Oxytocin receptor gene expression in rat uterus: regulation by ovarian steroids

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Abstract

The present study was designed to investigate a possible role for ovarian steroids in the regulation of rat uterine oxytocin receptor (OTR) mRNA expression before labour. By using a competitive RT-PCR system, we have previously reported that parturition was associated with high levels of uterine OTR mRNA in all the animals examined. On the other hand, near term, some rats showed high OTR mRNA levels while others did not. We therefore examined the changes in OTR mRNA expression before and during prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-induced parturition; a paradigm adopted to reduce the variation in the onset of parturition. Injection of $PGF_{2\alpha}$ on day 18 of pregnancy significantly increased OTR mRNA expression in all the rats within 24 h of treatment, suggesting that the variation in OTR mRNA levels during spontaneous parturition may be due to the difference in the timing of the onset of parturition. The increase in OTR mRNA was significantly abolished by injection of the anti-oestrogen compound, tamoxifen. The stimulatory action of oestrogen on OTR mRNA expression was then

examined in the presence or absence of ovarian factors. Pregnant rats were ovariectomized (OVX) or sham-operated on day 18 of pregnancy and either oestrogen or vehicle was administered 6 h after the surgical operation. Oestrogen increased OTR mRNA significantly in OVX rats 18 h after administration compared with sham-operated animals. Moreover, ovariectomy alone on day 18 of pregnancy increased OTR mRNA expression to a level which reached statistical significance 24 h after the operation. In addition, oestrogen treatment increased OTR mRNA levels in OVX virgin rats in which progesterone tubes were implanted for 1 week and removed 6 h before oestrogen injection. The stimulatory effect of oestrogen was not observed in rats in which the progesterone tubes were implanted for 1 week and not removed. These results suggest that the decline of progesterone is necessary for the expression of the stimulatory effects of oestrogen on uterine OTR mRNA.

Journal of Endocrinology (2000) **166**, 45–52

Introduction

Oxytocin (OT) was initially isolated as a neurohypophysial hormone which stimulates the contraction of the myometrium and myoepithelium to facilitate parturition and milk ejection respectively, and is considered to act on various reproductive functions as a neuroregulator or in a paracrine manner in the ovary or the uterus (Higuchi 1995). In the uterus, the near-term myometrium is extremely sensitive to OT and this increased uterine responsiveness to OT occurs in parallel with alteration in the number of uterine OT-binding sites in rats (Soloff *et al.* 1979, Fuchs *et al.* 1983), humans (Fuchs *et al.* 1984), rabbits (Maggi *et al.* 1988, 1991) and sheep (Wathes *et al.* 1996a, Wu *et al.* 1996a). The exact factors or sequence of events in late pregnancy which lead to the initiation of parturition and change in the dynamics of the OT-binding sites are not clearly understood at present. An understanding of the various physiological roles of OT, especially

in the initiation and progress of parturition, requires an elucidation of the mechanisms regulating uterine OT-receptor (OTR) synthesis near term.

Following cloning of the rat OTR gene (Rozen *et al.* 1995), several studies employing various methods have been reported on the regulation of uterine OTR mRNA expression during pregnancy and parturition (Larcher *et al.* 1995, Liu *et al.* 1996, Ou *et al.* 1998). A consistent finding among these studies is an increased OTR mRNA expression during delivery; however, the exact timing of the OTR mRNA increase is not still clearly understood. In two separate studies by Larcher *et al.* (1995) and Ou *et al.* (1998), no significant increase in OTR mRNA expression was observed before delivery. In our earlier study, using a competitive reverse transcription (RT)-PCR system, all parturient rats showed high levels of uterine OTR mRNA; while some near-term rats showed high OTR mRNA levels, others did not (Liu *et al.* 1996). In order to further understand how OTR mRNA expression is

regulated and how this is related to the onset of parturition, it is necessary to define why there is a variation in the increase in OTR mRNA expression before spontaneous parturition. One possible explanation is the difference in the timing of the onset of parturition, because rats used in our laboratory routinely start delivery from day 22 to day 23 of pregnancy. We have therefore examined the change in OTR mRNA expression before and during parturition induced by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) to reduce the variation in the onset of parturition.

