

Release of Luteinizing Hormone in Prepubertal and Middle-Aged Ovariectomized Rats<sup>1</sup> (41519)

GEORGE M. BUTTERSTEIN,<sup>2</sup> WILLIAM FRIEDMAN,  
MARY CATHERINE REHDER, AND FELICIA WILION

*Department of Biological Sciences, Union College, Schenectady, New York 12308*

---

**Abstract.** Concentrations of circulating LH were determined in conscious, free-moving ovariectomized rats. All of the animals had been ovariectomized at 24 days of age. Between 30 and 90 days there was an increase in mean blood LH concentrations; a more vigorous pulsatile release of LH characterized by an increase in amplitude and frequency of LH release; and an elevated responsiveness to LHRH administration. Rats which had been ovariectomized for 1 year still had elevated blood LH levels but had episodic pulses of reduced amplitude and a decrease in responsiveness to LHRH. These data suggest that important alterations occur with age in the neuroendocrine mechanisms responsible for the release of LH.

---

Ovariectomy (OVX) of the mature rat results in a rise in plasma luteinizing hormone (LH) and the establishment of an active pulsatile release of LH from the pituitary (1). This episodic pattern of LH release occurs in both OVX and intact prepubertal animals (2, 3) and continues in the aged animal (4). However, at about 10 months of age intact rats will begin to show signs of reproductive dysfunction (5-8) and eventually exhibit reproductive failure (9). A previous report indicated that aging also results in a modification in the episodic release of LH leading to a decrease in LH pulse amplitude without an alteration in pulse frequency (4). To date no study has examined the long-range neuroendocrine changes which occur as a result of gonadectomy at an early age. We therefore examined the pulsatile release of LH in OVX rats between 30 and 365 days of age in an effort to determine the changes that occur in the temporal release of LH in the absence of feedback control by the ovary.

**Materials and Methods.** Twenty-one-day-old Sprague-Dawley rats were purchased from Charles River Breeding Labs, Inc. The animals were caged in groups of three in a room with controlled temperature and lighting (L:D, 14:10) and were fed rat chow and water *ad libitum*. All the animals were ovariectomized at 24 days of age. Cannula were

implanted into the right atrium of each animal 2 days prior to the first blood collection. The animals were housed in individual cages and bled at 30 days, 90 days, or at 1 year of age. At the time of blood sampling each cannulated animal was connected to a cannula extension tube which protruded through the top of the cage and permitted free movement of the animal. The animals were then left undisturbed for 30 min prior to the start of the bleeding procedure. Over a period of 90 min, blood samples (75  $\mu$ l) were withdrawn at 10-min intervals in the 30-day-old animals, while samples (50  $\mu$ l) were taken at 5-min intervals in the older animals. An equal volume of physiological saline was returned to the animal following each bleed. After the 90-min samples were withdrawn a single injection of LHRH (20 ng/100 g body wt—Beckman Lt No. B90623) was administered to each animal through the cannula. Final blood samples were taken 10 and 20 min after LHRH administration. Whole blood samples were analyzed using the LH radioimmunoassay kit provided by NIAMDD. LH values (ng/ml whole blood) are expressed in terms of the NIAMDD rat LH-RP-1 reference preparation. The intraassay variation for the LH assay was 5.8% and the interassay variation for the last 7 assays was 4.8%.

Fluctuations in blood levels of LH for each animal were analyzed by a cycle detection program (10). Cycle frequency and amplitude were determined using an iterative pro-

---

<sup>1</sup> Supported by NIH Grant HD 13424.

<sup>2</sup> To whom all correspondence should be addressed.

TABLE I. CHARACTERISTICS OF PULSATILE LH RELEASE IN OVARECTOMIZED RATS AT 30 AND 90 DAYS OF AGE AND AT 1 YEAR

Age	Mean LH (ng/ml)	Amplitude (ng/ml)	Period (cycle length in min)
30 days (11)	96 ± 13	72 ± 12	34.3 ± 2.1
90 days (15)	219 ± 12*	140 ± 16**	25.5 ± 1.9**
1 year (6)	169 ± 15***	95 ± 10***	21.8 ± 2.8

Note. All of the rats were ovariectomized at 24 days of age. Values represent mean ± SEM. Number of animals are shown in parentheses.

\*  $P < 0.001$  vs 30-day-old animals.

