Peripheral plasma concentrations of LH, FSH, prolactin and GH from birth to puberty in male and female mice

Sandra D. Michael, S. B. Kaplan and B. T. Macmillan

Department of Biological Sciences, State University of New York, Binghamton, New York 13901, U.S.A.

Summary. Circulating concentrations of plasma LH, FSH, prolactin and GH in male and female mice were measured twice daily (12:00 and 24:00 h) by radioimmunoassay from birth to puberty. FSH concentrations in females were higher than in males from birth to about 16 days of age, then values in males rose while those in females fell. Prolactin levels in both sexes were low then increased slightly towards puberty with midnight levels being higher in the males and lower in the females. LH concentrations were relatively low in both sexes until Days 7–8 of age then showed distinct fluctuations. The fluctuations of GH in both sexes seemed to correspond to fluctuations in LH.

Introduction

The laboratory mouse is widely used for studies of cancer, genetics, immunology and reproductive physiology, but the hormonal changes during maturation are not fully understood. While some data are available on the levels of gonadotrophins (Stiff, Bronson & Stetson, 1974; Dullaart, Kent & Ryle, 1975; Selmanoff, Goldman & Ginsburg, 1977), prolactin (Sinha, Selby, Lewis & Vanderlaan, 1972a; Barkley, 1979) and growth hormone (Sinha et al., 1972b) during the prepubertal period in mice, the observations are not as detailed as for some other species. One reason for this lack of information is the small size of mice, which necessitates very large numbers of animals to obtain enough plasma for hormone analysis. We have now studied the concentrations of these hormones in mice from birth to puberty.

Materials and Methods

Animals. Mice of a random-bred stock derived from a four-way cross, Line C (Bradford, 1968), were utilized in this study. The animals were maintained in a room at 24°C with lights on from 05:00 to 19:00 h so that noon (12:00 h) and midnight (24:00 h) were the midpoints of the light and dark cycles respectively.

The mice were housed in stainless-steel cages and food and water were available ad libitum. Females were isolated when visibly pregnant and checked daily at 24:00 h. Animals which had not given birth by this time but had done so when checked at 08:00–10:00 h were used for the study with the day of birth being designated as Day 0 of age. The mice were weaned at 21 days of age and separated by sex.

After killing by decapitation, at 12:00–13:00 h or 24:00–01:00 h (in dim red light), trunk blood was collected over heparinized funnels and centrifuged. The plasma was stored at −30°C until assayed. Animals were killed twice daily from Day 0 to Day 22 and thereafter on Days 25.
30 and 35 for males, and 25, 30 and 34–36 for females. Females sampled on Days 34–36 were only those which had exhibited vaginal opening. The majority of animals had a dioestrous vaginal smear on the day of vaginal opening and only these animals were used. Male mice of this colony were fertile by 35 days of age as judged by their ability to sire litters.

Because of the small amounts of blood obtained, the samples had to be pooled at each sampling time. Each pool consisted of plasma from approximately 110 animals at Day 0 reducing to about 15 animals after Day 21. Plasma from a total of 1191 females and 1278 males was collected.

Radioimmunoassays

**LH and FSH.** LH concentrations were measured by the double-antibody ovine-ovine LH assay of Niswender, Midgley, Monroe & Reichert (1968), as verified for the mouse (Bronson & Stetson, 1973). FSH concentrations were determined with the NIAMDD-rat-FSH assay kit which has previously been verified for use in the mouse (Beamer, Murr & Geschwind, 1972). Crude freeze-dried mouse pituitary standards provided by Dr A. F. Parlow were tested for parallelism with the respective NIAMDD-rat-RP1 standards. Analysis of covariance showed that slopes were not significantly different for the two LH extracts (\( P > 0.80 \)) or for the two FSH extracts (\( P > 0.85 \)). Determinations of LH and FSH were each made on duplicate 100 μl aliquots of plasma. The coefficient of variation and the sensitivity were 4.7% and 2 ng/ml for LH, and 5.8% and 10 ng/ml for FSH. Values for the two assays were expressed in terms of the respective NIAMDD-RP1 reference standard.

