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Induction of the Self-Priming Effect of Luteinizing Hormone Releasing Hormone during the Sexual Maturation of the Male Rat (42107)

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Abstract. Pubertal and young adult male rats release more luteinizing hormone (LH) in response to luteinizing hormone releasing hormone (LHRH) if pretreated with LHRH than if pretreated with saline. Immature male rats do not show this self-priming effect. In order to examine the role of acute changes in testicular steroids in this process, immature (29–30 days old) or pubertal (50–51 days old) male rats were castrated or sham operated under ketamine HCl anesthesia. Beginning immediately after completion of the surgery, they were given three priming injections of 10 ng LHRH/100 g body wt or saline at 30-min intervals. Thirty minutes after the third priming injection, a blood sample was obtained by cardiac puncture followed immediately by a challenge injection of 50 ng LHRH/100 g body wt given to both saline and LHRH primed groups. Ten minutes after the challenge injection a final blood sample was obtained by heart puncture. Serum was assayed for LH concentration by radioimmunoassay. Sham-operated pubertal rats showed a typical self-priming effect. Animals pretreated with LHRH released significantly ($P < 0.01$) more LH in response to the challenge injection than did rats pretreated with saline. Acute castration also resulted in a significant ($P < 0.001$) self-priming effect in pubertal rats. As anticipated, sham castrated immature males did not show a self-priming effect. Acutely castrated immature rats however, showed a significant ($P < 0.05$) self-priming effect. These data provide support for the hypothesis that, prior to puberty, increases in testosterone during the priming process inhibit the expression of the self-priming effect.

A number of changes in hypothalamic–pituitary–gonadal function take place during the sexual maturation of the male rat (1). Among these are an alteration in gonadal responsiveness to gonadotropins (2), a decrease in the sensitivity of the hypothalamus/pituitary to the negative feedback effects of testosterone (3), and an alteration in the ability of the pituitary to release luteinizing hormone (LH) in response to exogenous luteinizing hormone releasing hormone (LHRH) (4).

More recent studies have described the development of a self-priming effect of LHRH on LH secretion during the sexual maturation of the male rat. This effect was first described in the female (5) and refers to the ability of small priming injections of LHRH to sensitize the anterior pituitary to subsequent LHRH administration. Pubertal or young adult male rats release more LH in response to LHRH if they are pretreated with LHRH than if they are pretreated with saline. Immature male rats do not show this self-priming effect (6). This effect appears to develop between the ages of 34 days, when no self-priming effect was evident, and 46 days, when a statistically significant self-priming was first apparent (7). When the hormonal responses during the priming process were examined at 15-min intervals (8), it appeared that serum testosterone concentrations were elevated sooner and relatively higher in immature male rats compared with those in pubertal animals. Since the immature male rat was known to be more sensitive to the negative feedback effects of testosterone (3, 9) and since androgens had been demonstrated to have a rapid, direct effect on the anterior pituitary (10), these observations suggested the possibility that the more rapid testosterone response seen in the immature male rat was responsible for the absence of a self-priming effect during this period (8). The experiments described here were designed to test this hypothesis.

Materials and Methods. Male rats of a Sprague–Dawley-derived strain were obtained from the Holtzman Company (Madison, Wisc.). They were housed four to five per cage in hanging wire mesh cages under standard conditions of temperature (24°C) and

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photoperiod (14L:10D, lights on at 0500). Food and water were available *ad libitum*. Immature rats were used at 29–30 days of age. Pubertal animals were used at 50–51 days of age. Developmental stages have been defined previously (6, 9). Rats were anesthetized with ketamine HCl (10–20 mg/100 g body wt im). Supplemental doses of this drug (5–10 mg/100 g body wt) were administered as needed to maintain an appropriate level of anesthesia throughout the experiment. Rats were castrated or sham operated via a single midscrotal incision. Immediately after removal of the testes, they received the first of three priming injections. Animals were primed with 10 ng LHRH/100 g body wt or saline at 30-min intervals via the right jugular vein. Thirty minutes after the third priming injection, a blood sample (~1.0 ml) was obtained by heart puncture. Immediately following the blood collection a challenge injection of 50 ng LHRH/100 g body wt was administered to all rats via the left jugular vein. Ten minutes after the challenge injection, a final blood sample was obtained, also by cardiac puncture. The number of animals in each group is shown in Fig. 1.

Appropriate aliquots of serum were diluted with assay buffer and stored frozen until assayed. LH concentrations in these samples were determined by radioimmunoassay using an antibody (No. 15) provided by Dr. Gordon Niswender (11) and radioiodinated rat LH as described previously (7). During the course of these studies the rat LH reference preparation supplied by the National Pituitary Agency changed from RP-1 to RP-2. Since RP-2 has a potency equivalent to 61 × RP-1, all data have been expressed in terms of RP-1 for consistency. Serum samples from a given developmental stage were all run in the same assay.

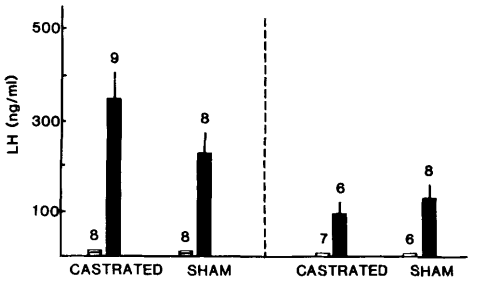

FIG. 1. Serum LH concentrations (Mean + SEM) just prior to the LHRH challenge injection in acutely castrated and sham-operated pubertal (left panel) and immature (right panel) male rats. Animals were treated iv with three priming injections of LHRH (solid bars) or saline (broken bars) at 30-min intervals beginning immediately after surgery.

