0013-7227/03/$15.00/0
Printed in U.S.A.

Endocrinology 144(5):1869–1875

doi: 10.1210/en.2002-220807

Estrogen Receptor β Messenger Ribonucleic Acid Expression in the Forebrain of Proestrous, Pregnant, and Lactating Female Rats

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Estrogen receptor (ER)β is present in hypothalamic and limbic neurons of female rat brains, but little is known about its regulation under physiological conditions. To determine whether ERβ expression varies during physiological conditions in which sex steroid hormone profiles are significantly different, we used *in situ* hybridization to assess ERβ mRNA expression in the periventricular preoptic area, bed nucleus of stria terminalis, paraventricular nucleus, supraoptic nucleus, and the posterodorsal medial amygdala of female rats on proestrus, on d 22 of pregnancy, or on d 10 of lactation (L10). In the periventricular preoptic area, d-22 pregnant females had fewer ERβ-mRNA-expressing cells than did females at proestrus, but the level of ERβ mRNA expression per cell in pregnant females was higher than in the two other groups. In the paraventricular nucleus, no changes in ERβ mRNA expression were observed; whereas in the supraoptic nucleus, proestrous females had fewer ERβ-mRNA-expressing cells than L10 females. In the posterodorsal medial amygdala, proestrous females had a greater number of ERβ-mRNA-expressing cells than did L10 females. These results demonstrate that ERβ mRNA expression is differentially regulated in a brain-region-specific and temporal manner under physiological conditions and suggest that ERβ may participate in the regulation of estrogen-sensitive reproductive functions in female rats. (*Endocrinology* 144: 1869–1875, 2003)

IN THE LAST few years, the novel estrogen receptor (ER), ERβ, has been cloned from prostate and ovary of several species (1, 2), and its expression has been reported in a variety of tissues, including the brain (3, 4). It is now well established that ERβ distribution in forebrain and limbic regions of female rat brains overlaps with the distribution of ERα and that, in many instances, ERβ-containing neurons coexpress ERα (5–8).

Although the hormonal regulation of ERα expression in brain has been well studied under manipulated or physiological conditions (9, 10), little information is available on the regulation of ERβ expression by sex steroid hormones in the brain (5, 11, 12). In some cases, the concentration of [^{3}H] estradiol (which binds to both ERs) in areas in which ERβ is present but ERα seems to be absent increases during reproductive states in which sex steroid hormones fluctuate (13). This suggests the possibility that ERβ expression could be regulated by these hormones.

Pregnancy and lactation are characterized by a complex series of behavioral and physiological events that are orchestrated, in part, by dramatic fluctuations in ovarian hormone levels and their receptors. In rats, estradiol levels begin to rise after day of pregnancy (P)16, and they peak on P22, around the time of parturition (14). In contrast, the level of plasma progesterone is elevated by P14 and remains elevated until P19, at which time it decreases to very low levels (15). Postpartum and during lactation, estradiol levels are low during the first week but increase, to return to a diestrous level by day of lactation (L)10 (16–18). By contrast, levels of progesterone are elevated from L4–L10 and then decline (16, 17). The rise in estradiol and simultaneous decline in progesterone before parturition and postpartum have been associated with both the induction of maternal behavior (19) and the increase in central secretion of oxytocin (20).

Changes in estradiol levels during pregnancy are preceded (and later paralleled) by increases in cell nuclear estradiol binding, as assessed by *in vitro* [^{3}H] estradiol binding, suggesting increased occupied ERs in brain regions, including the periventricular part of the preoptic area (PvPO); the medial preoptic area (MPOA); the paraventricular nucleus (PVN); the medial amygdala (MEA); and, in part, in the supraoptic nucleus (SON; Ref. 13). Each of these regions is important for either the display of maternal behavior (21–24) or synthesis and release of oxytocin (25). Increases in the level of occupied ERs start between P8 and P16, when levels of estradiol are still low (13), suggesting an up-regulation of ER expression in these brain regions during early to mid pregnancy. Though high levels of ERα are present in these hypothalamic and limbic regions, both mRNA and protein ERα levels do not increase in the rostral MPOA or the MEA between P8 and P16 (10, 26). Moreover, ERα does not seem to be present in the PVN (6, 27), where occupied ERs are also reported to increase until P22 (13). These discrepancies between changes in the levels of occupied ERs and the apparent lack of change in the level of ERα in some brain regions, as well as the absence of expression of ERα in others, suggest that expression of a different ER could be regulated during pregnancy.

Although ERα-immunoreactivity (ERα-ir) increases in many forebrain and limbic regions of female mice during

Abbreviations: BNSTpr, Principal nucleus of the bed nucleus of stria terminalis; ER, estrogen receptor; ERα-ir, ERα-immunoreactivity; EtOH, ethanol; L, day of lactation; MEA, medial amygdala; MEApd, posterodorsal nucleus of the MEA; MPOA, medial preoptic area; P, day of pregnancy; PVN, paraventricular nucleus; PvPO, periventricular preoptic area; SON, supraoptic nucleus; SSC, saline sodium citrate.

lactation (28), no information is available regarding changes in ER concentrations during lactation in female rats. Importantly, milk ejection requires the neurohypophyseal release of oxytocin, which is synthesized in the magnocellular neurons of the SON and PVN. *In vivo* and *in vitro*, the synthesis of oxytocin can be regulated by estradiol, and the oxytocin gene contains an estrogen response element (29), suggesting that its synthesis may be regulated directly or indirectly by an ER (30). The fact that ERβ is expressed by oxytocinergic cells in the SON and PVN (31–33) suggests that ERβ can influence the expression of estrogen-responsive genes within these cells. One means through which estradiol binding or expression of estrogen-responsive genes in these two regions could be influenced is through modulation of ERβ expression itself.

In the present study, we were interested in determining whether ERβ mRNA expression is modulated in the female rat brain under different reproductive conditions. Changes in ERβ mRNA expression were assessed during proestrus in virgin, estrous-cycling rats, on P22 and on L10. These conditions were chosen because of the extreme differences in profiles of hormonal and neuropeptide levels that are observed in these three reproductive states. The goal of this experiment was not do an exhaustive analysis of the regulation of ERβ mRNA during different physiological states; rather, the goal was to determine whether ERβ mRNA levels differ in response to vastly different physiological conditions.

