Serum LH, FSH, Prolactin and Progesterone from Birth to Puberty in Female and Male Rats

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ABSTRACT. Serum LH, FSH, prolactin and progesterone were determined in male and female prepuberal rats. Neonates of both sexes had high LH and FSH levels, the values in the females being higher than those in the males. Serum prolactin and progesterone were low in neonatal rats. A curious LH secretion pattern occurred in female rats between days 10 and 20; high LH levels were observed in 10–15% of the rats during this time, whereas the male rats showed only minor fluctuations. At the same time serum FSH was elevated in both sexes. Again, the values in the females were higher than in the males. Serum prolactin was still low, whereas progesterone rose slightly in both sexes. Serum LH and FSH remained low between days 21 and the immediate peripuberal period (i.e., days 37–45) in males and females, and serum prolactin rose steadily during this period. Progesterone also increased following the prolactin pattern. All hormones (LH, FSH, prolactin and progesterone) were increased during the peripuberal period with cyclic fluctuations in the females. The close relation of prolactin and progesterone secretion in the immature rat is discussed. (Endocrinology 94: 1003, 1974)

THE understanding of sexual development and maturation requires exact information concerning the time course of gonadotropic and steroid hormone secretion. The well-known masculinizing effect of perinatally injected testosterone (1) into female rats indicates that sex-dependent hormonal fluctuations occur in newborn rats. Androgen treatment of female rats up to the sixth day of life does not affect sexual development but it prevents normal estrous cycle activity (2), i.e., the animals are constantly estrous (for review see 1). These observations suggest again sex-specific developmental and possibly hormonal changes during early postnatal life in rats. The development of highly sensitive radioimmunoassays allowed measurements of gonadotropins and prolactin in small blood samples (3,4,5). Few observations are available about hormonal changes in prepuberal rats and most authors did not cover the interesting period of early postnatal life (6–10). We therefore studied the gonadotropin, prolactin and progesterone profiles of maturing rats from the day of birth until puberty.

Materials and Methods

The Sprague-Dawley rats used in these experiments in our own air-conditioned and light controlled (light period from 5 AM–7 PM) animal rooms. The litters were kept with their mothers until weaning on the 25th day. Groups of rats were separated according to their sex and killed by decapitation. More than 1500 pups were used in this study. Blood samples were pooled (3–8 rats/pool) at 1,2,4,6,8,10,11 and 12 days of age). Later blood was taken individually from each rat after decapitation. All blood samples were collected between 4 and 5 PM. Thus, all four hormones could be measured in most samples and 5–12 values per sex and day were used for statistical analysis. The sera were kept frozen and assayed in one assay for each hormone to avoid interassay variance. All hormones were measured by radioimmunoassay. The assay kits and the procedure for determination of the proteohormones were the same as described by others (3–5). LH and prolactin were determined in 50 and 100 ul, FSH in 200 ul of serum. The values of these hormones are expressed in terms of NIAMD-RP-1. The anti-progesterone was an anti-progesterone-11-BSA which significantly crossreacts with other steroids as follows: 11-oxy-progesterone, 35%; 11-β-hydroxy-progesterone, 1.4%; 5α-pregnan-3β-ol, 20-one, 4.7%. The equivalent of 200 ul petrol benzene (40–60 bp) extracted serum was assayed using the same method as described by Thorneycroft and Stone (11). The petrol benzene extraction achieved a reproducible recovery of 60–70% after evaporation of the organic solvent and resolution in 0.1% gelatin phosphate buffer. Water
FIG. 1. Serum LH levels in immature male and female rats. Note the higher values on days 1 and 2 in female newborn rats, although serum LH was increased in the males, too. Sporadic LH peaks are observed in female rats between days 10 and 20, whereas the males show only minor fluctuations. Preovulatory LH levels are seen on day 37. Day of vaginal opening = E. The following days of the estrous cycle are M, D, and P. The peripuberal time mark for male rats does not correspond to that of the females. Standard error of mean on top of each bar.

blanks were run with each assay; they rarely exceeded 10 pg, which was the lowest amount of progesterone detectable with this assay.

Results

High serum LH levels (Fig. 1) were found in both female and male newborn rats, the values being significantly higher in the females (p < 0.01). From the 3rd day to day 33 the levels of serum LH in the male rats showed only minor fluctuations. Between days 37 and 43 LH was significantly increased (p < 0.01) before falling to normal levels for adult male rats. This period is defined as the peripuberal time. Serum LH in the females similarly decreased after the postnatal elevation. Between the 10th and 20th day of life a curious LH pattern occurred in these female rats. Extremely high LH levels were found in some sera of individual rats, whereas others of the same age had low LH values. LH peaks were not found in all of the rats that had been decapitated between days 10 and 20, but they occurred randomly in approximately 10–15% of the rats of this age. From days 20 to 33 serum LH remained low in the female rats. In order to avoid complicated graphs the last blood samples from animals with closed vaginas were taken on day 37. Most rats had LH peaks on day 37 and only few rats had elevated LH levels on the day of vaginal opening, which was day 38 or 39.

Serum FSH levels in newborn rats (Fig. 2) were elevated for the first 2 days. They then fell for the next week; the levels in the females, however, remained higher than those in the males. Between days 10 and 21 the serum FSH values increased and then fell to almost undetectable levels until day 33. The FSH levels in the peripuberal male rats were again higher than in adult male rats (p < 0.01). Day 37 and the day of vaginal opening in the females were characterized by high FSH values. On the following days the rats displayed normal cyclic activity.

