Expression of Estrogen Receptor Subtypes in Rat Pituitary Gland during Pregnancy and Lactation

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The aim of this study was to examine whether the expression levels of mRNA of the three estrogen receptor (ER) subtypes, ERα, ERβ, and truncated ER product-1 (TERP-1) found in the rat pituitary gland were modified during gestation, lactation, and postlactation periods. By using relative quantitative RT-PCR, we found that ERα mRNA significantly peaked in mid-pregnancy. However, the ERα protein level remained constant. ERβ gene expression did not change throughout pregnancy, suggesting that it was not related to estradiol levels during this reproductive period. In contrast, both TERP-1 mRNA and protein levels dramatically increased throughout the second half of gestation, being faintly detectable in early pregnancy. TERP-1 expression was rapidly reversed by lactation, whereas neither pituitary ERα nor ERβ relative levels were significantly altered. In addition, pup removal for 24–96 h on d 9 postpartum significantly reduced the expression of both ERα and ERβ mRNA compared with that in lactating animals, but the expression of TERP-1 mRNA was no longer detected. Collectively, our data indicate that 1) TERP-1, ERα, and ERβ expression levels are differentially regulated in the pituitary; 2) TERP-1 is variably expressed depending on the hormonal environment related to the estrous cycle, pregnancy, and lactation; and 3) TERP-1/ERα ratios dramatically change depending on reproductive periods, suggesting a critical role for TERP-1 in reproductive events. (Endocrinology 143: 4249–4258, 2002)

Estradiol (E2) is well known as an important physiological regulator of the secretory activity of the pituitary gland. In lactotrope cells, estrogens play an important role in growth and differentiation; they also regulate the synthesis and secretion of PRL (1–4). Finally, they are involved in the development of PRL-secreting pituitary tumors. Most actions of estrogen are mediated by two estrogen receptors (ER), ERα and ERβ, encoded by two separate genes (5, 6). In the adult anterior pituitary, estrogen-binding sites are concentrated within gonadotrope, lactotrope, somatotrope, and thyrotrope cells, with the highest amounts found in gonadotrope cells (7–9). Several groups (10, 11) have demonstrated that ERα is the predominant ER subtype in the adult rat pituitary. Regarding the presence of ERβ in pituitary cells, conflicting data have been published (for review, see Refs. 12 and 13). Nevertheless, most studies agree that the ERβ-expressing cells are more gonadotrope than lactotrope cells (11, 14, 15). A natural ER variant, named truncated ER product-1 (TERP-1), has been shown to be specifically expressed in the rat lactotrope cells (16, 17). TERP-1 expression is tightly regulated throughout the estrous cycle and restricted to the proestrous stage. We have further demonstrated that an alternative intronic promoter in rats ERα gene controls TERP-1 expression (18). The activating signals required to drive the intronic TERP-1 promoter during the estrous cycle remain unknown. The lactotrope-specific expression of TERP-1 promoter agrees with the localization of the TERP-1 isoform in female lactotrope cell populations in vivo (17, 18). The truncated protein has been functionally characterized and has been shown to heterodimerize with ERα, ERβ, and androgen receptor (18, 19). Variations in the TERP-1/ERα ratios may be a critical parameter regulating lactotrope cell responsiveness to E2 through protein–protein interactions. TERP-1 may act as a negative regulator of ERα activity in vivo, exerting a protective role against the high level of E2 observed at the proestrous stage. TERP-1 could also contribute to regulate the E2-induced activation of proliferation of lactotrope cells (20, 21). The proliferation of lactotrope cells is a ubiquitous response to the physiological stimuli produced during pregnancy and lactation. However, no data are available concerning changes in ER subtype expression in the rat pituitary during pregnancy, lactation, and postlactation.