Materials and Methods

Animals

Virgin female Sprague Dawley rats (~200 g; Charles River Laboratories, Inc., Wilmington, MA) were group-housed for 1 wk in a 14-h light, 10-h dark cycle, with food and water available *ad libitum*. The animal use protocol was approved by the Institutional Animal Care and Use Committee of the University of Massachusetts, Amherst. In one group of females (n = 5), estrous cycles were monitored by daily vaginal smears for three cycles, and animals were decapitated on the morning of proestrus of the fourth cycle. A second group of females (n = 10) was housed with sexually active males and were examined daily for the presence of a seminal plug. Females were removed from males on the day that the seminal plug was observed, which was considered as P1. Half of the pregnant females were decapitated on the morning of P22 (n = 5). The remaining females were monitored for the day of parturition, left with their pups, and decapitated on the morning of L10 (n = 5).

Tissue preparation

Brains were removed immediately, rapidly frozen, and stored at −80 C. Sixteen-micrometer-thick sections, from the rostral aspect of the POA to the caudal aspect of the ventromedial hypothalamus, were cut on a cryostat, mounted onto gel-coated microscope slides, and stored at −80 C until hybridization.

In situ hybridization

Probe preparation and *in situ* hybridization histochemistry were performed as previously described (34, 35). ^{33}P-Uridine triphosphate-labeled cRNA probes were generated from pBluescript plasmids containing a 558-bp (bases 56–610) or a 285-bp (bases 1809–2094) fragment of the rat ERβ cDNA (gift from P. Shughrue, Wyeth-Ayerst Laboratories, Inc.), linearized with *Bam*H1 (antisense) or *Eco*RV (sense control). Before hybridization, brain sections were equilibrated to room temperature and incubated for 15 min in 4% formalin. Slides were incubated for 2 min in 2× saline sodium citrate (SSC), followed by 10 min in a triethanolamine HCl-acetic anhydride solution. After one rinse in 2× SSC, slides were incubated for 1 min in 70% ethanol (EtOH); followed by 1 min in 80% EtOH; then by 2 min in 95% EtOH; and finally, by 1 min in 100% EtOH. Slides were incubated for 5 min in chloroform, followed by 1 min each in 100% and 95% EtOH. Slides were air-dried, and 25 μl hybridization buffer, containing both the 558-bp and 285-bp ERβ antisense probes or sense cRNA probes (1×10^6 cpm/probe·section) and 5 M dithiothreitol (1:100 hybridization buffer) were applied to each section. Slides were incubated overnight at 55 C. Slides were then equilibrated to room temperature and washed twice in 1× SSC for 10 min each. Slides were washed in 50% formamide-2× SSC solution for 25 min at 52 C and then rinsed twice in 2× SSC at room temperature for 1 min each. Slides were then incubated in 2× SSC with 100 μg/ml ribonuclease A, at 37 C for 30 min, followed by two rinses in 2× SSC at room temperature. Slides were again incubated in 50% formamide-2× SSC at 52 C for 5 min, followed by dehydration in an ascending series of alcohol washes. Slides were air-dried, dipped in NTB-3 emulsion (Eastman Kodak Co., Rochester, NY) diluted 1:1 with distilled water, and were developed 10 d later.

Data analysis

Brain sections were matched, across groups, for neuroanatomical sites where ERβ mRNA hybridization signal was the most intense, including the PvPO, the dorsal principal nucleus of the bed nucleus of the stria terminalis (BNSTpr), the magnocellular part of the PVN, the rostral SON, and the posterodorsal nucleus of the MEA (MEApd; Fig. 1).

Numbers of cells and expression of ERβ mRNA within cells were measured using the NIH Image computer-assisted, image-analysis system (developed at the NIH and available on the Internet at http://rsb.info.nih.gov/nih-image/). Regions of interest were captured with a ×40 objective using darkfield illumination. Digitized images were inverted so as to have a white background and a black signal for each silver grain. As shown previously (34), the area of silver grains over labeled neurons is a good correlate for level of mRNA expression. Number of cells and grain area per cell were measured as follows. The gain and the black levels of the camera were set so that the gray level for the computerized image ranged from 1–255 pixel density for ERβ mRNA hybridization signal. Numbers of cells containing ERβ mRNA were quantified in each region by an observer blind to treatment groups. Area covered by silver grains over labeled cells and neighboring unlabeled cells (*i.e.* background value) was determined. Unlabeled cells (between 10–20 cells/brain area·animal) were defined as cell-sized areas, in the brain tissue but where clusters of silver grains (*i.e.* labeled cells) seemed virtually absent. Cells were considered labeled if the grain area over the cell exceeded three times the background grain area value (26). The mean grain area of hybridization signal per cell for each brain region was obtained by averaging the grain area measured cell-by-cell in all labeled cells/animal after subtracting background signal. Moreover, for the PvPO and MEApd, ERβ-mRNA-containing cells were grouped based on grain area of 500 pixel increments, and the percentage of cells expressing different levels of hybridization signal was assessed among groups (36).

For the PvPO, BNSTpr, PVN, and SON, one-way ANOVA was used to determine whether numbers of ERβ-mRNA-expressing cells, mean grain area of ERβ-mRNA-expressing cells, background values, and percentage of cells expressing different levels of hybridization signal varied among groups. Planned comparisons were made with *t* tests with significant differences assigned at $P < 0.01$ for ERβ-mRNA-expressing cells, mean grain area of ERβ-mRNA-expressing cells, and percentage of cells expressing different levels of hybridization signal and at $P < 0.05$ for the background values. For the MEApd, because data were available only for two conditions, *t* tests with $P < 0.01$ were used to determine whether numbers of ERβ-mRNA-expressing cells, mean grain area of ERβ-mRNA-expressing cells, and percentage of cells expressing different levels of hybridization signal differed between the proestrous group and the L10 group.

