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Delayed Puberty After Immunological Blockade of Endogenous LH-RH in Female Rats (40402)

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As shown by electrolytic lesion experiments the timing of the onset of puberty (1) and the regulation of gonadotropin secretion in the adult rat depends upon normal hypothalamic function (1, 2). The value of passive immunization as a means of studying the physiologic regulation of gonadal function in the female rat has been demonstrated by studies in which the administration of LH-RH antiserum into proestrus rats prevented the preovulatory surge of both LH and FSH and blocked ovulation (3, 4). Other recent studies utilizing passive immunization with antiserum to LH-RH (LH-RH-AS) have also shown the importance of LH-RH for the regulation of gonadotropin secretion in adults (5–7). Less is known about the role of the hypothalamus in prepubertal development. We have previously demonstrated that passive immunization with LH-RH-AS administered during the first 5 days of life in the male rat leads to decreased prepubertal growth of the testes persisting into adult life, and diminished fertility (8). In this experiment we sought to determine whether immunoneutralization of LH-RH activity at a critical developmental period in the female rat would exert a prolonged effect on reproductive function in the female adult as it does in the male.

Materials and methods. Sprague-Dawley CD Rats (Charles River Laboratories, Wilmington, Massachusetts) were housed in temperature-controlled rooms (25 ± 1°). The animals were kept in plastic cages and maintained on a 14h (light)/10h (dark) cycle with food (Agway Company RMH 3000 rat chow) and tap water available *ad libitum*. Newborn rats were kept with their mother in a single cage until weaned on the 25th day of life. At that age, the sexes were separated and females of the same age caged together (four animals/cage).

LH-RH-AS was generated in rabbits and characterized as described previously (8). In order to determine whether there was a "critical period for sexual development, a single injection of LH-RH-AS (0.25 ml s.c.) was administered to groups of rats (4–12 per group) on day 1, 3, 5 or 10 of life. Littermate controls were given an equal volume of normal rabbit serum (NRS) at comparable periods. Animals were examined daily after 25 days of age to detect vaginal opening, a finding that correlates with the increased estrogen secretion at the onset of puberty in the female rat (9).

In an attempt to maintain prolonged blockade of endogenous LH-RH secretion, a group of four animals was treated twice weekly with 0.25 ml ip injections of LH-RH-AS starting on day 1 and continuing through day 44. Control animals (4) received NRS. Another group of five animals was treated twice weekly with 0.25 ml ip injections of LHRH-AS until the day of vaginal opening. Six control animals were injected with NRS at the same time intervals. Fertility was evaluated in the females treated twice weekly by placing each female with a proven normal fertile male for 15 days. This study began at 96 days of age for all these females, and if they were not pregnant after 15 days, they were exposed to another fertile male for an additional 15 days.

Results. A single injection of LH-RH-AS failed to delay vaginal opening when admin-

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istered on day 1, 3 or 5 of life. However, when given on the 10th day of life, there was a delay of 3.9 days (38.2 ± 1.4 vs. 34.3 ± 0.4 days, $P < 0.02$). When LH-RH-AS was administered twice weekly until the age of 44 days (Group I), vaginal opening was strikingly delayed (74.0 ± 3.2 vs. 38.5 ± 0.5 days, $P < 0.001$) (Table I). With this dosage regimen it was not possible, however, to postpone puberty indefinitely. Continued twice weekly injections failed to result in a greater delay of vaginal opening. Whether animals were injected once or repeatedly, there was no difference between any treated group and its control group with respect to body weight suggesting that general health was unaltered. Animals in Group I, treated weekly until day 44 and studied after 96 days of age, achieved pregnancy during the first exposure to a fertile male. However, in Group II, animals which were treated with LH-RH-AS until vaginal opening occurred, one half (three out of six animals) required a second or third exposure to a fertile male before pregnancy was achieved. The litter size was similar in all groups (Group I, 12.8 ± 1.6 vs 13.0 ± 2.8 pups; Group II, 10.0 ± 1.1 vs 12.0 ± 1.5, treatment vs control).

