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THE ROLE OF TESTICULAR SENSITIVITY TO GONADOTROPINS IN SEXUAL MATURATION OF THE MALE RAT

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SUMMARY

The possible etiologies of sexual maturation have been systematically analyzed in the male rat. The changing patterns of LH and FSH in blood during maturation appear to be explained by changing feedback sensitivity to gonadal steroids and changing response of the pituitary to gonadotropin releasing hormone. The changing responses to GnRH are in turn explained by testosterone modulation at a pituitary level. LH is high in the 10-day-old animal, falls at 20 days, and then steadily increases during maturation. FSH, in contrast, is high at 10 and 20 days and falls progressively as maturation proceeds.

Testicular sensitivity or response to LH progressively increases during maturation and appears to be a major factor in increasing testosterone concentrations. Five days following hypophysectomy, the immature male rat shows little or no testosterone secretion in response to large doses of LH. Responsiveness may be restored by pretreatment with FSH. The magnitude of the response appears to be related to the duration of exposure to, as well as the dose of, FSH. FSH appears to induce responsiveness to LH.

The mechanism of this induction is uncertain. LH receptor populations do not appear to change during FSH exposure.

Thus a major factor in (sexual maturation in the male rat appears to be changing sensitivity of the testis to LH stimulation of testosterone secretion.

Until about ten years ago it was believed by most physicians that sexual maturation in children was caused by a "turn-on", or initiation of, pituitary gonadotropin secretion, with resultant increase in gonadal steroid secretion. In 1964–66, we developed radioimmunoassays for LH and applied these more sensitive gonadotropin assays to detection in prepubertal subjects; it became clear that this hormone was detectable in blood of all children prior to the age of puberty, and that the current hypothesis was incorrect (Odell *et al.*, 1966, 1967)[1, 2]. However, indirect data, from studies in parabiosed *sexually immature rodents* had previously suggested that the pituitary-gonadal axis was active prior to puberty. As early as 1929 (Kallas)[3] it was shown that if two sexually immature females were joined in parabiosis, and one castrated, the other underwent precocious puberty. Furthermore, in 1951, Byrnes and Meyer[4] demonstrated (also using parabiotic sexually immature female rats) that extremely small doses of estrogenic steroids could act in feedback suppression of gonadotropins without stimulating sex accessories as judged by uterine weight. Johnson (1966)[5] parabiosed immature hypophysectomized to immature intact rats, and demonstrated the effects of gonadotropins in the hypophysectomized partner. These studies indicated that the noncastrate immature female animal secreted gonadotropins, and that a dynamic-hypothalamic-pituitary-gonadal axis existed prior to puberty. To our knowledge, such parabiotic studies were not done in male animals. Ramirez and McCann (1965)[6] determined the dose of testosterone required to suppress LH in castrate immature male rats compared to castrate mature rats. They found a dose three to four times greater was required after sexual maturation rather than prior to maturation. Ultimately, these and other such studies led to an alternative hypothesis contending that sexual maturation caused by a progressive decrease in feedback sensitivity of the hypothalamic-pituitary unit to the inhibitory effects of circulating gonadal steroids; this in turn resulted in increased LH and/or FSH secretion and increased gonadal steroid secretion. This is the most commonly held hypothesis today. However, even this hypothesis, we believe, is too simple. Puberty appears to be caused by a series of related events, the final summation of which is increased gonadal (and adrenal) steroid secretion.

