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Alteration in Pulsatile Release of LH in Aging Female Rats[1] (40782)

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Reproductive senescence in the female rat is characterized by a gradual decline in ovarian cyclic activity. By 8 months of age, many previously normally cycling (NC) rats show irregular cycles interrupted by extended periods of estrus (1). In general, most of these irregularly cycling (IC) rats eventually enter a state of constant estrus, characterized by well-developed follicles, an absence of ovulation, and thus no formation of corpora lutea (1, 2).

In the rat, the initial decline in reproductive function appears to be due to a deficiency in the ability of the hypothalamo–hypophyseal system to secrete gonadotropin in response to centrally mediated stimuli. Thus castration (3), treatment with gonadal steroids (4), and acute restraint stress (5) induce less LH and FSH secretion in old than in young rats. It appears that this age-related decline in secretion of gonadotropins is due primarily to a defect in the central nervous system since chronic treatment with the centrally acting drugs, iproniazid and L-dihydroxyphenylalanine, can induce ovulation and a resumption of cyclicity in old constant estrous (CE) rats (6, 7).

In young ovariectomized (OVX) rats, LH is released from the pituitary gland in pulses (8) which appear to depend upon the norepinephrine-mediated pulsatile release of luteinizing hormone-releasing hormone (LHRH) from the hypothalamus (9, 10, 11). Because concentration and turnover of norepinephrine are decreased in old rats (12, 13), the present study was designed to determine if the age-related decrease in secretion of LH is due to a defect in its pulsatile release.

Materials and methods. Female rats (Sprague–Dawley, Charles River) 2–3, 8–10, and 16–17 months of age were maintained in a thermoregulated room (25 ± 2°) with a light–dark cycle (lights on 0600–2000 hr) and provided with Purina laboratory chow and water *ad libitum*. Daily vaginal smears were examined for at least 2 weeks to determine the reproductive state of each animal. Rats were then ovariectomized under ether anesthesia and 3 weeks later a Silastic catheter (Dow Corning, 0.025 in. i.d., 0.047 in. o.d.) was implanted by methods previously described (14). Two days following catheterization, a 30-cm-long Silastic tube was attached to the catheter and draped outside each animal's cage to allow repeated blood sampling. Thirty minutes prior to blood sampling, a single injection of 200 units of heparin was administered via the implanted catheter. Every 15 min for 3 hr (1230–1530 hr), the saline filling the catheter (about 100 μl) was removed and discarded and 300 μl of blood was removed for assay of serum LH. Following the removal of each sample, an equal volume of 0.9% saline was injected via the catheter. During the 3-hr period hematocrits decreased not more than 25%.

Upon completion of sampling of blood, animals were killed and necropsies were performed. Anterior pituitary and adrenal glands were examined, removed, and weighed. Other internal organs were examined for gross lesions. One 17- to 18-month CE rat had a small anterior pituitary adenoma; therefore, data from this animal were excluded from calculations.

Samples of 25 and 100 μl plasma were analyzed for LH using the radioimmunoassay methods described in the NIAMDD kits. Concentrations of the hormone are

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expressed in terms of the reference preparation NIAMDD rat LH-RP-1. Intraassay coefficient of variance was 15.6% and interassay coefficient of variance for seven assays was 17.9%. Mean concentration of LH was determined by averaging all LH values from each animal. The amplitude of pulses of LH was determined by the height of the increasing phase of each pulse. The decreasing phase of the pulse represents clearance of LH in the absence of pituitary secretion (9), and therefore was not included in calculation.

The significance of differences in pulse amplitude between groups was evaluated by least significant difference tests. The significance of differences among group mean concentration of plasma LH and organ weights was determined by one-way analysis of variance.

Results. Representative profiles of LH release patterns of each age and reproductive state used in this study are shown in Fig. 1. Pulse amplitude of LH in old CE rats was significantly lower than that in all other groups studied (Table I). Further 9- to 11-month-old IC rats had significantly lower LH pulse amplitude than 3- to 4-month-old NC rats. When LH pulse amplitude was compared by reproductive state alone, rather than by age, three significantly different populations were observed with LH pulse amplitudes of NC > IC > CE rats. Further, mean plasma LH concentrations showed an age-related decline (Table II).

Discussion. This study establishes that the inability of old CE rats to show a normal postcastration increase in secretion of LH is due to a relative absence of its pulsatile release. Further, the decrease in amplitude of pulses of LH appears to be closely associated with the progressive change in reproductive states which accompanies aging in the rat. Thus, the highest pulse amplitudes of LH were observed in 3- to 4- and 9- to 11-month NC rats and the lowest amplitude in 17- to 18-month CE animals. Mean amplitudes of LH pulses for IC rats (both 9- to 11- and 17- to 18-month groups) were between NE and CE values.

The observation that in the 9- to 11- and 17- to 18-month groups, multiple reproductive states were represented, indicates that the rate of reproductive aging varies among animals. Further, the amplitude of pulsatile release of LH is related more closely to state of reproductive function at the time of ovariectomy than to age of the animal. This

