Endocrine, *vol. 20, no. 1/2, 131–138, February 2003* 0969–711X/03/20:131–138/$20.00

Changes in Estrogen Receptor-α Expression in Hypothalamic Dopaminergic Neurons During Proestrous Prolactin Surge

Yueping Hou, Shu-Ping Yang, and James L. Voogt

Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS

A surge of prolactin (PRL) occurs in female rats during proestrus in response to elevated estradiol and progesterone levels. Dopamine is the primary hypothalamic inhibitor of PRL secretion from the pituitary. Using double-label immunocytochemistry, we investigated the pattern of estrogen receptor-α (ER-α) immunoreactivity in dopaminergic neurons in the arcuate nucleus (ARC) and the periventricular nucleus (PeVN) during the proestrous PRL surge and compared it to that during diestrus, when PRL levels are constantly low. Our results showed that during diestrus >80% of dopaminergic neurons in the ARC were also positive for ER-α, and this colocalization percentage decreased significantly during proestrus. By contrast, <15% of dopaminergic neurons in the PeVN expressed ER-α, and the low percentage of ER-α expression was unchanged throughout proestrus and diestrus. Results from estrogen plus progesterone–treated ovariectomized rats showed similar patterns of ER-α expression within the ARC and the PeVN and, once again, compared with the control group, had a significant reduction in ER-α immunoreactivity in dopaminergic neurons in the ARC, but not in the PeVN. These results provide an anatomic basis that dopaminergic neurons in the ARC and the PeVN are functionally different regarding to ER-α expression. Our study also supports the hypothesis that dopaminergic neurons in the ARC are an important neuronal population responsive to estrogen by changing the expression of ER-α in those neurons. This modification of sensitivity of dopaminergic neurons in the ARC in response to ovarian steroids may be an important molecular mechanism involved in PRL regulation, including the regulation of the proestrous surge of PRL.

Key Words: Estrogen receptor; prolactin; tuberoinfundibular dopaminergic; proestrus; ovarian steroids.

Introduction

The dependency of the preovulatory surge of prolactin (PRL) on circulating estrogen (E_2) and progesterone (P_4) levels is well established. The proestrous surge of PRL can be blocked by administration of E_2 antibody *(1)*. Although not indispensable, P_4 given following E_2 priming can strongly intensify E_2-induced PRL surges in ovariectomized rats (OVX) *(2,3)*.

Dopamine (DA) is the primary hypothalamic inhibitor of PRL secretion from the pituitary *(4)*. Hypothalamic DA is mainly provided by A12 and A14 neurons, which are located in the arcuate nucleus (ARC) and the periventricular nucleus (PeVN), respectively. A12 neurons give rise to the majority of tuberoinfundibular dopaminergic (TIDA) neurons that release DA to the anterior pituitary through long portal vessels *(5)*. A small population of rostral A12 neurons (the tuberohypophysial dopaminergic) terminate in the neurointermediate lobe of the pituitary gland *(6)*. The majority of the A14 cell group (periventricular hypothalamic dopaminergic neurons) projects to the intermediate lobe of the pituitary through the short portal vessels *(7)*.

Abundant evidence suggests that dopaminergic neuronal activity in the hypothalamus is influenced by ovarian steroids. Administration of E_2 downregulates the activity of tyrosine hydroxylase (TH), the rate-limiting enzyme for catecholamine synthesis in the ARC *(8)*. DA released from the hypothalamus decreases during proestrus *(9)* and during the ovarian steroid–induced PRL surge *(10)*.

It is generally accepted that E_2 functions primarily through specific intracellular E_2 receptors present in target cells *(11)*. The classic estrogen receptor-α (ER-α) is thought to mediate the effects of E_2 on neuronal morphology, neurotransmitter transition, neuroendocrine change, and reproductive behavior *(12–14)*. ER-α expression in the hypothalamus is also regulated by ovarian steroids *(15)*. The expression of ER-α mRNA and protein fluctuates across the estrous cycle *(16,17)*. Estradiol downregulates ER expression in the ARC *(18)*. The ER-immunoreactive neurons are markedly decreased in both number and signal intensity in the ARC by estradiol benzoate treatment *(19)*. A recent publication reported that TH neurons in the ARC also express ER-α *(20)*, suggesting a direct regulation of E_2 on A12 dopaminergic neurons. It is not known whether this modification of

---

Received October 4, 2002; Accepted December 4, 2002.

Author to whom all correspondence and reprint requests should be addressed: Dr. James L. Voogt, Department of Molecular and Integrative Physiology, University of Kansas Medical Center, 3901 Rainbow Boulevard, Kansas City, KS 66160. E-mail: jvoogt@kumc.edu

ER expression happens to all neuronal populations in the hypothalamus, or whether it takes place only in specific neuronal groups.

The present study investigated whether two dopaminergic neuronal populations, A12 and A14, respond similarly to ovarian steroids in terms of ER-α expression. Therefore, expression of ER-α in A12 and A14 dopaminergic neurons was compared in rats during proestrus and diestrus, and also in OVX rats with or without ovarian steroid treatment.

Results

Plasma PRL, E_2, and P_4 Levels in Proestrous and Diestrous Rats

Serum was collected from normal cycling rats at specific time points on days of diestrus and proestrus. As seen in Fig. 1, a surge of PRL secretion started at 3:00 PM and continued to 9:00 PM on the day of proestrus. E_2 levels were relatively low during diestrus, increased on proestrus at 9:00 AM, and returned to basal levels by 9:00 AM. Serum levels of P_4 were low during diestrus and the morning of proestrus, increased by 6:00 PM, and further increased on at 9:00 PM on proestrus.

