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Alterations in Proestrous LH, FSH, and Prolactin Surges in Middle-Aged Rats[1] (41356)

PHYLLIS M. WISE

Department of Physiology, School of Medicine, University of Maryland, Baltimore, Maryland 21201

Abstract. Plasma LH, FSH, and prolactin were measured in middle-aged (7- to 9-month-old) breeder and young (3–4 month old) female rats on proestrus. Sixty-eight percent of the middle-aged rats exhibited regular 4-day estrous cycles; the remainder showed irregular cycles 4–7 days in length. All young rats exhibited at least two consecutive 4-day cycles prior to use. The right atria of all rats were cannulated under ether anesthesia between 0700 and 0800 hr (14:10 light–dark cycle, lights on at 0400 hr). Blood was drawn at 0900 hr and at hourly intervals from 1200 to 2200 hr; each rat was bled a maximum of nine times. Plasma LH and FSH rose significantly later and peak concentrations were significantly lower in middle-aged rats. Baseline prolactin concentrations at 0900 and 2000–2200 hr were significantly higher in middle-aged female rats. These data indicate that alterations in the proestrous gonadotropin and prolactin surges occur early during the aging process in the female rat. Such changes may be important in the age-related transition to estrous acyclicity.

When female rats are between 10 and 18 months of age, the frequency of regular 4- to 5-day estrous cycles decreases. Many become acyclic and exhibit constant estrous (CE) or persistent vaginal diestrus (PD). The results of previous studies demonstrate that old CE and PD rats have altered hypothalamic (1–4) and pituitary function (5–7). The majority of the above studies were done using 2- to 3-year-old acyclic rats which had not exhibited estrous cyclicity for 6–24 months. Therefore, it is not possible to distinguish which changes occur prior to the onset of acyclicity, perhaps triggering the transition to acyclicity, from those alterations which are secondary effects of the acyclic state and appear after the transition to acyclicity is complete.

Results of recent studies using middle-aged female rats demonstrate that some changes at the hypothalamic–pituitary level are evident when animals are still cycling but when the transition to irregular cyclicity and acyclicity is imminent (8–13). Previous studies suggest that the proestrous LH surge in middle-aged rats may be altered in amplitude (9), time of onset (8), or both (10). Due to the experimental protocols used, the complete proestrous hormone pattern was not examined in the first two studies and no information is available on the effect of age on the secretion of FSH or prolactin on proestrus. The present study thoroughly characterizes the proestrous LH, FSH, and prolactin surges in middle-aged rats which are entering the initial stage of the transition to estrous acyclicity. The purpose of the study was to determine whether the timing of the initial hormone rise and/or the peak surge concentrations attained differ from those of young rats. Such changes may be important events which contribute to the transition to estrous acyclicity.

Materials and Methods. Female Sprague–Dawley rats were purchased from Zivic–Miller (Allison Park, Pa.). Middle-aged females, 7–9 months of age, weighed between 350 and 500 g. All of the middle-aged rats had been successfully used as breeders. Some had been purchased when young and were used as breeders in our animal facility while others were used as breeders by Zivic–Miller until the time of the experiment. These females, as opposed to "retired breeders," were used since the latter category includes some animals which never produced normal-sized litters or exhibited normal maternal behavior. By using females which had produced and