Ovarian steroids, oestrogen and progesterone have been reported to be involved in the regulation of OT function. In this context, oestrogen stimulated the number of uterine OT-binding sites (Soloff 1975, Fuchs *et al.* 1983, Soloff *et al.* 1983) and OTR mRNA expression in ovariectomized (OVX) virgin rats (Larcher *et al.* 1995, Liu *et al.* 1996). Concomitant injection of progesterone with oestrogen suppressed the stimulatory effect of oestrogen on OT binding (Soloff 1975, Fuchs *et al.* 1983, Soloff *et al.* 1983), probably via a direct effect of progesterone on the uterine OTR in part (Grazzini *et al.* 1998), but did not inhibit its effect on OTR mRNA expression (Larcher *et al.* 1995, Liu *et al.* 1996). On the other hand, treatment with the anti-progesterone compound, RU486, increased OTR mRNA expression in the rat uterus (Fang *et al.* 1997). The increased expression of OTR mRNA resulting from ovariectomy in late pregnancy was inhibited by progesterone injection (Ou *et al.* 1998). Thus, progesterone appears to be involved in the regulation of OTR mRNA expression, but its effect on the role of oestrogen in OTR mRNA expression is not clear. In this study, we have examined further the involvement of progesterone in the stimulatory action of oestrogen on OTR mRNA expression in both pregnant and OVX virgin rats.

Materials and Methods

Animals

Adult female Wistar rats (body weight 180–220 g) were obtained from Japan SLC (Hamamatsu, Japan). They were kept in an environmentally controlled room (temperature $23 \pm 3^\circ\text{C}$; lights on 0500–1900 h in experiments 1–3, lights on 0800–2000 h in experiment 4). They were allowed free access to tap water and pelleted rat food (NMF; Oriental Yeast Co. Ltd, Tokyo, Japan). Oestrous cycles were monitored by vaginal smears taken daily in the morning (0900–1000 h). Pro-oestrous females were allowed to mate with conspecific males housed under similar conditions and this was designated day 1 of pregnancy if spermatozoa were present in the vaginal smear the following morning.

Experiment 1: effects of $PGF_{2\alpha}$ in pregnant rats

At 1100–1130 h on day 18 of pregnancy, the rats were injected s.c. with 500 μg $PGF_{2\alpha}$ (Wako Chemicals Co.,

Osaka, Japan) in 0.2 ml 99% ethanol or vehicle alone. The rats delivered within 29 to 35 h after the $PGF_{2\alpha}$ treatment. The $PGF_{2\alpha}$ -treated rats were killed 6 ($n=3$), 12 ($n=4$), 18 ($n=6$), 24 ($n=6$), 30 ($n=6$) and 54 h ($n=3$) after the treatment and during delivery ($n=7$). Control rats ($n=4$) received vehicle alone and were killed 29–35 h after the treatment (day 19 of pregnancy). The uterine horns were quickly dissected, immediately frozen in liquid nitrogen and stored at -70°C until RNA extraction.

Experiment 2: effects of tamoxifen in $PGF_{2\alpha}$ -treated pregnant rats

At 1100–1130 h on day 18 of pregnancy, the rats were injected s.c. with 500 μg $PGF_{2\alpha}$ followed by 250 μg tamoxifen (Sigma, St Louis, MO, USA) in 0.25 ml sesame oil 6 h later ($n=6$). Control animals were injected with $PGF_{2\alpha}$ /sesame oil ($n=8$) and ethanol/sesame oil ($n=4$). The rats were killed at 1100–1130 h on day 19 of pregnancy.