The purpose of this study was therefore to examine whether the expression of ERα, ERβ, and TERP-1 mRNA in the rat pituitary was differentially regulated throughout gestation and lactation. A semiquantitative PCR method was used to investigate the changes in the expression of the ER mRNA subtypes in rat pituitaries of pregnant and lactating rats. In addition, we have determined the protein levels of ERα and TERP-1 in the rat pituitary during gestation and lactation. We found more dramatic variations in TERP-1 expression than in ERα and ERβ expression. Data herein suggest that cell-specific effects of estrogen probably depend on the differential expression of distinct ER subtypes, TERP-1, ERα, and ERβ, during pregnant and lactating periods.

Materials and Methods

Animals

Rats were obtained from the rodent facility of INSERM Laboratory (U435, University of Rennes). Animals were treated in accordance with

Abbreviations: E2, estradiol; ER, estrogen receptor; TERP-1, truncated estrogen receptor product-1.
the Guidelines for Care and Use of Experimental Animals. Mature female Sprague Dawley rats were group-housed (n = 5) in polycarbonate cages and maintained under a 14-h light, 10-h dark schedule (lights on at 0700 h) with food and water available ad libitum. The estrous cycles of female Sprague Dawley rats were monitored by daily cytological examination of vaginal smears. Each female was placed with a single male on the day of proestrus for mating purposes. On the next morning, animals were examined for vaginal plugs. This was designated d 0 of pregnancy, and parturition usually occurred on the morning of d 22 in our colony. Pregnant rats were housed individually and killed on d 7, 9, 10, 11, 13, 15, 17, 20, and 21 of pregnancy. The day of parturition was designated d 0 of lactation. The litter size was adjusted to eight pups per dam on postpartum d 2. Lactating animals were killed on d 1, 2, 3, 4, 5, 9, 10, and 20. In another group, pups were removed from their mothers on d 9 of lactation. These mothers were killed 24, 48, 72, or 96 h after weaning. At the indicated times, rats were killed by CO2 gas inhalation and decapitation, and the pituitary glands were quickly removed, frozen in liquid nitrogen, and stored at –80 C until RNA extraction. For the estrous cycle study, rats were killed during proestrus, estrus, metestrus, and diestrus between 0900 and 1000 h.

**Results**

were distributed in such a way that different experimental conditions for ER mRNAs subtypes, semiquantitative PCR was performed. Intraassay variations measured for PO were 8% (n = 5). All data are presented as the mean values of three or four individual controls, respectively.

**Western blot analyses**

Whole pituitaries were homogenized in extraction buffer [50 mm Tris-HCl (pH 7.5), 150 mm NaCl, 5 mm EDTA, 0.5% Nonidet P-40, and protease inhibitors (Roche, Indianapolis, IN)], briefly sonicated, and centrifuged at 14,000 × g for 10 min at 4 C. Supernatants containing similar amounts of proteins (~60–100 µg) were supplemented with 0.5 vol 3% Laemmli sample buffer [150 mm Tris-HCl (pH 7.5), 3% sodium dodecyl sulfate, 3 mm EDTA, 30% glycerol, 15% 2-mercaptoethanol, and bromophenol blue] and boiled for 5 min. Proteins were separated by SDS-PAGE and transferred to nitrocellulose membrane (Hybond C, Amersham Pharmacia Biotech, Arlington Heights, IL). Liquid transfer efficiency and protein loading were monitored by staining the membranes with Ponceau Red. Membranes were then blocked for 2 h with PBS containing 0.1% Tween 20 and 5% nonfat milk and subsequently probed overnight at 4 C with the ERα rabbit polyclonal antibody, MC-20 (1:800 dilution; Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Protein loading was normalized to β-tubulin using a monoclonal primary antibody (1:500 dilution; Sigma, Saint-Quentin, France). The membranes were then washed with six changes of PBS containing 0.1% Tween 20, after which a 1:6000 or 1:8000 dilution of the antirabbit or antimouse horseradish peroxidase-linked secondary antibody (Amersham Pharmacia Biotech), respectively, was incubated with the membranes for 2 h at room temperature. Antibody staining was detected using the enhanced chemiluminescence kit (Supersignal West Dura Extended Duration Substrate, Pierce Chemical Co., PerBio Science, Bezons, France). The intensities of signals captured on Kodak BioMax films (Sigma) were analyzed using relative ODs derived from a computer-assisted densitometry program (Molecular Analyst Software, Bio-Rad Laboratories, Inc.). For each SDS-PAGE, proteins from COS-7 cells transfected, or not, with rat ERα or TERP-1 were included on blots as positive and negative controls, respectively.

