Nuclear, Boston, MA, USA; specific activity 35.0 Ci/mmol). The assay was performed at room temperature and initiated by the addition of plasma membranes. At the end of the incubation period bound ligand was separated from unbound by filtration. Specific binding of [³H]-oxytocin was calculated by subtraction of

Figure 1 HPLC trace of PCR products. The internal standard RNA is indicated by the 240 bp product and represents 0.050 amol. The OTR product is represented as indicated. By comparing the areas under the curves of the two products it is possible to calculate the amount of OTR present in the tissue specimen.

binding in the presence of a 1000-fold excess of unlabeled oxytocin and was finally related to protein content *ad modum* Lowry *et al.* (1951). To ensure that the effect of isoproterenol infusion on the subsequent binding of ³H-oxytocin was not caused by residual isoproterenol in the plasma membrane preparation we investigated the ability of isoproterenol to affect ³H-oxytocin binding. ³H-oxytocin at concentrations of 6.05 and 12.10 nM was incubated in the presence and absence of 1 µM isoproterenol. Binding of the tritiated ligand was unaffected by isoproterenol.

In vitro examination of contractile force of myometrial strips

In vitro contractility was measured as described previously (Engstrøm *et al.* 1997). One uterine horn was opened longitudinally and a middle segment measuring 5 mm was mounted in an isometric myograph connected to a Grass force transducer, the resting tension being 1.5 g. The strip was placed in an organ bath containing 8 ml Munsick's buffer, 30 °C, pH 7.4 (Munsick 1960) and allowed to rest for 30 min. Every 10 min during this period the buffer was refreshed in order to wash out any isoproterenol potentially remaining in the tissue specimen. Before stimulation with oxytocin the specimens were further washed five times. The tissue was thereafter contracted with 50 mM KCl. Contractile responsiveness to oxytocin doses in the range

Figure 2 OTR mRNA (left panel) and OTR binding (right panel) following treatment for 3 days with estradiol-benzoate ($50 \mu\text{g} \times 1$, i.m.), progesterone ($100 \mu\text{g} \times 1$, i.m.) or a combination of both (same doses as in individual regimens). Crosshatched bars indicate additional treatment with isoproterenol ($10 \mu\text{g/h}$) for 3 days whereas grey bars indicate additional treatment with saline ($1 \mu\text{l/h}$) for 3 days. * $P<0.05$ and ** $P<0.001$ when isoproterenol was substituted with saline. Values are means $\pm$ S.E.M., $n=3-5$.

of 0.63×10^{-10} – 0.13×10^{-6} M was measured. Following stimulation with KCl and following every stimulation with oxytocin the chamber was washed five times with Munsick's buffer. During the experiment the buffer was constantly aerated with 5% CO_2 in O_2 . Responses following oxytocin stimulation were expressed as a percentage of the potassium induced contraction and were plotted against the logarithm to the agonist concentration.

Data analysis

A computer program (Fig. P., Biosoft, Cambridge, UK) was used for data analysis. A four-parameter non-linear curve-fitting model was used to evaluate myometrial responsiveness to oxytocin. Maximal contraction (E_{max}) and the agonist concentration giving half this effect (EC_{50}) were obtained from curve-fits of individual dose-response curves using the equation: $E = E_{\text{min}} + (E_{\text{max}} - E_{\text{min}}) / (1 + ([\text{OT}] / \text{EC}_{50})^{-p})$.

Specific binding data from saturation binding experiments were analyzed using the equation: specific binding = $[^3\text{H-OT}] \times B_{\text{max}} / ([^3\text{H-OT}] + K_D)$. Subsequently maximal specific binding (B_{max}) and dissociation constants (K_D) were obtained from linear regression of individual double reciprocal plots following the equation: $1/\text{specific binding} = 1/B_{\text{max}} + K_D/B_{\text{max}} \times 1/[^3\text{H-OT}]$.

One way analysis of variance or Kruskal-Wallis tests were used to compare multiple groups. Significant effects among individual means were subsequently separated using *post-hoc* tests for multiple comparison (Student-Newman-Keuls or Dunn's method). Student's *t*-test or the Mann-Whitney test were used to determine whether mRNA, receptor binding or *in vitro* contractility following isoproterenol treatment were statistically different from saline values. $P<0.05$ was considered statistically significant. Results are presented as means $\pm$ S.E.M. unless otherwise stated.

