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Plasma Prolactin Levels in Maturing Intact and Cryptorchid Male Rats: Development of Stress Response[1] (39199)

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Previous work has shown that plasma prolactin concentrations increased markedly in the male rat after the third week of life, reaching maximum values at about Day 90 (1). It has also been reported that plasma FSH concentration after reaching a peak around the fourth week of life, declines steadily as the animal approaches puberty (1, 2). This decline has been attributed to feedback inhibition exerted by a product of the germinal epithelium, since cryptorchidism, a condition that produces destruction of this epithelium, prevented the fall in FSH at a time prior to occurrence of Leydig cell dysfunction (2).

In view of the existence of this inverse pattern of plasma prolactin and FSH during sexual development in the male rat, it was of interest to study the possibility that the seminiferous tubules might exert a direct control over pituitary prolactin secretion. With this purpose in mind, cryptorchidism was produced at 22 days of age, and the developmental changes in plasma prolactin were compared to those of intact controls.

Materials and Methods. Holtzman male rats of different ages were used. Animals to be rendered cryptorchid were obtained with their mothers and arrived at the laboratory 4-6 days before the operation. All animals were housed in an air-conditioned room (24-26°) under controlled conditions of light (14 hr on, 10 hr off). Laboratory chow (Purina) and water were administered *ad libitum.*

Cryptorchidism was produced at Day 22 through bilateral abdominal incisions while the animal was anesthetized with ether; the testes were delivered into the abdominal cavity and the internal inguinal ring was closed with a purse-string suture. In sham-operated controls, the abdomen was opened, but the internal inguinal ring was not sutured.

In the first experiment, different groups of cryptorchid and intact animals were sacrificed by decapitation at 8-10 day intervals, from Day 32 to Day 70. Blood was collected from the trunk into heparinized tubes and plasma was separated by centrifugation and kept at −20° until assayed. In all cases; animals caged in groups of four or five were brought from the animal room to the laboratory at least 2 hr before sacrifice. At sacrifice the testes, seminal vesicles, and ventral prostate were dissected out and weighed. Testes were fixed in 10% formalin and stained with hematoxylin and eosin for subsequent examination.

As an additional control, some intact animals were provided with silastic jugular cannulae at Day 67, and blood samples were obtained 3 days later (Day 70) as previously described (3).

In a second experiment, 21-day-old males were made cryptorchid, and blood samples were taken from the jugular vein following a 3-min exposure to ether fumes at 10-day intervals from Day 30 to Day 70. Sham-operated controls were subjected to the same bleeding schedule.

In addition, a cross-sectional study was performed in which different groups of intact males were bled once from the jugular vein following a 3-min period of ether exposure at different ages from day 30 to Day 70. In all cases, animals were sacrificed in the morning.

Plasma concentrations of prolactin and FSH were assayed as recommended in the directions supplied with the NIAMDD kits[2]

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[2] Kits for determination of prolactin and FSH were provided through the NIAMD-NIH Pituitary Hormone Program.

with minor modifications, and results were expressed in terms of the NIAMDD rat pituitary RP-1 standards supplied with the kits. LH was assayed according to the method of Niswender *et al.* (4),[3] using the RP-1 rat pituitary LH reference standard and was expressed in terms of the NIH-LH-S1 reference preparation.

The statistical significance of the differences in plasma hormone titers between two groups of the same age was determined using Student's t test. Differences between groups of different ages subjected to the same experimental conditions were statistically evaluated using the Scheffe's multiple comparison test for unequal replications (5).

Results. Organ weights in intact and cryptorchid rats of different ages. Testis weight of intact animals increased markedly between day 32 and Day 70 ($P < 0.01$), the rate of increase being more pronounced between Day 32 and Day 50 (Table I). Testicular weight of cryptorchid rats was already significantly less ($P < 0.001$) than that of controls 10 days after surgery (Day 32). Thereafter, testicular weight remained almost unchanged in the cryptorchid animals, being at all ages significantly less ($P < 0.001$) than that of intact sham-operated controls (Table I).