**Statistical analysis**

All data are presented as the mean values of three or four individual experiments ± SEM. Statistical differences were assessed using one-way ANOVA, followed by Fisher’s protected least significant difference post hoc test.

| TABLE 1. Primers used for PCR amplification of ER subtypes and PRL cdNAs |
|---------------------------------|-----------------|-------|
| **Product** | **Direction** | **Primer sequence** | **TM** | **Size (bp)** |
| TERP-1 | Forward | CGGGATCCATTTCCCTTGAGCTTGGTGAACAGC | 60 | 805 |
| TERP-1 | Reverse | GGAATTCGATGTTGCAAGACGAACCTTGTTG | 60 | 567 |
| ERα | Forward | CAGCAGCGAGAAGGGGGAACA | 76 | 421 |
| ERα | Reverse | GGCGCGGGCCTTTCCAATTT | 60 | 421 |
| ERβ | Forward | TCCAGGAGCAGCGTCACTGCGGA | 60 | 400 |
| ERβ | Reverse | ACACCCGGCAACACACCCCC | 60 | 400 |
| PRL | Forward | CTTGAGAAGCAAGGAACAGG | 60 | 343 |
| PRL | Reverse | TGGAAAGCTTCTGGCAACCA | 60 | 343 |
| RL19 | Forward | GGACCGCGATGGAACAAACCG | 60 | 175 |
| RL19 | Reverse | CTTCGTTTTCTTGCATGGCC | 56 | 175 |
| PO | Forward | AAYTGGGTTGCCTAAGCCAGATG | 60 | 442 |
| PO | Reverse | GAGATGTTCCAGCATGTTACCA | 60 | 442 |
hoc test (StatView software for Windows 95, SAS, Cary, NC). In all cases, differences were considered significant when $P < 0.05$.

**Results**

**Validation of PCR method**

Conditions for semiquantitative RT-PCR were determined for rat pituitary samples. The linearity of PCR amplification reactions was assessed for each primer pair in a preliminary study, which determined the conditions under which PCR amplifications for ERα, ERβ, TERP-1, and PRL mRNA were in the logarithmic phase (data not shown). Thirty cycles were chosen to quantify ERα and TERP-1 levels, vs. 40 and 33 cycles for ERβ and PRL, respectively. In the case of RL19 ribosomal protein transcript (24) or PO (25), used as controls for the quality and quantity of RNA in each sample, 26 cycles were determined. The identity of the TERP-1 PCR product was validated by sequence analysis (data not shown), confirming that the RT-PCR-amplified product was TERP-1 specific.

**Changes in the expression of pituitary ERα and ERβ mRNA during the estrous cycle**

As TERP-1 mRNA has been previously shown to be transiently expressed during the estrous cycle, analyses were performed to examine whether pituitary ERα and β mRNA expression changes during estrous cycle. Pituitary glands were obtained from rats killed between 0900 and 1000 h on proestrus, estrus, metestrus, and diestrus. The results of three

![Graph A](image1.png)

**Fig. 1.** ER mRNA levels in pituitary glands of female rats on various days of the estrous cycle as measured by slot blot or PCR. A, ERα mRNA levels were measured by slot analysis and standardized relative to the amounts of β-actin. Slot results (■) are expressed in arbitrary units (AU) after setting the level in the proestrus stage to 100 and are shown as the mean ± SEM of three or four independent experiments [proestrus (P) and diestrus (D), $n = 4$; estrus (E) and metestrus (M), $n = 3$]. PCR results from a representative experiment are presented (□) and are standardized relative to the amounts of RL19 (ribosomal protein L19) (24). B, ERβ mRNA levels were measured by PCR analysis and standardized relative to the amounts of RL19 mRNA. PCR results are expressed in arbitrary units after setting the level in the proestrus stage at 100. Data are shown as the mean ± SEM of three independent experiments. *, $P < 0.05$. 

