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Inhibition of Prolactin Secretion Delays Extinction of Circadian LH Surges in Ovariectomized Rats Bearing Estradiol Implants[1] (41527)

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Abstract. Ovariectomized rats bearing Silastic capsules containing estradiol exhibit a daily afternoon surge of luteinizing hormone (LH) which decreases with time until it is undetectable by Day 10 after implantation of estradiol. Increases in basal prolactin levels as well as afternoon surges are also observed. To determine if increased prolactin secretion contributed to the extinction of the circadian LH surges, we examined the patterns of LH and prolactin secretion in rats in which prolactin was suppressed by bromocriptine treatment. In vehicle-treated control rats, the magnitude of the LH surges decreased with time. Large LH surges were observed on Days 2 and 4. A significant decrease in the surge occurred on Day 6, and it disappeared by Day 10. Animals treated with bromocriptine also exhibited large LH surges on Days 2 and 4, and in addition, secreted a greater amount of LH than the control group on Days 6, 8, and 10. In ovariectomized rats bearing estradiol implants, large afternoon surges in prolactin were observed and by Day 6, basal prolactin levels were also elevated. Bromocriptine treatment completely suppressed prolactin secretion through Day 6, but a small afternoon rise was observed on Days 8 and 10. These findings suggest that elevated prolactin secretion may be one factor contributing to the extinction of circadian LH surges in the estrogen-treated rat.

Ovariectomized rats bearing Silastic implants of estradiol 17β exhibit daily afternoon surges of luteinizing hormone (LH) which are believed to be analogous to the proestrous surge of LH. However, even when adequate estrogen levels are maintained, the magnitude of the LH surge gradually diminishes with time and finally disappears (1). Estrogen is also a potent stimulus for prolactin secretion, having both hypothalamic and pituitary sites of action. A bolus of estrogen will evoke an afternoon surge of prolactin in ovariectomized female rats (2) while chronic estrogen treatment will result in hyperprolactinemia associated with mammotroph hypertrophy (3).

Prolactin has been shown to depress LH secretion in both experimental and clinical paradigms, although the mechanism by which this occurs is still unknown. Injection of prolactin or implantation of prolactin into the median eminence prevents the postcastration rise of LH in male and female rats (4, 5). Elevated prolactin from pituitary transplants also has been observed to abolish the pulsatile release of LH in castrated female rats (6). In women, elevated prolactin is associated with suppressed LH secretion, and hyperprolactinemia is closely correlated with the cessation of ovulation in galactorrhea-amenorrhea (7).

In light of these observations, we postulated that elevated prolactin secretion due to the chronic estrogen treatment could play a role in the demise of the circadian LH surges. To test this hypothesis, we monitored the patterns of LH and prolactin secretion in rats bearing estrogen implants and in similarly prepared rats treated with bromocriptine to inhibit prolactin secretion. If prolactin contributes to extinction of the LH surge, then suppression of prolactin secretion should prevent this extinction.

Materials and Methods. Female Sprague–Dawley rats (Simonsen) weighing 250–300 g were individually housed in a 14:10 light–dark photoperiod and allowed free access to

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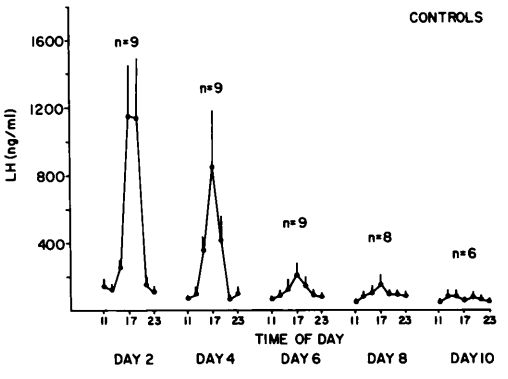

FIG. 1. Daily comparison of the mean patterns of LH secretion in ovariectomized rats with subcutaneous estradiol capsules. Female rats, ovariectomized 2 weeks previously, were fitted with carotid catheters and then implanted with estradiol capsules the next morning. Blood samples for hormone analysis were obtained at 2-hr intervals from 1100 to 2300 hr on Days 2, 4, 6, 8, and 10 after E_2 implantation. In this and subsequent figures each point on the graph represents the mean ± SEM and the numbers along the abscissa indicate time with reference to a 24-hr clock.