Experiment 3: effects of ovariectomy on oestrogen action in pregnant rats

On day 18 of pregnancy, the rats were ovariectomized or sham-operated under ether anaesthesia at 1030–1200 h and were then given an s.c. injection of 17β -oestradiol (Sigma; 12.5 μg in 0.2 ml sesame oil) or vehicle alone at 1700–1730 h ($n=5$ each group). The rats were killed at 1100–1200 h on day 19 of pregnancy.

Experiment 4: effects of progesterone on oestrogen action in virgin OVX rats

Virgin rats were ovariectomized under ether anaesthesia 2 weeks before the steroid treatments and then divided into six groups (groups 1–6, $n=5$). Silicon tubing (outside diameter, 3 mm; inside diameter, 2 mm) was filled with crystalline progesterone (Nacalai Tesque Inc., Kyoto, Japan), sealed with silicon glue and incubated with phosphate-buffered saline (pH 7.4) for 48 h. Groups 1 and 2 were implanted with three 30 mm empty tubes (day 1). Groups 3–6 were implanted with tubes containing progesterone (day 1). All the rats were then anaesthetised with ether at 1100–1130 h on day 8, and the tubes were removed from animals in groups 1–4 but not from animals in groups 5 and 6. The rats in groups 2, 4 and 6 were given an s.c. injection of 17β -oestradiol (12.5 μg in 0.2 ml sesame oil) at 1700–1730 h on day 8, and the rats in groups 1, 3 and 5 were given vehicle alone. All the rats were killed at 1100–1200 h on day 9.

Competitive RT-PCR analysis

Complementary DNA synthesis, PCR amplification and analysis of PCR products were performed as described

previously (Liu *et al.* 1996). Briefly, uterine tissue (50–100 mg) was homogenised with TRIzol reagent (Gibco BRL, Gaithersburg, MD, USA). Total RNA samples were prepared according to the acid guanidinium thiocyanate-phenol-chloroform extraction method (Chomczynski & Sacchi 1987) using TRIzol reagent and treated with RNase-free DNaseI (Gibco BRL) to exclude genomic DNA. The quantity of total RNA was assessed with a spectrophotometer at a wave length of 260 nm. Total RNA (1 µg) of samples and serial diluted total RNA from uterus during labour (1000, 250, 62.5, 15.6 and 3.9 ng/tube) as standards, together with 1×10^7 copies of internal cRNA, were reverse transcribed with 200 U SuperScript II reverse transcriptase according to the supplier's manual (Gibco BRL) and 10 pmol 9-mer random primer. All the samples and the standards in each experiment were assayed in a single run. Internal cRNA was constructed by the insertion of a 106 bp PvuII/SmaI fragment of pUC19 plasmid into a StuI site of 161 bp OTR DNA. Both cDNAs reverse-transcribed from native OTR mRNA and internal cRNA were amplified with same primers, 5'-CGATTGCTGGGCGGTCTT-3' and 5'-CCGCCGCTGCCGTCTTGA-3', to 161 bp and 267 bp respectively. The PCR amplification was carried out in PCR buffer (50 mM KCl, 2 mM MgCl₂ and 20 mM Tris-HCl, pH 8.4), with 0.2 µmol primers and 2.5 U Taq polymerase (Gibco BRL) in a total volume of 50 µl for 35 cycles consisting of denaturation at 94 °C for 30 s, annealing at 62 °C for 20 s and extension at 72 °C for 1 min after the initial denaturation at 94 °C for 10 min. Amplification was completed with an additional extension step at 72 °C for 10 min. The PCR products were separated on 2% agarose gel containing ethidium bromide (0.5 µg/ml) and fluorescence density was analysed using NIH Image software. The linear standard curve was obtained and no band was detected when the PCR reaction was carried out with total RNA sample without the reverse transcription to check contamination with genomic DNA or non-specific reaction in each experiment. The ratio of native OTR mRNA-derived DNA (161 bp) to internal cRNA-derived DNA (267 bp) of each sample was obtained and calibrated from a standard curve as the amount of standard total mRNA. Data are expressed as the relative amounts (%) by dividing the value of each sample with the mean value of the corresponding control group.