Results

OTR mRNA

Myometrial OTR mRNA results are shown in Fig. 2. Analysis of variance revealed an overall statistical difference between the steroid treatment groups not receiving isoproterenol ($P=0.003$). Subsequent *post-hoc* tests showed that estrogen treatment increased OTR mRNA when compared with the vehicle group ($P<0.05$). When estrogen was administered together with progesterone this effect did, however, disappear. Progesterone treatment did not in itself significantly change OTR mRNA when compared with vehicle.

Figure 3 Dose-response curves of isolated uterine strips challenged with oxytocin. Strips were obtained from rats treated with estradiol-benzoate ($50 \mu\text{g} \times 1$, i.m.), progesterone ($100 \mu\text{g} \times 1$, i.m.) or a combination of both (same doses as in individual regimens) for 3 days. The rats were additionally treated with saline ($1 \mu\text{l/h}$, ○) or isoproterenol ($10 \mu\text{g/h}$, ●) for 3 days. Values are means $\pm$ S.E.M., $n=3-4$.

Isoproterenol infusion increased OTR mRNA 13-fold in the progesterone treatment group ($P=0.047$) and 5-fold in the combined steroid treatment group ($P=0.040$). In contrast isoproterenol had no effect on OTR mRNA in the control group or the estrogen treatment group.

Binding of [^3H]-oxytocin to isolated myometrial plasma membranes

Specific binding of [^3H]-oxytocin to isolated plasma membranes was saturable and time-dependent. Equilibrium was attained after 15 min. Double reciprocal plots revealed binding sites of only a single affinity and they were used to calculate receptor affinity (K_D) and maximal specific binding (B_{max}) values.

B_{max} between steroid treatment groups not receiving isoproterenol were statistically different ($P<0.001$). *Post-hoc* analysis showed that estrogen treatment increased B_{max} when compared with the control group ($P<0.05$, Fig. 2). This increase was blocked by co-treatment with progesterone. Progesterone treatment alone did not alter B_{max} when compared with control (Fig. 2). Isoproterenol treatment enhanced B_{max} in both the progesterone ($P<0.001$) and the combined steroid group ($P=0.010$) but not in the control or estrogen group.

K_D values were in the nanomolar range and they varied from 0.67 ± 0.30 to 1.32 ± 0.79 nM. No statistical differences were found between the groups.

Contractile activity of isolated uterine strips

Dose-response curves from oxytocin stimulated uterine strips are shown in Fig. 3. Both maximal contractile effect (E_{max}) and the concentration giving half this effect (EC_{50}) were unaffected by any of the steroid treatments ($P=0.830$ and $P=0.252$ respectively, Table 1). Isoproterenol infusion significantly increased E_{max} by 30% in the estrogen treatment group ($P=0.029$). However, when both estrogen and progesterone were administered isoproterenol enhanced E_{max} further, namely by 40% ($P=0.004$). EC_{50} values were left unchanged following isoproterenol treatment in all groups.

To investigate whether the effects of β_2 -stimulation on myometrial contractility were merely caused by general metabolic changes induced by isoproterenol we compared uterine responses to potassium chloride among the different experimental groups. No significant changes were found between isoproterenol infused rats and control rats in either of the steroid treatment groups (3.03 ± 0.60 vs 2.91 ± 0.70 g (isoproterenol vs control, no steroid), 3.85 ± 0.73 vs 4.84 ± 1.28 g (isoproterenol vs control,

Table 1 EC50 and E_{max} values of isolated uterine strips challenged with oxytocin. Uteri were isolated from rats treated for 3 days with estradiol-benzoate (50 µg × 1), progesterone (100 µg × 1) and isoproterenol (10 µg/h) in the combinations indicated. Values are means ± S.E.M., n=3–4

