

Changes in Uterine Receptor mRNAs for Oxytocin and Estrogen in the Pseudopregnant Rat

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ABSTRACT. This study examined the uterine oxytocin- (OTR) and estrogen- (ER) receptor mRNA levels during and after pseudopregnancy (PSP) in rats. An increased OTR mRNA level was observed from day 14 of PSP, and the maximal level was attained during the following proestrus. The levels of ER α mRNA were low during PSP and significantly increased during the following estrus. The level of ER β mRNA was significantly decreased during proestrus and then returned to the values observed during days 7–14 of PSP by estrus. These results suggest i) suppression of ER α mRNA during the luteal phase and that ii) the changes in OTR, ER α and ER β mRNA levels during proestrus and estrus following PSP are similar to those during the normal estrous cycle.

KEY WORDS: estrogen receptor, oxytocin receptor, pseudopregnancy.

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Oxytocin (OT) was initially isolated as a neurohypophysial hormone that stimulates contraction of the myometrium and myoepithelium to facilitate parturition and milk ejection, respectively, and is considered to mediate various reproductive functions in the ovary, uterus and brain. In the uterus, the near-term myometrium is extremely sensitive to oxytocin. This increased uterine responsiveness to oxytocin occurs in parallel with an increase in the number of uterine oxytocin binding sites in rats [3, 21], humans [4], rabbits [13, 14] and cows [5]. Corresponding increases in uterine oxytocin receptor (OTR) mRNA expression in late pregnancy and parturition have been reported in rats [11, 12, 19], humans [9], cows [8] and sheep [24, 25].

Estrogen stimulates the number of uterine oxytocin binding sites [3, 20, 22] and OTR mRNA expression in ovariectomized (OVX) virgin rats [11, 12]. However, injection of estrogen does not stimulate oxytocin receptor mRNA expression in late pregnant rats or progesterone-primed OVX virgin rats, but is effective only after ovariectomy and removal of progesterone, respectively [17]. These results suggest that in addition to the increase in serum estrogen level near term in rats, regulation of the uterine responsiveness to estrogen is important for understanding the role of estrogen in the uterus. The actions of estrogen are mediated through estrogen receptors [1], and two types of estrogen receptor (ER), ER α and ER β , have been cloned from the human uterus [6] and rat prostate [10], respectively.

In the rat uterus at the end of pregnancy and during labor, the ER α mRNA level gradually increases simultaneously with the OTR mRNA level, while the ER β mRNA level does not show any significant changes; during this period, there is a positive correlation between the ER α and OTR mRNA levels [16]. However, while the OTR mRNA level

is increased during proestrus and then decreased during estrus in the estrous cycle, the ER α and ER β mRNA levels decrease during proestrus and return to the levels observed during diestrus at estrus [15, 16]. This indicates apparent reciprocal changes between OTR and ER (ER α and ER β) mRNA levels during proestrus and estrus. Furthermore, injection of 17 β -estradiol into the OVX rats increased OTR mRNA levels and decreased ER α and ER β mRNA levels [15, 16]. From these observations, understanding how ER α is regulated at the end of pregnancy appears to be important in understanding the mechanism of OTR dynamics and labor. In other studies, progesterone treatment blocked the increase in ER α mRNA level induced by estrogen in OVX rats [16]. Therefore ER α expression in the uterus may be affected by the luteal phase during pregnancy. The present study was designed to investigate whether the increase in the OTR and ER α mRNA levels in parturient rats is regulated by a specific mechanism during pregnancy or by the effect of progesterone secreted from corpora lutea by measuring changes in the OTR and ER α mRNA levels in pseudopregnant rats. In addition, the ER β mRNA level was measured because it is different at the end of pregnancy/during labor compared with during the estrous cycle.