Progesterone assay

Serum progesterone concentrations were measured using double-antibody assay kits purchased from Daiichi Radioisotope Laboratories Ltd (Tokyo, Japan) in duplicate on ether extracts of serum obtained from trunk blood.

Statistical analysis

The data are expressed as the mean $\pm$ s.e. and evaluated statistically using Student's *t*-test or one-way ANOVA

Figure 1 Relative amounts of uterine OTR mRNA levels in PGF_{2α}-treated pregnant rats. Circles and bars indicate individual and mean values respectively. Rats were injected with PGF_{2α} (500 µg, s.c.) at 1100–1130 h on day 18 of pregnancy. The value of control (cont.) rats which were treated with vehicle and killed 29–35 h after the treatment was defined as 100%. Values shown in parentheses are the number in each group. **P*<0.05, ***P*<0.01 compared with control group (Scheffe's *F* test).

followed by Scheffes's *F* test or Fisher's Protected Least Significant Difference (PLSD) test.

Results

Experiment 1: effect of PGF_{2α} in pregnant rats

The effects of PGF_{2α} treatment on OTR mRNA expression in pregnant rats were examined. There was an increase in the OTR mRNA level 12 h and 18 h after treatment. This increase in OTR mRNA expression reached a significant level 24 h after treatment and was maintained until during labour (Fig. 1). Serum progesterone levels began to decline from 12 h after the PGF_{2α} treatment and were maintained at low levels up to 30 h after the treatment (Table 1).

Experiment 2: effect of tamoxifen in PGF_{2α}-treated pregnant rats

Next, the involvement of oestrogen in the increase in OTR mRNA levels after PGF_{2α} treatment was examined. Tamoxifen, an anti-oestrogenic agent, was administered 6 h after the PGF_{2α} treatment. The PGF_{2α} treatment increased (*P*<0.01) OTR mRNA level at 24 h after treatment and this increase was significantly abolished

Table 1 Serum progesterone levels after PGF_{2α} treatment in pregnant rats. Data are expressed as the mean ± s.e. (n ≥ 3)

Serum progesterone (ng/ml)	
Treatment ^a	
PGF _{2α}	
6 h	28.0 ± 5.19
12 h	13.6 ± 3.99
18 h	7.43 ± 1.71
24 h	5.35 ± 0.97
30 h	4.58 ± 0.93
Vehicle	
30 h	29.2 ± 8.95

^aAnimals were injected s.c. with PGF_{2α} (500 µg) or vehicle at 1100–1130 h on day 18 of pregnancy.

Figure 2 Effect of tamoxifen on PGF_{2α}-induced increase of uterine OTR mRNA levels in pregnant rats. PGF_{2α} (500 µg, s.c.) was administered at 1100–1130 h on day 18 of pregnancy and tamoxifen (250 µg, s.c.) or vehicle was administered 6 h after the PGF_{2α} treatment. Rats were killed 24 h after the PGF_{2α} treatment. Data are expressed as the mean ± s.e. The value of control rats treated with vehicle alone was defined as 100%. Values shown in parentheses are the number in each group. Scheffe's F test was used for comparison of values.

(*P* < 0.05) by tamoxifen injection (Fig. 2). There was no significant difference between the control and the tamoxifen-treated rats.