**Prolactin and GH.** These were measured by homologous assays using the materials and protocol of Sinha et al. (1972a, b). For both hormones, determinations were made on duplicate 20 μl aliquots of plasma. The coefficient of variation and the sensitivity were 2.1% and 2 ng/ml for prolactin, and 3.4% and 2 ng/ml for GH. The biological potencies of the mouse standards are 25 i.u./mg for prolactin, as measured by the pigeon crop-sac assay, and 3.1 USP units/mg for GH, as measured by the rat tibia test. Values for each hormone were expressed as ng of the respective standard. All samples were run in one assay for each of the 4 hormones and routine dilutions yielded parallel curves.

The small size of the mice necessitated very large numbers of animals to yield even one pool of plasma of adequate volume. We therefore examined whether the hormone concentration in a pool was representative of the mean of the individual samples contributing to that pool. In 3 separate preliminary assays for each hormone, we found that the value for a pool did fall within the standard error of the mean of the separate determinations of samples from individual animals and, therefore, the data presented below are representative of the individuals comprising each pool.

For some hormones mean values of several days were compared using Student’s \( t \) test and were considered significantly different if \( P < 0.05 \).

**Results**

**FSH.** The hormone concentrations in male and female mice between birth and puberty are shown in Text-fig. 1. For each sex, combined noon versus midnight FSH values were not significantly different. Both 12:00 and 24:00 h levels were considerably higher in females than in males between Days 0 and 15 (\( P < 0.001 \)) while the reverse was true after Day 16 (\( P < 0.001 \)). In the females, the combined noon and midnight values from Days 0 to 15 were significantly higher than values after Day 16 (\( P < 0.001 \)), while in the males Day 0–15 levels were considerably lower than after Day 16 (\( P < 0.001 \)). In the females there was a pronounced noontime peak on Day 12.
**Text-fig. 1.** Plasma concentrations of LH, FSH, prolactin and GH at 12:00 h (---) and 24:00 h (-----) in maturing male (△) and female (○) mice. VO = day of vaginal opening. Symbols for LH values in the males between Days 1 and 7 have been omitted because of space.
LH. Plasma values were relatively low in both males and females until Days 7–8. Thereafter, in the females midnight levels of LH showed a fairly regular 2-day rhythm, with the greatest surge occurring at midnight on Day 12. There was no consistent pattern of LH surges in the male, and the surges ended after Day 18.

GH. Concentrations were similar in both sexes and showed irregular fluctuations. Many of the GH peaks seemed to correlate with peaks of LH. In the females, for example, of the 6 midnight LH surges which occurred at 2-day intervals between Days 10 and 20, 4 correlated with GH peaks ($P < 0.05$, $\chi^2$ analysis).

Prolactin. In both sexes prolactin values were uniformly low with only a slight increase towards the time of puberty. There were no marked fluctuations but combined midnight levels were higher than noon levels in the males ($P < 0.001$) and lower in the females ($P < 0.003$).

**Discussion**

Several recent reviews, including those of Odell & Swerdloff (1976) and Ramaley (1979), discuss the aetiology of sexual maturation in male and female rats. Since the general patterns of plasma levels of pituitary hormones are similar for rats and mice, this discussion is therefore mainly confined to comparisons with other studies of mice. To our knowledge there have been no other such detailed studies of mice before puberty.

In the two strains of mice (DBA/1/Bg and C57BL/10/Bg) studied by Selmanoff et al. (1977), the FSH patterns in males were similar to that found in the present study in that FSH levels increased towards puberty. We did not observe the single LH peak reported by Selmanoff et al. (1977) for Day 30, but several peaks were detected before Day 30, perhaps because of our more frequent sampling routine. The attainment of reproductive capacity was considered to be between Days 39 and 44 by Selmanoff et al. (1977) compared with Day 35 in the present study, but in both studies LH concentrations declined before this time. The increased concentrations of LH and FSH are presumably related to the testicular stimulation required to initiate androgen secretion and the onset of spermatogenesis.