Results

ERβ mRNA distribution

The distribution of ERβ mRNA was in good agreement with a previous report (6). We found intense ERβ mRNA hybridization signal in the PvPO (Fig. 2), the BNSTpr, the magnocellular part of the PVN, the SON, and the MEA. Moderate signal also was observed in the MPOA, the ventral

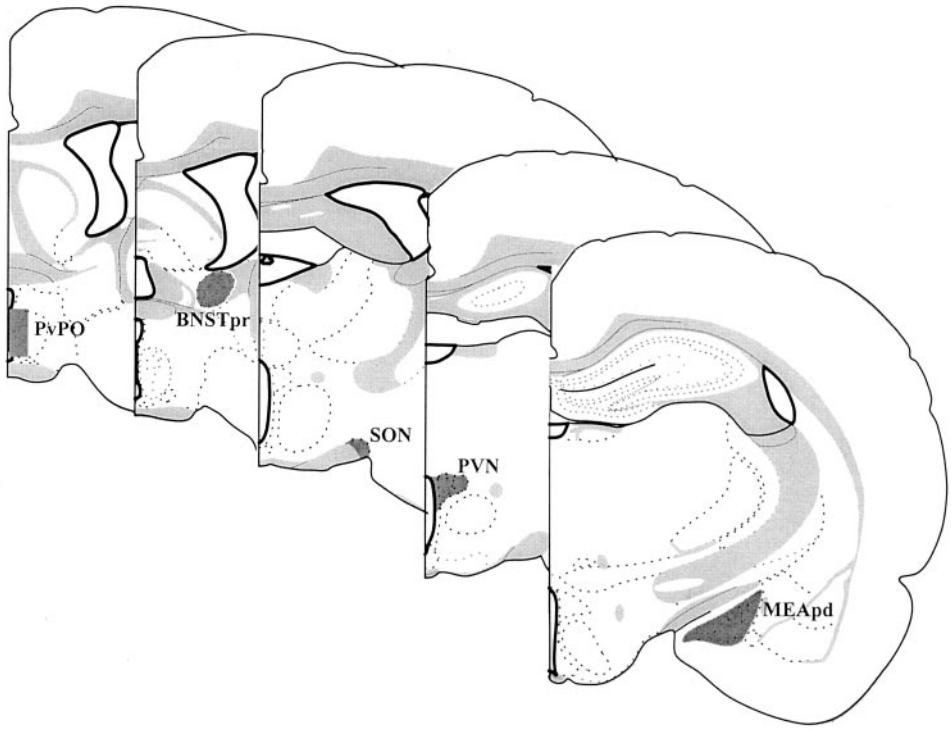

FIG. 1. Drawings of coronal brain sections representing the brain regions measured.

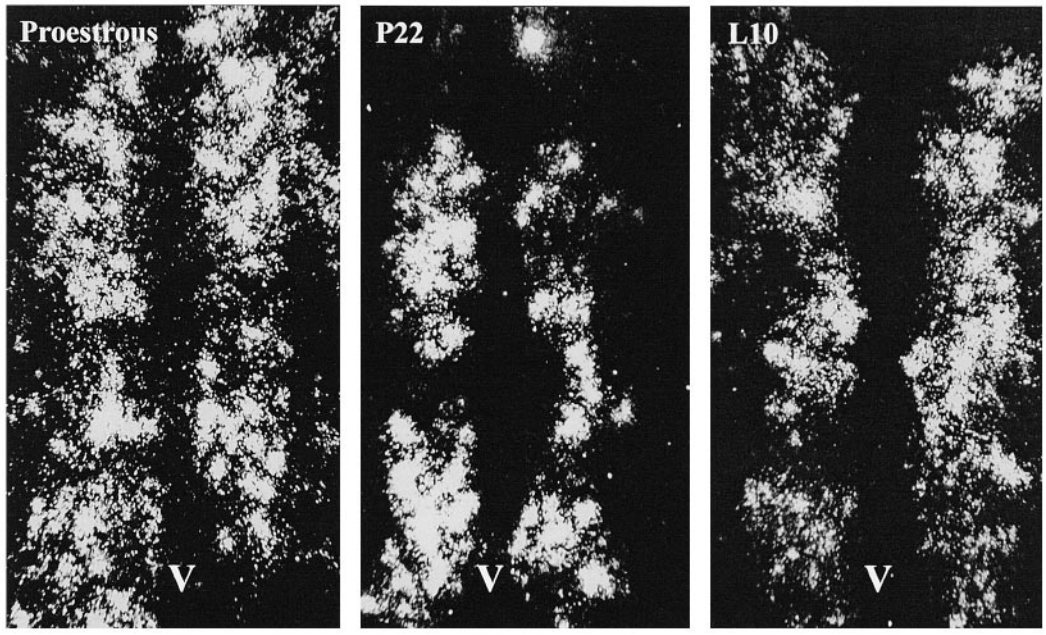

FIG. 2. Representative photomicrographs (×10, darkfield) of ERβ mRNA in the PvPO of proestrous, P22, and L10 females. V, Third ventricle.

nuclei of the bed nucleus of stria terminalis, and the anterior amygdala nuclei; and weak signal was observed in the hippocampus and the cortex. No hybridization signal was detected on tissue hybridized with sense strand ERβ probes.

Numbers of ERβ-mRNA-containing cells

The numbers of ERβ-mRNA-expressing cells differed among groups in a number of neuroanatomical regions (Fig. 3). In the PvPO, the number of ERβ-mRNA-expressing cells was significantly different among groups [$F(2,11) = 9.254$, $P < 0.01$]. P22 rats had significantly fewer ERβ-mRNA-positive cells than proestrous rats ($P < 0.01$). In the BNSTpr, the number of ERβ-mRNA-positive cells did not differ among groups [$F(2,9) = 0.23$; $P = 0.79$].

