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Increased Pituitary Sensitivity to Luteinizing Hormone Releasing Hormone at Puberty: An Event of Proestrus[1] (41192)

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Abstract. Groups of rats were studied between the ages of 36 and 43 days. Phenobarbital was injected at noon in order to remove the source of endogenous LH-RH. Beginning at 1400 hr, blood samples were obtained at ½-hr intervals during the 3-hr period when LH-RH was infused at 50 ng/hr through a double-lumen jugular cannula implanted 1–4 days earlier. Vaginal opening occurred at proestrus or estrus on Days 41–43. The peak level of circulating LH following LH-RH was significantly greater at age 42 than at 36, 38, 40, or 43. Responses at other ages were not statistically different from one another. However, five of the seven animals studied at 42 days were in proestrus, and the peak LH response was most significantly correlated with the stage of the estrous cycle: that observed at proestrus was significantly greater than those of diestrous, estrous, or anestrous (prepubertal) animals. These results fail to suggest an essential role of altered pituitary responsiveness in the onset of puberty. Rather, in agreement with the results of previous investigators, they reflect events at the initial proestrus, indicating that cyclic mechanisms have already been set in motion.

Circulating gonadotropin levels in female rats have been found to be high at birth, to exhibit large fluctuations in individual animals between Days 10 and 20, and to remain low from Day 21 until the first ovulation at puberty (1, 2). Initiation of luteinizing hormone (LH) surges may involve a surge of luteinizing hormone releasing hormone (LH-RH) release: Ojeda *et al.* (2) observed a decrease in hypothalamic content of LH-RH, but no increment in plasma levels was apparent. Alternatively, increased LH release at puberty may result from an increase in pituitary responsiveness. However, studies reported to date (3–5) indicate that pituitary responsiveness increases between ages 5 and 15 days but decreases thereafter, remaining low between Days 30 and 40. The only increase in pituitary responsiveness noted near the time of puberty has been associated with the initial proestrus (6). It should be noted that all of these studies have utilized pulse injections of LH-RH. Since both the duration and pattern of exposure to LH-RH may be important (7, 8) the present study was undertaken to define the response of the pituitary gland to a constant rate infusion during the peripubertal period.

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Materials and Methods. Female rats of known age were obtained from our breeding colony. Following weaning one to five animals were housed per cage and provided Purina Rat Chow and water *ad libitum*. The lights were controlled by a timer to provide 14 hr light per day. Times are expressed in terms of the colony schedule with noon at the midpoint of the light period.

One to five days prior to the experiment a double-lumen polyvinyl cannula was inserted via the external jugular vein and positioned in the right atrium of the heart. On the day of the experiment each animal was weighed, and phenobarbital (80 mg/kg, sc) was administered at noon. A dosage of 75 mg/kg administered at 1330 hr has been shown to eliminate or severely depress endogenous LH-RH release as judged by the blockade of the proestrous LH surge during the entire afternoon and evening (9). Blood samples (0.4 ml) were collected at ½-hr intervals from 1400 hr until 1700 hr through

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one lumen of the cannula. A Harvard infusion pump (Model 975) was used to deliver 1 ml/hr LH-RH (50 ng Beckman LH-RH/ml) or the heparinized saline vehicle through the second cannula lumen beginning immediately after the initial blood sample (1400 hr). Following the separation of plasma from each sample, the red cells were resuspended in saline and returned to the rat. At the conclusion of the experiment a vaginal smear was taken, the uteri were weighed, and the oviducts were searched for the presence of ova. The stage of the estrous cycle was judged on the basis of vaginal cytology and the presence or absence of ovulation. Groups of three to seven rats were studied at ages between 36 and 43 days. Undiluted plasma from each

TABLE I. DATA FOR INDIVIDUAL ANIMALS GROUPED BY AGE

Rat	Age	Vag. opn.[a]	Ut. wt[b] (mg/100 g)	Cycle[c]	Ova[d]	LH peak (ng/ml)
1	36	C	67.7	An		19.7
2	36	C	51.3	An		32.3
3	36	C	49.2	An		151.5
4	36	C	58.3	An		91.1
5	36	C	64.8	An		149.2
6	36	C	113.4	An		230.4
7	37	C	83.5	An		693.5
8	37	C	65.1	An		218.2
9	37	C	124.8	An		567.3
10	37	C	106.6	An		293.3
11	38	C	184.9	An		175.5
12	38	C	73.2	An		81.0
13	38	C	78.8	An		85.0
14	38	O	101.6	An		12.8
15	38	C	74.5	An		346.6
16	40	C	94.9	An		84.3
17	40	C	233.1	An		201.8
18	40	C	152.7	An		38.8
19	41	C	52.5	An		149.5
20	41	C	94.2	An		206.6
21	41	C	190.7	Pro		1801.5
22	41	O	100.6	Est	+	6.0
23	41	O	135.1	Est	+	13.3
24	41	O	106.5	Di		15.6
25	42	O	234.4	Est	+	8.4
26	42	C	162.1	Pro		1041.8
27	42	O	70.2	Pro		499.7
28	42	O	195.8	Pro		542.8
29	42	C	200.2	Pro		913.2
30	42	O	208.9	Pro		1928.2
31	42	C	79.9	Di		678.3
32	43	O	129.1	Est	+	46.4
33	43	O	84.4	Di		46.0
34	43	O	125.2	Di		48.6
35	43	O	152.8	Di		81.7
36	43	O	175.9	Est	+	2.8
37	43	O	260.1	Pro		377.2