PRL Levels Following Ovarian Steroid Treatment

Following injection of sex steroids into OVX rats, it was determined that either E_2 alone or E_2 plus P_4 treatments caused a significant elevation in serum PRL levels 6 h after the last injection, whereas P_4 alone had no effect on PRL levels compared to the vehicle-treated group (data not shown).

Changes in Number of TH-Expressing Neurons andPercentage of ER-α-Colabeled TH Neurons in ARC and PeVN in Rats Throughout Proestrus and Diestrus

The percentages of TH-ir (immunoreactive) neurons expressing ER-α and the number of TH-ir neurons in the ARC throughout the day on proestrus and diestrus are shown in Fig. 2. The percentage of TH/ER-α-colabeled neurons generally displayed an inverse relationship with serum PRL levels. The incidence of double-labeled TH/ER-α neurons did not differ at 9:00 AM in any of the three subdivisions of the ARC in rats in proestrus compared with rats in diestrus. Whereas the incidence did not change throughout the day of diestrus, the incidence of doubled-labeled neurons in the caudal ARC decreased at 3:00 PM on proestrus and remained low at 6:00 PM, at which time the incidence of double-labeled neurons also declined significantly in the rostral and middle portions of the ARC. There were no differences in the number of TH-ir cells in any of the three subdivisions of the ARC among experimental groups. In the PeVN, neither the percentage of TH-ir neurons expressing ER-α nor the number of TH-ir neurons in either rostral or caudal portion of the PeVN changed throughout the day of proestrus or diestrus (Fig. 3). There was no difference in either the percentage of ER-α-colabeled TH cells or the number of TH-ir-only cells comparing proestrus to diestrus.

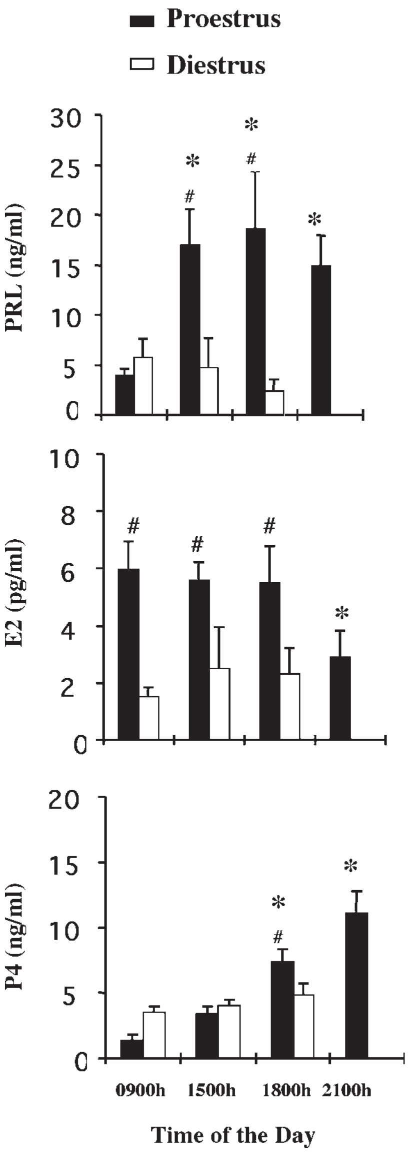

Fig. 1. Serum PRL, E_2, and P_4 concentrations (mean ± SE) obtained from proestrous (■) and diestrous (□) female rats. *Significantly different hormone levels than in proestrous 9:00 AM rats; #higher hormone levels than in diestrous rats ($p < 0.05$).

Examination of the data in Figs. 2 and 3 reveals that expression of ER-α in TH neurons in the ARC and the PeVN are different. TH/ER-α double-labeled neurons in the ARC are abundant. More than 80% of the TH-ir neurons expressed ER-α throughout the ARC, especially so during diestrus. In the PeVN, however, regardless of either diestrus or proestrus, <15% of the TH-ir neurons expressed ER-α, and most of the double-labeled neurons were found in the ventral portion of the PeVN. Few TH-ir neurons in the dorsal portion of the PeVN expressed ER-α.

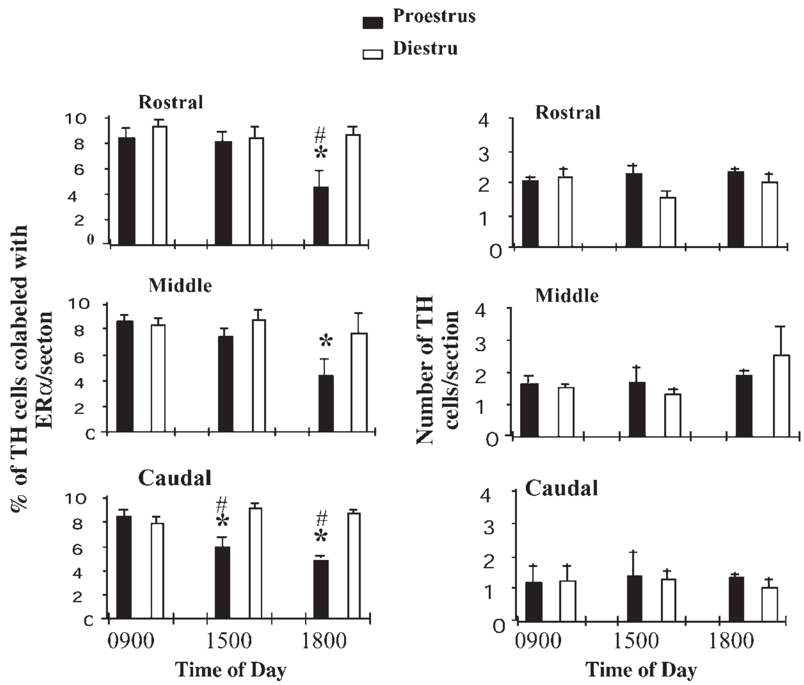

Fig. 2. Comparison of mean (±SE) percentage of ER-α-colabeled TH cells or number of TH-ir cells in three subdivisions (rostral, middle, and caudal) of ARC on day of proestrus (■) and diestrus (□). *Significantly lower number than in proestrous 9:00 AM rats; #significantly lower number than in diestrous rats at the same time point ($p < 0.05$).