Experiment 3: effect of ovariectomy on oestrogen action in pregnant rats

The stimulatory action of oestrogen on OTR mRNA expression was examined with or without ovarian factors. Rats were ovariectomized or sham-operated on day 18 of pregnancy. Oestrogen injection 6 h after the operation did

Figure 3 Effects of oestrogen on uterine OTR mRNA levels in OVX pregnant rats. Pregnant rats were ovariectomized or sham-operated at 1030–1200 h on day 18 of pregnancy and oestrogen (17β-estradiol, 12.5 µg, s.c.) or vehicle was administered 6 h after the operation. Rats were killed at 1100–1200 h on day 19 of pregnancy. Data are expressed as the mean ± s.e. (*n* = 5). The value of control rats was defined as 100%. **P* < 0.05 compared with the other three groups (Fisher's PLSD test).

not significantly increase OTR mRNA in sham-operated rats compared with the vehicle-treated rats (Fig. 3). Oestrogen treatment in OVX rats significantly increased OTR mRNA compared with either OVX or sham-operated rats without oestrogen treatment (Fig. 3). No significant difference (*P* = 0.08) was detected between the two vehicle-treated groups, the sham-operated rats and OVX rats without oestrogen treatment, when the data were analysed among the four groups using the PLSD test (Fig. 3). However, since there appeared to be a difference between these two vehicle-treated groups, the difference was re-examined between the groups using Student's *t*-test. This analysis revealed that ovariectomy in pregnant rats on day 18 alone had a small stimulatory effect on OTR mRNA expression (sham-operated 100 ± 5.1 (*n* = 9) versus OVX 165.8 ± 13.1 (%) (*n* = 10)).

Experiment 4: effects of progesterone on oestrogen action in virgin OVX rats

To understand the involvement of progesterone in the facilitation of oestrogen action after ovariectomy in pregnant rats, the effects of progesterone withdrawal on oestrogen action in OVX virgin rats were examined. The administration of oestrogen significantly increased OTR

Figure 4 Effects of progesterone on the stimulatory action of oestrogen in uterine OTR mRNA levels in OVX virgin rats. OVX virgin rats were divided into six groups (groups 1–6). Groups 1 and 2 were implanted with three empty tubes, and groups 3–6 were implanted with three tubes containing progesterone (day 1). The tubes were then removed at 1100–1130 h on day 8 in groups 1–4 and were not removed in groups 5 and 6. The rats in groups 2, 4 and 6 were given an s.c. injection of oestrogen (17 β -estradiol, 12.5 μ g) at 1700–1730 h on day 8, and the rats in groups 1, 3 and 5 were given vehicle alone. The rats were killed at 1100–1200 h on day 9. Data are expressed as the mean $\pm$ s.e. ($n=5$). The value of group 1 was defined as 100%. * $P<0.05$ compared with group 1 (Fisher's PLSD test).

mRNA levels in control rats implanted with empty tubes. In rats in which progesterone tubes were implanted for 1 week and removed 6 h before the oestrogen injection, the oestrogen treatment also increased OTR mRNA levels to the same level as that in the control rats. On the other hand, the stimulatory effect of oestrogen was not observed

in rats in which the progesterone tubes were implanted for 1 week without removal (Fig. 4). Serum progesterone levels of rats in each group at death are shown in Table 2.

Discussion

In a previous study (Liu *et al.* 1996), we reported that in late pregnant rats (on the late evening of day 21 of pregnancy), variable levels of uterine OTR mRNA were observed among the animals prior to spontaneous delivery. On the other hand, high levels of uterine OTR mRNA were observed in all parturient animals (on days 22 and 23 of pregnancy). To understand the relationship between the onset of parturition and the regulation of OTR expression, it is necessary to define the variation in OTR mRNA expression observed on day 21 of pregnancy. One possibility is that the variation results from the difference in the timing of the onset of parturition from one animal to the other. To compensate for this, it is necessary to adopt a paradigm in which the variation in the timing of the onset of parturition is kept to reasonably low limits. The change in OTR mRNA expression before and during parturition induced by PGF_{2 α} to reduce the variation in the onset of parturition was therefore examined. Delivery induced by PGF_{2 α} was routinely observed 29–35 h after treatment, while spontaneous delivery was observed from the evening of day 22 of pregnancy until the morning of day 23 (Liu *et al.* 1996). The present study demonstrated that OTR mRNA increased significantly within 24 h after PGF_{2 α} treatment, with all the rats showing high levels of OTR mRNA at 24 h. Since the variation observed in late pregnancy before spontaneous delivery was not observed before PGF_{2 α} -induced delivery, these results suggest that the variation observed in late pregnancy before spontaneous delivery may be due to the difference in the timing of parturition, and that the OTR mRNA levels change in a similar time-course once the mechanism regulating the onset of the parturition is turned on. Furthermore, PGF_{2 α} -treated pregnant rats (which showed a smaller limit of variation in OTR mRNA expression compared with the observation in spontaneously parturient

