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The First Proestrus in the Female Rat: Circulating Steroid Levels Preceding and Accompanying the Preovulatory LH Surge[1] (40767)

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It is now well established that the first ovulation in the female rat is triggered by a proestrus type of gonadotropin surge (1, 2) which in turn is preceded by an elevation not only in estradiol (E_2) levels (2–4) but also in serum progesterone (P) and testosterone (T) (3, 4). It is also known that the first LH surge takes place during the afternoon of the day in which both serum E_2 and accumulation of uterine fluid are maximal (1–4) and that it is preceded by remarkable changes in ovarian steroidal responsiveness to gonadotropins (3).

Although the changes in circulating steroid levels around the time of puberty have been studied by Meijs-Roelofs *et al.* (2), Parker and Mahesh (4), and ourselves (3), a more detailed characterization of the hormonal changes associated with the first preovulatory LH surge in the rat was considered to be of interest. Such a characterization will provide valuable information not only for a more complete understanding of the pubertal process but also for subsequent studies attempting to alter the onset of puberty by manipulating the prepubertal hormonal environment.

Materials and methods. Immature female rats (Holtzman Co., Madison, Wis.) were used. They were housed in group cages (five or six rats per cage) under controlled conditions of light (14 hr on, 10 hr off) and temperature (23–25°C). *Ad libitum* access to food and water was provided.

Beginning on Day 30, the animals were inspected daily for signs of imminent vaginal opening. Those animals showing edematization and reddening of the external genitalia were decapitated at different times of the day (every 2 hr) from 0800 to 1800 hr. Following decapitation, the blood was collected into centrifuge tubes and allowed to clot at room temperature. Thereafter, the sera were separated by centrifugation and stored at −20°C until assayed. Ovaries were removed, cleaned of adherent fat and their wet weight was recorded to the nearest 1 mg. Uteri were inspected for the presence of fluid, dissected out, drained of any fluid, and also weighed to the nearest 1 mg. According to a criteria previously published (1, 3), only those animals exhibiting ballooned uteri with a wet weight of 200 mg or more and ovaries with large follicles but no corpora lutea were considered to be in proestrus.

Radioimmunoassays.[4,5] Serum LH was measured by the method of Niswender *et al.* (5) using the RP-1 rat pituitary LH reference standard, and expressed in terms of the NIH-LH-S1 reference preparation.

Serum progesterone and testosterone were measured using antisera provided by Dr. G. D. Niswender, the specificity of which have been reported earlier (6, 7). Serum dihydrotestosterone was measured, after its chromatographic separation from

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[5] We are indebted to Dr. G. D. Niswender (Colorado State University) for the supply of antisera for progesterone, testosterone-dihydrotestosterone and LH radioimmunoassays. Serum estradiol was measured using an antiserum kindly provided by Drs. K. Wright and D. C. Collins (Emory University).

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testosterone (see below), using the same antiserum employed for testosterone measurements. This antiserum exhibits a 69% cross reactivity with DHT (7). The specificity of the antiserum used for measurement of serum estradiol also has been previously reported (8). In all cases, the steroids were measured following their chromatographic separation on celite columns according to the method described by Parker *et al.* (9). Total procedural losses were assessed by the recovery of tritiated steroids added to serum of ovariectomized–adrenalectomized rats. These recoveries were approximately 79% for E_2, 69% for testosterone, 81% for P, and 76% for DHT. The radioimmunoassay employed for all steroids was a modification of that reported by Hotchkiss *et al.* (10). Details of the procedure used and validation of the assays in our laboratory have been reported elsewhere (3, 11). In the case of DHT, the antiserum GDN No. S-250 was used at a dilution of 1:80,000. The sensitivity of the assay was 1.6 pg DHT/tube and the standard curve was linear between 1.6 to 100 pg DHT/tube. Intraassay variation was 12%. Sera from ovariectomized–adrenalectomized rats used for assay blanks were usually below the limit of sensitivity of the assay.

Statistics. The results were analyzed with a one-way analysis of variance, the Student/Newman–Keul's multiple comparison test for unequal replications and Dunnett's test for comparison of one mean to other group means (12).

Results. Serum levels of LH at 2-hr intervals from 0800 to 1800 hr on the day of the first proestrus of the rat are depicted in Fig. 1 (upper panel). Following verification of the time of the day at which peak LH levels occurred, the preceding and accompanying levels of E_2, P, T, and DHT were also determined. These values are depicted in Fig. 1 (middle and lower panel).