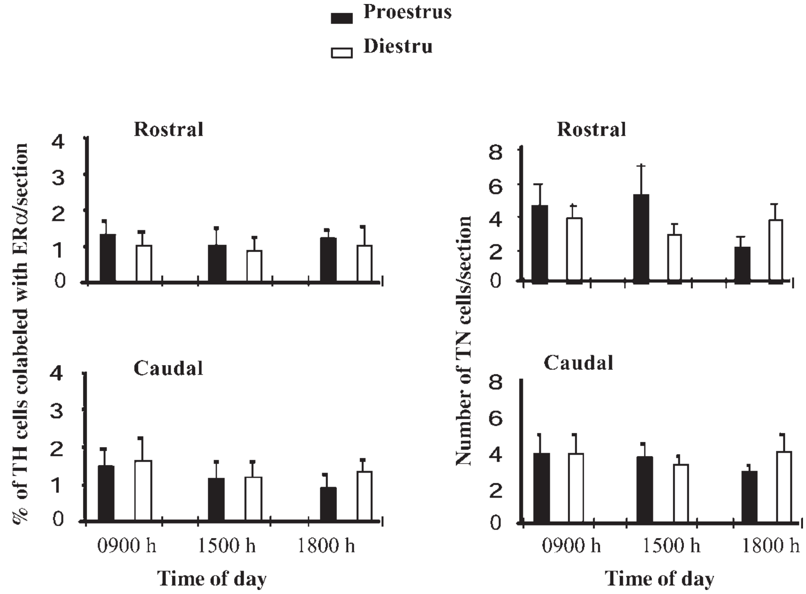

Fig. 3. Mean (±SE) percentage of TH/ER-α-colabeled cells or number of TH-ir cells in PVN neurons in proestrous and diestrous rats.

Changes in Number of ER-α- and TH-Immunoreactive Neurons and Percentage of ER-α-Labeled TH-Immunoreactive Neurons in ARC Following Ovarian Steroid Treatment

Compared to oil vehicle treatment, ER-α expression was decreased throughout the A12 (ARC) area under E_2 plus P_4 treatments (Fig. 4). Although E_2 alone suppressed ER-α expression to a certain degree, only when combined with P_4 was this decrease significant. P_4 alone did not change ER-α-ir to any significant extent. In all areas of the ARC, treatment with E_2 plus P_4 significantly reduced the portion of TH neurons that also expressed ER-α. Throughout much of the entire A12 area, E_2 and E_2 plus P_4 induced a strong increase in the number of TH-ir neurons. P_4 alone, however,

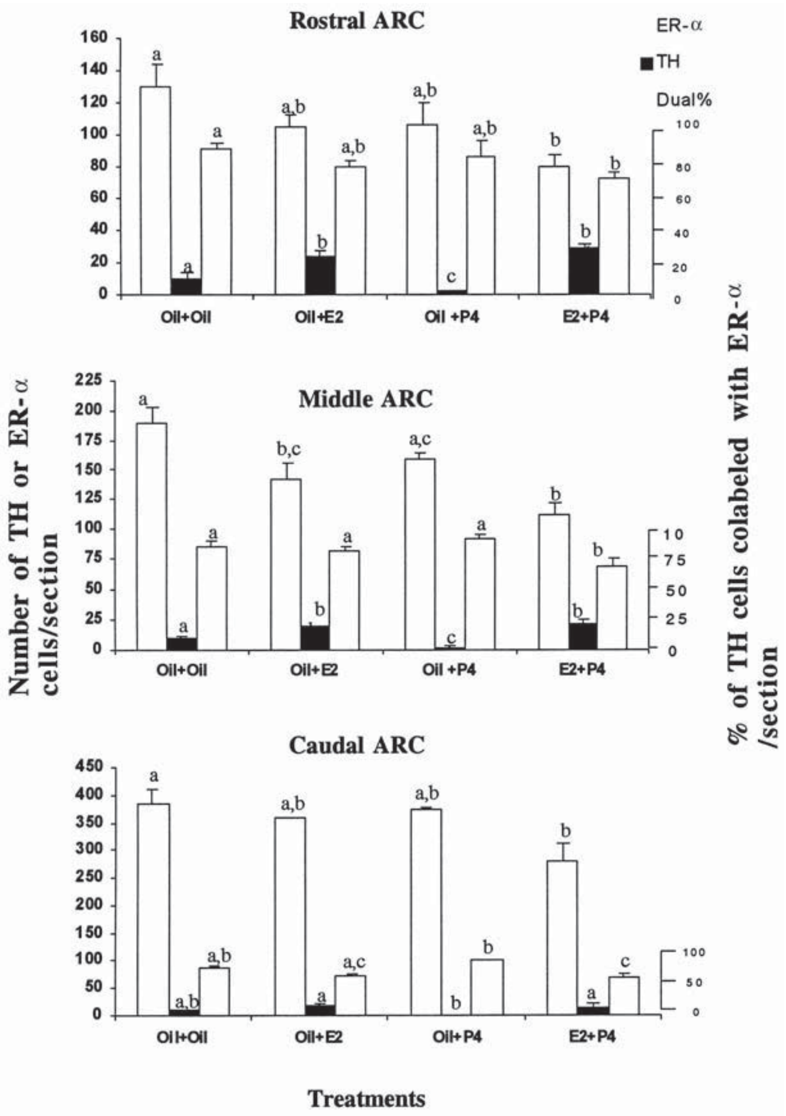

Fig. 4. Numbers of ER-α-ir, TH-ir neurons, and percentage of ER-α and TH dual-labeled TH neurons (mean ± SE) in ARC under different ovarian steroid treatments (oil vehicle, E_2, P_4, and E_2 plus P_4). a, b, c: Significantly different among different treatments within the same parameter group.