Some of the data were expressed as the increment in serum LH concentration, i.e., the concentration 10 min after the LHRH challenge minus the value just prior to releasing hormone administration. Expressing the data in this manner allows conclusions to be drawn about the capacity of the pituitary to respond, regardless of the basal hormone concentration. Data were analyzed and significance determined using Student's *t* test.

Results. Male rats pretreated with LHRH had significantly ($P < 0.01$) higher prechallenge LH concentrations than did saline pretreated animals (Fig. 1). This was true at both ages examined and in both castrated and sham-operated rats. When compared directly, castrated and sham-operated animals that had received similar priming had similar serum LH concentrations at a given age ($P > 0.05$).

Acutely castrated pubertal male rats (Fig. 2) released significantly ($P < 0.001$) more LH in response to the LHRH challenge if they had been pretreated with LHRH than if they were pretreated with saline. As was expected, sham-operated rats also showed a significant ($P < 0.01$) self-priming effect.

Sham-operated immature male rats (Fig. 2) released similar amounts of LH in response to the LHRH challenge regardless of pretreatment ($P > 0.05$). When immature male rats were castrated immediately prior to the start of priming, they showed a self-priming effect. In response to the LHRH challenge, these animals released significantly ($P < 0.05$) more LH if pretreated with LHRH than if pretreated with saline.

Discussion. One of the changes that takes place during the sexual maturation of the male rat is the development of a self-priming effect of LHRH on LH secretion (6). Pubertal male rats respond to LHRH priming with an increase in the ability of the anterior pituitary gland to release LH in response to subsequent LHRH administration. Such a

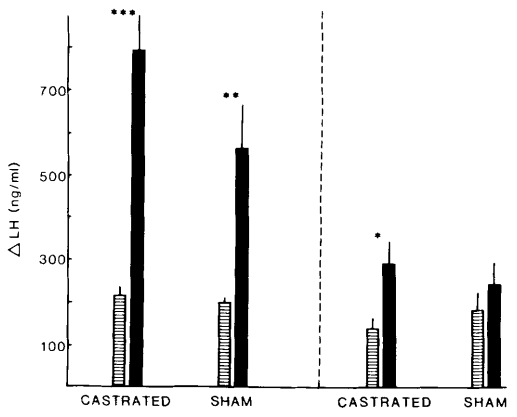

FIG. 2. The increment (mean ± SEM) in serum LH concentrations 10 min after the challenge injection of LHRH in acutely castrated and sham-operated pubertal (left panel) and immature (right panel) male rats. Animals were pretreated iv with three priming injections of LHRH (solid bars) or saline (broken bars) beginning immediately after surgery. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

response is not evident in immature male rats (6–8). A recent study that examined serum and pituitary hormone concentrations during the priming process suggested that one possible cause of this difference was the difference in the serum testosterone response (8). Although not ruling out a role for testicular factors other than testosterone, the results reported here provide support for this hypothesis.

Previous studies reported that immature male rats responded to LHRH priming with faster and relatively larger increases in serum testosterone than did pubertal animals (8). Additionally, it has been suggested that testosterone has a rapid and direct negative feedback effect on the ability of the anterior pituitary to secrete LH, possibly by altering the self-priming effect (10). These reports, coupled with the observation that the "gonadostat" is more sensitive to androgens prior to puberty (3, 6), suggested that the testosterone secreted in response to LHRH priming inhibited the self-priming effect in immature male rats. In older animals, the response was greater in absolute terms but still slower, and smaller in relative terms and thus was not sufficient to inhibit the expression of the self-priming effect. If this reasoning is true, then removal of the testes prior to the initiation of priming should permit the expression of a self-priming effect in the immature male rat but have little or no effect in older animals. Precisely these results are reported here.

An alternative explanation for the lack of a self-priming effect in immature male rats would be an inability of the hypothalamus to release LHRH in an episodic or pulsatile fashion, thus resulting in a lack of endogenous priming. That this is not true is suggested by the observations that castrated immature male rats released LH in this fashion (12, 13). Additionally, the observation that as many as six priming injections of LHRH did not induce a self-priming effect in the immature male rat suggests that the anterior pituitary is the site of the developmental difference (14).

A possible alternative explanation for the results reported here is that castration brings about an increase in the frequency and/or amplitude of endogenous LHRH pulses. This is not likely since it has been reported that the frequency of pulsatile LHRH release was not altered in acutely castrated male rats (15). Neither did acute castration appear to increase the amplitude or frequency of pulsatile LH release at least during the initial 5–7 hr (16).

It is conceivable that a self-priming effect might have been missed in the immature male rats. Serum concentrations of LH just prior to the LHRH challenge were higher in those animals pretreated with LHRH than in those pretreated with saline. This elevated LH would be subjected to constant reduction due to normal metabolic clearance. It might, therefore, have been more difficult to elevate the LH levels in these rats, resulting in an underestimate of the LH response to the LHRH challenge. This possibility is partially offset by expressing the data as the increment in LH after challenge. Additionally, there was no difficulty in demonstrating a self-priming effect in the pubertal rats. At this age also, the LHRH primed groups had higher prechallenge LH levels than did saline pretreated rats.

Thus it appears reasonable to suggest that the absence of a LHRH self-priming effect in immature male rats is due to a combination of the greater sensitivity of their anterior pituitary glands to the negative feedback

effects of testosterone and their faster and relatively greater testosterone response to LHRH priming. It is possible that, as sexual maturation proceeds, this effect is unmasked in the intact rat as the result of the rising testosterone concentrations altering pituitary function. It has been reported that increases in serum testosterone precedes the development of the self-priming effect (7) and that the development of this effect can be blocked with androstenedione (17).

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