LH. Mean serum LH levels were low in prepubertal rats (less than 1 ng/ml) and remained very low throughout the morning of proestrus until 1200 hr. At the next interval studied (1400 hr) LH values were already elevated with maximal values being ob-

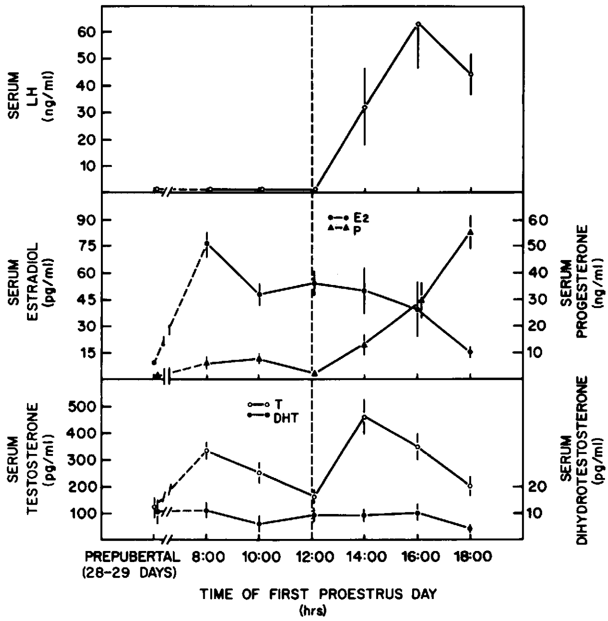

FIG. 1. Changes in serum levels of LH (upper panel), E_2, and P (middle panel), and T and DHT (lower panel) during the day of the first proestrus in the female rat. Levels of these hormones measured in a group of prepubertal rats are also depicted. In this and the next figure, vertical lines represent SEM. Each point is the mean of 6 to 11 animals.

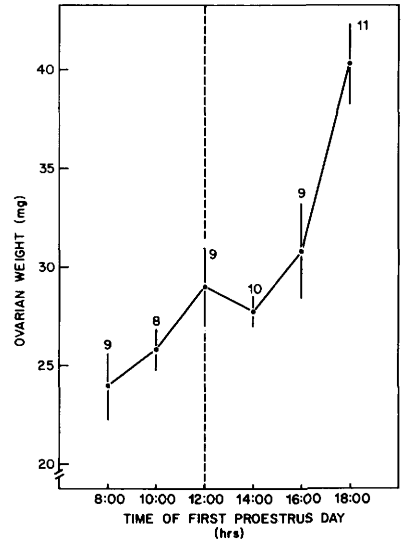

FIG. 2. Changes in ovarian weight during the day of the first proestrus in the female rat. Numbers next to means indicate number of animals per group.

served at 1600 hr ($P < 0.001$) (63 ± 17 ng/ml).

E_2. Serum estradiol was distinctly ($P < 0.001$) higher in the morning of the first proestrus than in prepubertal rats. The more elevated levels were observed at 0800 hr (76 ± 7 pg/ml). Thereafter, E_2 values decreased somewhat, but declined to basal values (16 ± 2 pg/ml) only after the LH surge had already become established.

P. Progesterone levels in prepubertal rats were lower than 1 ng/ml (600 ± 200 pg/ml) and increased during the morning of proestrus to variable levels which ranged between 6.2 ± 2.3 ng/ml at 0800 hr and 7.9 ± 2.8 ng/ml at 1000 hr to 2.7 ± 0.6 ng/ml at 1200 hr. Once serum LH started to increase, there was a much more pronounced elevation in serum P ($P < 0.01$) with maximal levels (56 ± 7 ng/ml) being observed at 1800 hr, i.e., at a time subsequent to that in which peak LH levels occurred.

T. Serum T exhibited a biphasic pattern on the day of first proestrus. Levels were greater ($P < 0.01$) at 0800 hr of proestrus than in prepubertal rats (334 ± 29 vs 123 ± 31 pg/ml). Thereafter, they declined to reach a nadir (162 ± 19 pg/ml) at noontime, and increased again (459 ± 66 pg/ml, $P < 0.001$) concomitant with the initiation of the LH surge. This elevation, however, was brief with values starting to decrease at the time of the peak of the LH surge.

DHT. Serum DHT titers did not vary throughout the day of the first proestrus nor were they different from those of prepubertal animals. In all cases DHT values fluctuated around 10 pg/ml.

Changes in ovarian weight during the first proestrus. The ovaries of proestrus rats almost doubled their weight in 10 hr (from 0800 to 1800 hr) with the steeper increase being observed at the time of the LH surge ($P < 0.01$). Interestingly enough, ovarian weight had already begun to increase before any change in mean basal LH levels could be discerned (from 0800 to 1200 hr, $P = 0.05$).

Discussion. These results confirm and extend those of Meijs-Roelofs *et al.* (2), Parker and Mahesh (4), Osman (13), and ourselves (1, 3). In addition, they complement the extensive study by Knudsen *et al.* (14) dealing with the morphological and hormonal changes occurring on the days preceding the first proestrus. The present, more detailed characterization of some of the hormonal events that occur during the first proestrus in female rats provides further support for the view that the onset of puberty in this species is ultimately a consequence of the maturation and expression of the positive feedback of estrogen on LH release (2, 15). In close resemblance to adult and to PMS-primed immature rats (16–18), proestrus E_2 levels remain elevated until maximal LH values are reached, and decrease rapidly thereafter. Of greater interest, however, is the observation that throughout the morning of proestrus, E_2 levels are elevated even though serum LH values are low. Indeed, the presence of low LH levels during the morning of proestrus has been observed in earlier experiments involving single (1) or multiple (2) morning blood samples.