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nursed litters previously, I hoped to eliminate those animals which were reproductively abnormal when young. One is thus less likely to attribute congenital reproductive abnormalities to the aging process. Young, 3- to 4-month-old females weighing between 200 and 250 g, were used as controls. All animals were maintained in temperature- and humidity-controlled rooms with a 14:10 light dark cycle (lights on at 0400 hr) and were fed Purina rat chow and water *ad libitum*. Under these lighting conditions the critical period during which the proestrous LH surge begins in young rats is between 1300 and 1500 hr. Estrous cyclicity was monitored by daily vaginal lavage. Young rats were used on proestrus after demonstrating two consecutive 4-day cycles; middle-aged rats were used if they had exhibited regular 4-day or irregular (4- to 7-day) cycles. Rats were etherized between 0700 and 0800 hr and external jugular veins were cannulated to the level of the right atrium using PE 50 (o.d. 9.65 mm). Sequential blood samples (0.5 ml) were drawn at 0900 hr and at hourly intervals between 1200 and 2200 hr. Between 1900 and 2200 hr, blood was drawn under red light (one 25-W bulb) since others have found that continued illumination can affect the LH surge (14). The volume of blood was replaced with saline. Each individual was bled hourly between 1400 and 1800 hr and at four other times during the day. Thus a total of 4.5 ml of blood representing approximately 15–25% of the total blood volume was drawn over a 13-hr period. Mattheiz and Van Pijkeren (15) have previously demonstrated that a similar blood collection protocol does not disturb prolactin concentrations and we have found that LH concentrations measured in similarly sequentially bled young animals do not differ from those attained from decapitated young animals (16, 17). That middle-aged rats do not react differently to the stress of cannulation than young rats is demonstrated by the fact that LH concentrations reported below do not differ significantly from those of middle-aged decapitated rats (16). In addition Cooper *et al.* (10) found that the stress of withdrawing blood from the tail vein eight times on proestrus from middle-aged rats did not affect subsequent estrous cycle length.

LH (RP-1) was measured by radioimmunoassay using antibody supplied by Dr. G. D. Niswender and LH to be iodinated was supplied by Dr. L. E. Reichert (19). FSH (RP-1) and prolactin (RP-2) were measured by radioimmunoassay using kits donated by the NIAMDD pituitary hormone distribution program.

Cochran's test (20) for homogeneity of variance was applied to all raw data and revealed heterogeneity; therefore, all data were $\log_e$ transformed. When two-way analysis of variance with repeated measure was performed (time of day vs age) for each hormone, it revealed a significant effect of time ($P < 0.001$; $P < 0.001$; $P < 0.001$), for LH, FSH, and prolactin, respectively, and a significant interaction term ($P < 0.001$; $P < 0.01$) for LH and FSH, respectively. Therefore, the effect of time on hormone concentrations was compared by one-way analysis of variance followed by Newman–Keuls multiple range test. The difference in hormone concentration between young and middle-aged rats at a given time was compared using the t (separate) statistical test.

Results. Sixty-eight percent of the middle-aged females exhibited regular 4-day estrous cyclicity; the remaining animals had irregular estrous cycles between 4 and 7 days in length. All animals which demonstrated a rise in plasma LH concentrations during the afternoon to at least twice baseline concentrations measured at 0900 hr were included in all calculations. One of forty-seven regular 4-day cyclers and 3 of 22 irregular cyclers (4- to 7-day estrous cycles) did not surge on proestrus using these criteria. LH, FSH, and prolactin data from middle-aged rats were first calculated as two separate groups: regular 4-day cyclers and irregular cyclers (Table I). The timing of the first significant rise and peak concentrations attained were the same in both groups of rats for each hormone; therefore, data from regularly and irregularly cycling middle-aged rats were combined for all comparisons with young animals.