\*\*  $P < 0.01$  vs 30-day-old animals.

\*\*\*  $P < 0.05$  vs 90-day-old animals.

cedure that scanned the data to locate sequential increases and decreases which were greater than a predetermined threshold value. The cycle detection program provided good estimation of cycle frequency and amplitude with signal-to-noise ratios as low as 2:1. Statistical comparisons were made using the Student's  $t$  test, and a  $P < 0.05$  was considered significant.

**Results.** Between 30 and 90 days of age there was approximately a 100% increase in the mean concentration of circulating LH

(Table I). Although animals which had been OVX for nearly 1 year still had elevated mean blood levels of LH compared to prepubertal castrates, the levels were significantly less ( $P < 0.05$  than those observed at 90 days of age. A pulsatile pattern of LH release was observed for all the OVX rats in the three age groups examined. The episodic release of LH of 12 representative animals is given in Fig. 1. The animals selected were chosen to illustrate the variation observed in LH secretion at each representative age. Analysis of the cycles of LH release showed that while the average pulse amplitude was increased by approximately 100% between 30 and 90 days of age (Table I), the periodicity of the pulses was greatly reduced ( $P < 0.01$ ). Long-term OVX animals had a significantly ( $P < 0.05$ ) reduced average pulse amplitude compared to the animals at 90 days but the values did not differ from those originally observed at 30 days of age. Although there appeared to be a further reduction in pulse periodicity in the older animals, the values were not significantly different from those observed in the younger animals.

LH levels rose sharply in all the animals in response to LHRH treatment but the

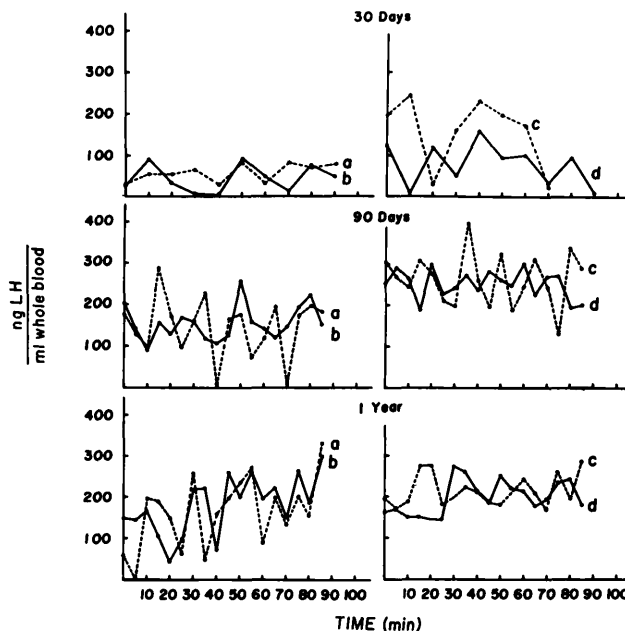


FIG. 1. The episodic release pattern of LH for twelve representative ovariectomized rats (a-d) at three different ages.

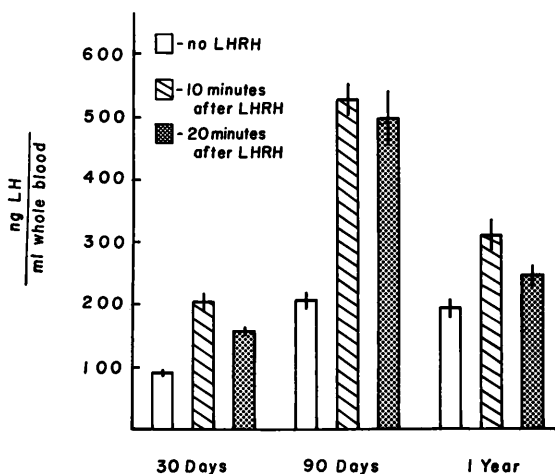


FIG. 2. LH release following LHRH administration in ovariectomized rats. Dose of LHRH = 20 ng/100 g body wt. Same rats as in Table 1. Blood levels of LH differed significantly ( $P < 0.001$ ) from preinjection values at each age. LH concentrations following LHRH administration were significantly higher at 90 days ( $P < 0.001$ ) and 1 year ( $P < 0.01$ ) when compared to those values observed at 30 days of age.

greatest increase (157%) occurred in 90-day-old animals (Fig. 2). The smallest increase (59%) in circulating LH was found in the oldest animals.