dramatically reduced the number of TH-ir neurons in all of A12 (see Fig. 5 for photomicrographs of each treatment group).

Discussion

E_2 plays a critical role in the regulation of PRL secretion. In the absence of E_2, plasma PRL levels are low and relatively stable throughout the day *(2,21)*, and estradiol initiates small daily diurnal PRL surges *(22)*. In addition to a direct regulation by E_2 in the pituitary to influence lactotroph recruitment *(23)*, PRL synthesis *(24)*, PRL secretion *(25)*, and the responsiveness of the anterior pituitary to PRL-releasing and -inhibiting hormones *(26)*, E_2 also has a profound influence on dopaminergic neurons, particularly TIDA neurons in the hypothalamus. Previous studies have reported the existence of both ER-α and the recently found ER-β in dopaminergic neurons. In the ARC, >50–80% of the dopaminergic neurons, depending on different reports, are positive for ER-α immunoreactivity *(20,27)*. E_2 decreases TH activity, TH mRNA levels, and DA metabolism in the hypothalamus *(8,10,27)*. An inhibitory effect of E_2 on both basal and stimulated TH activity has been observed in hypothalamic cell cultures, suggesting that E_2 appears to affect hypothalamic dopaminergic neurons directly *(28)*. Consistent with an earlier study *(20)*, the present results show that the majority of dopaminergic neurons in the ARC express ER-α, supporting the basis of a direct E_2 effect on this neuronal population.

The activity of TIDA neurons fluctuates throughout the estrous cycle in rats, being the highest during diestrus and the lowest during proestrus *(9,29)*. Activity of TIDA neurons decreases coincident with the onset of the proestrous surge of PRL or during the E_2 or E_2/P_4-induced PRL surge

