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Reproductive Experience Reduces Circulating 17β-Estradiol and Prolactin Levels during Proestrus and Alters Estrogen Sensitivity in Female Rats

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The reproductive experiences of pregnancy, parturition, and lactation affect a range of neural and endocrine processes after the end of lactation. In women, previous parity results in reduced circulating prolactin (PRL) and androgen levels years after giving birth. Reductions in PRL secretion also occur in reproductively experienced, female rats. In the present study we examined the status and regulation of estradiol (E_2) and PRL during the reproductive cycle after reproductive experience. These hormones regulate one another and have been implicated in a number of disease and aging processes. Using a rat model, the patterns of E_2 and PRL secretion, pituitary PRL content, and estrogen receptor α expression were characterized from 1200–1800 h on proestrus in age-matched, primiparous and nulliparous animals. The possible effect of parity on estrogen sensitivity was then examined by challenging nonlactating, ovariectomized, age-matched, multiparous and nulliparous rats with estradiol benzoate (EB; 0, 1, 5, 25, and 125 μg/kg) and measuring PRL responses 24 and 48 h later. Previous parity resulted in modest, yet significant, reductions in E_2 and PRL levels on proestrus, a limited increase in pituitary estrogen receptor α expression, and a significant shift in estrogen sensitivity, as measured by EB-induced PRL secretion. Nulliparous animals were more sensitive than multiparous rats to the two lower doses of EB, whereas multiparous animals were more responsive to the highest EB dose. These unique parity-induced alterations in the female's endocrine state that persist beyond lactation may impact a multitude of estrogen-mediated processes over the female's adult life span. (*Endocrinology* 147: 2575–2582, 2006)

DURING THE COURSE of a life span, reproductively successful, female mammals experience either a single or multiple pregnancies and lactations. The endocrine changes accompanying gestation, parturition, and lactation are dynamic and extensive. For example, during gestation, females are exposed to prolonged elevations of progesterone; the lactogenic hormones, prolactin (PRL) and placental lactogen; increasing titers of estrogens; and altered glucocorticoid secretion and sensitivity, whereas parturition and lactation are states typified by increased oxytocin and PRL activities. Recent work in rodents has supported previous observations in women that these physiological changes associated with previous parity produce long-term changes in hormone secretion (1–6). Specifically, plasma PRL levels as well as androgen levels are reduced for years in women after an initial pregnancy and lactation (1–3). In rats, shifts in both PRL secretion and its regulation can be demonstrated during both a second pregnancy and lactation (4–6). More recent work, which examined the effects of duration of lactation in primiparous rats on possible changes in circulating hormone levels, revealed that pregnancy plus a full 21-d lactation, but not pregnancy alone, produced significant reductions in plasma PRL levels after the resumption of cyclicity on the afternoon of proestrus (7). Although estradiol (E_2) levels in these reproductively experienced females tended to be lower on proestrus than those in nulliparous controls, E_2 levels did not differ statistically among the age-matched, nulliparous, and parous groups (7). This tendency for an effect of reproductive experience on plasma E_2 levels was based on one or two sampling times during proestrus. Hence, it is possible that a more thorough examination of E_2 levels across proestrus may reveal an effect of reproductive experience on circulating E_2.

Given the plethora of physiological and disease-related roles that has been reported for PRL in female mammals that, for example, include normal and pathogenic stimulation of cell differentiation and proliferation (8), stimulation of mammogenesis, lactogenesis (9), stimulation of maternal behavior (10, 11), and regulation of postpartum stress (12), we decided to systematically examine the effects of reproductive experience throughout proestrus in the female rat. In the present study in addition to determining the effects of reproductive experience on PRL secretion and its regulation, we also measured circulating E_2, a known regulator of PRL (8, 13), during proestrus and evaluated possible changes in estrogenic regulation of PRL, comparing responses between nonlactating parous and age-matched nulliparous rats.

The results demonstrate that circulating titers of PRL and E_2 on proestrus are reduced in reproductively experienced rats. These reductions in circulating hormone levels are accompanied by a shift in pituitary estrogen receptor α (ERα) expression and alterations in the ability of estrogen to stimulate PRL secretion.

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Abbreviations: CT, Cycle threshold; EB, estradiol benzoate; ER, estrogen receptor; HPRT, hypoxanthine guanine phosphoribosyl transferase 1; PRL, prolactin.

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Materials and Methods

Animals

Adult female Sprague Dawley female rats (Crl:CD[SD]BR; 201–225 g body weight) were purchased from Charles River Laboratories (Kingston, NY) and housed in our animal facility. Animals were maintained in temperature- (21–25 C) and light (14-h light, 10-h dark cycle)-controlled rooms, and food and water were available *ad libitum* throughout the study. The animals used in these experiments were maintained in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals (National Academy of Science). The research protocol was approved by Tufts University-Cummings School of Veterinary Medicine's institutional animal care and use committee.