The theoretical possible causes of sexual maturation can be easily understood by reference to Fig. 1. Changes in the hormonal sets or control systems involving each facet of the hypothalamic-pituitary-gonadal unit could theoretically be a cause of sexual maturation; these are listed in Table 1. It is to be noted that factors, I to III, would result in increasing LH and/or FSH concentrations during sexual maturation. Such increases, while of relatively small magnitude, are observed in children. However, their presence in animal models is not so clear-cut. Figure 2a,b depicts LH and FSH concentrations in female and male rats, between 10 days of age and sexual maturity. Note that in the female, sexual maturation is associated with *falling* FSH concentrations, and LH concentrations, which initially fall and then remain fairly

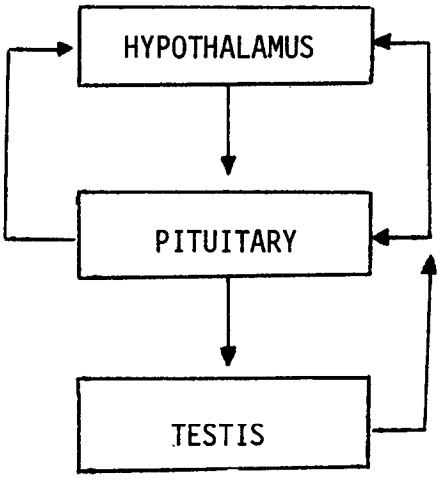

Fig. 1. Schematogram of the central nervous system: pituitary-testicular axis. Analysis of this figure reveals the possible course of sexual maturation which are listed in Table 1.

steady between 20 and 37 days of age, Swerdloff *et al.*, 1972[7], and Odell and Swerdloff, 1972[8]. In the male, LH falls between 10 and 20 days of age, then rises slowly as further maturation proceeds, Swerdloff *et al.*, 1971[9], and Odell and Swerdloff, 1972[8]. These observations shed doubt on the hypothesis that decreasing feedback sensitivity of the hypothalamic-pituitary unit to gonadal steroids is the sole cause of sexual maturation in the rat. In other studies, we showed (Odell *et al.*, 1970)[10] that LH does not increase in cattle between 15 days of age and sexual maturity (which occurs about 12 months of age). Additional studies in our laboratory have been designed to systematically examine each of the possible causes of sexual maturation listed in Table 1, using the immature and maturing rat as a model for study. In this presentation, we wish to emphasize the role of gonadal sensitivity to gonadotropins, and therefore shall only summarize the results of some of our studies of other facets of the system described in Fig. 1.

Table 1. Possible etiologies of sexual maturation

I. Extrahypothalamic-central nervous system areas:
  A. Increasing extrahypothalamic-central nervous system stimulation of hypothalamic centers governing LH and/or FSH secretion.
  B. Decreasing extrahypothalamic-central nervous system inhibition of hypothalamic centers governing LH and/or FSH secretion.
  C. Decreasing sensitivity to gonadal steroid feedback suppressive effects.
II. Hypothalamic areas:
  A. Increasing hypothalamic stimulation of LH and/or FSH secretion ("maturation process").
  B. Decreasing feedback sensitivity to gonadal steroid feedback suppressive effects.
III. Pituitary:
  A. Increasing LH and/or FSH secretion to constant LRH stimulation.
IV. Gonads:
  A. Increasing response to LH and/or FSH stimulation.
V. Sex accessory organs:
  A. Increasing sensitivity to gonadal steroid stimulation.

From Odell and Swerdloff, 1974[8].

1. Detailed studies (Swerdloff *et al.*, in preparation) of feedback sensitivity to testosterone propionate in male rats castrated the day of initiation of treatment at ages of 10 days, 21 days (immature) and 75 days (mature), revealed a difference in feedback sensitivity. The dose of testosterone required to suppress LH to approximately 50% of control (castrate) concentrations was 1·0 μg at 20 days, 5 μg at 10 days and 20 μg at 75 days. For FSH, the dose was 9·0 μg at 10 days, and 20 μg at both 20 days and 75 days. These 20 day old males are sexually immature and roughly equivalent to the animals used in the parabiotic studies previously reviewed. Since laboratory experimental rats mature earlier now than they did in the 1930's and 40's, exact equivalence in age is uncertain. These studies confirm those of Ramirez and McCann[4] and add further details.