The gradual increase in prolactin levels as the male mice approached puberty is similar to that reported by Barkley (1979). The temporal difference observed in males and females for the samples taken at noon and midnight has also been found by Sinha, Salocks, Wickes & Vanderlaan (1977). In genetically prolactin-deficient mice, treatment with prolactin induces fertility and stimulates growth of the testes, testosterone production and spermatogenesis (Bartke, Goldman, Bex & Dalterio, 1977). Increased testosterone production at 30 days of age preceded a dramatic increase in prolactin at 35 days of age (Barkley, 1979) which coincided with rapid growth of the accessory organs in mice (Barkley & Goldman, 1977). We did not observe this dramatic increase at any time although our mice were fertile by Day 35.

Sinha et al. (1972b), who combined the data for males and females because there were no apparent differences between the sexes, reported a general decline in concentrations of GH to weaning (Day 20), but we found irregular fluctuations in both sexes after Day 7. Several studies (reviewed by Bartke, Hafez, Bex & Dalterio, 1978) indicate that GH may be of physiological importance during puberty in the male. There is evidence that GH and/or prolactin can act synergistically with gonadal steroids in promoting the growth and maintaining the structure of accessory reproductive organs in rats (Chase, Geschwind & Bern, 1957).

The FSH and LH patterns in the female mice in the present study are similar to those reported by Dullaart et al. (1975). In both studies FSH values increased to peak levels by Day 12–13 then remained basal after about Day 18 throughout the pubertal period. However, in our mice values declined immediately after the peak while Dullaart et al. (1975) found that high levels persisted until Day 18. The LH data of our study and that of Dullaart et al. (1975) are also similar, with an initial peak on Day 7–8 and dramatic fluctuations thereafter. Stiff et al. (1974)
found peak titres of LH and FSH on Day 10, but they sampled only at 5-day intervals. Several studies involving the use of antisera to FSH or LH in the prepubertal mouse show that gonadotrophins are essential for normal ovarian development (Eshkol, Lunenfeld & Peters, 1970; Hardy, Danon, Eshkol & Lunenfeld, 1974; Purandare, Munshi & Rao, 1976). The sexual dimorphism of plasma FSH concentrations found in mice is probably due to the oestrogen-binding protein, alpha-fetoprotein (AFP). Studies with immature rats have shown that AFP limits the biological expression of oestrogen (not androgen) thereby preventing the full negative feedback effect of this steroid on FSH levels, thus leading to higher FSH levels in females than in males during the early juvenile phase (see Meijls-Roelofs & Kramer, 1979).

Although there were large fluctuations throughout the period of study, we observed the highest level of daytime GH concentration at 30 days of age, 5 days before vaginal opening. Ojeda & Jameson (1977) also found increasing titres of GH as juvenile female rats approached puberty. The increases in GH occurred several days before the gonadotrophin release that normally precedes the first ovulation, suggesting that GH may play a role in this process. GH treatment of mice deficient in GH and TSH resulted in ovarian development (Bartke, 1964).

Daytime levels of prolactin gradually rose as the female mice approached puberty. This pattern is also found in rats (Dohler & Wuttke, 1975), and male-induced or oestradiol-induced precocious puberty in female mice is preceded by elevated levels of prolactin (Bronson & Maruniak, 1976). However, prolactin may not be essentially involved in the onset of puberty because there is no prepubertal prolactin surge in adrenalectomized rats and yet corticosterone treatment leads to normal puberty onset (Ramaley & Campbell, 1977).

Many studies of the rat and the studies of Sinha et al. (1977) of the mouse indicate the existence of daily rhythms in the secretion of prolactin and GH with marked differences between males and females. In the present study the fluctuations of GH in both sexes seemed to correspond to fluctuations in LH. We have also observed this correspondence between GH and LH in prepubertal female and male Peromyscus leucopus (unpublished data). We know of no other report in which this relationship has been observed. Dohler & Wuttke (1975) suggested that the pulsatile secretion of LH in female rats between Days 10 and 20 of age was due to the stimulating effect of oestradiol which can be abolished by antiserum to oestradiol (Kronibus & Wuttke, 1977). We are currently studying the possibility that oestradiol is also responsible for the GH pulses which rise and fall concomitantly with the LH pulses during the prepubertal period.

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References


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