Experiment 1: effects of reproductive experience on plasma PRL and E_2 concentrations, pituitary PRL content, and pituitary ERα expression

Seventy adult female rats were assigned to one of two main treatments. Half of these animals were mated to males in our animal facility and allowed to give birth. Their litters were culled to 10 pups/litter, and dams raised litters for 21 d at which time the pups were weaned. Animals in this group were designated as primiparous subjects. The remaining females were not bred and were designated as nulliparous subjects. At least 2 wk after weaning litters, vaginal smears were taken from the primiparous animals and the age-matched, nulliparous females for a minimum of 2 wk to characterize estrous cyclicity. Only females displaying regular 4-d estrous cycles were assigned to a sampling time on proestrus. Cycling, primiparous and age-matched, nulliparous animals were assigned to groups that were killed by rapid decapitation at 1200, 1400, 1600, and 1800 h on proestrus. Blood was collected in heparinized test tubes and then centrifuged. Plasma samples were stored frozen at −20 C until assayed for PRL and E_2 concentrations. At the time of death, pituitary glands were removed, and the adenohypophysis was dissected, divided, and frozen at −80 C. Half of each adenohypophysis was subsequently sent to the Lucy Whittier Molecular and Diagnostic Core Facility (University of California-Davis), where it was processed for real-time PCR detection using TaqMan to measure ERα mRNA. The remaining portion of the anterior pituitary gland was used to measure PRL content by RIA. PRL concentrations in the anterior pituitary were adjusted for weight.

Experiment 2: effects of reproductive experience on E_2-induced PRL secretion

One hundred and 20 age-matched, nulliparous female rats were separated into two groups of 60. One group of rats was mated to males in our facility and allowed to give birth and raise litters of 10 pups for 21 d, at which time the pups were weaned. These females were bred with males for a second time approximately 2 wk after weaning their first litters. These rats gave birth to a second litter that was culled to 10 pups and then was weaned at 21 d postpartum. Females in this group were designated multiparous subjects. The remaining 60 females were not bred and were designated nulliparous subjects. At least 2 wk after weaning, the second litters the multiparous animals together with age-matched, nulliparous females were anesthetized with ketamine and xylazine and bilaterally ovariectomized. Subjects were then individually housed and allowed to recover for 2 wk, at which time they were again anesthetized and surgically implanted with intraatrial catheters (14).

The day after catheterization, catheters were rinsed, and subjects were weighed, then injected sc with one of four doses (1, 5, 25, and 125 μg/kg) of estradiol benzoate (EB; Sigma-Aldrich Corp., St. Louis, MO) or vehicle (peanut oil) between 0900 and 0930 h. One and 2 d later, blood samples were collected at 2-h intervals from 1200–1800 h during the expected estrogen-induced increase in PRL secretion (15, 16). Blood volumes of approximately 500 μl were collected in heparinized test tubes at each sampling time. Samples were centrifuged, and plasma was frozen for subsequent PRL measurement. Red blood cells were resuspended in physiological saline and returned to each subject after the 1800 h collection on sampling d 1. At the completion of the sampling, subjects were killed with CO_2.

Finally, to determine the levels of E_2 generated by the EB injections, an additional set of 25 nulliparous rats (225–250 g) were ovariectomized 1 wk after arrival in our animal facility. Two weeks later, groups of rats (n = 5/group) were injected with one of two doses of EB (1 or 125 μg/kg) or vehicle between 1000 and 1100 h. These low and high dose EB-injected rats were then killed by decapitation 24 or 48 h later, between 1000 and 1100 h, whereas the vehicle group was sampled only 48 h after injection. Plasma samples were subsequently processed for PRL concentrations by RIA.

PRL assay

Plasma concentrations of PRL were measured by RIA in duplicate using the National Institute of Diabetes and Digestive and Kidney Diseases rat PRL kit supplied by Dr. A. F. Parlow through the National Hormone Pituitary Program. This kit includes reference preparation National Institute of Diabetes and Digestive and Kidney Diseases rat PRL RP-3 and antirat PRL S-9. The [^{125}I]rat PRL used in the RIA was purchased from PerkinElmer (Boston, MA). Samples were run in duplicate at volumes ranging from 1–50 μl. Inter- and intraassay coefficients of variation for the PRL assay in our laboratory are 11% and 9%, respectively.

E_2 assay

Plasma E_2 was assayed using a Coat-A-Count Kit for E_2 purchased from Diagnostic Products Corp. (Los Angeles, CA). Samples were assayed in duplicate at a volume of 100 μl. The sensitivity of the assay was equivalent to 1 pg/ml, and the intraassay coefficient of variation was 12%. All samples were measured in a single assay. Plasma E_2 levels for ovariectomized rats are routinely less than 2 pg/ml.

Real-time quantitative TaqMan PCR for pituitary ERα mRNA

Sample preparation. Each anterior pituitary hemisection was placed into 500 μl 1× AB lysis buffer (Applied Biosystems, Foster City, CA), stored at −20 C, and shipped on dry ice to the Lucy Whittier Molecular and Diagnostic Core Facility. Before analysis, proteinase K and two grinding beads (4 mm diameter; SpexCertiprep, Metuchen, NJ) were added to each sample, and the tissues were homogenized in a GenoGrinder2000 (SpexCertiprep) for 2 min at 1000 strokes/min. Protein digest was performed at 56 C for 30 min, followed by a 30-min period at −20 C to reduce foam. Total RNA was extracted from the tissue lysates using a 6700 automated nucleic acid workstation (Applied Biosystems) according to the manufacturer's instructions.