food and water. All surgical procedures were performed using halothane (Fluothane) anesthesia. The ovaries were removed through bilateral abdominal incisions. Two weeks after ovariectomy, an indwelling carotid catheter (PE 50) was inserted into each rat. The next morning (0800 hr) each animal received a 5-mm Silastic capsule containing estradiol, implanted subcutaneously (Day 0). Also beginning on Day 0, the experimental animals were injected ip with 1 mg/day of bromocriptine. The injection regimen consisted of 0.5 mg/0.2 ml 3% ethanol at 0900 and 1900 hr. Control animals were injected with an equal volume of vehicle. Blood samples (0.3 ml) for LH and PRL analysis were obtained at 2-hr intervals from 1100 to 2300 hr on Days 2, 4, 6, 8, and 10 after estradiol implantation. All blood samples were centrifuged immediately and the serum was harvested. The red blood cells were resuspended in 20% heparinized saline and returned to each animal after the next blood sample was obtained. The serum was frozen at −20° until assayed for LH and prolactin.

Serum prolactin concentrations were determined by radioimmunoassay with reagents provided by the NIAMDD. Anti-rat prolactin serum S-7 was used at a dilution of 1:5000 and rat prolactin I-3 was radioiodinated. Rat prolactin RP-1 (11 IU/mg) served as the reference preparation.

Serum LH measurements were obtained with anti-ovine LH serum No. 15 provided by Dr. Gordon Niswender (diluted 1:40,000) and with radioiodinated ovine LH G3-222B from Dr. Harold Papkoff. NIAMDD rat LH RP-1 (0.3 × NIH · LH · S1) was used as the standard. The buffer (0.1 g gelatin/100 ml phosphate-buffered saline, pH 7.4) and the precipitating antibody (goat anti-rabbit γ globulin provided by Dr. Scott Monroe) were identical in both assays.

Statistical differences between the groups were determined by comparing the peak LH and prolactin values and the total LH and prolactin secreted on each day with Student's t test. One-way analysis of variance was applied across time to the prolactin values in the bromocriptine-treated group.

Results. Figure 1 illustrates the serum LH concentrations in ovariectomized rats which had been implanted with estradiol capsules on Day 0. As previously reported (1), the magnitude of the LH surge gradually decreases with time. Large LH surges were present on Days 2 and 4, but the LH surge was significantly smaller on Day 6 (Student's t

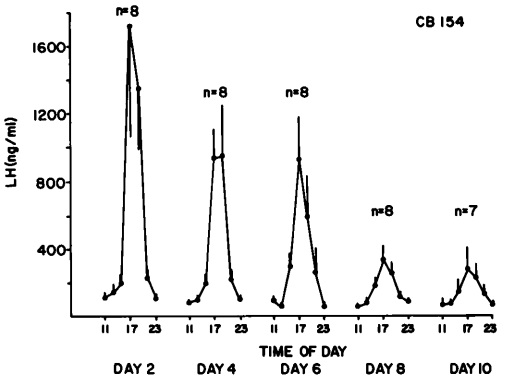

FIG. 2. Daily comparison of the mean patterns of LH secretion in ovariectomized rats with subcutaneous estradiol capsules and injected with bromocriptine (CB154). These animals were treated identically to the control animals shown in Fig. 1, except in addition, they received injections of bromocriptine.

TABLE I. DAILY PEAK HORMONE LEVELS (ng/ml) OF LH AND PROLACTIN (MEAN ± SEM)[a]

		Day 2	Day 4	Day 6	Day 8	Day 10
LH	Controls	1230 ± 327	898 ± 319	246 ± 82	153 ± 55	108 ± 35
		$P > 0.1$	$P > 0.1$	$P < 0.02$	$P < 0.05$	$P > 0.1$
	CB154	1896 ± 635	1143 ± 248	960 ± 245	372 ± 69	108 ± 35
Prolactin	Controls	862 ± 95	1181 ± 84	875 ± 26	951 ± 117	899 ± 159
		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
	CB154	69 ± 20	101 ± 23	102 ± 20	145 ± 31	255 ± 60

[a] The highest value exhibited by each individual animal was designated as that individual's peak value for the day. The mean of the individual values is represented here.

test, $P < 0.01$; Day 4 vs Day 6). On Day 10 the LH surge is no longer detectable.