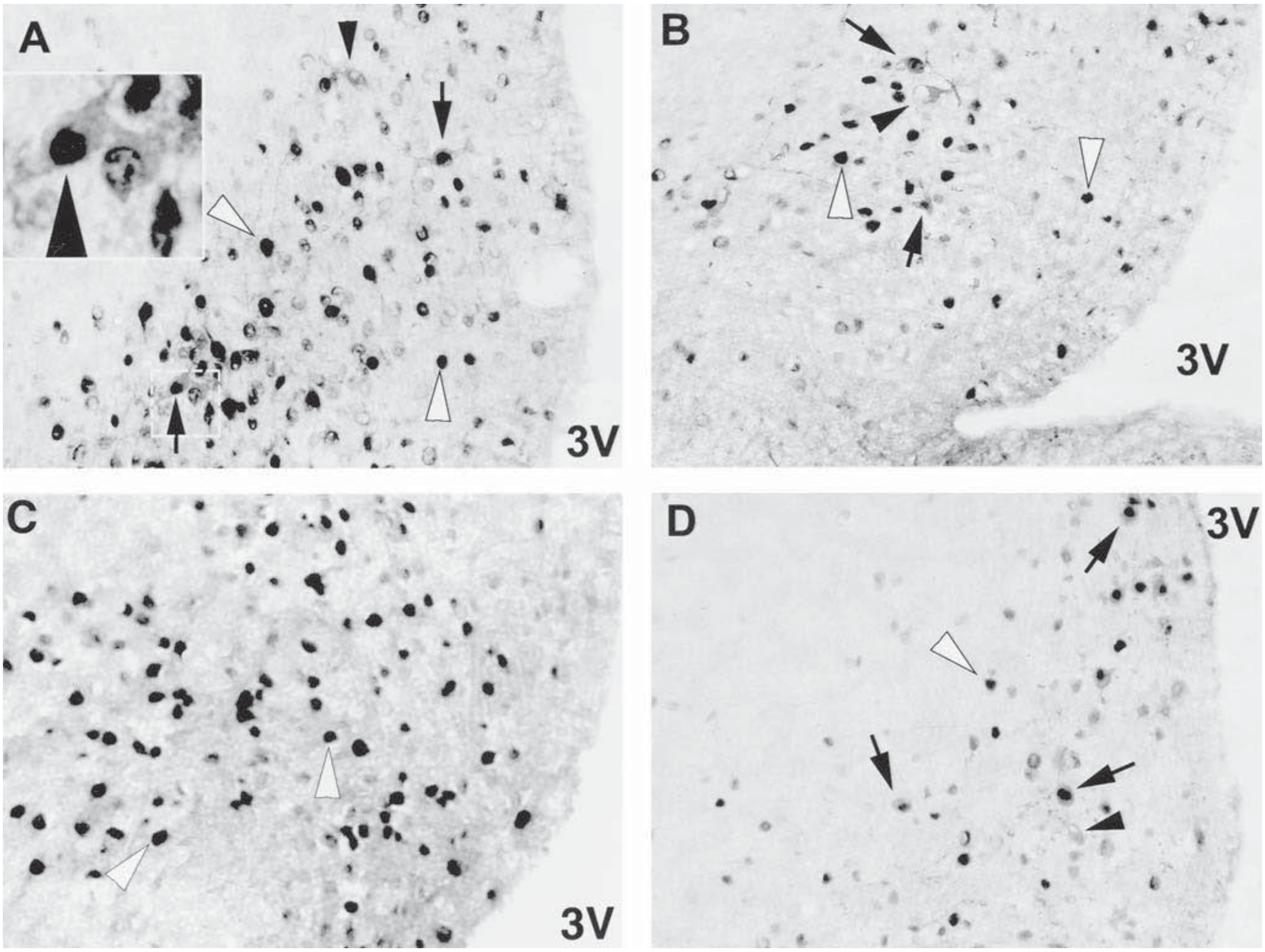

Fig. 5. Representative photomicrographs of ER-α and TH colocalization staining in middle ARC under different ovarian steroid treatments (×400). (**A**) Oil vehicle alone; (**B**) E_2 alone; (**C**) P_4 alone; (**D**) E_2 plus P_4. 3V: third ventricle; open arrowhead: ER-α-ir neurons; solid arrowhead: TH-ir neurons; long arrow: ER-α and TH colocalized neurons. Note the enlargement in (A) demonstrating colocalization of TH and ER-α.

(10). The concentration of DA decreases in the median eminence *(30)* and anterior pituitary *(31)* during the proestrous PRL surge. Our results indicate that ER-α expression in A12 neurons decreased in the afternoon of proestrus and after E_2 and P_4 treatment in OVX rats. The incidence of ER-α-expressing neurons in A12 displayed an inverse relationship to PRL levels.

The change in ER-α expression in A12 neurons in cycling and steroid-treated rats may result from changes in the level of circulating ovarian steroids. The decrease in the percentage of ER-α-colabeled TH neurons in the afternoon of proestrus may be a response to elevated levels of E_2 that occurred in the morning of proestrus. This interpretation is generally consistent with an inhibitory influence of E_2 on ER expression in the hypothalamus. However, the real mechanism underlying the reduction in ER-α immunoreactivity is still very controversial. The attenuation of ER-α immunoreactivity within the brain could be a result of ligand-induced receptor immunoreactivity loss, or ligand-influenced receptor protein turnover, or the repression of ER-α gene expression and the subsequent synthesis of ER-α protein, or a combination of all three different mechanisms.

The loss of ER-α immunoreactivity after it binds to its ligand may have nothing to do with the turnover of ER-α protein or the expression of ER-α gene and the subsequent synthesis of ER-α. The binding of ER-α and its ligand alone might be attributed to the loss of the immunoreactivity of the receptor. This possibility has been suggested by several investigators *(32–35)*. There are several possible explanations for this. Firstly, the binding site of ER-α to its ligand may be the same epitope against which the antiserum was made. Thus, the binding of ligand may block the antiserum from binding to the epitope it was meant to bind, resulting in the loss of immunoreactivity *(32,33)*. This possibility can be excluded by carefully choosing the antibody. The antibody we used in our study is a polyclonal antibody designed to bind to the epitope outside the ligand-binding pocket of ER-α. Secondly, the dimerization of ER-α *(34)*, the conformational change in the receptor following binding *(35)*, or the binding of ligand-receptor complex to target DNA may also reduce the accessibility of the antibody to ER *(34)*, and thus to reduce the immunoreactivity of the receptor. However, since all the possible aforementioned mechanisms are shorttime events in comparison to the longtime steroid effects as seen in our study, these mechanisms are not likely explanations for our observed reduction in ER-α immunoreactivity.

Ubiquitination-proteasome-medicated degradation of ER after estrogen has also been reported as a way of downregulating ER protein *(36)*. However, this is not likely to be the mechanism in the present study. First, estrogen-dependent proteasome-mediated pathway is more likely to be a mechanism used to keep the dynamic balance of cellular ER concentration as well as to recycle cellular ER. It works rapidly after administration of exogenous ligand, mostly within 2 h, with no change in ER mRNA levels, and with a half-life of the ligand-binding ER reduced to 3 to 4 h *(36–38)*. Our measurements were made 6 h after administration of estradiol, at which time decreased ER mRNA has been expected *(39, 40)*. Second, ER-α immunoreactivity downregulation has been shown to be regional specific within the brain in our study as well as in other reports. There are no reports showing regional specificity of proteasome-mediated ER downregulation.

E_2 downregulates the expression of ER mRNA in several brain areas of the hypothalamus including the ARC *(39,40)*. It also has been reported that the changes in ER protein appeared to lag behind that of mRNA *(17)*. The decrease in ER-α expression in A12 neurons during the afternoon of proestrus also could be owing to the inhibitory effect of elevated P_4, since P_4 has been shown to attenuate ER synthesis in the brain *(41,42)*. The preovulatory P_4 surge may act synergistically with E_2 to reduce the expression of ER-α in A12 neurons. In our study, plasma levels of P_4 were low in the morning and were increased at 6:00 PM, when a profound decrease in ER-α expression in A12 cells was observed.