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*Vaillant et al. Pituitary ER Subtype Expression in Pregnant and Lactating Rats* Endocrinology, November 2002, 143(11):4249–4258
Differential expression of the ER mRNA subtypes during pregnancy and lactation

To investigate whether the expression of ER mRNA subtypes may be differentially regulated under other physiological conditions, we studied the pituitary mRNA expression of ERα, ERβ, and TERP-1 in both gestation and lactation conditions. Figure 2A shows typical RT-PCR analyses of ER subtypes during the pregnant and lactating periods. ERα (yielding a 421-bp PCR product) was expressed throughout both gestation and lactation in the pituitary gland (Fig. 2A and B). The levels of ERα mRNA expression during the 21 d of pregnancy slightly varied (Fig. 2B). A significant increase in the level of ERα mRNA expression occurred on d 13 of gestation compared with that on d 7 (P < 0.05). On d 13, the level of ERα mRNA expression was significantly higher (P < 0.05) than those on d 15, 17, and 21 of the gestation period (Fig. 2B). There was no significant change in ERα gene expression on the first day after parturition compared with that during pregnancy, except for d 13 of gestation (P < 0.05). ERα mRNA levels remained constant during the 20 d of lactation (Fig. 2B). ERβ mRNA levels did not significantly change in the pituitary gland from rats at any stage of pregnancy or lactation (Fig. 2C). However, larger fluctuations of ERβ mRNA levels were observed among individuals during the lactating period. The primers specific for PO mRNA amplified a PCR product of 175 bp with a similar intensity in all the situations examined (Fig. 2A).

Figure 2 (A and D) shows the expression of TERP-1 (yielding a 805-bp PCR product) during pregnancy. Positive signals, even if relatively faint, were detected in the early phase of pregnancy and progressively dropped to very low levels on d 9 (P < 0.05) and d 11 (P < 0.005). TERP-1 expression levels dramatically increased on d 13 of pregnancy (P < 0.0001). They increased significantly through late gestation, with peak levels occurring by d 21 of pregnancy (d 21 vs. d 17, P < 0.0005; d 21 vs. d 15 and d 13, P < 0.0001). Interestingly, the relative expression levels of TERP-1 abruptly decreased from d 21 of pregnancy to the first day of the lactating period (24 h later; Fig. 2, A and D). With 30 cycles of PCR amplification, no signal was detected in the pituitary gland during the lactating stage (Fig. 2, A and D), except for a very weak signal on d 20. Thus, TERP-1 mRNA levels increase dramatically in the rat pituitary gland at midpregnancy, followed by an abrupt disappearance immediately after parturition.

As a control, we also analyzed pituitary samples for PRL expression (Fig. 2E). The level of PRL expression during the first 13 d of pregnancy was relatively constant. It progressively decreased up to d 17 and then remained low until the day of parturition (d 13 vs. 15, P < 0.05; d 13 vs. 17 and 21, P < 0.0005; d 15 vs. 17 P < 0.05). Lactating PRL mRNA levels were significantly higher compared with late gestation levels (P < 0.05) and similar to those in the early phase of pregnancy. All of these data confirm previous observations (27, 28). During lactation, no significant changes were detected. Data are in total agreement with those reported by Ren et al. (29) and confirm the validity of the RT-PCR method used in the present study.