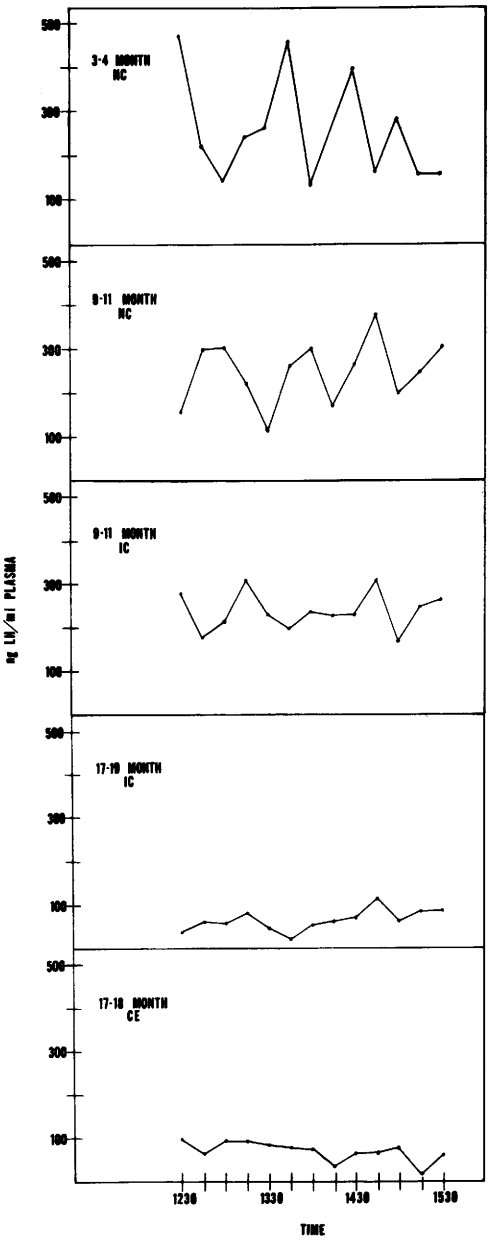

FIG. 1. Representative LH release patterns in OVX rats from each age group and reproductive state studied. NC, IC, and CE indicate normal cycling, irregular cycling, and constant estrus rats, respectively.

TABLE I. AMPLITUDE OF LH PULSES IN OVARIECTOMIZED RATS OF SEVERAL AGES AND REPRODUCTIVE STATES

Age (months)	LH pulse amplitude (ng increase/ml plasma)		
	Normal cycle	Irregular cycle	Constant estrus
3–4	124 ± 12 (40)*	—	—
9–11	117 ± 16 (13)	86 ± 16 (14)[a]	—
17–18	—	90 ± 25 (10)	37 ± 4 (28)[b]
Total	122 ± 9 (53)	89 ± 13 (24)[c]	37 ± 4 (28)[d]

* Means ± SEM (number of pulses/group).
[a] Significantly different from 3- to 4-month NC group.
[b] Significantly different from all other groups.
[c] Significantly different from total NC and total CE.
[d] Significantly different from total NC and total IC.

relationship between the amplitude of pulses of LH and reproductive state suggests that a central deficiency, progressive in nature and variable among rats of the same age, may be responsible for the age-related changes in estrous cycles.

In the young OVX rat, the pulsatile release pattern of LH results in part from norepinephrine-mediated pulsatile secretion of LHRH from the hypothalamus (9–11). In old rats, hypothalamic concentration and turnover of norepinephrine are lower than in young animals (12). Further, in response to ovariectomy, old CE rats do not show the normally observed increase in turnover of hypothalamic norepinephrine (13). Thus, the decreased amplitude of pulses of LH in CE rats may result from a decreased response of central noradrenergic neurons to ovariectomy. Arendash and Gallo (15) have recently reported that deafferentation of the medial basal hypothalamus, which depletes hypothalamic concentration of norepinephrine, results in abated or reduced pulsatile secretion of LH in OVX animals.

TABLE II. AGE-RELATED CHANGES IN MEAN CONCENTRATION OF LH

Age (months)	Number of rats	LH (ng/ml)
3–4	10	263 ± 16*
9–11	7	193 ± 22[a]
17–18	9	85 ± 15[b]

* Means ± SEM.
[a] Significantly different from 3- to 4- and 17- to 18-month groups ($P < 0.05$).
[b] Significantly different from 3- to 4- and 9- to 11-month groups ($P < 0.05$).

Alternatively, high circulating levels of prolactin, which inhibit secretion of LH (16), may be involved in dampening the amplitude of surges of LH. In human subjects, high concentrations of prolactin in plasma appear to inhibit pulsatile release of LH, whereas inhibition of prolactin secretion results in a resumption of the normal LH release pattern (17). Further, Beck *et al.* (18) observed that elevation of serum prolactin following implantation of pituitaries abolished the pulsatile secretion of LH in ovariectomized rats. Thus the elevated serum prolactin observed in old CE rats in the present study (data not shown) and by others (19) may also contribute to the dampening of the pulses of LH in these old rats.

The possibility that the age-related decline in the amplitude of LH pulses might simply reflect an increase in volume of distribution of LH due to the observed age-related increase in body weight appears unlikely for two reasons. First, groups with the same body weight (i.e., 17- to 18-month-old IC and CE rats) showed significantly different LH pulse amplitudes, whereas groups with different body weights showed similar LH pulse amplitudes (i.e., 3- to 4-month-old NC rats and 9- to 11-month-old NC rats). Thus, factors other than simply the age-related increase in body weight are responsible for the presently observed alterations in LH pulse amplitude.

Summary. Amplitude of pulsatile release of LH was examined in OVX rats of ages 3–4, 9–11, and 17–18, months, serially bled via chronic jugular catheters. At the time of ovariectomy, rats showed normally cycling (NC), irregularly cycling (IC), or constant estrus (CE) ovarian activity. Amplitude of pulsatile release of LH was significantly reduced in CE rats as compared to all other groups. Further, LH pulse amplitude appeared to be more closely associated with reproductive state at the time of ovariectomy than with the age of the animal. Thus, LH pulse amplitude was greatest in NC rats, intermediate in IC rats, and lowest in CE rats, regardless of age. These data suggest that a common, central mechanism may be responsible for both the changes in reproductive pattern and the changes in pulsatile release of LH which accompany aging in the rat.

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