Treatment	EC50 (nM)	E _{max} (% of KCl response)
Vehicle	0.75 ± 0.34 ^{n.s.}	94.3 ± 3.82
Vehicle+isoproterenol	0.51 ± 0.27	94.7 ± 6.88 ^{n.s.}
Estrogen	0.42 ± 0.14	93.3 ± 2.59
Estrogen+isoproterenol	0.47 ± 0.25 ^{n.s.}	122.0 ± 13.1*
Progesterone	0.18 ± 0.10	98.0 ± 12.2
Progesterone+isoproterenol	0.17 ± 0.06 ^{n.s.}	91.0 ± 4.34 ^{n.s.}
Estrogen+progesterone	0.22 ± 0.09	89.3 ± 6.75
Estrogen+progesterone+isoproterenol	0.15 ± 0.03 ^{n.s.}	123.0 ± 3.46**

*P<0.05, **P<0.005; n.s.: statistically insignificant.

estrogen treatment), 1.90 ± 0.37 vs 2.28 ± 0.33 g (isoproterenol vs control, progesterone treatment) and 3.71 ± 0.38 vs 4.13 ± 0.67 g (isoproterenol vs control, progesterone plus estrogen treatment)).

Discussion

The present study shows that rat myometrial OTR gene expression and OTR binding are under the influence of β₂-adrenoceptor activation and gonadal steroids. Estrogen treatment alone increased both parameters but did not affect myometrial contractility when evaluated by means of isolated uterine strips stimulated with oxytocin. On the other hand, when estrogen was combined with isoproterenol an augmented E_{max} was observed although no further increase in OTR mRNA and binding was seen. Progesterone did not in itself alter OTR mRNA, OTR binding or maximal contractility of isolated uterine strips. However, OTR formation was induced at the level of gene expression when progesterone injections were supplemented with isoproterenol infusion. Finally progesterone suppressed the effect of estrogen on mRNA production and binding when the two compounds were administered together. However, when isoproterenol treatment was added this effect was abolished and the maximal contractile ability of oxytocin on isolated strips was enhanced more than that seen following treatment with estrogen alone.

The induction of OTR expression by estrogen shown in the present study is in accordance with previous findings (Alexandrova & Soloff 1980, Fuchs *et al.* 1983a,b). It is promoted by activation of an estrogen response element within the OTR gene (Bale & Dorsa 1997). The contractile ability of isolated uterine strips challenged with oxytocin, however, remained unchanged in estrogen treated rats unless the treatment was supplemented with isoproterenol infusion. Because isoproterenol did not alter the binding of [³H]-oxytocin, the effect of isoproterenol

appears not to be mediated by a direct isoproterenol–OTR interaction. In addition, in the estrogen treated group, OTR mRNA and binding were unaffected by isoproterenol when compared with the estrogen treatment alone. We thus suggest that β₂-adrenoceptor activation may augment the coupling between OTR occupancy and contractile responsiveness. Since β₂-adrenoceptor stimulation raises intracellular cAMP and OTR activation increases phospholipase C activity the mechanism described constitutes an example of cross-talk between two different receptor–effector systems. Likewise, Pittner & Fain (1989, 1990) observed that intracellular signaling of another phospholipase C linked receptor, namely the hepatic vasopressin receptor, was enhanced by cAMP-dependent kinase (protein kinase A).

The ability of oxytocin to induce contractions of isolated uterine strips is dependent on intact intercellular communications, which are mainly controlled by the presence of gap junctions (Garfield *et al.* 1988). Gap junction-mediated intercellular communication was enhanced following treatment with the cAMP analogue 8-bromo-cAMP of both human (Burghardt *et al.* 1996) and rabbit (Nnamani *et al.* 1994) myometrial cells. In addition, the gap junction protein connexin-43 was regulated independently of estrogen receptor concentrations in human myometrium (Geimonen *et al.* 1998) and we therefore speculate whether the increase in E_{max} following isoproterenol infusion in the estrogen treated group may originate from a rise in the number of gap junctions.