In the SON, the number of ERβ-mRNA-expressing cells differed significantly among groups [$F(2,9) = 5.201$, $P <$

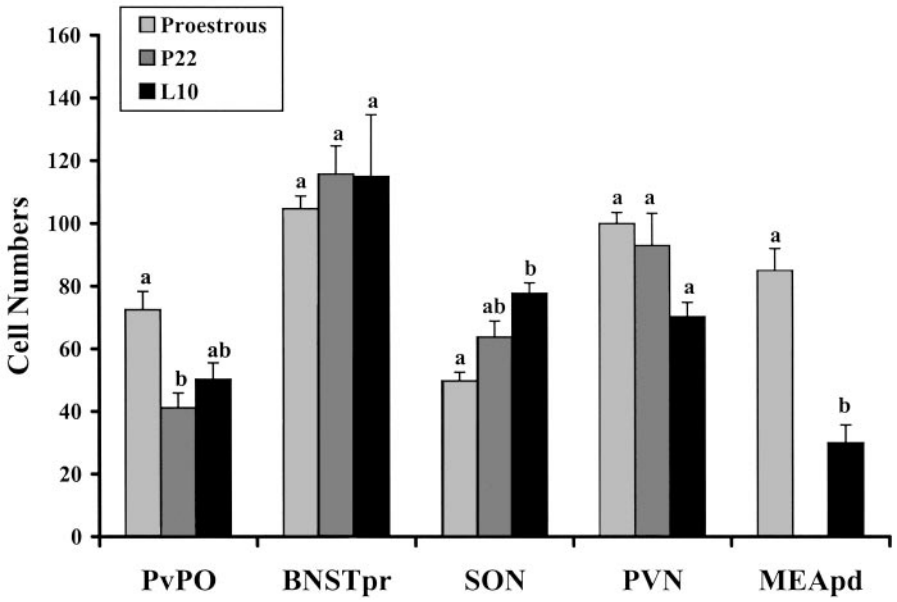

FIG. 3. Mean (± SEM) number of ERβ-mRNA-expressing cells in the PvPO, BNSTpr, SON, PVN, and MEApd of proestrous, P22, and L10 females. a and b, Significantly different from other groups; ab, not significantly different from other groups.

TABLE 1. Mean (± SEM) background grain area in the PvPO, BNSTpr, SON, PVN, and MEApd of proestrous, P22, and L10 females

Regions	Proestrous	P22	L10
PvPO	18 ± 2.4	15.7 ± 2.2	14.6 ± 2.4
BNSTpr	18.3 ± 3.8	23.5 ± 5.6	16.6 ± 2.9
SON	29.5 ± 6.7	22.2 ± 5.9	18.4 ± 4.3
PVN	19 ± 4.8	16.5 ± 2.3	18.6 ± 3.4
MEApd	23.6 ± 2.9	NA	23 ± 4.5

NA, Not applicable.

0.01]. L10 rats had significantly more ERβ-mRNA-containing cells than proestrous rats ($P < 0.01$). The number of ERβ-mRNA-positive cells in P22 rats did not differ from proestrous rats and L10 rats. In the PVN, although there was a trend, the number of ERβ-mRNA-expressing cells did not differ significantly between groups [$F(2,9) = 5.201$; $P = 0.03$].

In the MEApd, quantification of the number of ERβ mRNA cells was performed only in rats in proestrus and L10 because of problems with residual emulsion on matched sections of the P22 rats. L10 rats had significantly fewer ERβ-mRNA-containing cells than proestrous rats ($P < 0.01$).

ERβ mRNA expression

Grain area. For each brain area, background values measured on neighboring unlabeled cells did not differ significantly among groups [see Table 1. PvPO, $F(2,65) = 0.59$, $P \geq 0.05$; BNSTpr, $F(2,19) = 0.56$, $P \geq 0.05$; PVN, $F(2,26) = 0.91$, $P \geq 0.05$; SON, $F(2,19) = 0.79$, $P \geq 0.05$; MEA, $P \geq 0.05$]. In the PvPO, the mean grain area of labeled cells was significantly different among groups [$F(2,10) = 15.2$; $P < 0.01$]. Mean grain area was significantly increased in P22 rats, compared with proestrous and L10 rats ($P < 0.01$; Fig. 4). Although there was a trend toward significance in the MEApd, the mean grain area of labeled cells did not differ significantly between proestrous and L10 rats ($P = 0.016$; Fig. 4). The mean grain area of ERβ-mRNA-labeled cells was not significantly different among groups in the BNSTpr [$F(2,8) = 0.15$; $P \geq 0.01$], the PVN [$F(2,8) = 2.55$; $P \geq 0.01$], or the SON [$F(2,9) = 3.05$; $P \geq 0.01$] (Fig. 4).

Percentage of cells expressing different levels of hybridization signal. In the PvPO, the percentage of cells containing various levels of mRNA differed significantly among groups (Fig. 5). P22 rats had significantly lower percent of cells expressing between 500 and 1000 pixels/cell than proestrous and L10 rats [$F(2,10) = 20.8$; $P < 0.01$], and P22 had a significantly higher percent of cells expressing between 2000 and 2500 pixels/cell than proestrous and L10 rats. There was no significant difference among groups for cells expressing less than 500, between 1000 and 1500, or between 1500 and 2000 pixels/cell. Overall, about 78% and 82% of cells expressed less than 1500 pixels/cell in the proestrous and L10 groups, respectively, whereas only 39% of cells expressed less than 1500 pixels/cell in the P22 group. In contrast, 22% and 17.8% of cells expressed above 1500 pixels/cell in the proestrous and L10 group respectively, whereas 61% of cells expressed above 1500 pixels/cell in the P22 group.

In the MEApd, distributions of cells containing various levels of mRNA differed significantly between the proestrous and the L10 females ($P < 0.01$; Fig. 6). L10 females had a significantly lower percent of cells expressing less than 1000 pixels/cell but had a significantly higher percent of cells expressing between 1500 and 2000 pixels/cell than proestrous females ($P < 0.01$). In the proestrous group, 86% of cells expressed less than 1500 pixels/cells; whereas in the L10 group, only 50% of cells expressed less than 1500 pixels/cells. In contrast, only 14% of cells expressed above 1500 pixel/cells in the proestrous group, whereas about 50% of cells expressed above 1500 pixels/cells in the L10 group (Fig. 6).