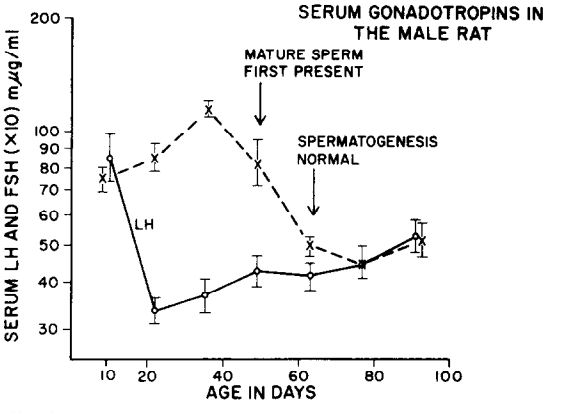

Fig. 2a. LH and FSH concentrations in blood during sexual maturation in the male rat. Reproduced from Odell and Swerdloff, 1972[8].

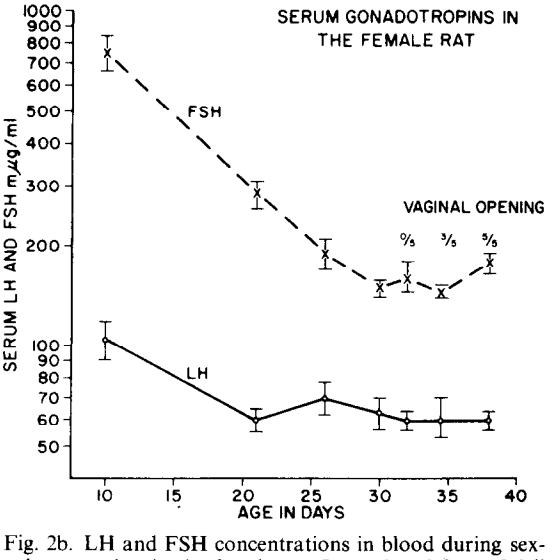

Fig. 2b. LH and FSH concentrations in blood during sexual maturation in the female rat. Reproduced from Odell and Swerdloff, 1972[8].

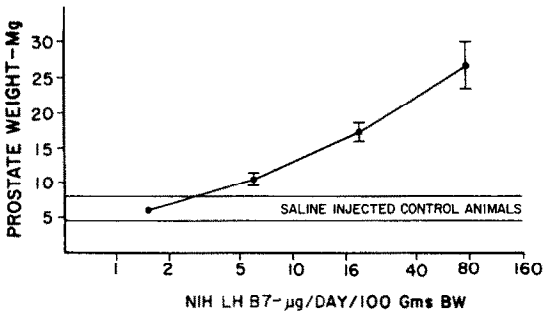

Fig. 3. Response of 21 day old hypophysectomized immature male rat to LH when treatment is initiated within 24 h of hypophysectomy. LH was administered for five days. Reproduced from Odell *et al.*, 1973 (with permission) [12].

2. Studies of sensitivity to gonadotropin releasing hormone (GnRH) Swerdloff *et al.*, 1972[11] administered to intact male rats in doses of 3 and 30 μg/100 gm BW revealed that there is no difference in sensitivity for LH release at 10, 21 and 60 days of age. In contrast, GnRH effects on FSH were affected by age. The 60 day old animal had no significant (<10%) rise in FSH, while the 10 and 21 day old animal showed a very significant rise (~300%) in FSH. Recall that concentrations in blood FSH are higher in the immature rat than in the adult.

3. Patterns of change in LH and FSH in blood, during maturation, particularly in females, do not offer strong support to the hypothesis that the decrease in feedback sensitivity to gonadal steroids is essential for maturation of the rat.

4. Assessment of sex accessory response to estrogens in females and androgens in males failed to reveal any difference between the immature and mature rat [8].

If gonadotropin concentrations do not progressively increase with sexual maturation, then to explain maturation, gonadal sensitivity to gonadotropins must change. We have systematically evaluated that possibility [7, 8, 12].