Housekeeping gene validation experiment. To determine the most stably transcribed housekeeping gene, a housekeeping gene validation experiment was run on six representative samples from the anterior pituitary glands. Three commonly used housekeeping genes were used for this experiment: a TaqMan PCR system recognizing rat 18S ribosomal RNA, rat glyceraldehyde-3-phosphate dehydrogenase, and mouse hypoxanthine guanine phosphoribosyl transferase 1 (HPRT). Mouse HPRT1 is highly sequence identical with rat HPRT1, and the system amplifies cDNA from rat with high amplification efficiency (95%). Based on these preliminary data, mouse HPRT1 was chosen as the most appropriate housekeeping gene for the subsequent analyses.

RT reaction and real-time TaqMan PCR. cDNA was synthesized using 100 U SuperScript III (Invitrogen Life Technologies, Inc., Grand Island, NY), 600 ng random hexadeoxyribonucleotide ($pd(N)_6$) primers (random hexamer primer), 10 U RNaseOut (ribonuclease inhibitor), and 1 mM deoxy-NTPs (all from Invitrogen Life Technologies, Inc., Carlsbad, CA) in a final volume of 40 μl. The RT reaction proceeded for 120 min at 50 C. After the addition of 60 μl water, the reaction was terminated by heating for 5 min to 95 C and cooling on ice.

Each PCR contained 20× Assay-on-Demand primer and probes for the respective TaqMan system (ERα: ABI system no. Rn00562166m1; reference sequence ID NM012689.1: HPRT1: ABI system no. Mm00446968m1; reference sequence ID M013556),and commercially available PCR Master Mix (TaqMan Universal PCR Master Mix, Applied Biosystems) containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 5 mM $MgCl_2$, 2.5 mM deoxynucleotide triphosphates, 5 U AmpliTaq Gold DNA polymerase/reaction, 0.25 U AmpErase uracil-N-glycosylase/re-

action, and 5 μl diluted cDNA sample in a final volume of 12 μl. The samples were placed in 96-well plates and amplified in an automated fluorometer (ABI PRISM 7700 Sequence Detection System, Applied Biosystems). The standard amplification conditions were used: 2 min at 50 C, 10 min at 95 C, 40 cycles of 15 sec at 95 C, and 60 sec at 60 C. Fluorescent signals were collected during the annealing temperature, and cycle threshold (CT) values were extracted using a threshold of 0.04 and baseline values of 3–15.

Relative quantification of gene transcription. Final quantification was performed using the comparative CT method (User Bulletin 2, Applied Biosystems) and is reported as the relative transcription or the n-fold difference relative to a calibrator cDNA (*i.e.* lowest ERα transcription). In brief, the housekeeping gene for the rat brain tissue, HPRT1, was used to normalize the target raw signals (ΔCT). The ΔCT was calibrated against the weakest signal of ERα. The relative linear amount of target molecules relative to the calibrator was calculated by $2^{-\Delta\Delta Ct}$. Therefore, all gene transcription is expressed as an n-fold difference relative to the calibrator (17).

Statistical analyses

Both hormone and mRNA data were analyzed using a two-way factorial ANOVA, with cycle stage and reproductive experience as independent factors. *Post hoc* tests were performed using Tukey's test.

Results

Experiment 1: effects of reproductive experience on plasma PRL and E_2 concentrations, pituitary PRL content, and pituitary ERα expression

Plasma PRL and E_2. The effects of a previous pregnancy and lactation on plasma PRL concentrations during the afternoon of proestrus are shown in Fig. 1A. ANOVA revealed both significant overall effects of time of day ($P < 0.001$) and reproductive experience ($P = 0.015$).

Comparisons between sampling times for both groups combined on the afternoon of proestrus found that PRL levels were significantly elevated at 1400, 1600, and 1800 h compared with PRL levels at 1200 h (all $P < 0.001$). Moreover, when nulliparous and primiparous groups were separately analyzed, PRL levels increased significantly between the 1200 and the 1400, 1600, and 1800 h times (all $P < 0.001$). Although there were no differences among PRL levels within either the reproductive group from 1400–1800 h, the difference in PRL levels in the primiparous group approached significance when the 1400 and 1800 h points were compared ($P = 0.08$).

Comparisons between treatment groups across proestrus revealed that plasma PRL levels were significantly lower in primiparous than age-matched, nulliparous rats at 1400 h ($P = 0.004$). Mean plasma PRL levels at 1400 h were 88 and 32 ng/ml for the nulliparous and primiparous rats, respectively. Differences between treatments were not detected at 1200, 1600, or 1800 h.