Although the functional importance of the decrease in ER-α expression in A12 neurons remains to be established, it may provide a molecular mechanism in the feedback regulation of E_2 on dopaminergic neuronal activity in the hypothalamus. Because of the prominent role of dopaminergic neurons in regulating PRL release and its established relationship with E_2, we hypothesize that E_2 may influence the responsiveness of dopamine neurons to further influx of E_2 by modifying E_2 receptor expression in those neurons. E_2 may reduce its receptor expression directly in its target neurons *(40)*. It is also possible that E_2 may act on ER-β, which, in turn, may modulate the action of ER-α on dopaminergic neurons. Meanwhile, E_2 may enforce the change in ER expression by an indirect way, such as by increasing P_4 receptor expression. Reports have shown that P_4 is capable of reducing ER concentration in rat hypothalamus and pituitary *(42)*. By acting via elevated levels of P_4 receptors, P_4 may strongly decrease the expression of ER. A former study as well as the present work suggests that E_2 combined with P_4 is more effective in damping ER expression *(43)*. Another report also showed extensive overlapping of ER and P_4 receptor expressions *(44)*. ER-immunoreactive neurons are distributed extensively in the hypothalamus in rats. The reduction in E_2 receptor expression takes place throughout the entire ARC in many different neuronal populations besides dopaminergic neurons. Therefore, changes in neuronal responsiveness in the hypothalamus to ovarian steroids by changing ER expression could be a commonly employed mechanism in the hypothalamus.

In contrast to the changes in ER-α expression in TIDA neurons in the ARC during proestrus, ER-α expression did not change in PeVN TH-ir neurons throughout proestrus or diestrus (Figs. 2 and 3). This result is consistent with previous reports that showed regional specificity in the regulation of ER gene expression in OVX or intact rats during the estrous cycle *(16,39)*. Earlier studies suggest an important role for the intermediate lobe of the pituitary in PRL regulation, presumably by receiving DA terminals from the tuberohypophysial and periventricular hypothalamic dopaminergic neurons *(45)*. However, the relatively small population of ER-containing neurons in the PeVN (A14) that are colocalized with TH in the present study suggests that dopaminergic neurons in A14 may be less important than that in A12 regarding the regulatory effect of E_2. The absence of changes in the number of ER-α/TH-colabeled neurons in the PeVN during proestrus further suggests that dopaminergic neurons in A14 may not be as important as those in A12 in the feedback mechanism of E_2 on dopaminergic neurons. Thus, it is likely that this population of neurons plays a relatively minor role in the regulation of the preovulatory PRL surge. However these neurons may exert a tonic influence on PRL secretion or play a role in E_2-independent PRL secretion, i.e., mating or stress-induced PRL release.

In contrast to our data obtained from intact rats, we found that the number of dopaminergic neurons in A12 was changed significantly while undergoing ovarian steroid treatments. In comparison with oil-treated OVX animals, E_2 alone and E_2-primed P_4-treated rats displayed a robust increase in TH-ir in A12 (Fig. 4). P_4 treatment alone, however, resulted in an obvious absence of TH-ir (Fig. 5). Although elevated E_2 and P_4 could have inhibitory effects on dopaminergic neurons, the strong increase in PRL secretion from the anterior pituitary may override the inhibitory effects from ovarian steroid and stimulate TH synthesis instead. The stimulatory effect of PRL on dopaminergic neurons has been reported by many investigators *(46)*.

In summary, our study revealed that the majority of dopaminergic neurons in A12 expressed ER-α, whereas <15%

of dopaminergic neurons in A14 contained ER-α. ER-α expression in A12 dopaminergic neurons changed after ovarian steroid administration, whereas expression in A14 dopaminergic neurons remained unchanged. This observation suggests that dopaminergic neurons in A12 and A14 are functionally heterogeneous, at least in the regulation of PRL release from the pituitary. Moreover, the present study demonstrated, for the first time, that ER-α expression in A12 dopaminergic neurons decreased during the proestrous PRL surge and after ovarian steroid treatment. These results support the concept that there may be a feedback mechanism by which E_2 regulates A12 dopaminergic neuronal responsiveness to ovarian steroids. This mechanism may be involved in many neuroendocrine events in the hypothalamus, including the regulation of the proestrous PRL surge.

Materials and Methods

Animals

Adult female Sprague-Dawley rats were obtained from Sasco (Omaha, NE) and housed in an AALAC-accredited facility maintained in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Animals were acclimated to controlled temperature (22°C) and light (12-h/12-h light/dark cycle, with lights on at 8:00 AM). Animals were provided rat chow and tap water ad libitum. Daily vaginal smears were obtained by lavage to monitor estrous cycles.

Intact female rats were sacrificed at 9:00 AM, 3:00 PM, and 6:00 PM on the day of proestrus and d 1 of diestrus. Blood samples were collected at these three times each day and also at 9:00 PM on proestrus to examine the changing hormonal profile. After the animals were deeply anesthetized with an overdose of sodium pentobarbital, sodium heparin (0.1 mL of 1000 U) was injected into the left ventricle. Blood samples were collected directly from the heart. Animals were subsequently perfused transcardialy with 150 mL of saline followed by 350 mL of 4% paraformaldehyde in 0.05 *M* potassium phosphate-buffered saline (KPBS) pH 7.6. Brains were removed, postfixed in the same fixative overnight at 4°C, saturated in 25% buffered sucrose solution, quickly frozen in HistoFreezeTM-2000 (Fisher, Pittsburgh, PA) and stored at −80°C until the initiation of immunocytochemistry (ICC).

For ovarian steroid treatment, 20 young female Sprague-Dawley rats weighing 200–250 g were OVX bilaterally under ketamine (Phoenix Pharmaceutical, St. Joseph, MO) (0.25 mL/rat) anesthesia. Seven days following OVX, rats were divided into four treatment groups. Rats in group 1 were given one injection of sesame oil vehicle (subcutaneously 0.2 mL/rat) at 10:00 AM, followed by another oil injection 24 h later. Rats in groups 2 and 3 rats also were given one injection of oil at 10:00 AM the first day and 24 h later were injected subcutaneously with either 17-β-estradiol benzoate (EB; Amersham Life Science) (30 µg/rat in 0.2 mL of sesame oil) or progesterone (P_4, Amersham Life Science) (2 mg/rat in 0.2 mL of sesame oil), respectively. Rats in group 4 were administered EB on the first day at 10:00 AM followed 24 h later with P_4 injection. All four groups were deeply anesthetized with an overdose of sodium pentobarbital 6 h after the second injection. Blood samples were collected directly from the heart before transcardial perfusion. Animals were perfused and brains were collected as mentioned previously.