Figure 2 shows the serum LH concentrations of ovariectomized animals bearing estradiol implants and receiving bromocriptine treatment. Like the control group, large surges of LH were observed on Days 2 and 4. However, unlike the control group, a large LH surge was also present on Day 6 and significant LH surges were still present on Days 8 and 10. Between group comparison of the total LH secreted on each day with Student's t test revealed that significantly more LH was present in the bromocriptine-treated group than in the control group on Days 6, 8, and 10 ($P < 0.01$, $P < 0.01$, and $P < 0.025$, respectively). When the peak LH values were compared between groups with Student's t test, a significant difference was present on Days 6 and 8 ($P < 0.02$ and $P < 0.05$, respectively; Table I).

The estrogen implants caused a significant elevation of prolactin secretion (Fig. 3, top panel) in the form of large afternoon surges. In addition, by Day 6, the baseline values of prolactin became elevated. The increase in basal prolactin levels continued until by Day 10, high levels were present throughout the sampling period. Also, the elevation in basal prolactin on Day 6 is coincident with the first significant decrease in the LH surge. Bromocriptine effectively suppressed prolactin secretion in the experimental group (Fig. 3, bottom panel). Prolactin levels were significantly lower in the bromocriptine-treated group than in the control group on each day ($P < 0.0001$). However, prolactin secretion did gradually "escape" from bromocriptine treatment as evidenced by the small afternoon rise in prolactin which is most prominent on Day 10. One-way ANOVA of the bromocriptine-treated group revealed there was a significant increase in prolactin secretion by Day 10 ($P < 0.01$).

Discussion. Ovariectomized rats treated with estrogen implants will initially exhibit daily afternoon surges of LH (1) and prolactin (2). These surges are thought to be cir-

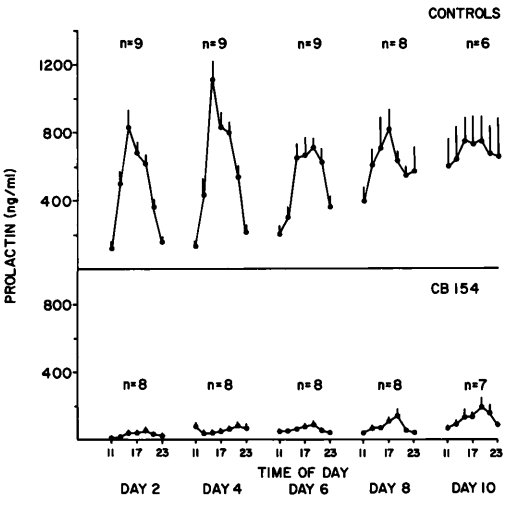

FIG. 3. The mean patterns of prolactin secretion in the same animals for which the LH values are shown in Fig. 1 and 2. All animals were ovariectomized, catheterized, and carried subcutaneous capsules of estradiol. The control group, in the top panel, received twice daily injections of vehicle. The experimental group, in the bottom panel, received twice daily injections of bromocriptine (CB154).

cadian rhythms generated by the SCN and require estrogen to be expressed (8). Hence, LH and prolactin surges are manifested only once during the 4-day estrous cycle when adequate estrogen levels have been reached. However, if serum estrogen levels are chronically maintained with Silastic implants, a large LH surge is secreted for several days and then begins to decrease in magnitude until it finally disappears (1, 9). This daily decrease in the LH surge has been termed *extinction*, and the factors contributing to this phenomenon are unresolved.

The current study demonstrates that the coincidental estrogen-induced prolactin secretion correlates with the early demise of the LH surge. When prolactin secretion was inhibited with bromocriptine, a large LH surge was present for 6 days, whereas in the untreated group, a large LH surge was observed for only 4 days. In addition, more LH was secreted on Days 8 and 10 in the bromocriptine-treated group than in the control group.

The role of dopamine in the regulation of LH secretion remains controversial, and the possibility exists that bromocriptine could directly facilitate the LH surge by acting at CNS dopamine receptors. However, there is currently no evidence that bromocriptine has a stimulatory effect on LH secretion. Rather, it has been suggested to depress LH secretion in humans (10, 11).