Discussion

It has been shown previously that expression of ERα is modulated by sex steroid hormones in a number of brain regions during pregnancy (10, 26) and lactation (28). The

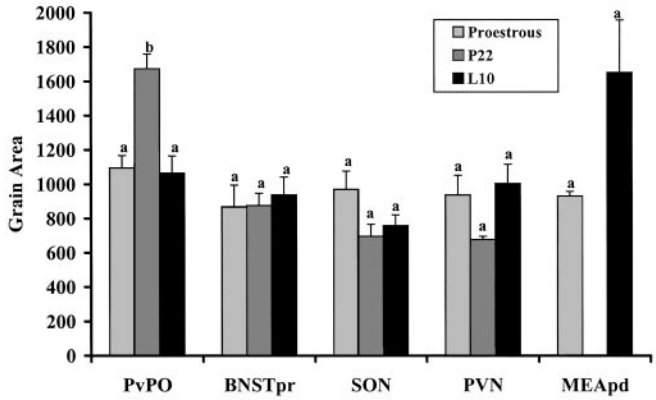

FIG. 4. Mean (± SEM) grain area of ERβ-mRNA-expressing cells in the PvPO, BNSTpr, SON, PVN, and MEApd of proestrous, P22, and L10 females. a and b, Significantly different from other groups.

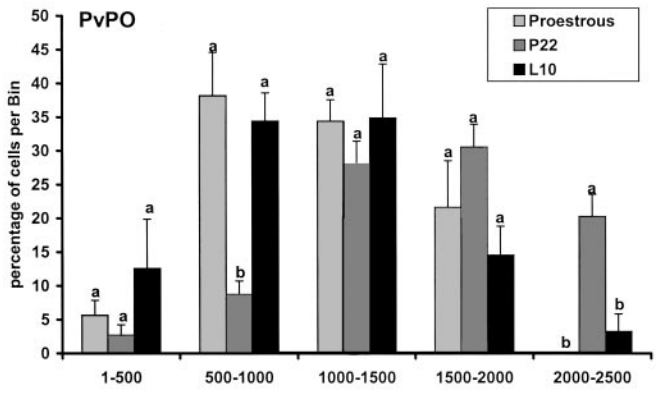

FIG. 5. Histogram of mean (± SEM) percentage of labeled cells in the PvPO according to pixel/cell in proestrous, P22, and L10 females. The range values of pixel/cell are divided into bins of 500 pixels. a and b, Significantly different from other groups.

present study provides support for the idea that ERβ expression also varies in various brain regions under disparate reproductive conditions.

The distribution of ERβ mRNA in the brain of proestrous, pregnant, and lactating rats corresponds well with that described for ovariectomized rats (6). ERβ mRNA was found in many hypothalamic and limbic regions that express ERα mRNA (6), as well as in the magnocellular nucleus of the PVN and the SON, where ERα mRNA is not found. It is noteworthy that the distribution of ERβ-mRNA-expressing cells within most regions seemed heterogeneous with clusters of cells containing high amounts of ERβ mRNA and scattered cells with lower amounts of ERβ mRNA. These patterns of expression may reflect differential regulation of one or multiple ERβ mRNA splice variants or phenotypically distinct populations of ERβ-expressing cells (37–41).

The expression of ERβ mRNA varied in a brain-region-specific manner and was differentially regulated during the different reproductive states. Indeed, the number of ERβ-mRNA-expressing cells in the PvPO was lower on P22 than during proestrus (Fig. 3), but the overall level of ERβ mRNA expression was elevated in cells on P22, compared with proestrus and L10 (Fig. 4). This increase in hybridization signal on P22 represented a shift in cell population, with a significant decrease in the percent of low ERβ-mRNA-expressing cells (about 39%) and a significant increase in the percent of high ERβ-mRNA-expressing cells (about 61%), in comparison with proestrous (78% of cells with low levels, 22% of cells with high levels) and L10 females (83% of cells with low levels, 17% of cells with high levels; Fig. 5). Thus, although fewer ERβ-mRNA-expressing cells were found at d 22 of pregnancy, the majority of these cells expressed a higher level of ERβ mRNA than during proestrus or lactation. The number of ERβ-mRNA-expressing cells did not differ significantly in the BNSTpr, across the reproductive states studied (Fig. 3).

In the SON, the number of cells expressing ERβ mRNA during lactation was increased, in comparison with proestrus (Fig. 3), but the overall level of ERβ mRNA (Fig. 4) did not vary among groups. In contrast, in the PVN, neither the number of ERβ-positive cells nor the overall level of ERβ mRNA varied significantly among groups (Figs. 3 and 4). Although ERβ mRNA signals in the SON and the PVN were measured over a mixed population of oxytocin- and vasopressin-containing cells, variations in levels of ERβ mRNA in cells, as in the PvPO, were not observed in these two regions.

In the MEApd, the number of ERβ-mRNA-expressing cells on L10 was lower than on proestrus (Fig. 3), but the overall level of ERβ mRNA expression was higher than during proestrus (Fig. 4). Although not reaching statistical significance, this increase in hybridization signal on L10 represented a significant shift in the number of cells with particular levels of mRNA expression. That is, we observed a statistically significant decrease in the percent of cells expressing low levels of ERβ mRNA (about 50%) and a comparable increase in the percent of cells expressing high levels of ERβ mRNA, in contrast to the results obtained during proestrus (84% of cells with low levels, 14% of cells with high levels; Fig. 6). Thus, although fewer ERβ-mRNA-expressing cells were found at L10 in the MEApd, the majority of these cells expressed a higher level of ERβ mRNA than during proestrus.