Reproductive experience also affected plasma E_2 concentrations in proestrous rats (see Fig. 1B). Because no significant differences were found as a function of time of proestrus within each treatment group or between treatment groups at a given time during proestrus, the data were collapsed across

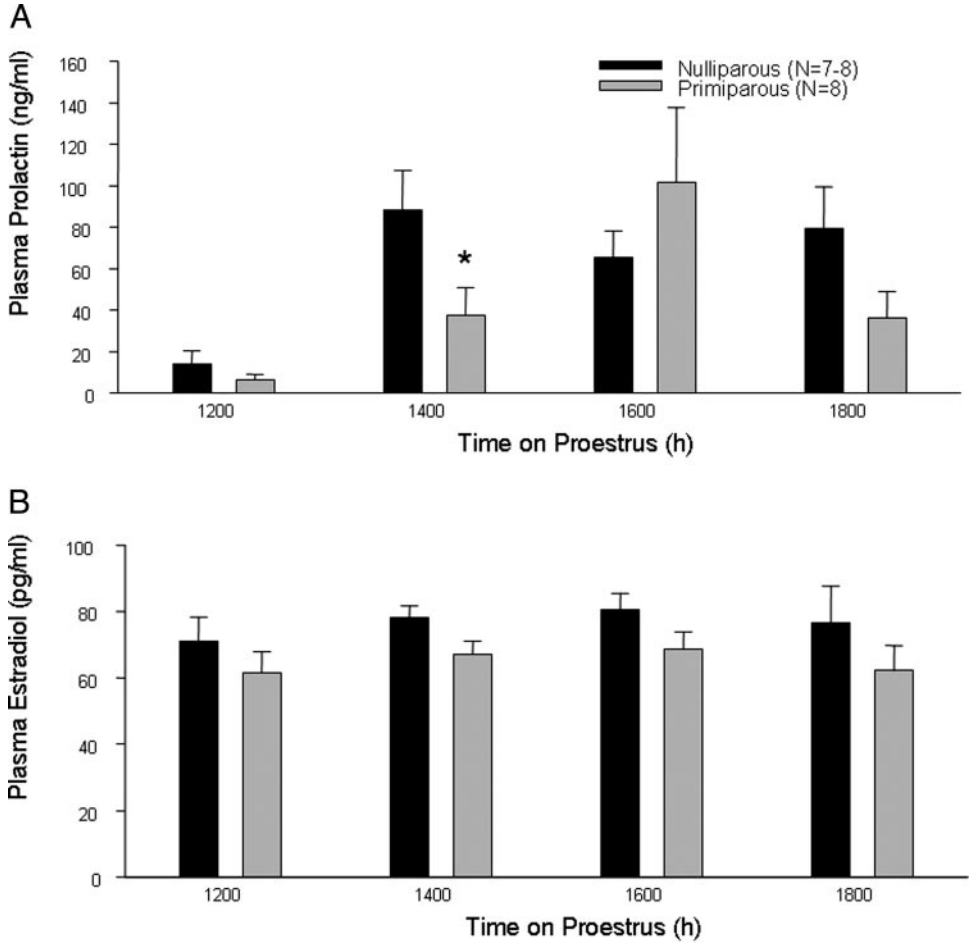

FIG. 1. Plasma PRL (A) and E_2 (B) concentrations during proestrus in cycling, nonlactating, primiparous and age-matched, nulliparous rats. *, $P < 0.01$ *vs.* nulliparous PRL values. Overall analysis revealed that E_2 levels were significantly reduced in the primiparous group, although no differences were detected at individual time points. See the text for other statistical comparisons.

time to compare plasma E_2 concentrations between the nulliparous and primiparous subjects. This comparison revealed that plasma E_2 levels were significantly reduced during proestrus in primiparous animals ($P = 0.015$). The average plasma E_2 concentrations across proestrus was reduced 16% in primiparous females, declining from a mean of 77 pg/ml in nulliparous animals to 65 pg/ml in age-matched, primiparous rats.

Pituitary PRL content and ERα mRNA expression. Pituitary PRL content in the adenohypophysis of nulliparous and primiparous rats during proestrus is shown in Fig. 2. Using a two-way ANOVA, no overall differences in pituitary PRL content were found between the two reproductive conditions. However, a subsequent one-way ANOVA for each group found that although there were no differences in pituitary PRL content across proestrus in nulliparous animals, there was a time of day effect for the primiparous groups ($P = 0.031$). Individual comparisons of sampling times for the primiparous groups revealed that pituitary PRL content was higher at 1200 h than at either the 1400 h ($P < 0.05$) or 1800 h ($P < 0.05$) samplings.

Expression of ERα message in the anterior pituitary changed significantly as a function of time during proestrus ($P < 0.001$) and as an interaction between sampling time and reproductive experience ($P = 0.03$; see Fig. 3). Overall expression of ERα mRNA was higher during early proestrus and declined thereafter. This effect of time of proestrus was solely accounted for by primiparous animals that had higher levels of receptor message expression at 1200 h than at 1400 ($P = 0.046$), 1600 ($P < 0.001$), and 1800 h ($P < 0.001$). In addition, at 1200 h on proestrus, primiparous rats expressed higher levels of ERα mRNA than age-matched, nulliparous animals ($P = 0.006$). Finally, the expression of ERα mRNA in the anterior pituitary gland did not change across the proestrous sampling points in nulliparous rats.