Since the LH surge did ultimately decrease, even though prolactin was suppressed, it is unlikely that elevated prolactin is the only factor contributing to LH extinction. Additional mechanisms involved in the extinction of the LH surge could include chronic estrogen-induced changes in pituitary responsiveness to GnRH or a central action of estrogen on neural systems involved in rhythm regulation.

Earlier experiments demonstrated that the amount of LH released in response to a bolus of GnRH decreased with long-term estradiol administration (9). The decreased response in "releasable" LH could be due to a direct long-term estrogen effect on gonadotrophs or an indirect effect via estrogen-stimulated prolactin secretion. GnRH challenges to ovariectomized rats with estrogen implants and receiving bromocriptine to suppress prolactin will resolve this question.

Chronic estrogen treatment has also been shown to abolish the circadian rhythm in hypothalamic serotonin (12). Since serotonergic-mediated neural events appear to be involved in the generation of LH surges (13–15), the disruption of serotonin rhythms by estradiol may be another factor contributing to extinction of the circadian LH hormone pattern.

In conclusion, extinction of the circadian LH surges may involve multiple factors such as elevated prolactin secretion, changes in pituitary LH reserves, and loss of the hypothalamic serotonin rhythm. The mechanisms by which prolactin inhibits LH secretion are the subjects of further studies.

1. Legan SJ, Karsh FJ. A daily signal for the LH surge in the rat. Endocrinology **96**:57–62, 1975.
2. Neill JD. Sexual differences in the hypothalamic regulation of prolactin secretion. Endocrinology **90**:1154, 1972.
3. Gersten BE, Baker BL. Local action of intrahypophyseal implants of estrogen as revealed by staining with peroxidase labeled antibody. Amer J Anat **128**:1–20, 1970.
4. Grandison L, Hodson C, Chen HT, Adirs J, Simpkins J, Meites J. Inhibition by prolactin of post-castration rise in LH. Neuroendocrinology **23**:312–322, 1977.
5. Gudelsky GA, Simpkins J, Mueller GP, Meites J, Moore KE. Selective actions of prolactin on catecholamine turnover in the hypothalamus and on serum LH and FSH. Neuroendocrinology **22**:206–215, 1976.
6. Beck W, Englebart S, Gelato M, Wuttke W. Antigonadotrophic effect of prolactin in adult castrated and in immature female rats. Acta Endocrinol **84**:62–71, 1977.
7. Keye WR, Chang RJ, Jaffe RD. Prolactin secreting pituitary adenomas in women with amenorrhea or galactorrhea. Obstet Gynecol Surv **32**:727–738, 1977.
8. Brown-Grant K, Murray MAF, Raisman G, Sood MC. Reproductive function in male and female rats following extra- and intra-hypothalamic lesions. Proc R Soc London **198**:267–278, 1977.
9. Chazal G, Faudon M, Gogan F, Hery M, Kordon C, Laplante E. Circadian rhythm of luteinizing hormone secretion in the ovariectomized rat implanted with oestradiol. J Endocrinol **75**:251–260, 1977.

10. Lachelin GCL, Leblanc H, Yen SSC. The inhibitory effect of dopamine agonists on LH release in women. J Clin Endocrinol Metab **44**:728–732, 1977.
11. Leebaw WF, Lee LA, Woolfe PD. Dopamine affects basal and augmented pituitary hormone secretion. J Clin Endocrinol Metab **47**:480–487, 1978.
12. Yates CA, Herbert J. The effects of different photoperiods on circadian 5HT rhythms in regional brain areas and their modulation by pinealectomy, melatonin and estradiol. Brain Res **176**:311–326, 1979.
13. Hery M, Laplante E, Kordon C. Participation of serotonin in the phasic release of LH. I. Evidence from pharmacological experiments. Endocrinology **99**:496–503, 1976.
14. Walker RF. Serotonin neuroleptics change pattern of preovulatory secretion of luteinizing hormone in rats. Life Sci **27**:1063–1068, 1980.
15. Walker RF. Serotonin circadian rhythm as a pacemaker for reproductive cycles in the female rat. In: Brambilla F, Racagni G, deWild D, eds. Progress in Psychoneuroendocrinology. Amsterdam/New York, Elsevier/North-Holland, pp591–600, 1980.

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