Figure 3 depicts the prostate weight response of the acutely hypophysectomized (1 day) immature 21 day old male rat to purified LH. A significant increase in prostate weight was observed at doses as low as 6 μg/100 g BW/day. In contrast, Fig. 4, shows that five days after hypophysectomy, the same 21 day old male fails to significantly increase prostate weight to much greater doses of LH; doses up to 400 μg/100 g BW/day failed to produce a detectable response. In contrast, in the sexually *mature* male rat, five days after hypophysectomy, doses as low as 0·8 g/day increased prostate weight. Furthermore, in contrast to the ineffectiveness *of LH* in the immature male after hypophysectomy, FSH is active. Figure 5 shows the increment in testis weight produced by treatment of immature and mature rats five days after hypophysectomy with purified FSH.

Since our studies showed that five days after hypophysectomy immature male rats lost their ability to respond to LH, but retained the ability to respond to FSH, we asked whether FSH pretreatment might restore responsiveness to LH. Table 2 shows the data that demonstrate this is indeed the case. All groups of animals receiving LH received the same dose over the five day period prior to sacrifice. LH (NIH-LH-B7) had little or no effect on prostate weight, as shown earlier when given alone. FSH given alone (NIH-FSH-S4) had a small effect on prostate weight when administered for 25 and 30 days time. Perhaps it could be restated that FSH appeared to prevent the slow atrophy that occurred in control groups over the 30 days period. If however a constant dose of LH was preceded by five days, 20 days or 25 days *pretreatment with FSH**, a progressive increment in prostate weight occurred. This appeared to be related to the duration of a time-related pretreatment with FSH. We concluded that FSH induced responsiveness to LH in the maturing rat, and such induction is related to the duration of exposure to FSH as well as, in all probability, to the dose administered [7, 8, 12].

To further assess the role of FSH in sensitivity to LH, we treated groups of immature 21 day old hypophysectomized male rats with FSH (80 μg/100 g BW/day of NIH-FSH-S4) beginning the day of hypophysectomy and then tested the response to a single injection of LH (30 μg/100 g BW given IP). Table 3 shows these results. In control studies, we determined that the maximal increment in serum testosterone occurred by one hour after LH was administered intraperitoneally in both mature and immature animals. When animals receiving saline injections daily for five days after hypophysectomy were treated with a single dose of LH (on day five) serum testosterone doubled. FSH given alone daily beginning the day of hypophysectomy failed to increase serum testosterone by day five. However, when daily FSH was given followed

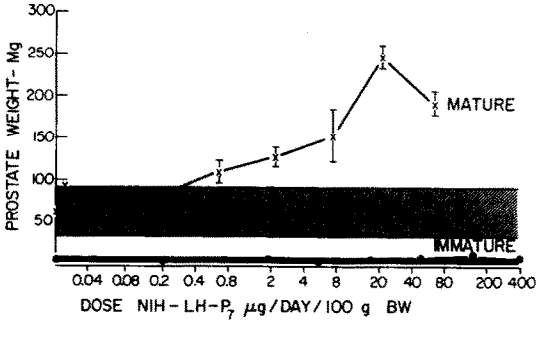

Fig. 4. Response of the immature male rat and mature male rat to LH administered daily for five days beginning five days after hypophysectomy. Immature animals were hypophysectomized at 21 days of age; mature animals at 87 days of age. Reproduced from Swerdloff *et al.*, 1972; and Odell *et al.*, 1973, Refs. 7 and 12, respectively.

* The 25-day group received 20 days' pretreatment with FSH followed by five days of FSH and LH. The 30-day group received 25 days' pretreatment with FSH followed by five days' treatment with FSH and LH.