Plasma LH concentrations on proestrus

TABLE I. PLASMA HORMONE (ng/ml) CONCENTRATIONS ON PROESTRUS IN REGULARLY AND IRREGULARLY CYCLING MIDDLE-AGED RATS

	Time (hr)											
	0900	1200	1300	1400	1500	1600	1700	1800	1900	2000	2100	2200
LH												
Regular 4-day cyclers	57 ± 4	62 ± 4	50 ± 2	144 ± 15	608 ± 155	1780 ± 241	2307 ± 239	1793 ± 210	965 ± 137	620 ± 99	345 ± 117	332 ± 174
Irregular 4- to 7-day cyclers	49 ± 2	63 ± 9	50 ± 3	197 ± 94	384 ± 250	1413 ± 336	1868 ± 389	1390 ± 292	1103 ± 275	674 ± 145	227 ± 83	149 ± 26
FSH												
Regular 4-day cyclers	149 ± 15	147 ± 14	163 ± 14	156 ± 18	175 ± 14	344 ± 26	359 ± 23	358 ± 21	322 ± 26	335 ± 19	294 ± 35	291 ± 23
Irregular 4- to 7-day cyclers	142 ± 17	142 ± 15	160 ± 16	165 ± 13	186 ± 30	269 ± 53	377 ± 56	375 ± 34	371 ± 53	328 ± 39	310 ± 26	296 ± 41
Prolactin												
Regular 4-day cyclers	39 ± 5	29 ± 4	45 ± 6	99 ± 15	167 ± 19	173 ± 19	159 ± 15	130 ± 15	74 ± 15	86 ± 17	39 ± 6	38 ± 7
Irregular 4- to 7-day cyclers	34 ± 4	25 ± 3	55 ± 7	73 ± 15	126 ± 30	165 ± 29	138 ± 28	126 ± 27	68 ± 17	73 ± 27	48 ± 8	53 ± 11

in young and middle-aged females are shown in Fig. 1. Baseline plasma LH concentrations in young and middle-aged rats were the same at 0900 and 1200 hr. In young rats, the first significant increase in plasma LH was at 1300 hr, peak concentrations were attained between 1600 and 1700 hr and returned to baseline by 2200 hr. In contrast, LH in middle-aged rats showed the first significant increase at 1500 hr, peaked between 1600 and 1900 hr and returned to baseline concentrations at 2100 hr. Hormone concentrations were significantly lower in ($P < 0.01$) middle-aged animals during the surge at 1300, 1500, 1600, and 1700 hr; but concentrations remained elevated longer and were significantly higher ($P < 0.01$) than in young rats at 2000 and 2200 hr.

Figure 2 depicts FSH concentrations in young and middle-aged proestrous rats. In young rats, FSH rose at 1500 hr. Proestrous peak concentrations were achieved between 1600 and 1900 hr; a secondary estrus rise was apparent beginning at 2100 hr. The FSH surge began later (1600 hr) and peaks were reached by 1700 hr in middle-aged animals. A separate secondary estrous FSH rise was not apparent in these animals, although hormone levels remained significantly elevated at 2200 hr when bleeding was terminated. FSH concentrations were significantly higher ($P < 0.01$) in young animals than in middle-aged rats between 1500 and 1800 hr and at 2200 hr.

Figure 3 shows plasma prolactin concentrations in young and middle-aged proestrous animals. Two-way analysis of variance revealed a significant effect of time of day. In young rats prolactin levels were elevated by 1400 hr and peak concentrations were attained between 1500 and 1800 hr. At 1900 hr, prolactin levels were not significantly different from morning baselines at 0900 and 1200 hr. Prolactin levels in middle-aged rats were significantly elevated at 1500 hr, attained peak concentrations between 1600 and 1800 hr, and returned to baseline concentrations by 1900 hr. Prolactin levels were higher in middle-aged animals ($P < 0.01$) before the proestrous surge (0900 hr) and after the surge (2000 and 2200 hr).