Adult female Wistar Imamichi rats (body weight: 180–220 g) were obtained from the Institute for Animal Reproduction (Ibaragi, Japan) and were kept in an environmentally-controlled room (temperature 23 \pm 3°C; lights on 0600–1800 hr) with free access to tap water and pelleted rat food (NMF; Oriental Yeast Co., Ltd., Tokyo, Japan). The estrous cycle and pseudopregnancy (PSP) were monitored by vaginal smears taken each morning (0900–1000 hr). In the evening on the day of proestrus (1700–1800 hr), the rats were cervically stimulated with a glass rod to induce PSP, and the day after stimulation was designated as day 1 of PSP. Rats were euthanized at 1000–1130 hr on days 7, 10, 12, 13 and 14 of PSP and during the first proestrus and estrus after PSP. The uteri were collected and frozen at

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–70°C until RNA extraction. Animal care, maintenance and treatment were approved by the Animal Care and Use Committee and were conducted according to Guidelines for Animal Experiments of the University of Fukui.

Synthesis of complementary DNA was performed as described previously [15–17]. Real time PCR was performed using SYBR Green master mix and an ABI PRISM 7000 sequence detector (Applied Biosystems, Foster City, CA, U.S.A.). Previously described reaction protocols and primers [15–17] were used for each PCR. Each value for OTR, ER α and ER β mRNA was standardized by dividing it by the value for β -actin in the same sample. The data were converted into the relative amounts (%) by dividing the value of each sample with the mean value of the corresponding control group and expressed as the mean \pm SEM; they were then evaluated statistically using one-way ANOVA followed by the Student-Newman-Keuls test.

OTR mRNA levels during PSP increased gradually, and the level was significantly higher on day 14 of PSP than the value on day 7 (Fig. 1). During proestrus following PSP, the OTR mRNA level attained a peak value and was significantly higher than on any other day examined (Fig. 1). These changes mimicked those during pregnancy, with increased expression at the end of pregnancy and during labor [12, 17]. During estrus following PSP, the OTR mRNA level decreased significantly. The increase in the OTR mRNA level during proestrus and decrease during estrus were similar to those observed during the normal estrous cycle; that is, the OTR mRNA levels are high during proestrus and low during estrus in the normal estrous cycle [15]. Since estrogen is a potent stimulator of OTR expression, particularly in the uterus, these results indicate that estrogen is the major factor regulating OTR mRNA expression at the end of pregnancy/labor, during the proestrus phase of the estrous cycle and at the end of PSP.

The ER α mRNA level increased from day 12 to day 14 compared with day 7, but no significant difference was detected between days 7 and 14 (Fig. 2). The ER α mRNA level during the following proestrus was still low and was similar to that on day 7; it then increased during the following estrus (Fig. 2). On the other hand, the ER α mRNA level during estrus was significantly higher than on any other day examined. Thus, during PSP, the ER α mRNA level did not dynamically change and was relatively low compared with the value during estrus. The level of ER α mRNA in OVX rats is suppressed by chronic treatment with progesterone [16]. Therefore, it is likely that the ER α mRNA level was suppressed by the progesterone secreted by corpora lutea during PSP. In addition to the effect of progesterone, ER α mRNA is also suppressed by the action of estrogen in OVX rats [16] and is low during the proestrus phase of the estrous cycle in rats [15]. Therefore, it is conceivable that the increase in the plasma estrogen level may account for the low level of ER α mRNA during proestrus following PSP. It is worth mentioning that the changes in the ER α mRNA level after PSP, which was low during proestrus and high at estrus, were similar to those during the normal estrus cycle.

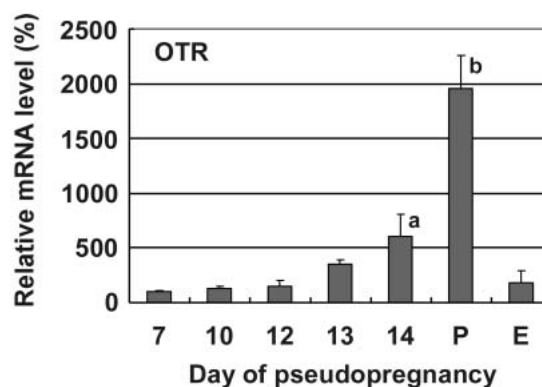


Fig. 1. Changes in the relative OTR mRNA levels in the uteri of pseudopregnant rats. Samples were obtained from rats at 1000–1030 hr on days 7, 10, 12, 13 and 14 of PSP and during proestrus (P) and estrus (E) following PSP. The data are expressed as means \pm SEM (n=5). The value on day 7 was defined as 100%. $P < 0.05$, a vs day 7; b vs all other days (one-way ANOVA followed by Student-Newman-Keuls test).