The effects of isoproterenol on OTR mRNA and binding in the progesterone treated group are in agreement with the finding by Bale & Dorsa (1998) who found the OTR gene to contain a cAMP response element which enhances OTR transcription. However, in the presence of progesterone, isoproterenol did not increase the myometrial response to oxytocin, suggesting that newly formed OTRs remain uncoupled in the absence of other stimuli. Several mechanisms may account for the

lack of coupling of those OTRs induced by combined treatment with isoproterenol and progesterone. As observed by Lécivain *et al.* (1998) G_{α} is increased by isoproterenol in pregnant rat myometrium and thus OTR signaling may be altered. Other explanations may include progesterone-induced alterations in myosin light-chain kinase activity promoted by cAMP dependent phosphorylation of this enzyme (Badia *et al.* 1986) or a negative effect of progesterone on gap junction formation as suggested by Hendrix *et al.* (1995).

When both estrogen and progesterone were administered the enhancing effect of isoproterenol on OTR production was paralleled by an increase in $E_{\max}$, which was more pronounced than the effect of isoproterenol on $E_{\max}$ when estrogen was the only steroid given. This might indicate that the enhancing effect of β_2 -adrenoceptor activation on the synthesis of functional OTR operates only in the presence of both steroids. Nevertheless, the fact that isoproterenol was not able to increase OTR mRNA and binding among estrogen treated rats is apparently intriguing due to the presence of the cAMP response element within the OTR gene. However, estrogen is, in itself, a strong promoter of OTR expression, and the relatively high dose of estrogen administered might therefore increase OTR transcription rate to an extent which blunts a qualitatively similar effect of β_2 -adrenoceptor activation.

A proposed role of β_2 -adrenoceptor stimulation in the up-regulation of OTR may appear contradictory, since we have earlier published that isoproterenol *in vitro* attenuates myometrial contractility (Engstrøm *et al.* 1997). Indeed, obstetricians use β_2 -stimulation to prevent pre-term labour (Caritis *et al.* 1983). Several studies have, however, shown that continuous exposure to tocolytics of this kind results in a loss of myometrial responsiveness to subsequent β_2 -adrenoceptor activation (Berg *et al.* 1985, Abel & Hollingsworth 1986). Based on the present results we suggest that this effect may involve the induction of OTR expression.

Myometrial sensitivity to oxytocin is increased at term primarily due to a rise in the number of OTRs (Hirata *et al.* 1996). Some studies have shown that rat (Larcher *et al.* 1995, Quinones Jenab *et al.* 1997), rabbit (Jacobson *et al.* 1987) and ewe (Crankshaw *et al.* 1982) OTR are up-regulated by estrogens and down-regulated by progesterone. It has thus been proposed that the withdrawal of progesterone observed in rodents prior to term constitute the signal responsible for the up-regulation of OTR and the subsequent initiation of parturition (Csapo 1956). The idea of progesterone withdrawal as a prerequisite for parturition is, however, not valid in all species. In guinea pig (Porter 1970) and human pregnancy (Yannone *et al.* 1968, Tulchinsky *et al.* 1972) progesterone levels do not decline before the onset of labor and in human pregnancy (Lofgren & Backstrom 1997) high progesterone concentrations during parturition appeared

to be related to effective labor. Interestingly, Vallet *et al.* (1990) observed an increase in ewe uterine OTR following 12 days progesterone treatment and in accordance with the present results, Ruzicky & Crankshaw (1988) observed no inhibitory effect of progesterone on oxytocin induced uterine contractility in estrogen dominated rats. Even within the same species the effects of progesterone on OTR differ in different tissues (Kremarik *et al.* 1995). These observations altogether suggest that other factors than progesterone and estrogen influence myometrial OTR expression and function. In this context, and in view of the remarkable increase in maternal catecholamines (Suzuki *et al.* 1989) during labor, our results suggest that β_2 -adrenoceptor activation may be of importance.