Table 2 Serum progesterone levels in rats treated with progesterone. Data are expressed as the mean $\pm$ s.e. ($n=5$)

Treatment		Serum progesterone (ng/ml)
Day 1–day 8	Day 8–day 9	
Empty tubes ^a	—	2.22 $\pm$ 1.06
Progesterone tubes ^b	—	6.79 $\pm$ 1.51
Progesterone tubes	Progesterone tubes ^c	16.8 $\pm$ 1.28

Virgin rats were ovariectomized 2 weeks before the treatments and were implanted with empty tubes or tubes containing progesterone (progesterone tubes) (day 1). ^aEmpty tubes were implanted and removed at 1100–1130 h on day 8; ^bprogesterone tubes were implanted and removed on day 8; ^cprogesterone tubes were implanted and not removed. All the rats were killed at 1100–1200 h on day 9 and blood samples were collected from trunk blood.

rats) may constitute a useful model to understand the mechanism(s) which are involved in the regulation of parturition.

Oestrogen treatment has been reported to cause increased uterine OTR mRNA in OVX virgin rats (Larcher *et al.* 1995, Liu *et al.* 1996). To ascertain whether endogenous oestrogen is actually involved in the increased OTR mRNA expression during parturition, the effect of the anti-oestrogenic agent, tamoxifen, on OTR mRNA levels in PGF_{2α}-treated rats was examined. Tamoxifen treatment suppressed the increase in OTR mRNA levels observed at 24 h after PGF_{2α} treatment on day 18 of pregnancy, suggesting that the increased endogenous oestrogen levels after luteolysis is necessary for the induction of OTR mRNA during parturition. In addition, this observation indicated that the PGF_{2α}-induced increase in OTR mRNA levels is mediated by endogenous oestrogen, but not by the direct effect of PGF_{2α} *per se*.

The importance of the decline in progesterone levels in late pregnancy for the action of oestrogen in OT expression in the hypothalamus of female rats (Crowley *et al.* 1995) and the induction of maternal behaviour (Bridges 1984) has been reported. However, a concomitant administration of progesterone with oestrogen does not suppress the stimulatory effect of oestrogen on OTR mRNA expression in OVX non-pregnant rats (Larcher *et al.* 1995, Liu *et al.* 1996), although progesterone administration prolongs pregnancy and suppresses the increase in OTR mRNA expression (Ou *et al.* 1998). Thus, the effect of progesterone on the role of oestrogen in the expression of OTR mRNA and whether the withdrawal of progesterone is necessary for the action of oestrogen on OTR mRNA expression have not yet been clarified. To investigate the nature of the effect of progesterone withdrawal on oestrogen action, we therefore examined the effects of ovariectomy (on day 18 of pregnancy) on oestrogen-induced OTR mRNA expression in pregnant rats. Administration of oestrogen 6 h after ovariectomy significantly increased OTR mRNA in OVX pregnant rats to a level similar to that observed in PGF_{2α}-treated rats and during spontaneous labour. However, oestrogen treatment failed to significantly increase the OTR mRNA in sham-operated (control) pregnant rats compared with vehicle-treated control pregnant rats. Thus, the removal of the ovary during pregnancy, as in this study, facilitated the action of oestrogen on OTR mRNA expression, indicating that some ovarian factor(s) suppressed the induction of OTR mRNA by oestrogen in pregnant rats. Taken together with the results of PGF_{2α} treatment, these results suggest that progesterone secreted from the corpora lutea during pregnancy suppresses the action of oestrogen, and that luteolysis coupled with the decline in serum progesterone levels may be necessary for the induction of OTR mRNA by oestrogen in parturition.