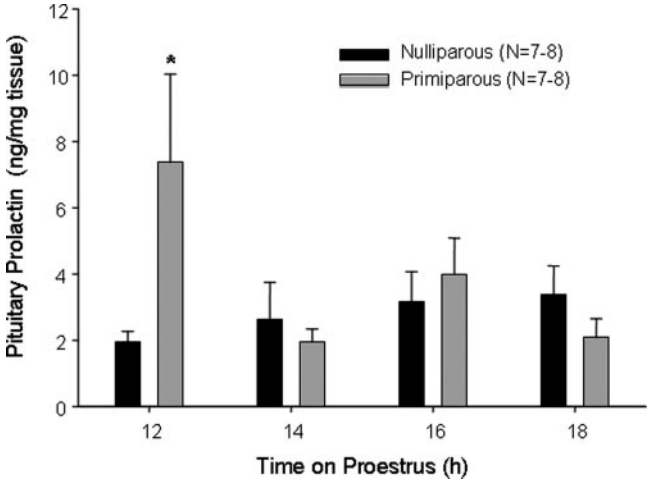

FIG. 2. Pituitary PRL content across proestrus in cycling, age-matched, primiparous and nulliparous rats. Pituitary PRL content was similar as a function of reproductive experience. *, $P < 0.05$ *vs.* 1400 and 1800 h primiparous samples.

Experiment 2: effects of reproductive experience on E_2-induced PRL secretion

The effects of reproductive experience on estrogen-induced PRL secretion are shown in Fig. 4. Statistical analyses revealed significant overall effects for time ($P < 0.001$), time by EB dose ($P < 0.001$), and time by EB dose by parity interaction ($P < 0.005$). As expected, plasma PRL concentrations in ovariectomized, vehicle-injected rats were uniformly low, ranging from 2–3 ng/ml, and did not change over the 6-h bleeding period. However, after a challenge with EB, the average plasma PRL concentration increased as the dose of EB increased ($P < 0.001$). On d 2 after EB treatment, plasma PRL levels were significantly higher after the 5, 25, or 125 μg/kg dose of EB compared with concentrations after either vehicle or the 1 μg/kg dose (all $P < 0.01$ to $P < 0.001$). PRL levels also increased significantly for all EB groups within the 6-h bleeding period, with maximal levels generally found at the 1600 and 1800 h samplings.

Perhaps, the most striking finding is the differential response to EB administration detected between the nulliparous and age-matched, nonlactating, multiparous rats. At the lowest 1 μg/kg dose of EB, nulliparous females displayed a significant increase in plasma PRL at 1600 h on d 2 after EB treatment compared with that found in multiparous animals ($P < 0.04$). Likewise, nulliparous females were more responsive after the 5 μg/kg dose at 1600 and 1800 h on d 1 after EB treatment ($P < 0.05$). This difference was no longer present on d 2 after the 5 μg/kg EB treatment. The patterns and absolute levels of PRL in the plasma after the 25 μg/kg dose of EB were comparable in the nulliparous and multiparous subjects and did not differ from each other at any sampling time. In contrast, at the highest EB dose of 125 μg/kg, the multiparous animals displayed a greater sensitivity to EB than the nulliparous group on d 2 after EB. Plasma PRL concentrations on this day were significantly higher in multiparous animals throughout the estrogen-induced PRL increase at 1400, 1600, and 1800 h ($P < 0.01$).

E_2 levels 24 and 48 h after animals were injected with either the lowest or the highest dose of EB are shown in Table 1. Overall, plasma E_2 concentrations were basal (3–4 pg/ml) at both 24 and 48 h after injection of the lowest EB dose (1 μg/kg), whereas plasma E_2 levels were well above physiological concentrations in rats injected with the 125 μg/kg at both times. Plasma E_2 concentrations in the highest EB dose group averaged 377 and 195 pg/ml 24 and 48 h after injection, respectively.

Discussion

The present study demonstrates that pregnancy and lactation result in reductions in circulating E_2 and PRL during proestrus after lactation and the resumption of estrous cyclicity in the rat. These hormonal changes are accompanied by both an increase in the expression of ERα in the anterior pituitary gland and a shift in estrogen sensitivity. Gonadectomized, nulliparous rats were more sensitive to the lower doses of EB in the induction of PRL secretion than were age-matched, multiparous animals. This effect was reversed after treatment with the high superphysiological EB dose.