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References

- Abel MH & Hollingsworth M 1986 Comparison of nifedipine and diltiazem with salbutamol for prevention of preterm delivery in the ovariectomized, oestrogen-treated late pregnant rat. *Journal of Reproduction and Fertility* **77** 559–568.
- Alexandrova M & Soloff MS 1980 Oxytocin receptors and parturition. I. Control of oxytocin receptor concentration in the rat myometrium at term. *Endocrinology* **106** 730–735.
- Altschul SF, Gish W, Miller W, Myers EW & Lipman DJ 1990 Basic local alignment search tool. *Journal of Molecular Biology* **215** 403–410.
- Anwer K, Hovington JA & Sanborn BM 1989 Antagonism of contractants and relaxants at the level of intracellular calcium and phosphoinositide turnover in the rat uterus. *Endocrinology*, **124** 2995–3002.
- Anwer K, Hovington JA & Sanborn BM 1990 Involvement of protein kinase A in the regulation of intracellular free calcium and phosphoinositide turnover in rat myometrium. *Biology of Reproduction* **43** 851–859.
- Badia E, Nicolas JC, Haiech J & Crastes de Paulet A 1986 Effect of steroid hormones on the regulation of uterine contractility. *Pflugers Archiv* **407** 670–676.
- Bale T & Dorsa D 1997 Cloning, novel promoter sequence, and estrogen regulation of a rat oxytocin receptor gene. *Endocrinology* **138** 1151–1158.
- Bale TL & Dorsa DM 1998 NGF, cyclic AMP, and phorbol esters regulate oxytocin receptor gene transcription in SK-N-SH and MCF7 cells. *Molecular Brain Research* **53** 130–137.