**Discussion.** Pulsatile gonadotropin secretion is important and may be essential to the establishment of puberty and the initiation and maintenance of cyclic reproductive patterns. Periodic LHRH release is responsible for this episodic secretion of LH from the pituitary (11) indicating that the pituitary is under rhythmic hypothalamic control. Moreover, the pulsatile nature of LH release is influenced by a variety of factors which can impinge on the hypothalamus to modify either the frequency or amplitude of LHRH release. Previous studies have shown that estrogen treatment led to a decrease in serum LH (12, 13) and a reduction in LH pulse frequency (14). Ovariectomy of a mature rat, on the other hand, prompts an increase in the magnitude of the LH pulses rather than a change in frequency (15). In this study we have shown that OVX of prepubertal animals led to a dramatic increase in mean concentrations of blood LH between 30 and 90 days of age. This was the result of a combination of an increase in pulse amplitude and frequency. Long-term gonadectomized animals which had been OVX when 24 days of age

demonstrated a decrease in LH pulse amplitude when compared to 90-day-old animals and showed no significant alteration in pulse frequency. The finding that pulse amplitude is changed rather than pulse frequency in the aged animal is in agreement with a previous report (4). Thus it appears that the most vigorous release of LH occurs at an age when a rat would be expected to be most reproductively active.

In addition to age-related alterations in the episodic release of LH there is ample evidence to suggest that there are changes in the neuroendocrine responsiveness to gonadectomy and LHRH stimulation. Young cycling rats and middle-aged rats (8–12 months old) showed no differences in blood LH concentrations (7), but in older rats (16–36) LH concentrations have been reported to be either elevated or lower than younger control animals (5, 8, 16). More striking is the fact that ovariectomy resulted in a 44-fold increase in plasma LH in young rats but only a 4- to 8-fold increase in middle-aged animals (7). The ability of the pituitary gland to secrete LH in response to OVX is also markedly depressed in much older animals (14 months old) (17). Our study shows that the elevation in LH concentrations seen in 90-day-old OVX animals was retained for 1 year. In con-

trast was the finding that older rats showed a smaller increase in blood LH following LHRH injection than the 90-day-old animals (59% vs 157%). In support of this finding Watkins *et al.* (18) have also shown that pituitaries of aged rats are less capable of releasing LH in response to acute LHRH administration.

These results indicate that the following changes occur in the neuroendocrine system between 30 and 90 days: (i) elevation in mean blood LH concentrations, (ii) more frequent LH pulses of high amplitude, and (iii) a heightened responsiveness to LHRH. One-year-old OVX rats maintain increased levels of LH in response to castration, but have LH pulses of reduced amplitude and a comparatively sluggish response to acute LHRH administration. These data suggest that the time-related alterations in LH secretion in the OVX rat may be due to several different mechanisms. Since pulsatile LHRH release is responsible for the pulsatile nature of plasma LH in OVX rats (11), an initial and prolonged increase in hypothalamic stimulation probably occurs in the 90-day-old following OVX as a prepubertal animal. The lower LH pulse amplitude observed in 1-year-old OVX animals could result from an attenuated hypothalamic release of LHRH. An alternative explanation for the heightened response of LH release at 90 days and subsequent decrease at 1 year could be a reduction in the pituitary's ability to store or to synthesize LH. Other studies have shown that pituitaries of old female rats contain less LH than pituitaries of corresponding younger animals (19). In addition, a change in a neuroendocrine set-point (sensitivity) in the hypothalamus could produce a chronic change in pituitary hormone output over time. Finally, recent studies (20–22) also indicate that the number of pituitary receptors for LHRH are altered in response to castration and aging. Thus, additional information is necessary to elucidate the precise neuroendocrine mechanisms responsible for age-related alterations in LH release. The changes in the episodic release of LH and responsiveness to LHRH administration with age suggest that the aging process may have an influence on both the hypothalamus and the pituitary.

We are grateful to Kim Evans for technical assistance. The authors wish to thank the NIAMDD for the reagents used in the LH assays.