Two further possibilities which are not precluded from the result and require clarification are whether the effect of

ovariectomy on oestrogen action is specific in pregnant rats and whether ovarian factor(s) other than progesterone are involved. Hence it was deemed necessary to determine whether the decline in progesterone levels prior to the increase in oestrogen level is important for the action of oestrogen in OVX virgin rats. A single administration of oestrogen increased OTR mRNA levels in OVX control rats, but this effect was suppressed by exposure to high levels of serum progesterone for 8 days in rats whose progesterone tubes were not removed. When the tubes containing progesterone were removed before oestrogen injection, the oestrogen treatment caused an increased OTR mRNA similar to the level observed in the oestrogen-only group. These results suggest that a decline in the progesterone level is necessary for the expression of the stimulatory effects of oestrogen on uterine OTR mRNA even in OVX virgin rats. Thus, although progesterone did not suppress the stimulatory effect of oestrogen on OTR mRNA when both steroids were concomitantly injected into OVX virgin rats (Larcher *et al.* 1995, Liu *et al.* 1996), our present observation showed that exposure to progesterone before the increase in oestrogen level in the circulation resulted in a suppression of oestrogen action on OTR mRNA expression.

The inhibitory effects of progesterone on OTR expression have been reported in OVX ewes (Leavitt *et al.* 1985, Lau *et al.* 1992, 1993, Zhang *et al.* 1992). The decline in serum progesterone levels in the presence of oestrogen resulted in OTR increases in OVX sheep (Leavitt *et al.* 1985, Zhang *et al.* 1992), accompanied by increased nuclear oestrogen receptor (ER) in both endometrium and myometrium while cytosolic ER and progesterone receptor (PR) increased only in endometrium (Leavitt *et al.* 1985). Progesterone also blocked oestrogen-induced ER gene expression and ER synthesis in non-pregnant sheep myometrium and endometrium (Wu *et al.* 1996b). Long-term treatment with progesterone in OVX sheep caused the down-regulation of PR (Vallet *et al.* 1990, Wathes *et al.* 1996b), followed by increased ER and OTR (Wathes *et al.* 1996b). Progesterone interferes with oestrogen-induced replenishment of uterine cytosolic ER in immature rats (Hsueh *et al.* 1976) and both cytosolic and nuclear ER in OVX rats (Zhou *et al.* 1993). Synthetic progestin increased ER turnover and blocked oestrogen-induced ER synthesis in hamster uterine decidual cells (Takeda & Leavitt 1986). These effects of progesterone on ER regulation in the uterus may involve a suppressive effect of progesterone on oestrogen action in the OTR mRNA expression mentioned above. While RU486 (an anti-progesterone compound) treatment in late pregnant rats did not result in a significant increase in uterine cytosolic ER and ER mRNA levels (Fang *et al.* 1997), it increased cytosolic and nuclear ER in decidua and myometrium of rhesus macaques in late pregnancy (Haluska *et al.* 1990). Therefore, whether progesterone regulates uterine responsiveness to oestrogen by decreasing ER levels is not clear.

However, the evidence that progesterone slightly inhibited the ovariectomy-induced increase in uterine ER mRNA levels in rats (Rosser *et al.* 1993) may support the hypothesis that progesterone may regulate uterine responsiveness to oestrogen by decreasing ER levels.