The present study is the first to demonstrate that circu-

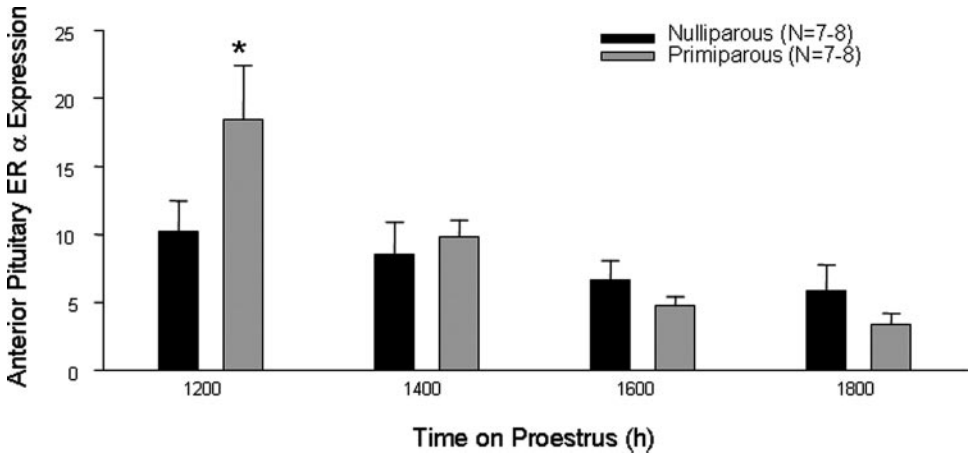

FIG. 3. Relative expression of ERα in the anterior pituitary across proestrus in cycling, age-matched, nulliparous and primiparous rats. $P < 0.01$ *vs.* nulliparous animals. See text for additional statistical comparisons.

lating E_2 levels are reduced as a function of previous parity in the rat. A previous examination in the rat of the impact of a prior pregnancy and lactation on plasma E_2 levels across the estrous cycle failed to detect significant reductions in E_2 levels, although there was a trend toward significance in reproductively experienced rats on the afternoon of proestrus (7). By comparing nulliparous with parous rats across multiple time points on the afternoon of proestrus, the significant shift in circulating E_2 levels emerged.

The effects of parity on circulating PRL levels presented in this study confirm and extend previous findings in female rats. These results characterize the pattern of plasma PRL levels across the afternoon of proestrus and demonstrate that a significant reduction in PRL is detected at 1400 h, but not earlier, or at 1600 and 1800 h. Previous investigations in which PRL levels were collected at 1000 and 1500 h on proestrus found significantly lower plasma PRL levels at 1500 h, but not at 1000 h (7). Therefore, it appears that this difference in PRL levels between parous and nulliparous animals occurs over a 2- to 4-h period on the afternoon of proestrus. Similar parity-associated reductions in circulating PRL levels have been found during early and midgestation (4, 5) and in response to suckling stimuli (6). In women, previous parity is associated with reductions in circulating PRL during the follicular phase of the menstrual cycle up to 10 yr after giving birth (2). Thus, changes in circulating PRL secretion are reflected broadly across a number of reproductive states in the species studied to date.

An examination of pituitary PRL content across proestrus in nulliparous and reproductively experienced rats indicated that pituitary content was not affected by reproductive experience, although within the primiparous animals, PRL content was higher at 1200 h than later during proestrus. In contrast, the expression of message for ERα was altered by reproductive experience; ERα mRNA was significantly increased in parous rats above levels of expression measured in nulliparous animals at 1200 h. Although the factors responsible for increased ERα expression are not known, it may be a response to the overall levels of circulating E_2 or other regulatory factors (18).

Functionally, reproductive experience alters estrogen sensitivity. This shift in sensitivity is reflected in the ability of EB to stimulate diurnal PRL secretion. At the two lower doses, EB stimulated greater elevations in plasma PRL in nulliparous females than in age-matched, multiparous animals. This effect was evident during the afternoon at 1600 h, 2 d after EB administration in animals treated with the lowest 1 μg/kg dose of EB and again at 1600 and 1800 h, 1 d after administration of the 5 μg/kg dose of EB. Based on the results of these EB challenges, it appears that reproductively experienced rats are rendered less sensitive to estrogen. This shift in homeostatic control of estrogen action may involve a combination of reduced circulating E_2 levels together with a decreased sensitivity to this hormone. It should be noted that at the highest EB dose, multiparous animals were more responsive to EB from 1400–1800 h 2 d after treatment. This biphasic response to EB, with nulliparous females being more responsive at low doses of estrogen and multiparous rats being more responsive to a superphysiological doses of EB, may be a reflection of ER availability and occupancy. Therefore, it appears that reproductively experienced, parous females are less responsive than nulliparous females to physiological levels of E_2. Examination of ERα and ERβ subtypes should help elucidate possible shifts in hormone response substrates that underlie this differential response to an estrogen challenge.

What biological factors account for this shift in basal and estrogen-stimulated PRL secretion? The decrease in basal PRL secretion during proestrus may be due to reductions in estrogen levels or alterations in neurotransmitter activity. Dopamine is an established PRL-inhibiting factor (19). Moreover, numerous physiological studies have correlated increases in activity in the tuberoinfundibular dopaminergic system with reductions in PRL secretion (8, 20). It has also been suggested that one outcome of previous parity is an up-regulation of neural dopaminergic activity, *i.e.* increased dopaminergic tone (21, 22). In both rats (21) and women (23), reproductive experience results in decreased PRL secretion after treatment with dopamine antagonists. This change in sensitivity to a given dose of a dopamine antagonist may reflect increased endogenous dopaminergic activity and, hence, an attenuated PRL response. Although dopaminergic activity in the tuberoinfundibular dopaminergic system has not been examined directly, this possibility is supported by the finding that reproductive experience is associated with increased dopamine release in the brain, *i.e.* elevations in