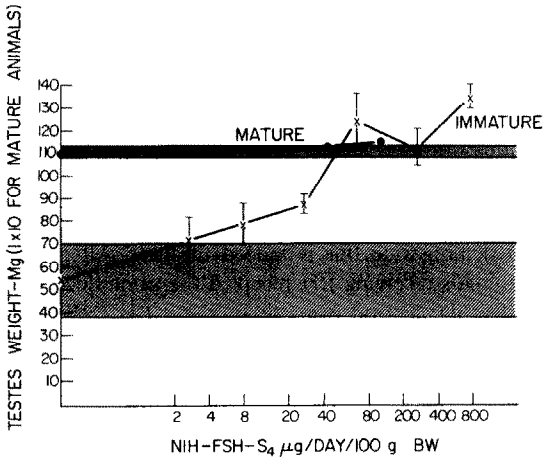

Fig. 5. Response of the five day hypophysectomized sexually immature and mature male rat to ovine follicle-stimulating hormone (NIH-FSH-S4) administered over five days. The immature animal responded to doses as low as 8 g. Because testicular atrophy has not been completed in this five day hypophysectomized animal model, a response to FSH of the sexually mature male is difficult to demonstrate. Only at doses of 80 g did testicular wt of the mature animal increase. Note that scale for testes wt of mature animals is 10 times greater than the immature animal. Reproduced from Swerdloff *et al.*, 1972; and Odell *et al.*, 1973, Refs. 7 and 12, respectively.

by the single injection of LH, serum testosterone increased by greater than 1000% [8, 12].

Most recently, we have attempted to determine how FSH induces sensitivity to LH. As an initial possibility, it appeared possible that new receptors for LH were appearing during sexual maturation under the influence of FSH. Accordingly, using the method of Leidenberger and Reichert[13], we evaluated receptor population and affinity in testicular homogenates from immature control animals (21 day old), five days after hypophysectomy, and in similar animals, receiving FSH 100 μg/100 g BW/day for five days after hypophysectomy. Recall the former respond very minimally to LH as judged by increment in blood testosterone, while the latter have a brisk response. When homogenates were prepared on an equal testicular weight basis, LH receptor populations were identical in the two groups. However, FSH treatment doubled testes weight, therefore per testes,

Table 2. Restoration of LH response in five day hypophysectomized immature male rat

	Duration FSH Treatment		
	10 days	25 days	30 days
Saline + LH	124 ± 6	107 ± 8	104 ± 6
FSH	128 ± 7	128 ± 8	131 ± 9
FSH + LH	135 ± 4	163 ± 2	287 ± 51

* Per cent increase over saline treated controls.

No treatment was given until five days after hypophysectomy. Animals receiving FSH received 60 μg/day of NIH-FSH-S4 for 10, 20 or 30 days. Animals receiving LH all received the same dose, 20 μg/day NIH-LH-B7 for five days prior to sacrifice.

From Odell *et al.*, 1973[12]; Swerdloff *et al.* 1972[7].

Table 3. Immature hypox rat - Response to LH

Treatment	Serum T (pg/ml)
Saline – 5 days	<166
FSH* – 5 days	<166
Saline + LH†	310 ± 43
FSH + LH	1852 ± 191

* 50 μg/day/100 gms BW (NIH-LH-S9).

† Single I.P. injection 30 μg/100 g BW (NIH-FSH-S17).

From Odell and Swerdloff 1974[14].

LH receptors increased by this amount. However, this alone may not explain the enhanced LH response which increased over one thousand fold. FSH has a preferential action on testicular tubules, and LH receptors are not usually considered to be present in tubules. At present, our data do not distinguish whether there are increased numbers of receptors on existing Leydig cells or formation of additional Leydig cells or development of new LH receptors in some other portion of the testis. Suffice it to say that expressed on a weight basis, LH receptor populations do not increase with FSH treatment. These data are summarized in Fig. 6 [14].