Western blot analysis of TERP-1 and ERα

To examine TERP-1 and ERα protein expression during gestation and lactation, immunoblot studies with the polyclonal Ab MC20, which is directed against the carboxyl terminus of ERα and recognizes both proteins, were performed. Whole cell extracts of 11 female pituitaries collected during mid- and late pregnancy and throughout lactation (d 3, 4, 9, and 20) were analyzed. The antibody revealed in all 11 tested lysates a single protein, with an apparent molecular mass of 66,000, that comigrated with ERα overexpressed in COS-7 cells (Fig. 3A, bottom panel). The TERP-1 protein was detected as a doublet with an apparent molecular mass of about 26,000 in TERP-expressing COS-7 as well as in some pituitary lysates, essentially from rats in late gestation (Fig. 3A, top panel). The membranes were probed with an anti-β tubulin antibody to ensure equivalent loading among pituitary tissue samples. Densitometric analysis of TERP-1 and ERα bands and normalization to β-tubulin showed that ERα protein remained relatively constant throughout the various endocrine states of the pituitary (Fig. 3B, bottom panel) as the slight increase measured between d 10–11 and d 20 of pregnancy was not significant. In contrast, a 10-fold increase in the pituitary amount of TERP-1 protein was observed from mid- to late pregnancy (d 10–11 vs. d 20, P < 0.005). No TERP-1 protein was later detected in pituitaries from lactating rats (Fig. 3B, top panel). Therefore, the expression levels of ERα and TERP-1 protein were consistent with the mRNA data.

Expression of ER mRNA subtypes after pup removal

The experiments were conducted to determine whether pup removal could alter the expression of ER mRNA subtypes in the mother pituitary. Lactating rats suckled by eight pups were studied on d 9 post partum. The data presented in Fig. 4 indicate that pup removal results in decreased levels of both pituitary ERα and ERβ mRNA (for ERα, gestating and lactating vs. postlactating, P < 0.001; for ERβ, lactating d 10 and 20 vs. postlactating 24, 48, and 72 h, P < 0.05). The level further decreased for ERα mRNA from 24 to 48–72 h

or four independent experiments are shown in Fig. 1A, using two different methods, PCR and slot blot, for ERα. Data show strictly similar patterns using both PCR and slot blot techniques. In all subsequent experiments we used the semi-quantitative RT-PCR method to examine changes in mRNA levels from individual rat pituitary glands.

The relative ERα mRNA levels were the highest during proestrus, significantly decreased on estrus and metestrus by 28% and 26%, respectively (proestrus vs. estrus or metestrus, P < 0.05), and again significantly increased on diestrus by 22% (metestrus vs. diestrus, P < 0.05; Fig. 1A). In contrast, no significant changes were observed for ERβ mRNA (Fig. 1B). These changes suggest a relationship between pituitary ERα mRNA levels and estrogen status in normal females during the estrous cycle. A similar correlation has been previously found between pituitary TERP-1 mRNA and estrogen levels (17, 26). Therefore, significant changes in pituitary ERα and TERP-1 mRNA levels occur during the different stages of the estrous cycle in females, whereas ERβ mRNA levels remain constant.

significant changes were observed for ERα vs. 22% (metestrus during pregnancy compared with that on d7( lactation (Fig. 2C). However, larger fluctuations of ERα levels dramatically increased on d 13 of pregnancy (P < 0.0001). They increased significantly through late gestation, with peak levels occurring by d 21 of pregnancy (d 21 vs. d 17, P < 0.0005; d 21 vs. d 15 and d 13, P < 0.0001). Interestingly, the relative expression levels of TERP-1 abruptly decreased from d 21 of pregnancy to the first day of the lactating stage (Fig. 2, A and D). With 30 cycles of PCR amplification, no signal was detected in the pituitary gland during the lactating stage (Fig. 2, A and D), except for a very weak signal on d 20. Thus, TERP-1 mRNA levels increase dramatically in the rat pituitary gland at midpregnancy, followed by an abrupt disappearance immediately after parturition.