Discussion. The effect of antiserum to LH-RH in a dose of 1.0 ml of antiserum was shown to depress serum testosterone for 4 days in adult male animals (8). Therefore, in these much smaller animals, twice weekly injections of 0.25 ml of LH-RH-AS should have been sufficient to neutralize LH-RH, as long as treatment was continued. It was observed indeed, that vaginal opening in the treated pups was markedly delayed thus indicating the importance of LH-RH for pubertal development. A single injection of LH-RH-AS given at 10 days of age delayed puberty by almost 4 days in comparison to animals treated at an earlier age when no delay occurred. These data indicate that LH-RH secretion prior to day 10 has little effect on sexual maturation, and that a critical period for development begins on or around the 10th day of life.

TABLE I.[a]

Group	Treatment	Day of vaginal opening (mean ± SE*)
I	LH-RH-AS ($n = 4$)	74.0 ± 3.2
I	NRS ($n = 4$)	38.5 ± 0.5
P value		<0.001
II	LH-RH-AS ($n = 6$)	67.2 ± 7.6
II	NRS ($n = 5$)	35.2 ± 0.5
P value		<0.01

[a] Day of vaginal opening of female rats treated twice weekly from the first day of life with 0.25 ml ip of luteinizing hormone releasing hormone antiserum (LH-RH-AS) or normal rabbit serum (NRS). Group I was treated until 44 days of age only and Group II was treated until vaginal opening occurred.

* SE (standard error of mean). Group comparisons were analyzed using unpaired *t* tests.

It is not possible to determine whether the delay of puberty by administration of LH-RH-AS was due to a temporary decrease in the secretion of FSH or LH because the levels of both fall after giving LH-RH-AS (4, 5). Ultimate fertility was not impaired in animals treated until day 44 suggesting complete recovery from the effects of antiserum prior to age 96 days. Possibly, recovery was delayed in the animals treated until vaginal opening took place because one half of the animals required a second or third exposure to a fertile male before becoming pregnant. Nonetheless, in both groups of animals treated with LH-RH-AS for an extended period the reproductive capability suggests restoration of a functioning hypothalamic-pituitary-ovarian axis. This is in contrast to our previous observations that a single neonatal dose of the same LH-RH-AS during neonatal life in the male rat caused permanent decrease in fertility (8) and impaired hypothalamic–pituitary gonadotropin function (10).

Vaginal opening occurred in spite of continued LH-RH-AS administration. This observation, in addition to the finding of normal fertility, suggests that the hypothalamic-pituitary-gonad axis of the female is more resistant to immunologic blockade than that of the male. It is possible that antibodies to rabbit LH-RH antiserum formed in the rats, and thus made the LH-RH-AS no longer effective in blocking endogenous LH-RH.

These studies indicate that the normal timing of pubertal development in the female rat depends on the hypothalamic secretion of LH-RH from an early age. However, in contrast to male rats which suffer persisting hypothalamic-pituitary-gonadal abnormalities after transient LH-RH blockade during the neonatal period (8, 10), female rats treated

prepubertally with LH-RH-AS eventually achieved fertility. However, the fact that a second or third exposure to a male was required to achieve pregnancy suggests that there may have been a persistant alteration in the function of the hypothalamic–pituitary–ovarian axis. Further studies are required to determine the nature of these abnormalities.

Summary. In order to evaluate the role of luteninizing hormone releasing hormone (LH-RH) secretion in the early postnatal development of gonadal function in the female rat, groups of animals received a single injection of a highly potent LH-RH antiserum (LH-RH-AS) on day 1, 3, 5 or 10 days of life or were given injections twice weekly from day 1 until the time of vaginal opening. Control rats were given normal rabbit serum (NRS). Vaginal opening was delayed 4 days in animals treated with a single injection of LH-RH-AS on day 10 of life compared to littermate controls. In animals treated twice weekly, vaginal opening was delayed more than 32 days. These studies indicate that the normal timing of pubertal development in the female rat requires LH-RH from an early age.

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