If FSH induction of sensitivity is a major factor in sexual maturation, in the male rat and the duration of exposure is important, the sexually immature animal should be less sensitive to LH than the mature animal. This is, of course, in opposition to time honored use of sexually immature animals as bioassay subjects for gonadotropins. We made a direct test of sensitivity of the intact rat to LH during sexual maturation. Single injections of purified LH (NIH-LH-B7) adjusted on a body weight basis, were administered intraperitoneally to intact male rats at 10, 21, 41 and 62 days of age. Serum testosterone was measured 1 h later, the time of maximal response. Figure 7 and Table 4 depict the per cent change in serum testosterone for two doses studies. Note that for both doses, the change in testosterone was progressively greater

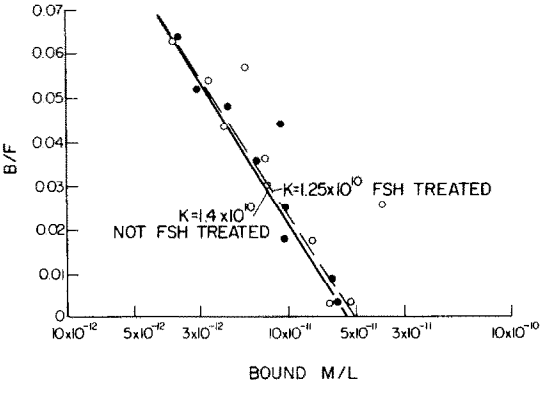

Fig. 6. Scatchard plot of receptor populations in immature control male rats five days after hypophysectomy and in similar animals receiving FSH 80 μg NIH-FSH-S4 daily for five days after hypophysectomy. Testis weight was doubled (159 ± 7 to 305 ± 10 mg) by this treatment. Reproduced from Odell *et al.*, 1974a (in preparation).

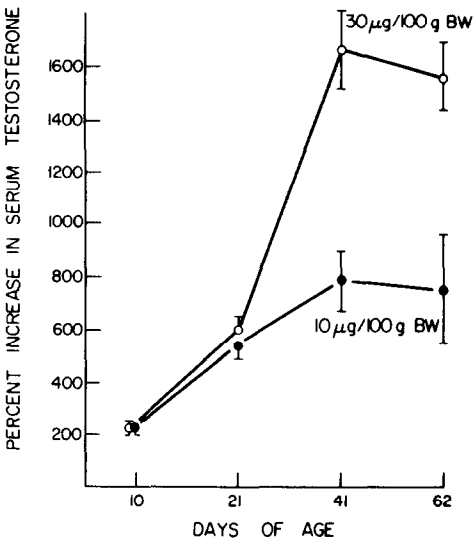

Fig. 7. Per cent increase in serum testosterone plotted against age of animals for two doses of NIH-LH-B7. Note that for both doses a greater change in serum testosterone occurred with increasing age in response to a constant dose of LH.

with age between 10 and 41 days. The changes at 41 days and 62 days were indistinguishable [8, 15].

We conclude from these studies that: (1) changing testicular response to LH is a major factor in sexual maturation in the male rat, (2) the response to LH is determined by time of exposure and possibly dose of FSH.

These studies may bring to focus an explanation for the high FSH concentrations observed prior to maturation in rats. It appears possible that FSH is secreted in unrestrained fashion during the first 10–30 days of life, inducing testicular sensitivity to LH. As sensitivity develops and gonadal feedback steroids are secreted, FSH concentrations fall. The LH change shown in Fig. 2a,b do not appear to be explained by the same mechanism, but would be explained by decreasing feedback sensitivity. However, from a theoretical standpoint, if gonadal sensitivity to LH is a major factor, puberty could occur without any changes in blood LH. These considerations superimposed upon our findings of changing sensitivity to GnRH are sufficient to explain maturation in the male rat.

Table 4. Responses of serum testosterone to LH injection as a function of age of male rats

Dose of NIH-LH-B7 (μg/100 g)	Serum testosterone (pg/ml ± SEM)			
	10 days old	21 days old	41 days old	62 days old
Control	440 ± 58 (18)†	344 ± 31 (20)	1,076 ± 127 (23)	2,222 ± 204 (22)
10	939 ± 52 (10)	1,865 ± 166 (15)	8,465 ± 1,305 (12)	16,783 ± 4,594 (11)
30	941 ± 60 (9)	2,072 ± 164 (14)	18,005 ± 1,566 (12)	34,893 ± 2,882 (12)

† Numbers in parentheses indicate number of observations.

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