- Berg G, Andersson RG & Ryden G 1985 Beta-adrenergic receptors in human myometrium during pregnancy: changes in the number of receptors after beta-mimetic treatment. *American Journal of Obstetrics and Gynecology* **151** 392–396.
- Burghardt RC, Barhoumi R, Stickney M, Monga M, Ku CY & Sanborn BM 1996 Correlation between connexin43 expression, cell-cell communication, and oxytocin-induced Ca^{2+} responses in an immortalized human myometrial cell line. *Biology of Reproduction* **55** 433–438.
- Caritis SN, Lin LS, Toig G & Wong LK 1983 Pharmacodynamics of ritodrine in pregnant women during preterm labor. *American Journal of Obstetrics and Gynecology* **147** 752–759.
- Crankshaw DJ, Romaniuk E & Branda LA 1982 Identification and characterization of receptors for oxytocin in the myometrium of the pregnant ewe. *Gynecologic and Obstetric Investigation* **14** 202–213.
- Csapo AI 1956 Progesterone block. *American Journal of Anatomy* **98** 273–291.
- Engstrom T, Atke A & Vilhardt H 1988a Receptor-binding characteristics and contractile responsiveness of the myometrium following prolonged infusion of bradykinin and oxytocin in rats. *Journal of Endocrinology* **118** 81–85.
- Engström T, Atke A & Vilhardt H 1988b Oxytocin receptors and contractile response of the myometrium after long term infusion of prostaglandin F2 alpha, indomethacin, oxytocin and an oxytocin antagonist in rats. *Regulatory Peptides* **20** 65–72.
- Engström T, Bratholm P, Vilhardt H & Christensen NJ 1997 Effect of pregnancy on rat myometrial beta 2-adrenoceptor mRNA and isoproterenol-induced relaxation of isolated uterine strips. *Journal of Endocrinology* **153** 393–399.
- Engström T, Bratholm P, Vilhardt H & Christensen NJ 1998 β_2 -desensitization in non-pregnant estrogen primed rat myometrium involves modulation of oxytocin receptor gene expression. *Journal of Molecular Endocrinology* **20** 261–270.
- Faure C, Gouhier C, Langer SZ & Graham D 1995 Quantification of alpha-1-adrenoceptor subtypes in human tissues by competitive RT-PCR analysis. *Biochemical and Biophysical Research Communications* **213** 935–943.
- Fuchs AR, Periyasamy S, Alexandrova M & Soloff MS 1983a Correlation between oxytocin receptor concentration and responsiveness to oxytocin in pregnant rat myometrium: effects of ovarian steroids. *Endocrinology* **113** 742–749.
- Fuchs AR, Periyasamy S & Soloff MS 1983b Systemic and local regulation of oxytocin receptors in the rat uterus, and their functional significance. *Canadian Journal of Biochemistry and Cell Biology* **61** 615–624.
- Fuchs AR, Helmer H, Behrens O, Liu HC, Antonian L, Chang SM & Fields MJ 1992 Oxytocin and bovine parturition: a steep rise in endometrial oxytocin receptors precedes onset of labor. *Biology of Reproduction* **47** 937–944.
- Ganjam VK, Kenney RM & Flickinger G 1975 Effect of exogenous progesterone on its endogenous levels: biological half-life of progesterone and lack of progesterone binding in mares. *Journal of Reproduction and Fertility* **23** 183–188.
- Garfield RE, Blennerhassett MG & Miller SM 1988 Control of myometrial contractility: role and regulation of gap junctions. *Oxford Review of Reproductive Biology* **10** 436–490.
- Geimonen E, Boylston E, Royek A & Andersen J 1998 Elevated connexin-43 expression in term human myometrium correlates with elevated c-Jun expression and is independent of myometrial estrogen receptors. *Journal of Clinical Endocrinology and Metabolism* **83** 1177–1185.
- Grazzini E, Guillon G, Mouillac B & Zingg HH 1998 Inhibition of oxytocin receptor function by direct binding of progesterone. *Nature* **392** 509–512.
- Hendrix EM, Myatt L, Sellers S, Russell PT & Larsen WJ 1995 Steroid hormone regulation of rat myometrial gap junction formation: effects on cx43 levels and trafficking. *Biology of Reproduction* **52** 547–560.
- Hilliard J, Spies HG & Sawyer CH 1968 Cholesterol storage and progesterin secretion during pregnancy and pseudopregnancy in the rabbit. *Endocrinology* **82** 157–165.
- Hirata J, Kikuchi Y, Imaizumi E, Furuya K & Nagata I 1996 Correlation between the stimulatory effect of oxytocin on the formation of inositol phosphates and the oxytocin receptor level in the pregnant rabbit myometrium. *Journal of Obstetrics and Gynaecology* **22** 497–506.
- Hixon JE & Flint AP 1987 Effects of a luteolytic dose of oestradiol benzoate on uterine oxytocin receptor concentrations, phosphoinositide turnover and prostaglandin F-2 alpha secretion in sheep. *Journal of Reproduction and Fertility* **79** 457–467.
- Jacobson L, Riemer RK, Goldfien AC, Lykins D, Siiteri PK & Roberts JM 1987 Rabbit myometrial oxytocin and alpha 2-adrenergic receptors are increased by estrogen but are differentially regulated by progesterone. *Endocrinology* **120** 1184–1189.
- Jeng YJ, Hinko A & Soloff MS 1995 Effectors of cyclic adenosine 5'-monophosphate up-regulating oxytocin receptors in rabbit amnion cells: isoproterenol, parathyroid hormone-related protein, and potentiation by cortisol. *Biology of Reproduction* **53** 1051–1056.
- Kremarik P, Freund Mercier MJ & Stoeckel ME 1995 Estrogen-sensitive oxytocin binding sites are differently regulated by progesterone in the telencephalon and the hypothalamus of the rat. *Journal of Neuroendocrinology* **7** 281–289.
- Larcher A, Neculcea J, Breton C, Arslan A, Rozen F, Russo C & Zingg HH 1995 Oxytocin receptor gene expression in the rat uterus during pregnancy and the estrous cycle and in response to gonadal steroid treatment. *Endocrinology* **136** 5350–5356.
- Lau TM, Kerton DJ, Gow CB & Fairclough RJ 1992 Increase in concentration of uterine oxytocin receptors and decrease in response to 13,14-dihydro-15-keto prostaglandin F2 alpha in ewes after withdrawal of exogenous progesterone. *Journal of Reproduction and Fertility* **95** 885–893.
- Lécrivain JL, Cohen Tannoudji J, Robin MT, Coudouel N, Legrand C & Maltier JP 1998 Molecular mechanisms of adenyl cyclase desensitization in pregnant rat myometrium following *in vivo* administration of the beta-adrenergic agonist, isoproterenol. *Biology of Reproduction* **59** 45–52.
- Lofgren M & Backstrom T 1997 High progesterone is related to effective human labor. Study of serum progesterone and 5alpha-pregnane-3,20-dione in normal and abnormal deliveries. *Acta Obstetrica et Gynecologica Scandinavica* **76** 423–430.
- Lowry OH, Rosebrough NJ, Farr AL & Randall RJ 1951 Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193** 265–275.
- Munsick RA 1960 Effect of magnesium ion on the response of the rat uterus to neurohypophysial hormones and analogues. *Endocrinology* **66** 451–457.
- Nnamani C, Godwin A, Ducsay CA, Longo LD & Fletcher WH 1994 Regulation of cell-cell communication mediated by connexin 43 in rabbit myometrial cells. *Biology of Reproduction* **50** 377–389.
- Pittner RA & Fain JN 1989 Exposure of cultured hepatocytes to cyclic AMP enhances the vasopressin-mediated stimulation of inositol phosphate production. *Biochemical Journal* **257** 455–460.
- Pittner RA & Fain JN 1990 Vasopressin and norepinephrine stimulation of inositol phosphate accumulation in rat hepatocytes are modified differently by protein kinase C and protein kinase A. *Biochimica et Biophysica Acta* **1043** 211–217.
- Porter DG 1970 The failure of progesterone to affect myometrial activity in the guinea-pig. *Journal of Endocrinology* **46** 425–434.
- Quinones Jenab V, Jenab S, Ogawa S, Adan RA, Burbach JP & Pfaff DW 1997 Effects of estrogen on oxytocin receptor messenger ribonucleic acid expression in the uterus, pituitary, and forebrain of the female rat. *Neuroendocrinology* **65** 9–17.