Differential expression of ERα among brain regions has also been reported during pregnancy and lactation. Other investigators have noted that during pregnancy in the rat, the total number of ERα-ir cells in the rostral preoptic area remains constant, but the number of a darkly stained cells increases between d 16 and d 22. In parallel, the overall number of ERα-ir cells in the bed nucleus of stria terminalis decreases between P16 and P22, but the number of darkly stained cells increases (10, 26). Although data are lacking in the rat, during lactation in female mice, ERα-ir expression also varies by neuroanatomical area (28). Moreover, we now report that in female rats, the percentage of cells expressing different levels of ERβ mRNA signal vary considerably in function of the neuroanatomic regions and the reproductive status of the females (Figs. 5 and 6). The present data, together with these earlier studies, suggest that specific subpopulations of ERα- and ERβ-containing cells are differentially regulated in brain regions critical to behaviors or physiological events associated with pregnancy and lactation.

The significance of the changes in ERβ mRNA or ERα in specific brain areas remains unclear. ERβ is expressed in ERα-ir cells in a number of areas, including the PvPO, the POA, the bed nucleus of stria terminalis, and the MEA (5, 8).

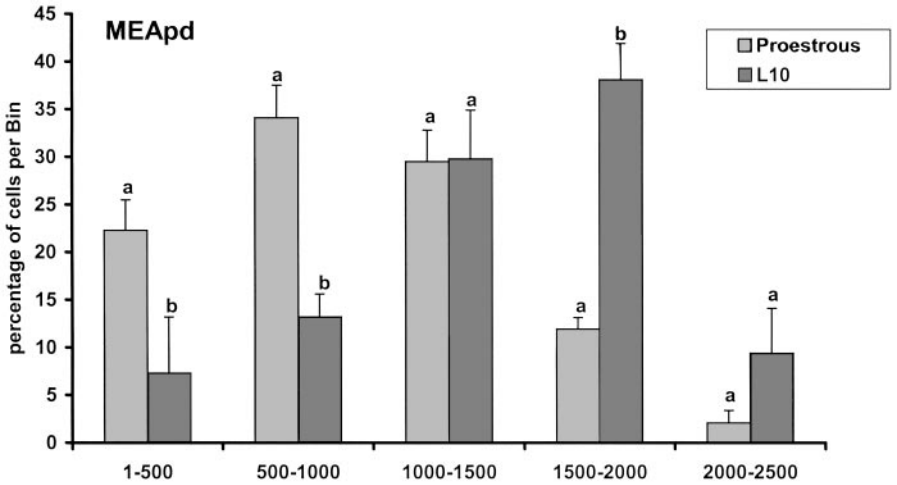

FIG. 6. Histogram of mean (± SEM) percentage of labeled cells in the MEApd according to pixel/cell in proestrous and L10 females. The range values of pixel/cell are divided into bins of 500 pixels. a and b, Significantly different from other groups.

Thus, it is plausible that changes in ERβ expression during pregnancy and lactation could occur within ERα cells in these regions. *In vitro* studies show that cotransfection of different isoforms of ERβ with ERα can influence estradiol-induced gene transcription (42–44). Specifically, ERβ negatively modulates ERα transcriptional activity when estradiol concentrations are low but not high (45). Collectively, the level of estradiol, as well as the interaction and ratio of ERs within cells, could alter, in a brain-region-specific manner, the way in which estrogenic signals are processed and, subsequently, the expression of genes associated with late pregnancy and lactation.

ERβ mRNA expression was modulated in some of the brain regions involved in the regulation of maternal behavior and the neuroendocrine functions associated with parturition and lactation. The action of estradiol on neurons of the MPOA is critical for the induction of maternal behavior (21, 24). The increase in ERβ mRNA expression (Figs. 4 and 5) and ERα protein (10) in neurons of the preoptic region suggests that both forms of ER may be involved in the increase in occupied ERs observed in this area during late pregnancy (13) and may contribute to increases in sensitivity to estradiol that are required for induction of maternal behavior during late pregnancy (46).

The concentration of occupied ERs in the MEA increases significantly during pregnancy, up until parturition (13), but ERα expression remains constant (10, 26). Unfortunately, we were not able to gather data on ERβ mRNA expression during pregnancy; therefore, we cannot discern the contribution that ERβ might make to the previously noted increases in occupied ERs in this area during pregnancy. In lactating rats, we found that expression of ERβ increases in some cells in the MEApd (Figs. 3, 4, and 6). Although unexplored in rats, the expression of ERα increases in the MEA of lactating female mice (28). Therefore, during lactation, changes in expression of both ERs could result in a differential sensitivity of the MEA to estradiol.

We examined the PVN and SON because cells in these areas synthesize oxytocin, which plays a major role in female rodent maternal behavior, parturition, and lactation (47–49). The synthesis of oxytocin is regulated by changes in gonadal hormone levels (20) and can be driven *in vitro* by estradiol (29), suggesting a regulation of oxytocin expression through an ER. Because ERβ alone is expressed in oxytocin-containing cells in the magnocellular neurons of the PVN and SON of rats (6, 31, 32), most estradiol binding and most effects of estradiol on oxytocin synthesis in these regions may be attributable to interactions with ERβ.

During pregnancy in rats, levels of oxytocin mRNA, in the PVN and SON, peak 24 h before parturition. This increase is induced by an extended exposure to sex steroid hormones followed by a peak in estradiol levels and a concomitant decline in progesterone levels (18, 50). Changes in oxytocin mRNA expression before parturition may be mediated, in part, through ERβ, but they are not paralleled by a change in ERβ mRNA expression in either PVN or SON (Figs. 3 and 4).

Levels of gonadal steroid hormones vary during the first weeks of lactation, and they contribute to changes in oxytocin mRNA expression in the PVN and SON (16, 17, 20, 50). Levels of oxytocin mRNA, which are low during the first weeks of lactation, increase in the PVN and SON, by L10, to high levels equivalent to those observed at the end of pregnancy (18). We found that on L10 the number of ERβ mRNA-containing cells increased significantly in the SON (Fig. 3), suggesting that changes observed in ERβ mRNA expression in some of the magnocellular cells (Fig. 3) could be one means through which estradiol acts to modulate oxytocin mRNA expression during lactation (18).