[a] Vagina: C = closed; O = open.
[b] Ut wt: uterine weight in mg per 100 g body wt.
[c] Cycle classification: An = anestrus; Pro = proestrus; Est = estrus; Di = diestrus.
[d] Presence of ova in oviducts at the conclusion of the infusion indicated the occurrence of a proestrous LH surge on the previous afternoon.

blood sample was measured in duplicate (except for the 1400-hr sample which required more plasma in a single determination), and all samples were included in a single double antibody radioimmunoassay performed by the method of Monroe *et al.* (10). Plasma samples from saline-infused animals were estimated in a second assay. Since it was unnecessary to make comparisons of results in the two assays the consequences of interassay variability of 17–21% (B/BO 20, 50, or 80%) were avoided. Intra-assay variability was found to be 3.75%. Group mean values of peak LH concentration and area under the LH curve were compared using Duncan's multiple range test (11).

Results. The results for individual animals are presented in Table I. Vaginal opening occurred primarily on Days 41–43, and represented either the day of proestrus or estrus. LH remained at basal levels in all samples collected from animals infused with the saline vehicle. This group of 14 animals ranged from 39 to 42 days of age and included six proestrous, four estrous, one diestrous, and three anestrous rats. In contrast, infusion of LH-RH was followed by increased levels of circulating LH which were greatest in 42-day-old rats. Uterine weight also varied with age: uteri on Day 42 were significantly heavier than those on Days 36–38 ($P < 0.05$). In addition, the stage of the estrous cycle varied with age, ranging from anestrus in the younger rats through the cycle at puberty. Uterine weight at proestrus (184 ± 22 mg/100 g body wt, mean ± SE; $n = 7$) was significantly greater than that at either diestrus (110 ± 13 mg/100 g, $n = 5$; $P < 0.013$) or anestrus (96 ± 11 mg/100 g, $n = 20$, $P < 0.0002$), but was not different from that at estrus (155 ± 23 mg/100 g, $n = 5$). Thus it was not unexpected that an analysis of covariance indicated that both the height of the LH peak and the area under the LH-versus-time curve following a 3-hr infusion of LH-RH were most significantly related to the stage of the estrous cycle and to uterine weight. The response profiles calculated by stage of the cycle are shown in Fig. 1.

Discussion. In the present experiment the data were gathered in terms of age, and were analyzed to account for other factors. No age-related trend or progressive change in pituitary responsiveness was observed

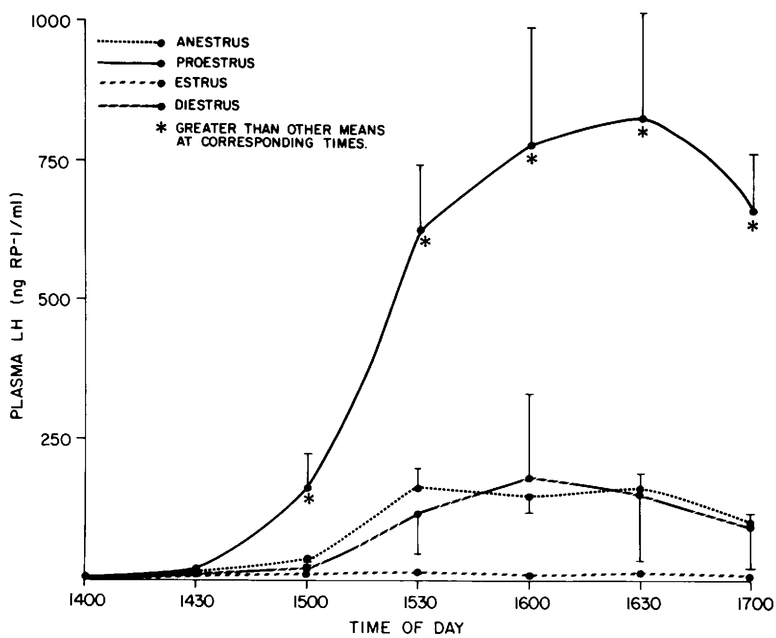

FIG. 1. Time-sequence profiles of the mean LH response for each stage of the initial estrous cycle. The 3-hr infusion of LH-RH (50 ng/hr) began immediately after the 1400-hr sample.

over the range of ages studied. In contrast, the only significantly different response observed, that on Day 42, can be accounted for by the fact that five of the seven animals studied at this age were in proestrus. Indeed, the effect of the stage of the estrous cycle on the peak LH response remained even after adjustment for age and uterine weight. However, the dramatic rise in LH levels observed in proestrous animals was not due to incomplete blockade of endogenous LH-RH release since no change in LH occurred in proestrous animals infused with saline alone. These results obtained using a 3-hr constant rate infusion of LH-RH are in agreement with those reported by Castro-Vazquez and Ojeda (6) that the greatest response to a pulse injection of LH-RH occurred on the afternoon of proestrus. The rapidly accelerating rate of release of LH between 1430 and 1530 hr in proestrous animals is indicative of the priming effect observed by these same authors (6) in which exposure to LH-RH increases the responsiveness of the pituitary gland to subsequent stimulation (12). The present results show no systematic changes in pituitary responsiveness over the week prior to vaginal opening suggestive of a mechanism triggering the onset of puberty. Thus these results are consistent with a mechanism involving increased sensitivity of the central nervous system to the positive feedback of estrogen and augmented ability of the hypothalamus to release LH-RH as suggested by Andrews and Ojeda (13). In this regard, both pulse injection and constant rate infusion of LH-RH lead to the same conclusion.

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