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FIG. 2. Relative quantitative RT-PCR analysis of ER subtypes and PRL mRNA contents in pituitary glands throughout gestation and lactation. Total RNA from individual rat pituitary at various gestational (left panel) or lactating (right panel) periods was isolated and reverse transcribed. Relative quantitative PCR analysis was performed. The primers used to amplify the different receptor subunits and PRL control are listed in Table 1. The intensity of the bands was determined by densitometry as described in Materials and Methods. A, Representative ethidium bromide-stained gels of the results observed for gestating or lactating period with each ER subtype, PRL, and PO (ribosomal protein P0) (25) are presented. A graphic compilation of the PCR data are shown: B, ERα; C, ERβ; D, TERP-1; E, PRL. The data are represented as relative units of a particular ER subtype at each time relative to the expression of P0, which was constant throughout reproductive periods. Results are the mean ± SEM of at least two independent RT for each sample of three independent animals. ****, P < 0.0001; ***, P < 0.001; **, P < 0.01; *, P < 0.05.
after pup removal (24 h pup removal vs. 48 and 72 h pup removal, *P* < 0.05) and thereafter remained constant (Fig. 4B). In contrast, the relative levels of ERα mRNA did not change significantly after pup removal (Fig. 4C). TERP-1 mRNA expression was only slightly detected at 24 and 48 h after pup removal (Fig. 4D). Pituitary PRL mRNA levels were significantly higher at 72 and 96 h after pup removal compared with those in the 24 and 48 h groups (*P* < 0.005; Fig. 4E). These results are in accordance with those previously described (27). Thus, the expression of ERα and β mRNA subtypes were reduced during postlactation compared with pregnant and lactating animals, whereas TERP-1 expression was barely detectable.

**Discussion**

The physiological effects of estrogen on the pituitary, including cell proliferation and regulation of hormone synthesis, are mediated by the nuclear ER. The recent cloning of a second form of ER (ERβ) (6) and of a new ER variant, TERP-1 (16, 17), have prompted us to perform an analysis of the expression of pituitary ER subtypes during reproductive functions. This is the first report investigating the span of ERα, ERβ, and TERP-1 in the rat pituitary gland during the physiological stages of pregnancy and lactation and during the postlactation period. Our study demonstrates that the ER subtypes are differentially expressed in rat pituitary depending upon reproductive stages.

Our results show that the expression of ERα mRNA in the anterior pituitary gland varies during the estrous cycle, with the lowest expression measured during estrus and metestrus. Expression levels increased during diestrus, reaching the highest values in proestrus. However, the absolute amplitude of the ERα mRNA peaks remains relatively weak (<30%) in pituitary gland compared with uterine tissue (30, 31). Recently, Schreihofer *et al.* (32) failed to detect any change in pituitary ERα mRNA throughout the estrous cycle. Alterations that we observed in ERα mRNA expression are in agreement with changes in steroid binding capacity reported in the pituitary gland during the estrous cycle (33). The observed pattern was similar to the reported changes in ERα in the uterus during the estrous cycle (30, 31). Thus, the pattern of ERα gene expression in the pituitary during the estrous cycle could be positively related to circulating E2 levels. Moreover, the data showed a correlation between the highest ERα mRNA expression and the induction of TERP-1 mRNA expression in lactotrope cells (17, 26). Like Schreihofer *et al.* (32), we observed up-regulation of TERP-1 level on the morning of proestrus and a decline by the afternoon of proestrus (data not shown). Our observations indicating that TERP-1 protein is up-regulated during the proestrous period (kilodaltons) are indicated. The nitrocellulose membranes were also probed with anti-β-tubulin, as an indicator of equal loading. B, Densitometric analysis of TERP-1 (top) and ERα (bottom) immunoblots of 11 individual pituitary extracts. Signals have been normalized to β-tubulin levels and expressed as arbitrary units (AU) after setting at 100 the mean levels measured at d 20 of pregnancy (P20). Histograms represent the mean ± SEM from three or four different rats in each group. **, *P* < 0.005. P, Pregnant; L, lactating.
stage (unpublished data) suggest a similar expression pattern of mRNA and protein.

Regarding ERβ, our experiments indicate that relative mRNA levels did not change at any of the stages studied, whereas Schreihofer et al. (32) observed a significant decrease on the morning of proestrus. In addition, ERβ mRNA expression remains relatively constant during pregnancy and lactation. Relative ERα mRNA levels significantly increased up to midpregnancy. During late pregnancy, there was a slight, but significant, decrease in pituitary ERα mRNA levels on d 15, 17, and 21 compared with d 13. However, we failed to detect any significant change in ERα protein levels. During lactation, no significant change could be detected in ERα mRNA and protein levels.