The present study demonstrates that ERβ mRNA expression can be regulated under physiological conditions in specific brain regions of female rats. These data, however, do not directly address regulation of ERβ during different stages of the estrous cycle, pregnancy, or lactation. To accomplish that, extensive time-course experiments during the estrous cycle, pregnancy, and lactation would be necessary. Rather, these data support the idea that ERβ mRNA is regulated in some brain regions during disparate hormonal conditions. The fact that differences in mRNA for ERβ were observed in brain regions of physiological importance during pregnancy and lactation further suggests that ERβ may contribute to the regulation of neural circuits associated with different reproductive states.

Acknowledgments

We want to thank Dr. Jun Xu for his technical assistance, and Dr. Christine Wagner for her helpful comments on the writing of this manuscript.

Received August 5, 2002. Accepted January 10, 2003.

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This work was supported by NS-19327, MH-56187, and Senior Scientist Award MH-01312, all from the NIH (to J.D.B.) and by USDA 98-35203-6309 (to L.S.L.).

References

1. **Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA** 1996 Cloning of a novel receptor expressed in rat prostate and ovary. Proc Natl Acad Sci USA 93:5925–5930
2. **Tremblay GB, Tremblay A, Copeland NG, Gilbert DJ, Jenkins NA, Labrie F, Giguere V** 1997 Cloning, chromosomal localization, and functional analysis of the murine estrogen receptor β. Mol Endocrinol 11:353–365
3. **Shughrue P, Scrimo P, Lane M, Askew R, Merchenthaler I** 1997 The distribution of estrogen receptor-β mRNA in forebrain regions of the estrogen receptor-α knockout mouse. Endocrinology 138:5649–5652
4. **Kuiper GG, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S, Gustafsson JA** 1997 Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β. Endocrinology 138: 863–870
5. **Greco B, Allegretto EA, Tetel MJ, Blaustein JD** 2001 Coexpression of ERbeta with ERalpha and progestin receptor proteins in the female rat forebrain: effects of estradiol treatment. Endocrinology 142:5172–5181
6. **Shughrue PJ, Lane MV, Merchenthaler I** 1997 Comparative distribution of estrogen receptor-α and -β mRNA in the rat central nervous system. J Comp Neurol 388:507–525
7. **Shughrue PJ, Merchenthaler I** 2001 Distribution of estrogen receptor β immunoreactivity in the rat central nervous system. J Comp Neurol 436:64–81
8. **Shughrue PJ, Scrimo PJ, Merchenthaler I** 1998 Evidence for the colocalization of estrogen receptor-β mRNA and estrogen receptor-α immunoreactivity in neurons of the rat forebrain. Endocrinology 139:5267–5270
9. **Blaustein JD** 1994 Estrogen receptors in neurons: new subcellular locations and functional implications. Endocr J 2:249–258
10. **Wagner CK, Morrell JI** 1996 Levels of estrogen receptor immunoreactivity are altered in behaviorally-relevant brain regions in female rats during pregnancy. Brain Res Mol Brain Res 42:328–336
11. **Patisaul HB, Whitten PL, Young LJ** 1999 Regulation of estrogen receptor β mRNA in the brain: opposite effects of 17β-estradiol and the phytoestrogen, coumestrol. Brain Res Mol Brain Res 67:165–171
12. **Osterlund M, Kuiper GG, Gustafsson JA, Hurd YL** 1998 Differential distribution and regulation of estrogen receptor-α and -β mRNA within the female rat brain. Brain Res Mol Brain Res 54:175–180
13. **Giordano AL, Siegel HI, Rosenblatt JS** 1991 Nuclear estrogen receptor binding in microdissected brain regions of female rats during pregnancy: implications for maternal and sexual behavior. Physiol Behav 50:1263–1267
14. **Shaikh AA** 1971 Estrone and estradiol levels in the ovarian venous blood from rats during the estrous cycle and pregnancy. Biol Reprod 5:297–307
15. **Sanyal MK** 1978 Secretion of progesterone during gestation in the rat. J Endocrinol 79:179–190
16. **Smith MS, Neill JD** 1977 Inhibition of gonadotropin secretion during lactation in the rat: relative contribution of suckling and ovarian steroids. Biol Reprod 17:255–261
17. **Smith MS** 1984 Effects of the intensity of the suckling stimulus and ovarian steroids on pituitary gonadotropin-releasing hormone receptors during lactation. Biol Reprod 31:548–555
18. **Crowley RS, Insel TR, O'Keefe JA, Amico JA** 1993 Cytoplasmic oxytocin and vasopressin gene transcripts decline postpartum in the hypothalamus of the lactating rat. Endocrinology 133:2704–2710
19. **Bridges RS** 1984 A quantitative analysis of the roles of dosage, sequence, and duration of estradiol and progesterone exposure in the regulation of maternal behavior in the rat. Endocrinology 114:930–940
20. **Amico JA, Crowley RS, Insel TR, Thomas A, O'Keefe JA** 1995 Effect of gonadal steroids upon hypothalamic oxytocin expression. Adv Exp Med Biol 395:23–35
21. **Fahrbach SE, Pfaff DW** 1986 Effect of preoptic region implants of dilute estradiol on the maternal behavior of ovariectomized, nulliparous rats. Horm Behav 20:354–363
22. **Gray P, Brooks PJ** 1984 Effect of lesion location within the medial preoptic-anterior hypothalamic continuum on maternal and male sexual behaviors in female rats. Behav Neurosci 98:703–711
23. **Numan M, Numan MJ, English JB** 1993 Excitotoxic amino acid injections into the medial amygdala facilitate maternal behavior in virgin female rats. Horm Behav 27:56–81
24. **Numan M, Rosenblatt JS, Komisaruk BR** 1977 Medial preoptic area and onset of maternal behavior in the rat. J Comp Physiol Psychol 91:146–164
25. **Silverman AJ, Zimmerman EA** 1983 Magnocellular neurosecretory system. Annu Rev Neurosci 6:357–380