- Rozen F, Russo C, Banville D & Zingg HH 1995 Structure, characterization, and expression of the rat oxytocin receptor gene (published erratum appears in *Proceedings of the National Academy of Sciences of the USA* 1996 **93** 12051). *Proceedings of the National Academy of Sciences of the USA* **92** 200–204.
- Ruzycky AL & Crankshaw DJ 1988 Role of inositol phospholipid hydrolysis in the initiation of agonist-induced contractions of rat uterus: effects of domination by 17 beta-estradiol and progesterone. *Canadian Journal of Physiology and Pharmacology* **66** 10–17.
- Sheldrick EL & Flint AP 1985 Endocrine control of uterine oxytocin receptors in the ewe. *Journal of Endocrinology* **106** 249–258.
- Sheldrick EL & Flick Smith HC 1993 Effect of ovarian hormones on oxytocin receptor concentrations in explants of uterus from ovariectomized ewes. *Journal of Reproduction and Fertility* **97** 241–245.
- Silvia WJ, Lewis GS, McCracken JA, Thatcher WW & Wilson L Jr. 1991 Hormonal regulation of uterine secretion of prostaglandin F2 alpha during luteolysis in ruminants. *Biology of Reproduction* **45** 655–663.
- Stürmer E 1968 The rat uterus assay procedure. In *Handbook of Experimental Pharmacology*, pp 130–189. Ed B Berde. Berlin, Heidelberg, New York: Springer Verlag.
- Suzuki A, Hashino M, Chiba H, Saito H, Notake Y, Yanaihara T & Nakayama T 1989 Correlation between the levels of catecholamines (noradrenaline, adrenaline) and adrenal steroids (DHA-S, cortisol) in maternal and fetal blood during pregnancy and labor. *Journal of the Japan Veterinary Medical Association* **65** 704–714.
- Tulchinsky D, Hobel CJ, Yeager E & Marshall JR 1972 Plasma estrone, estradiol, estriol, progesterone, and 17-hydroxyprogesterone in human pregnancy. I. Normal pregnancy. *American Journal of Obstetrics and Gynecology* **112** 1095–1100.
- Vallet JL, Lamming GE & Batten M 1990 Control of endometrial oxytocin receptor and uterine response to oxytocin by progesterone and oestradiol in the ewe. *Journal of Reproduction and Fertility* **90** 625–634.
- Yannone ME, McCurdy JR & Goldfien A 1968 Plasma progesterone levels in normal pregnancy, labor, and the puerperium. II. Clinical data. *American Journal of Obstetrics and Gynecology* **101** 1058–1061.

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