1. Gay VL, Sheth NA. Evidence for a periodic release of LH in castrated male and female rats. *Endocrinology* **90**:158–162, 1972.
2. Andrews, WW, Ojeda SR. A detailed analysis of the serum luteinizing hormone secretory profile in conscious, free-moving female rats during the time of puberty. *Endocrinology* **109**:2032–2039, 1981.
3. Butterstein GM, Whitmoyer D, Sawyer CH. Episodic LH release in prepubertal ovariectomized rats. *Fed Proc* **39** (Abstract 2934), 1980.
4. Steger RW, Huang HH, Meites J. Pulsatile LH release in old and young ovariectomized rats. 61st Annual Meeting of the Endocrine Society. 1979 (Abstract 135).
5. McPherson III JC, Costoff A, Mahesh VB. Effects of aging of the hypothalamic-hypophysial-gonadal axis in female rats. *Fertil Steril* **28**:1365–1371, 1977.
6. Wilkes MM, Lu KH, Hopper BR, Yen SSC. Altered neuroendocrine status of middle-age rats prior to the onset of senescent anovulation. *Neuroendocrinology* **29**:255–261, 1979.
7. Wise PM, Ratner A. Effect of ovariectomy on plasma LH, FSH, Estradiol and progesterone and medial basal hypothalamic LHRH concentration in old and young rats. *Neuroendocrinology* **30**:15–19, 1980.
8. Huang HH, Marshall S, Meites J. Capacity of old versus young female rats to secrete LH, FSH and prolactin. *Biol Reprod* **14**:538–543, 1976.
9. Ingram DL. The vaginal smear of senile laboratory rats. *J Endocrinol* **19**:182–188, 1959.
10. Clifton DK, Steiner RA. A new generalized method for analyzing episodic hormone secretion. 61st Annual Meeting of the Endocrine Society. 1981 (Abstract 940).
11. Blake CA, Sridaran R, Elias KA, Ashiru DA, Rush ME. Plasma LH patterns after LHRH infusion in long-term, unanesthetized ovariectomized rats. *Neuroendocrinology* **30**:45–51, 1980.
12. Blake CA, Norman L, Sawyer CH. Localization of the inhibitory actions of estrogen and nicotine on release of luteinizing hormone in rats. *Neuroendocrinology* **16**:22–35, 1974.
13. Weick RF. Effects of estrogen and progesterone on pulsatile discharges of luteinizing hormone in the ovariectomized rat. *Canad J Physiol Pharmacol* **35**:226–233, 1977.
14. Butterstein GM, Whitmoyer DI. Estrogen modulation of pulsatile release of luteinizing hormone in the long-term ovariectomized rat. 13th Annual Meeting of the Society for the Study of Reproduction. 1980 (Abstract 204).
15. Weick RF, Soper BD, Greenberg D. Development

- of pulsatile LH release as a function of time following ovariectomy in the rat. *Neuroendocrinology* 32:78-81, 1981.
16. Shaar CJ, Euker JS, Riegler GD, Meites J. Effects of castration and gonadal steroids on serum luteinizing hormone and prolactin in old and young rats. *J Endocrinol* 66:45-51, 1975.
  17. Howland BE, Preiss C. Effects of aging on basal levels of serum gonadotropins, ovarian compensatory hypertrophy and hypersecretion of gonadotropins after ovariectomy in female rats. *Fert Steril* 26:271-276, 1975.
  18. Watkins BE, Meites J, Riegler GD. Age-related changes in pituitary responsiveness to LH-RH in the female rat. *Endocrinology* 97:543-548, 1975.
  19. Clemens JA, Meites J. Neuroendocrine status of old constant estrous rats. *Neuroendocrinology* 7:249-256, 1971.
  20. Clayton RN, Solano AR, Garcia-Vela A, Dufau ML, Catt KJ. Regulation of pituitary receptors for gonadotropin-releasing hormone during the rat estrous cycle. *Endocrinology* 107:699-706, 1980.
  21. Conne BS, Scaglioni S, Lang U, Sizonenko PC, Aubert ML. Pituitary receptor sites for gonadotropin-releasing hormone: Effect of castration and substitutive therapy with sex steroids in the male rat. *Endocrinology* 110:70-79, 1982.
  22. Pieper DR, Gala RR, Regiani SR, Marshall JC. Dependence of pituitary gonadotropin-releasing hormone (GnRH) receptors on GnRH secretion from the hypothalamus. *Endocrinology* 110:749-753, 1982.
- 

Received May 24, 1982. P.S.E.B.M. 1983, Vol. 172.