26. **Wagner CK, Morrell JI** 1995 *In situ* analysis of estrogen receptor mRNA expression in the brain of female rats during pregnancy. Brain Res Mol Brain Res 33:127–135
27. **Simerly RB, Chang C, Muramatsu M, Swanson LW** 1990 Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: an in situ hybridization study. J Comp Neurol 294:76–95
28. **Koch M, Ehret G** 1989 Immunocytochemical localization and quantitation of estrogen-binding cells in the male and female (virgin, pregnant, lactating) mouse brain. Brain Res 489:101–112
29. **Burbach JPH, Adan RAH, Van Tol HHM, Verbeeck MAE, Axelson JF, Van Leeuwen FW, Beeckman JMG** 1990 Regulation of the rat oxytocin gene by estradiol. J Neuroendocrinol 2:633–639
30. **Stedronsky K, Telgmann R, Tillmann G, Walther N, Ivell R** 2002 The affinity and activity of the multiple hormone response element in the proximal promoter of the human oxytocin gene. J Neuroendocrinol 14:472–485
31. **Alves SE, Lopez V, McEwen BS, Weiland NG** 1998 Differential colocalization of estrogen receptor β (ERβ) with oxytocin and vasopressin in the paraventricular and supraoptic nuclei of the female rat brain: an immunocytochemical study. Proc Natl Acad Sci USA 95:3281–3286
32. **Simonian SX, Herbison AE** 1997 Differential expression of estrogen receptor α and β immunoreactivity by oxytocin neurons of rat paraventricular nucleus. J Neuroendocrinol 9:803–806
33. **Hrabovszky E, Kallo I, Hajszan T, Shughrue PJ, Merchenthaler I, Liposits Z** 1998 Expression of estrogen receptor-β messenger ribonucleic acid in oxytocin and vasopressin neurons of the rat supraoptic and paraventricular nuclei. Endocrinology 139:2600–2604
34. **Petersen SL, McCrone S, Keller M, Shores S** 1995 Effects of estrogen and progesterone on luteinizing hormone-releasing hormone messenger ribonucleic acid levels: consideration of temporal and neuroanatomical variables. Endocrinology 136:3604–3610
35. **Petersen SL, McCrone S** 1993 Use of dual-label *in situ* hybridization histochemistry to determine the receptor complement of specific neurons. In: Valentino KL, Eberwine JJ, Barchas JD, eds. *In situ* hybridization applications to neurobiology. New York: Oxford University Press; 78
36. **Bergen HT, Hejtmancik JF, Pfaff DW** 1991 Effects of γ-aminobutyric acid receptor agonists and antagonist on LHRH-synthesizing neurons as detected by immunocytochemistry and *in situ* hybridization. Exp Brain Res 87:46–56
37. **Hrabovszky E, Shughrue PJ, Merchenthaler I, Hajszan T, Carpenter CD, Liposits Z, Petersen SL** 2000 Detection of estrogen receptor-β messenger ribonucleic acid and 125I-estrogen binding sites in luteinizing hormone-releasing hormone neurons of the rat brain. Endocrinology 141:3506–3509
38. **Laflamme N, Nappi RE, Drolet G, Labrie C, Rivest S** 1998 Expression and neuropeptidergic characterization of estrogen receptors (ERα and ERβ) throughout the rat brain: anatomical evidence of distinct roles of each subtype. J Neurobiol 36:357–378
39. **Petersen DN, Tkalcevic GT, Koza-Taylor PH, Turi TG, Brown TA** 1998 Identification of estrogen receptor β2, a functional variant of estrogen receptor beta expressed in normal rat tissues. Endocrinology 139:1082–1092
40. **Maruyama K, Endoh H, Sasaki-Iwaoka H, Kanou H, Shimaya E, Hashimoto S, Kato S, Kawashima H** 1998 A novel isoform of rat estrogen receptor β with 18 amino acid insertion in the ligand binding domain as a putative dominant negative regular of estrogen action. Biochem Biophys Res Commun 246:142–147
41. **Price RH, Lorenzon N, Handa RJ** 2000 Differential expression of estrogen receptor β splice variants in rat brain: identification and characterization of a novel variant missing exon 4. Brain Res Mol Brain Res 80:260–268
42. **Paech K, Webb P, Kuiper GG, Nilsson S, Gustafsson J, Kushner PJ, Scanlan TS** 1997 Differential ligand activation of estrogen receptors ERα and ERβ at AP1 sites. Science 277:1508–1510
43. **Watanabe T, Inoue S, Ogawa S, Ishii Y, Hiroi H, Ikeda K, Orimo A, Muramatsu M** 1997 Agonistic effect of tamoxifen is dependent on cell type, ERE-promoter context, and estrogen receptor subtype: functional difference between estrogen receptors α and β. Biochem Biophys Res Commun 236:140–145
44. **Hyder SM, Chiappetta C, Stancel GM** 1999 Interaction of human estrogen receptors α and β with the same naturally occurring estrogen response elements. Biochem Pharmacol 57:597–601
45. **Hall JM, McDonnell DP** 1999 The estrogen receptor beta-isoform (ERβ) of the human estrogen receptor modulates ERα transcriptional activity and is a key regulator of the cellular response to estrogens and antiestrogens. Endocrinology 140:5566–5578
46. **Rosenblatt JS, Olufowobi A, Siegel HI** 1998 Effects of pregnancy hormones on maternal responsiveness, responsiveness to estrogen stimulation of maternal behavior, and the lordosis response to estrogen stimulation. Horm Behav 33:104–114
47. **Challis JRG, Lye SJ** 1994 Parturition. In: Knobil E, Neill JD, eds. The physiology of reproduction. New York: Raven Press; 985–1031
48. **Russell JA, Leng G** 1998 Sex, parturition and motherhood without oxytocin? J Endocrinol 157:343–359
49. **Young WS, Shepard E, Amico J, Hennighausen L, Wagner KU, LaMarca ME, McKinney C, Ginns EI** 1996 Deficiency in mouse oxytocin prevents milk ejection, but not fertility or parturition. J Neuroendocrinol 8:847–853
50. **Amico JA, Thomas A, Hollingshead DJ** 1997 The duration of estradiol and progesterone exposure prior to progesterone withdrawal regulates oxytocin mRNA levels in the paraventricular nucleus of the rat. Endocr Res 23:141–156