Serum prolactin levels in female and male newborn rats were uniformly low for the first three weeks (Fig. 3). Later the levels gradually increased in both sexes to peak values between days 37 and 45 (p < 0.001) with typical cyclic fluctuation in the females.

The serum progesterone values in newborn rats were low (Fig. 4), in both males and females and remained low for the first 10 days of life. The progesterone levels then increased slowly during the next 10 days in both sexes without any dramatic fluctuations. During the third and fourth week of life the progesterone values increased progressively in both sexes (p < 0.001). The levels in the males then dropped soon to the normal levels of adult male rats.

Between days 10 and 20 serum values of LH and FSH did not correlate with each
other, nor did they correlate with serum progesterone. It was never observed that progesterone levels were increased when one or both of the gonadotropins were elevated. However, a tendency for high serum prolactin levels to be accompanied by increased progesterone levels was observed.

**Discussion**

The present study shows that newborn rats have high serum LH and FSH levels during the first 2 days of life, the serum value being higher in the females than in the males. No information about metabolic clearance rates of gonadotropins in newborn rats is available. The half-life of LH in adult rats is approximately 23 min (12). If one assumes a similar \( t_{1/2} \) in newborn rats, the elevated LH levels in these animals cannot be of maternal origin. This speculation is relevant since pregnant rats ovulate shortly after delivery and have high gonadotropin levels during delivery (13).

Serum prolactin and progesterone levels
are low in newborn rats, and serum testosterone values in male pups have been reported to be high at this stage of postnatal life (14). Goldman et al. (8) also reported high LH and FSH levels at this time and it can be assumed that high gonadotropin levels cause the increased testosterone levels (14) in the males and thus imprint the male hypothalamus. It also has been shown that suppression of testosterone during the first days of life by the use of antibodies (15,16) or by prenatal administration of antiandrogens (17) severely interferes with sexual development. To the authors' knowledge no information is yet available about estrogen levels in newborn female rats. Serum progesterone and prolactin do not seem to be of any importance during this stage of sexual development; their levels were uniformly low during the first 10 days and 3 weeks, respectively.

Between days 10 and 20 the hypothalamus of both male and female rats seems to reach a certain maturity. Extremely high LH levels were observed in about 10–15% of the young female rats decapitated at this age. This observation could indicate that high LH levels are events, which may happen only once in an individual animal of this age. Episodic LH fluctuations in female animals at a certain stage of development have also been observed in immature female cows and were considered to reflect "training" of the hypothalamus for future cyclic activity (18). Another explanation could be a dissociation between hypothalamic, adenohypophyseal and ovarian maturation. A possible lack of ovarian hormonal feedback in female rats between days 10 and 20 may result in pulsatile pituitary LH secretion. Thus, the explanation of Bhattacharya et al. (20) for pulsatile LH secretion in ovariectomized monkeys (21) could possibly be applied to these maturing female rats. Ojeda and Ramirez (7) and Goldman et al. (8) demonstrated generally increased LH levels between days 10 and 20 in female rats. This difference could result from the use of pooled blood samples and too long intersample intervals. Goldman and Girski (19) also observed a large degree of variability in serum LH levels of intact immature female rats of this age. But as these authors stated, their number of animals was too small to draw any firm conclusions. Ojeda and Ramirez (7) observed also increased serum LH levels in 10-day-old male rats. Crim and Geschwind (22) also observed occasionally high LH levels in prepuberal rams at a certain stage.
of development. We observed no marked increases in serum LH levels in any of our male rats. We only found slightly and inconsistently elevated FSH levels in male rats between days 10 and 20. Highly increased FSH levels in female rats between day 10 and day 20 are consistent with observations by other authors (7–9).

The ovaries of young female rats may not yet respond with increased progestagen secretion to the LH-FSH stimulus at this time since serum progesterone rose only slightly while serum LH and FSH levels were high. It is possible that the ovaries are not the only source of this slowly increasing serum progesterone level. A first indication is given by the fact that progesterone levels measured in individual rats do not correlate with increased LH or FSH levels or both. Further experiments with adrenalectomized immature rats are necessary to clarify this point.

The 2 weeks following the first 20 days of life are characterized by very low gonadotropin levels. At this time serum prolactin levels rose gradually first in the females and then in the males to reach peak levels in both sexes on day 37. Our observation in female rats is generally in agreement with results reported by Voogt et al. (23). The steadily increasing serum prolactin levels in males and females between days 20 and 40 are also accompanied by steadily increasing serum progesterone levels. We are not implying that these increased serum progesterone values are due to the increasing prolactin levels, but the temporal coincidence is worth noting. The source of this increased serum progesterone remains unknown.

It is difficult to draw firm conclusions upon sexual development on the basis of the results presented in this paper. The high gonadotropin levels in the newborn rats seem to be responsible for high circulating testosterone levels in the male rat as reported by Resko et al. (14).

Whether the gonadotropins stimulate estrogen secretion in the neonatal females is not known; however, they do not stimulate progesterone secretion at this age. The occurrence of high LH levels in female rats between days 10 and 20 coincides well with the time when testosterone administration no longer affects the so-called “cyclic center” in the female rat brain. This can be interpreted as a sign of maturity of the hypothalamus. The increased gonadotropin levels prior to puberty may be necessary for increased estrogen or testosterone release. The progesterone levels in the immature male and female rat do not follow this gonadotropin pattern. If one of the pituitary hormones is involved with increasing release of progesterone in the premature male and female rat, it could be prolactin. Further experiments, possibly with simultaneous measurements of estrogens and androgens, are necessary to fully understand these mechanisms.

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References