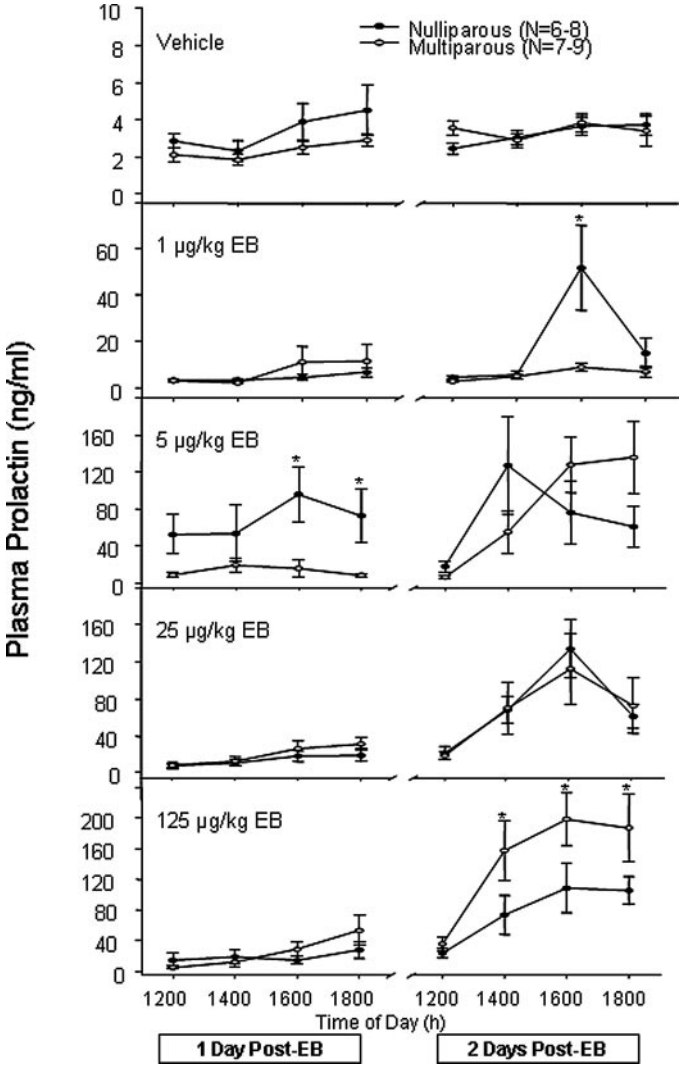

FIG. 4. Effects of reproductive experience on estrogen-induced PRL secretion 24 and 48 h after EB administration. See text for statistical comparisons. Overall, the primiparous females were more sensitive to EB-stimulated PRL than nulliparous animals 48 h after treatment.

striatal 3,4-dihydroxyphenylacetate concentrations (22, 24). Thus, an increase in neural dopaminergic activity may help account for this change in estrogen sensitivity in multiparous rats. Estrogen has been shown to stimulate PRL, acting at both hypothalamic and pituitary levels (8). Moreover, hypothalamic actions of estrogens in PRL stimulation are mediated in part through estrogen's inhibitory action on dopaminergic neurons (25–27). Therefore, increased dopaminergic activity that might develop upon a background of reduced estrogen secretion would be expected to result in reductions in PRL levels. Such reductions are present in rats as well as women (2, 4–7). It should also be noted that the reported increase in striatal dopamine turnover in reproductively experienced rats (22, 24) is somewhat unexpected given that circulating E_2 is reduced. However, because acute E_2 treatment appears to down-regulate D2 receptors in the striatum (28, 29), a reduction in circulating E_2 may conversely up-regulate D2 receptors and, hence, DA sensitivity. Additional studies are needed, however, to establish how estrogens interact in these different neural regions to modulate the activity of neural dopamine.

TABLE 1. Plasma E_2 concentrations in trunk blood collected from ovariectomized, nulliparous rats 24 and 48 h after injection of the lowest and highest doses of EB or 48 h after the injection of vehicle

EB dose (μg/kg)	Plasma E_2 (pg/ml)	
	24 h	48 h
0		3.5 ± 0.5
1	4.2 ± 1.0	3.8 ± 0.9
125	377.5 ± 29.4	195.4 ± 33.0

Sample sizes were five animals per group.

Whether the differential circulating levels of PRL result from a difference in pituitary responsiveness is unknown. That ERα mRNA is altered by reproductive experience makes this a feasible possibility. However, future *in vitro* studies using pituitary cultures from nulliparous and parous females may be needed to determine whether the reduced PRL during proestrus in parous rats results from direct changes in pituitary responsiveness to estrogen.