The patterns of serum E_2 and LH during the hours preceding the first preovulatory LH surge raise the question as to how the ovary is activated for the first time to produce a preovulatory elevation in serum estrogen levels. It is possible that subtle sporadic changes in pulsatile LH release occur during the late prepubertal period which may activate the ovary. However, we have presented evidence that marked changes in ovarian steroidal responsiveness to gonadotropin precede the first gonadotropin surge (3) and that pharmacologically induced hyperprolactinemia advances puberty by enhancing ovarian responsiveness to gonadotropins (11). The finding that the ovarian weight "spurt" seen during first proestrus (13) is initiated at a time when LH levels are still basal, further supports the importance of changes in ovarian responsiveness to gonadotropins for the onset of puberty. Recently, Uilenbroek and Richards (19) reported that during the estrous cycle of the adult rat there is an increase in follicular estradiol responsiveness to LH from diestrus to proestrus and that this enhanced response correlates well with an increase in LH receptors and occurs in the presence of low, unchanging serum LH levels.

Thus, it would not be unreasonable to conclude that unless the radioimmunoassayable LH levels detected in pre- and peripubertal rats do not accurately reflect the serum levels and/or the biological activity of all the circulating LH, no elevation in mean basal gonadotropin levels precedes the first LH surge. Therefore, a change in responsiveness of the hypothalamic-pituitary unit to steroidal negative feedback may not be the primary factor responsible for the initiation of puberty. In fact, we recently have found (20) that the effectiveness of E_2 in suppressing the postcastration elevation in LH and FSH decreases abruptly only after the first preovulatory LH surge occurs.

Prior to the LH surge, serum P levels become maximally elevated during the day(s) immediately preceding the first proestrus (3). At this time uterine weight, accumulation of uterine fluid, and serum E_2 levels already are increasing. In the morning of proestrus serum P values, though greater than in prepubertal rats, are much more variable and decline prior to the serum rise of this steroid which is evoked by the LH surge. A similar finding has been reported by Meijs-Roelofs *et al.* (2). This tendency of P to decrease at a time in which E_2 levels are maximally elevated might be due to the intraovarian inhibitory effect of E_2 on P release recently reported by Fortune and Hansel (21). Nevertheless, this possibility becomes less likely if it is considered that a major portion of serum P in prepubertal rats may be of adrenal origin (22). On the other hand, the observations that serum P increases as the animal approaches puberty (3, 4) and that P administration can advance the age at which E_2 becomes capable of eliciting an LH surge (23) strongly suggest that P may play a stimulatory role in the process of puberty, possibly by facilitating the expression of E_2 positive feedback on gonadotropin release.

Parker and Mahesh (4) have previously shown that not only serum E_2 and P levels increase before puberty but serum testosterone also rises. The present results indicate that during the morning of proestrus, serum T is lower at 1200 than at 0800 hr, which may represent an increased utilization of T for E_2 synthesis. Moreover, the initiation of the LH surge is accompanied by an increase in T which is short lasting, perhaps due to the enhanced release of P that ensued.

While these changes in serum T appear mainly to reflect the shifts in secretion of E_2 and P which occur during the first proestrus, the lack of a major change in circulating DHT suggests that this steroid may not have relevance in the genesis of the first gonadotropin surge.

Summary. On the day of the first proestrus in the female rat, serum LH levels were very low throughout the morning and increased markedly between 1200 and 1600 hr to reach maximum values at this time. By 1800 hr the LH titers, though still elevated, were already declining. Serum estradiol (E_2) levels were maximally increased at 0800 hr (76 pg/ml) and decreased somewhat between this time and 1600 hr to finally return to prepubertal values by 1800 hr. Although morning progesterone (P) levels were higher than those of prepubertal rats, the greatest increase in serum P occurred in the afternoon of proestrus with a time course that slightly lagged behind that of LH, so that maximal P values (56 ng/ml) were observed by 1800 hr. At 0800 hr on

proestrus serum testosterone (T) was higher than in prepubertal rats, declined thereafter to a nadir at 1200 hr and increased again briefly at the time of the LH surge. Serum dihydrotestosterone (DHT) did not vary throughout the proestrus day and did not differ from levels in prepubertal animals. Ovarian weight increased dramatically between 0800 and 1800 hr, with some increment already being observed at a time in which serum LH was still at basal levels.

The occurrence of elevated E_2 levels, in the absence of any apparent elevation in mean basal radioimmunoassayable LH levels prior to the afternoon LH surge, supports the view that the onset of puberty in the female rat is a consequence of the triggering of E_2 positive feedback on LH release.

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