Discussion. The effect of age on the

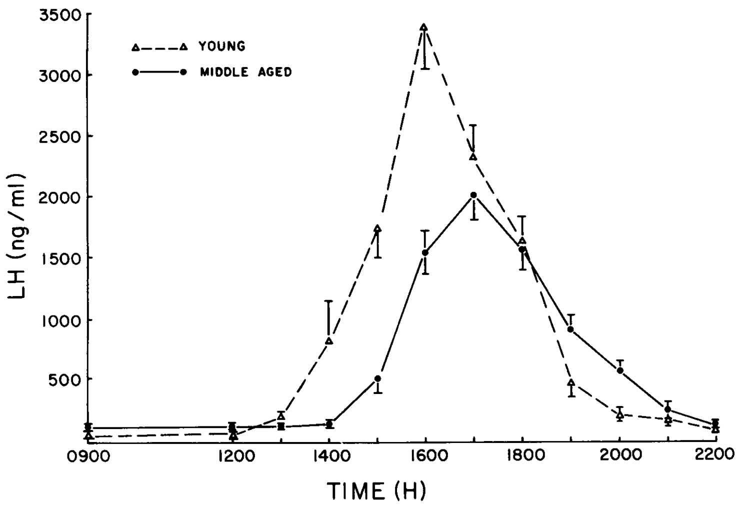

FIG. 1. Plasma LH (RP-1) concentrations in young and middle-aged rats on proestrus. Rats were bled from right atrial cannulae a maximum of nine times during the day; all rats were bled at hourly intervals between 1400 and 1800 hr. Values represent means ± SE.

proestrous patterns of LH secretion is uncertain and its effect on proestrous FSH and prolactin secretion is unknown. Our knowledge about the proestrous hormone profiles is incomplete because previous attempts to characterize hormone profiles have used blood sampling methods which entailed restraint of the animals (10), administration of anesthesia (9), or barbiturates (8). Such stressful methods prevent multiple sequential samples from one animal over a short time interval and make it impossible to estimate prolactin concentrations since this hormone responds to acute stress within 2 min of initial handling (15, 21). In the present study, blood was collected from unrestrained animals which had been cannulated under light ether anesthesia at least 5 hr prior to the onset of the proestrous hormone surge. There is dis-

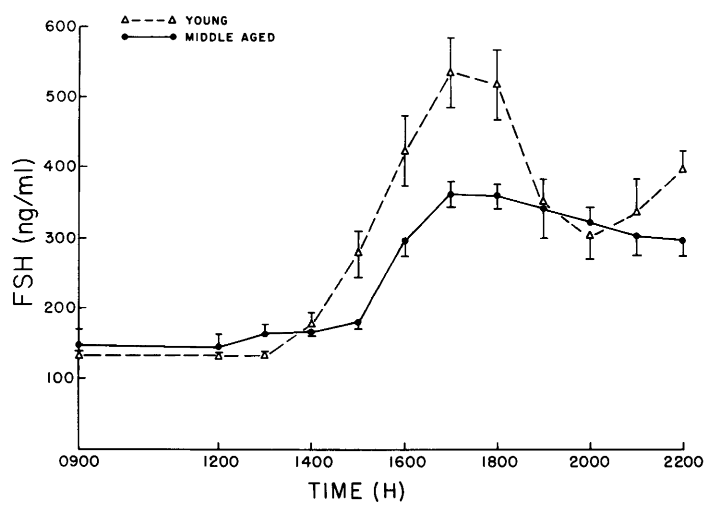

FIG. 2. Plasma FSH (RP-1) concentrations in young and middle-aged rats on proestrus. Values represent means ± SE.

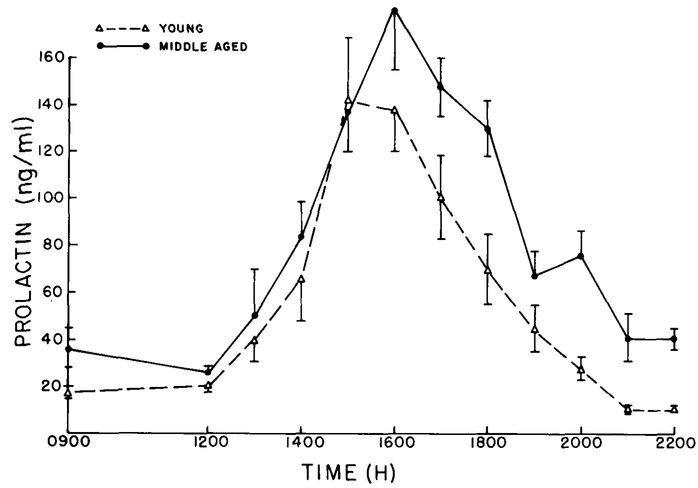

FIG. 3. Plasma PRL (RP-2) concentrations in young and middle-aged rats on proestrus. Values represent mean ± SE.

agreement on the effect of such temporally removed experimental manipulations. Previous studies suggest that the hormone returns to baseline concentrations within 15–40 min of such a stress (15, 21). In addition, the present prolactin data do not differ significantly from concentrations measured in naive animals decapitated at the same time on proestrus (unpublished observations). However, Neill (22) reported that proestrous LH and prolactin surges are significantly different in cannulated and decapitated rats. Thus, the present experimental protocol allows us to examine the gonadotropin and prolactin surge in unrestrained animals to more thoroughly characterize and compare the changes in each of the hormones during the afternoon and evening of proestrus in middle-aged and young cycling rats.