OTR mRNA expression in rats was detected in myometrium but not in endometrium (Larcher *et al.* 1995), while it was observed in sheep endometrium and myometrium (Wathes *et al.* 1996a) and in human myometrium, decidua and chorion (Takemura *et al.* 1994, Kimura *et al.* 1996). Human OTR mRNA expression in the myometrium increased in late pregnancy while decidual expression did not rise at term. Sheep OTR mRNA expression increased at labour in endometrium and myometrium (Wathes *et al.* 1996a), but ER and PR are likely to be regulated differently in endometrium and myometrium in sheep (Leavitt *et al.* 1985). Thus, the regulation of OTR expression and the involvement of ovarian steroids appears to be different among tissues in different species. A further examination of the involvement of ovarian steroids is therefore necessary for a precise understanding of OTR regulation and the onset of parturition.

It has been shown that treatment with RU486 increased OTR mRNA expression in pregnant rat uterus (Fang *et al.* 1997) and ovariectomy in late pregnancy resulted in an increased OTR mRNA expression 48 h and 96 h afterwards; progesterone injection suppressed this increase (Ou *et al.* 1998). Thus, progesterone, in itself, has a suppressive effect on OTR mRNA expression in pregnant rats. The findings of the present study confirm this action of progesterone and further demonstrate that its action is already present at about 24 h after ovariectomy. It should be noted that OTR mRNA in PGF_{2α}-treated pregnant rats also significantly increased 24 h after the treatment. It is conceivable, therefore, that the decline in progesterone levels results in the induction of OTR mRNA expression possibly through two different mechanisms. One is mediated via the stimulatory action of oestrogen in OTR mRNA expression and the other is independent of this stimulatory action. But whether, in the later mechanism, progesterone directly affects the OTR gene is difficult to say, because the promoter region of the rat OTR gene does not possess a consensus progesterone response element (Rozen *et al.* 1995, Bale & Dorsa 1997). An apparent paradox is that this induction of OTR mRNA was not observed in OVX virgin rats after the removal of the tubes containing progesterone. Two plausible ideas may be proposed as an explanation of this disparity. First, a specific endocrine environment in late pregnancy is necessary for the expression of OTR mRNA induced by the removal of the ovaries. In this context, since administration of progesterone after ovariectomy in late pregnancy suppressed the effect of ovariectomy on OTR expression after 48 h and 96 h (Ou *et al.* 1998), the decline of progesterone in itself is a trigger for the

expression. It is also possible that some factors derived from tissues other than the ovary, such as the placenta, fetus or uterus, appear to be involved in the induction of OTR mRNA. Secondly, a 1-week exposure to progesterone may not have been enough to simulate the effect of progesterone withdrawal on OTR mRNA expression. It has been reported that there is a difference in OT mRNA expression in the paraventricular nucleus of the rat hypothalamus as determined by *in situ* hybridisation between exposure to progesterone for 1 and 2 weeks (Amico *et al.* 1997). In PGF_{2α}-deficient mice, ovariectomy also increased uterine OTR mRNA levels (Sugimoto *et al.* 1997), but the ratio of increase was much larger than that observed in this study. It is difficult to proffer a suitable explanation for this difference at present, but it may be due to the influence of PGF_{2α} deficiency on OTR mRNA expression or the species difference between rats and mice.

In summary, this study has demonstrated that both the decline of progesterone resulting from luteolysis and the increase in oestrogen in late pregnancy are important for the expression of OTR mRNA. Once luteolysis occurs, the OTR mRNA increases in a similar time-course. Moreover, PGF_{2α}-treated pregnant rats, OVX pregnant rats, and steroid-treated OVX virgin rats together constitute appropriate models which may be useful in the attempt to gain full understanding of the mechanism(s) which regulate OTR synthesis and the onset of parturition in mammals.

Acknowledgements

We thank Dr Chuma Okere for reviewing this manuscript. This work was supported by the Yokoyama Foundation for Clinical Pharmacology and by a grant-in-aid for Scientific Research from the Ministry of Education, Sports, Science and Culture of Japan.

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Received 9 November 1999

Revised manuscript received 8 February 2000

Accepted 24 February 2000