Immunocytochemistry

Brains were sectioned using a freezing microtome at 20 µm and every third section was collected. Sections were rinsed with KPBS followed by incubation with 0.6% hydrogen peroxide in methanol to quench endogenous peroxide activity and with 10% normal goat serum in KPBS to reduce nonspecific binding. The sections were then incubated with ER-α polyclonal rabbit antibody (diluted 1:500) for 24 h at room temperature in KBPS containing 2% normal goat serum and 0.01% thimerosal. The ER-α antibody (sc-542; Santa Cruz Biotechnology, Santa Cruz, CA) was raised in rabbits against the carboxy terminus of ER-α of mouse origin, specific for ER-α. This antibody does not crossreact with ER-β. Tissues were subsequently incubated with biotinylated goat anti–rabbit serum (1:600, Vectastain Elite Kit; Vector, Burlingame, CA) in KPBS with 0.4% Triton-X 100 for 1 h at room temperature, followed by incubation with avidin DH-biotinylated horseradish peroxidase-H complex (4.5 µL of each A and B reagents/mL of KPBS-Triton-X 100; Vector). After rinsing in KPBS followed by 0.175 *M* sodium acetate solution, sections were stained with nickel sulfate (25 mg/mL)–diaminobenzidine (0.2 mg/mL) in sodium acetate solution containing 0.83 µL/mL of 3% hydrogen peroxide for 15 min. After these procedures, the ER-α-labeled sections were washed with KPBS extensively and treated with 0.6% hydrogen peroxide in methanol to quench a surplus of peroxidase from ER-α ICC, followed by 10% normal horse serum in KPBS. Brain sections were incubated with monoclonal TH antibody (1:50,000) (Chemicon, Temecula, CA) for 24 h at room temperature. After rinsing in KPBS, sections were incubated with horse anti–mouse IgGs and avidin-biotinylated horseradish peroxidase complex. Following rinses in 0.1 *M* Tris buffer (pH 7.4), the peroxidase activity was revealed in Tris containing 0.03% hydrogen peroxide and 0.4% 3-amino-9-ethylcarbazole. Using these procedures, TH-ir cells were stained brownish red and nuclei of ER-α were dark blue. TH/ER-α double-labeled cells were defined by the presence of a dark blue nucleus surrounded by red cytoplasm.

Cell Counting

The ARC and PeVN were identified according to the rat brain atlas of Paxinos and Watson *(47)* under phase contrast with a Nikon Optiphot microscope. The ARC (A12) and PeVN (A14) were divided into three and two portions, respectively, as described by Lerant and Freeman *(29)*. The rostral PeVN was from 900 to 1800 mm postbregma and caudal PeVN from 1800 to 2100 mm postbregma. The rostral ARC was from 1800 to 2100 mm, the middle ARC from 2100 to 3600 mm, and the caudal ARC from 3600 to 4200 mm postbregma. Sections were anatomically matched across animals and groups. The number of TH-ir and colabeled TH/ER-α neurons were counted bilaterally on sections in each portion, and a mean count per section was generated for each animal.

Measurement of PRL, E_2 and P_4

Plasma PRL levels were determined by radioimmunoassay (RIA) with materials kindly provided by Dr. Albert F. Parlow (National Hormone and Pituitary Program; UCLA-Harbor Medical Center) and National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), and ^{125}I-PRL was obtained from Dupont (Boston, MA). Plasma E_2 and P_4 levels were determined by methods described previously *(48)*.

Statistical Analyses

All data are expressed as means ± SEM. The number of single- or double-labeled cells in each brain area and plasma hormone levels were analyzed by analysis of variance followed by a post-hoc comparison using the Fisher protected least significant difference test. These statistical tests were conducted using the Statview Program, version 4.0 (Abacus Concepts). Differences were considered significant at the level of $p < 0.05$.

Acknowledgments

We wish to thank Dr. Albert F. Parlow and the National Hormone and Pituitary Program, NIDDK for the gift of PRL RIA material. We also thank Darlene Limback for her help in estradiol and P_4 assays. This work was supported by National Institutes of Health grant HD24190 and through cooperative agreement U54 HD33994 as part of the Specialized Cooperative Center Program in Reproduction Research.