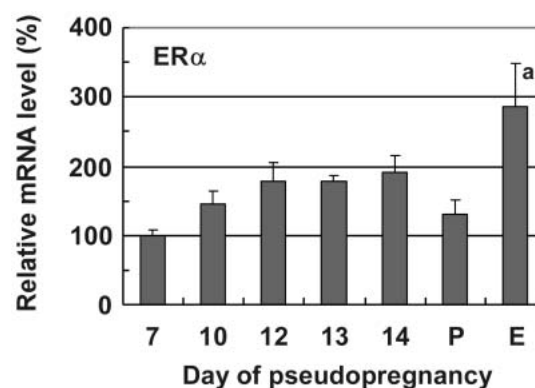


Fig. 2. Changes in the relative ER α mRNA levels in the uteri of pseudopregnant rats. Samples were obtained from rats at 1000–1030 hr on days 7, 10, 12, 13 and 14 of PSP and during proestrus (P) and estrus (E) following PSP. The data are expressed as means \pm SEM (n=5). The value on day 7 was defined as 100%. $P < 0.05$, a vs all other days (one-way ANOVA followed by Student-Newman-Keuls test).

The level of ER β mRNA was unchanged from day 7 to day 14 of PSP, decreased significantly during proestrus and then returned to the values observed during days 7–14 of PSP by estrus (Fig. 3). Thus, the ER β mRNA level was low during proestrus and high during estrus following the PSP; these levels are similar to those during the proestrus and estrus phases of the normal estrous cycle (Fig. 3). At the midpoint of PSP, the ER β mRNA level was similar to that during estrus, indicating that the luteal phase did not affect the ER β level as it did the ER α mRNA level.

While OTR is expressed in the rat uterine myometrium [11], immunohistochemical and *in situ* hybridization studies have shown that ER α and ER β expression occur in the

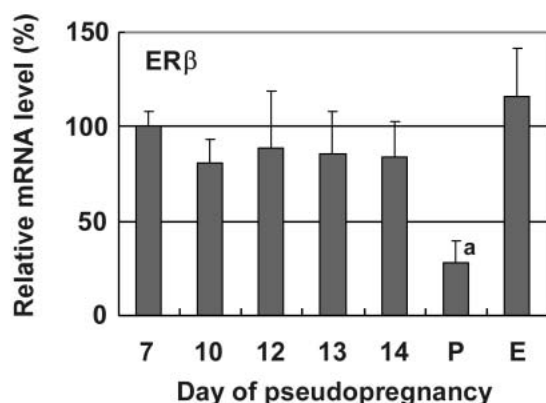


Fig. 3. Changes in the relative ER β mRNA levels in the uteri of pseudopregnant rats. Samples were obtained from rats at 1000–1030 hr on days 7, 10, 12, 13 and 14 of PSP and during proestrus (P) and estrus (E) following PSP. The data are expressed as means \pm SEM (n=5). The value on day 7 was defined as 100%. P<0.05, a vs days except for day 10 (one-way ANOVA followed by Student-Newman-Keuls test).

stroma, glandular epithelium, luminal epithelium and myometrium [2, 7, 18, 23]. These studies have also shown that estrogen increases the ER α levels in the myometrium [2], epithelium [18, 23] and stroma [18] or decreases its expression [18, 23] in the epithelia of OVX rats. Therefore, differential expression of ER α in uterine cells might be important in regulation of uterine function.

This study showed the changes in the OTR, ER α and ER β mRNA levels during PSP and the reciprocal changes occurring in the OTR and ER α /ER β mRNA levels during proestrus and estrus following PSP, which were similar to those of the normal estrous cycle. These results suggest that changes during the luteal phase and regression of the corpora lutea alone cannot totally account for the concurrent increases in the OTR and ER α mRNA levels during labor. This observation also suggests the possibility of specific regulation of ER α mRNA expression at the end of pregnancy and labor.

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