It is noteworthy that ERα and ERβ subtypes are highly expressed during pregnancy and lactation, whereas postlactating rats exhibit low levels of both ERα and ERβ mRNA. Interestingly, pituitary TERP-1 mRNA was slightly or not detected in early pregnancy, but it was highly expressed in mid- to late pregnancy, i.e. from d 13-21 of pregnancy. In contrast, TERP-1 mRNA was not detectable during the 3 wk of the lactating period under appropriate amplification conditions. Pup removal did not result in a significant elevation of TERP-1 mRNA level. Likewise, the expression of TERP-1 protein was highest during late gestation, whereas the protein was barely detected in pituitary during early gestation and was absent during the lactating period. The data show that TERP-1 protein in both pituitary extracts and COS-7 cells transfected with rat TERP-1 cDNA migrates as a doublet. This suggests that it is translated from the first and/or second of the possible ATG codon start sites of rat TERP sequence as previously suggested by Mitchner et al. (11).

Several factors may account for the differential regulation of ER isoforms in the pituitary, including ovarian steroids, hypothalamic peptides, and intrapituitary factors. As we and others have found that TERP-1 mRNA expression was induced dramatically by E2 in the pituitary (16, 17), the increase in the expression of TERP-1 mRNA and protein at proestrus and late gestation may be due to the high estrogen concentration at this period. Higher E2 levels during late pregnancy (34) are accompanied by increased TERP-1 levels. Low levels of TERP-1 expression coincide with lower plasma E2 concentrations during both early pregnancy and lactation (28). However, serum α-fetoprotein levels in pregnant rats raise significantly after d 12, reaching the maximum level on d 21 (35). We did not
know the concentration of E2 not bound to plasma proteins in the pregnant rat. The presence of a consensus palindrome estrogen response element sequence in TERP promoter sequence (18) also suggests that estrogen may exert a direct effect on TERP-1 promoter and play a role in the regulation of TERP-1. A potentially complex cascade of events could occur: ERα with other factors may activate the internal promoter and bind to estrogen response element, triggering TERP-1 expression, which may interact with ER to modulate its action, suggesting a regulating feedback loop.

Previous data indicated that ERα mRNA is not highly regulated by E2. Several reports suggested that E2 could not be a direct effector of pituitary ERα regulation. Sprangers et al. (36) showed that E2 treatment did not affect the ERα mRNA level in cultured monkey pituitary cells. Schreihofer et al. (32) also indicated that ERα mRNA levels did not change in response to most treatments in vivo or in cell lines. In addition, we showed that short- or long-term exposure to either E2 alone or in combination with progesterone did not exert any significant effect on ERα mRNA levels of primary cultured pituitary cells (unpublished data). Thus, our data indicating no (protein data) or discrete (mRNA) changes in ERα levels during pregnancy and lactation are not surprising. However, discrete ERα changes in a particular cell type could not be detected. Concerning ERβ, the data correlate with the absence of reports of function in reproductive stages. Hence, the maintenance of a high level of ERα and ERβ expression in the anterior pituitary during gestation and lactation could be independent of estrogen regulation. Alternative activation of ERα via phosphorylation by growth factor-stimulated pathways or GnRH is suggested. Pulsatile GnRH treatment of pituitary cell aggregates indeed increased ERα mRNA levels (37). In a more recent paper we reported that in the absence of E2, ERα can be transcriptionally activated in gonadotrope cells in an estrogen-independent manner, and GnRH may serve as an important signal in the regulation of pituitary ERα trans-activation (38).