The results demonstrate that the proestrous LH, FSH, and prolactin surges are altered during the early stages of the age-related transition to estrous cyclicity. Some of the rats used in these studies were not consistently exhibiting 4-day estrous cycles. Of those rats exhibiting a proestrous vaginal smear, a greater proportion of irregular cyclers, (3/22) than regular 4-day cyclers (1/47) did not exhibit any rise in plasma LH, FSH, or prolactin during proestrous afternoon. The timing and duration of the LH surges of those irregular cyclers that did surge, were not significantly different from those of regular 4-day cyclers. Thus, the data suggest that changes in the timing and/or amplitude of the proestrous surge occur in aging animals and are not closely tied to the regularity of the cycle. Instead, it appears that the hormonal alterations do in fact precede the appearance of irregular cycles and may be causally related to further perturbations at the ovarian and hypothalamic level in older animals which ultimately result in total acyclicity.

The onset of LH surges is delayed approximately 1 hr, peak concentrations are significantly later, and are attenuated in middle-aged rats. Cooper *et al.* (10) found delayed and attenuated proestrous LH surges in 10-month-old Long–Evans female rats showing regular 4-day estrous cycles. A delay in the neural trigger for the LH surge was suggested by the finding that the critical period during which pentobarbital blocked the LH surge extended 1–2 hr beyond that of young cycling females (8). Gray *et al.* (9) bled middle-aged females at two times during proestrous afternoon and found that LH concentrations were significantly lower at both times. Their data gave no indication of a delay in the surge.

The present data indicate a similar effect of age on the proestrous FSH surge. Peak concentrations were significantly lower and occurred later in middle-aged rats compared to young animals although the age-related difference was not as dramatic as that seen in LH concentrations. FSH concentrations were attenuated at the beginning of the secondary estrous surge (2200 hr). Since blood was not drawn at later times, it is impossible to determine whether FSH secretion during the entire secondary estrous surge is affected in middle-aged animals.

Attenuation and delay of both the LH and FSH surges in middle-aged rats may be caused by changes in the neural trigger which regulate gonadotropin release. Changes in neurochemical balance (3, 11, 24) and brain LHRH concentrations (4, 24, 25) are evident in older rats and may occur at this early stage of the transition to acyclicity (11). In addition, other investigators have demonstrated that the ability of estradiol to induce an LH surge is altered in middle-aged rats (9, 12, 13). Thus, altered proestrous LH surges may be due to an age-related change in the positive feedback action of this steroid at the hypothalamic and/or pituitary level.

Prolactin concentrations are significantly elevated during the morning prior to the proestrous surge and in the evening after the surge in middle-aged rats. The present data indicate that baseline prolactin concentrations are elevated early during the aging process, prior to the onset of acyclicity. However, prolactin concentrations during the surge are not significantly altered in middle-aged rats, though concentrations tend to be higher in these animals from 1500 to 1900 hr. We have previously shown that 8- to 12-month-old CE females exhibit elevated prolactin concentrations compared to cycling rats on estrus (26). And others have observed increased prolactin levels in older female (27) and male (28) rats. This elevation in basal prolactin secretion may be due to a decrease in prolactin inhibitory factor influence (24, 28) and/or to increased release of a prolactin stimulatory factor. Since high prolactin levels are known to inhibit LH surges and estrous cyclicity (29), elevated prolactin may be one of several changes that eventually leads to age-related acyclicity.

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