A second neurotransmitter system that may contribute to reduced PRL levels after reproductive experience is that of the endogenous opioids. This system becomes dampened in experienced females. It is established that the endogenous opioid, β-endorphin, can mimic the effects of suckling in stimulating PRL secretion in lactating rats (30). Moreover, endogenous opioids are involved in the control of both the nocturnal PRL surge during pregnancy (31) and the prepartum PRL rise (32). Reproductive experience itself results in an attenuated rise in PRL in response to suckling (6) as well as a dampened nocturnal PRL surge (5) in rats. The suckling-induced PRL surge can be partially blocked by previous treatment with the opiate antagonist naloxone in primiparous rats, but does not affect PRL release in age-matched, multiparous dams (6). What might account for this shift in opioid function? Desjardins and Brawer (33) reported that treatment with estrogen valerate resulted in opioid neuron apoptosis in the hypothalamus. Increased opioid cell death or alteration in opioid activity induced by the combination of high levels of exposure to estrogens during pregnancy and the sensory and endocrine events associated with lactation might contribute to both increased dopaminergic tone, because opioids are generally inhibitory to dopamine neurons (34), and the reductions in basal and opioid-stimulated PRL secretion.

The events that transpire during gestation and lactation appear to induce these shifts in endocrine set points. Previous studies have shown that pregnancy accompanied by a full lactational period is needed to produce shifts in circulating PRL levels (7). When parous rats were challenged with haloperidol, an attenuated PRL response only occurred in females that had experienced pregnancy plus lactation; females that had given birth, but not raised young, did not show an attenuated response to this dopamine antagonist (21). Likewise, rats that give birth and did not raise young or raised young for 10 d failed to exhibit a reduction in circulating PRL on the afternoon of proestrus, whereas dams that raised a litter for 21 d displayed reduced PRL levels (7). Interestingly, the dampened PRL response to metoclopro-

mide reported in parous women was found in subjects that had nursed their babies for a minimum of 3–4 months. Therefore, based upon these studies, it appears that events associated with a prolonged lactation after pregnancy are crucial to inducing these endocrine and neuroendocrine changes. These events may include the prolonged endocrine state of hyperprolactinemia experienced during pregnancy and lactation or perhaps the prolonged sensory stimulation the female receives from her young. The involvement of these factors in bringing about the changes in neuroendocrine and endocrine states remains to be determined.

How might these biological changes brought about by reproductive experience affect the female's reproductive condition and health status? In rats, it is established that the experiences of pregnancy and lactation can extend reproductive fecundity (35, 36). The signal in the female rat that triggers reproductive senescence involves a shift in neural regulation of the hypophyseal-ovarian axis, whereas in women, the determinant factor appears to reside in the ovary (37). Based upon the present set of results, it seems feasible that part of this prolongation of estrous cyclicity in the rat involves an alteration in estrogenic regulation of GnRH and gonadotropin secretion. The precise mechanisms associated with reproductive experience that mediate this prolongation of estrus, however, remain to be established and could involve a combination of changes in endocrine and neurochemical factors that include dopamine, opioids, and steroid feedback.

The physiological significance of the 16% reduction is plasma E_2 in reproductively experienced primiparous female rats across proestrus is unknown, although an alteration in circulating estrogen levels probably affects ER expression and action. It is possible that females with greater amounts of reproductive experience, *i.e.* multiparous animals, would demonstrate a progressive reduction in circulating E_2 levels. The consequences of a reduction in E_2 for subsequent estrous cycles during the female's lifetime after giving birth may be cumulative and alter a broad set of estrogen-sensitive responses. It is noted that in the present study, which examined the effects of EB on PRL secretion, that females had raised two litters. Additional studies involving age-matched subjects are needed to explore the effects of repeated births on subsequent endocrine and neuroendocrine functions and the female's health.

The finding of reduced circulating levels of E_2 and PRL after reproductive experience is of particular interest in light of the protective role that an early pregnancy confers upon the subsequent incidence of breast cancer in women (38, 39). Both E_2 and PRL are associated with increases in cancer in rodents and women (3, 40–42). Perhaps, lower circulating levels of estrogens and/or PRL after pregnancy and lactation over the subsequent reproductive life span may function to reduce the incidence of this disease (43).

A third possible impact of reproductive experience appears to be a long-term increase in central dopaminergic activity that could affect behavioral processes. In rats, increases in striatal dopaminergic activity as well as increased sensitivity to dopamine agonists have been reported (21, 22, 24), whereas in women as well as rats, reproductive experience reduces sensitivity to dopamine antagonist-induced PRL release (23). Because one treatment for mental illness, *i.e.* schizophrenia, involves the administration of estrogen to inhibit dopamine activity (44), it raises the issue of whether a psychiatric disorder, such as postpartum depression, may involve an alteration in neural dopamine activity in response to the changing steroid levels associated with reproductive experience (45, 46). The mechanisms that mediate these alterations in dopamine activity have not yet been established, but may include shifts in both estrogen and PRL actions. Specifically, given the ability of E_2 to inhibit dopamine activity (25–27), reductions in endogenous estrogens brought about by reproductive experience might result in both the stimulation of neural dopamine function as well as a reduction in PRL secretion. Additional studies are needed to elucidate the relationships among the changes in hormone release, the alterations in hormone and neurotransmitter sensitivities, and these or similar physiological and clinical states.

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