A major difference between early and late pregnancy concerns dopamine and PRL secretion. Dopamine, which is released from the tuberoinfundibular dopaminergic neurons, activates D2 dopamine receptors on lactotropes to tonically inhibit the release of PRL. A diminution of dopamine activity during early pregnancy (28) and an increase at midpregnancy (39) have been observed. Dopamine levels in the portal blood slightly decreased on the day before parturition (40). Both early pregnancy and lactation are physiological hyperprolactinemic states. However, the regulation of PRL secretion during late pregnancy is different from that in early pregnancy. During early pregnancy in rats, pituitary PRL secretion is relatively high and occurs in a semicircadian rhythm (for review, see Ref. 41). The magnitudes of these PRL surges start to decline on d 8 of pregnancy and are completely inhibited by d 10. In contrast, during late pregnancy, plasma PRL levels remain low until a large PRL surge occurs during the dark period immediately preceding parturition (40). The results herein support these data, and we confirm significantly diminished amounts of PRL mRNA in mid- to late pregnancy (28). In contrast, low levels of hypothalamic dopamine are present during lactation. Concurrently, pituitary PRL mRNA levels increased as previously observed (Ref. 28 and our results). On the other hand, it is known that dopamine secretion is increased after sustained pup separation (39). Previous data have suggested that an alteration in dopaminergic activity may result in a significant change in the positive regulation of ERα in the pituitary gland (42). Thus, it cannot be ruled out that dopamine may be one of the factors involved in triggering pituitary TERP-1 mRNA expression during the late pregnancy and proestrous stage.

Lactation in the rat also results in the suppression of pulsatile LH secretion, which is accompanied by a large decrease in pituitary GnRH receptor content (27). Removal of the suckling stimulus results in a significant increase in GnRH receptor expression and pulsatile LH secretion (reviewed in Refs. 43 and 44). As we did not observe any TERP-1 expression during lactation and in the postlactation period, the GnRH receptor is probably not involved in TERP-1 expression, at least during those times.

The highly induced expression of TERP-1 in mid- to late pregnancy and in the proestrous stage and the relatively constant levels of ERα and ERβ suggest a physiological role of the truncated protein. We have previously shown in the rat pituitary gland that one of the roles of TERP-1 is to act as a negative regulatory partner of ERα in lactotrope cells (18).

The capacity of TERP-1 to regulate the function of ER suggests that the relative ratio of ER and TERP-1 proteins in vivo may be a critical factor regulating lactotrope cell responsiveness to E2. Even if a relatively low level of TERP-1 protein is detected, it is important to note that TERP-1 is only expressed in lactotropes of the rat pituitary gland, whereas ERβ is restricted to gonadotropes (15), and ERα has been characterized in both populations. The data presented in this report show a marked increase in the TERP-1/ER mRNA ratio in lactotrope cells in mid- to late pregnancy. In addition, a high degree of TERP-1 expression occurs during the proestrous stage (17, 26). This is consistent with the fact that the increase in progesterone receptor, a typical estrogenic response, is not observed in rat lactotrope cells (45). It is possible that the ERα/TERP-1 heterodimer can inhibit this action. From our results, it also appears that TERP-1 could prevent an accumulation of ERα protein despite an increase in its mRNA.

During both the estrous cycle and gestation, TERP-1 expression declines just at the onset of lactotrope proliferation, which requires both a hypophysiotropic stimulatory input from the hypothalamus and a sensitizing action of E2 (for review, see Ref. 46). Only lactotrope cells among the anterior pituitary cells exhibit an increase in proliferation rates on the morning of estrous (20). Yin and Arita (47) showed that the proliferative activity of lactotropes was selectively repressed during early pregnancy. In addition, they observed a marked increase in proliferative activity of lactotropes on late parturition. Although E2 is responsible for cell expansion during pregnancy, this steroid is not responsible for the continued maintenance of lactotropes throughout lactation (48). No cell proliferation has been detected during lactation despite elevated PRL release (47). Therefore, we suggested that most ERα activity in late gestation or in proestrous stage may be
agonized by TERP-1 expressed in the same cells. TERP-1 may modulate lactotrope hormonal responsiveness through protein-protein interaction. However, the initiating mechanism of TERP-1 up-regulation and the precise role of TERP-1 in the pituitary need further investigation.

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