References

1. Neill, J. D., Freeman, M. E., and Tillson, S. A. (1971). *Endocrinology* **89,** 1448–1453.
2. Caligaris, L., Astrada, J. J., and Taleisnik, S. (1974). *J. Endocrinol.* **60,** 205–215.
3. Yen, S. H. and Pan, J. T. (1998). *Endocrinology* **139,** 1602–1609.
4. Ben-Jonathan, N. (1985). *Endocr. Rev.* **6,** 564–589.
5. Fuxe, K. (1964). *Acta Physiol. Scand.* **58,** 383–384.
6. Bjorklund, A., Moore, R. Y., Nobin, A., and Stenevi, U. (1973). *Brain Res.* **51,** 171–191.
7. Goudreau, J. L., Lindley, S. E., Lookingland, K. J., and Moore, K. E. (1992). *Neuroendocrinology* **56,** 100–105.
8. Arbogast, L. A. and Voogt, J. L. (1993). *Neuroendocrinology* **58,** 501–510.
9. DeMaria, J. E., Livingstone, J. D., and Freeman, M. E. (1998). *Neuroendocrinology* **67,** 377–383.
10. DeMaria, J. E., Livingstone, J. D., and Freeman, M. E. (2000). *Brain Res.* **879,** 139–147.
11. Blaustein, J. D. and Olster, D. H. (1989). In: *Advances in comparative and environmental physiology*. Balthazart, J. (ed.). Springer: Berlin.
12. Kumar, V., Green, S., Stack, G., Berry, M., Jin, J. R., and Chambon, P. (1987). *Cell* **51,** 941–951.
13. Carson-Jurica, M. A., Schrader, W. T., and O'Malley, B. W. (1990). *Endocr. Rev.* **11,** 201–220.
14. Couse, J. F. and Korach, K. S. (1999). *Endocr. Rev.* **20,** 358–417.
15. Simerly, R. B. (1993). In: *Advance in neurology.* Seil, F. J. (ed.). Raven: New York.
16. Shughrue, P. J., Bushnell, C. D., and Dorsa, D. M. (1992). *Endocrinology* **131,** 381–388.
17. Zhou, Y., Shughrue, P. J., and Dorsa, D. M. (1995). *Neuroendocrinology* **61,** 276–283.
18. Brown, T. J., Scherz, B., Hochberg, R. B., and MacLusky, N. J. (1996). *Neuroendocrinology* **63(1),** 53–60.
19. Orikasa, C., Mizuno, K., Sakuma, Y., and Hayashi, S. (1996). *Neurosci. Res.* **25,** 247–254.
20. Simonian, S. X., Spratt, D. P., and Herbison, A. E. (1999). *J. Comp. Neurol.* **411,** 346–358.
21. Jimenez, A. E., Voogt, J. L., and Carr, L. A. (1977). *Neuroendocrinology* **23,** 341–351.
22. Caligaris, L. and Taleisnik, S. (1977). *Neuroendocrinology* **23,** 323–329.
23. Lieberman, M. E., Maurer, R. A., Claude, P., Wiklund, J., Wertz, N., and Gorski, J. (1981). *Adv. Exp. Med. Biol.* **138,** 151–163.
24. Lieberman, M. E., Maurer, R. A., Claude, P., and Gorski, J. (1982). *Mol. Cell. Endocrinol.* **25,** 277–294.
25. Lieberman, M. E., Maurer, R. A., and Gorski, J. (1978). *Proc. Natl. Acad. Sci. USA* **75,** 5946–5949.
26. Pilotte, N. S., Burt, D. R., and Barraclough, C. A. (1984). *Endocrinology* **114,** 2306–2311.
27. Blum, M., McEwen, B. S., and Roberts, J. L. (1987). *J. Biol. Chem.* **262,** 817–821.
28. Arbogast, L. A. and Hyde, J. F. (2000). *Neuroendocrinology* **71,** 219–227.
29. Lerant, A. and Freeman, M. E. (1997). *Neuroendocrinology* **65,** 436–445.
30. Kerdelhue, B., Bojda, F., Lesieur, P., et al. (1989). *Neuroendocrinology* **49,** 176–180.
31. Mulchahey, J. J. and Neill, J. D. (1986). *Brain Res.* **386,** 332–340.
32. Meredith, J. M., Auger, C. J., and Blaustein, J. D. (1994). *J. Neuroendocrinol.* **6,** 639–648.
33. Blaustein, J. D. (1993). *Endocrinology* **132,** 1218–1224.
34. Kumar, V. and Chambon, P. (1988). *Cell* **55,** 145–156.
35. Fritsch, M., Welch, R. D., Murdoch, F. E., Anderson, I., and Gorski, J. (1992). *J. Biol. Chem.* **267,** 1823–1828.
36. Alarid, E. T., Bakopoulos, N., and Solodin, N. (1999). *Mol. Endocrinol.* **13,** 1522–1534.
37. Preisler, M., Mara, T., Solodin, N., Stark, B. L., Tyriver, M. K., and Alarid, E. T. (2002). *Am. J. Physiol.* **282(4),** E891–E898.
38. Nawaz, Z., Lonard, D. M., Dennis, A. P., Smith, C. L., and O'Malley, B. M. (1999). *Proc. Natl. Acad. Sci. USA* **96(5),** 1858–1862.
39. Simerly, R. B. and Young, B. J. (1991). *Mol. Endocrinol.* **5,** 424–432.
40. Berthois, Y., Dong, X. F., Roux, D. M., and Martin, P. M. (1990). *Mol. Cell. Endocrinol.* **74(1),** 11–20.
41. Blaustein, J. D. and Brown, T. J. (1984). *Brain Res.* **304,** 224–236.
42. Brown, T. J. and MacLusky, N. J. (1994). *Mol. Cell. Neurosci.* **5,** 283–290.
43. Smanik, E. J., Young, H. K., Muldoon, T. G., and Mahesh, V. B. (1983). *Endocrinology* **113(1),** 15–22.
44. Warembourg, M., Jolivet, A., and Milgrom, E. (1989). *Brain Res.* **480(1–2),** 1–15.
45. Frawley, L. S. (1994). *Trends Endocrinol. Metab.* **5,** 107–112.
46. Ben-Jonathan, N. and Hnasko, R. (2001). *Endocr. Rev.* **22(6),** 724–763.
47. Paxinos, G. and Watson, C. (eds.). (1986). *The rat brain in stereotaxic coordinates.* 2nd ed. Academic: New York.
48. Terranova, P. F. and Garza, F. (1983). *Biol. Reprod.* **29,** 630–636.