Seminal vesicle weight (SVW) of intact animals increased steadily throughout the period studied (Table I). In age-matched cryptorchid rats, the SVW was similar to that in control animals, except at Day 50 and at Day 70, ages at which it was significantly lower ($P < 0.01$ and $P < 0.005$, respectively). Ventral prostate weight showed the same rate of increase in both intact and cryptorchid animals (Table I).

Plasma prolactin, LH, and FSH concentrations in decapitated intact or cryptorchid rats of different ages. Plasma prolactin levels varied very little, remaining constantly low during the entire period studied (Day 32 to Day 70) in both the intact and the cryptorchid group (Fig. 1). Furthermore, the levels were not different from those detected in intact male rats bled at Day 70 through a permanent jugular cannula.

Except on Day 32, plasma LH was slightly but significantly ($P < 0.05$-$P < 0.001$) elevated in cryptorchid animals at all ages studied when compared with age-matched controls. Plasma FSH, on the other hand, was elevated at Day 32 in intact controls and markedly declined thereafter ($P < 0.01$) to reach adult levels around Day 60. In cryptorchid rats, plasma FSH, although declining slightly after Day 40, was significantly higher than levels in normal rats at all ages studied ($P < 0.005$-$P < 0.001$).

Effect of ether stress on plasma prolactin levels of intact and cryptorchid rats of different ages. Longitudinal study. At Day 20, the age at which a separate group of intact rats was ether-stressed, plasma prolactin was slightly but significantly elevated ($P < 0.05$) with respect to levels in decapitated controls of the same age (Fig. 2). When intact rats were subjected to repeated ether stress between 30 to 70 days of age, the stress-induced elevation in plasma prolactin greatly increased with age, reaching a plateau around Day 60. Cryptorchidism did not alter this response since no differences were observed in plasma prolactin concentrations between the two groups at any of the ages studied. At all ages, plasma prolactin titers in both groups were significantly ($P < 0.005$-$P < 0.001$) higher than prolactin levels in intact, age-matched, decapitated animals.

Cross-sectional study. Since this apparent age-dependent increase in prolactin response to ether stress could be attributed to sensitization of the prolactin-releasing apparatus to the previous stresses of exposure to ether and bleeding, different groups of animals of various ages were subjected to a single exposure to ether and then sacrificed. As before, the prolactin response to ether stress, although less striking, increased from Day 20 to Day 60, reaching a plateau at this later age (Fig. 3). At all ages, plasma prolactin values were significantly higher ($P < 0.05$-$P < 0.001$) than those in decapitated controls.

Discussion. The earlier findings that plasma FSH concentrations markedly declined as male rats approached puberty (1, 2) and that prolactin levels increased during the same period of sexual development (1) raised the possibility that the same factor(s)

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[3] Antiovine LH serum was provided through the courtesy of Dr. G. Niswender (Colorado State University). Dr. L. Reichert (Emory University) generously provided the purified ovine LH for radioiodination.

TABLE I. TESTES, SEMINAL VESICLES, AND VENTRAL PROSTATE WEIGHTS OF CRYPTORCHID AND INTACT SHAM-OPERATED RATS SACRIFICED AT DIFFERENT AGES.

Age at sacrifice[a] (days)	Testes weight (g)		Seminal vesicle weight (mg)		Ventral prostate weight (mg)	
	Sham	Cryptorchid	Sham	Cryptorchid	Sham	Cryptorchid
32	1009 ± 29[b] (10)*	740 ± 15** (13)	22 ± 1	22 ± 1	52 ± 4	61 ± 2
40	1599 ± 43 (10)	724 ± 28** (15)	46 ± 5	52 ± 4	80 ± 5	81 ± 7
50	2563 ± 48 (10)	708 ± 29** (15)	161 ± 8	125 ± 8***	160 ± 12	147 ± 8
60	2971 ± 74 (10)	734 ± 23** (7)	225 ± 10	220 ± 12	250 ± 15	238 ± 16
70	3254 ± 50 (9)	789 ± 31** (13)	302 ± 8	255 ± 9****	350 ± 30	367 ± 17

[a] Cryptorchidism was produced at Day 22.
[b] Mean ± SE.
* Number of animals per group.
** $P < 0.001$ compared with the intact control group.
*** $P < 0.01$ compared with the intact control group.
**** $P < 0.005$ compared with the intact control group.

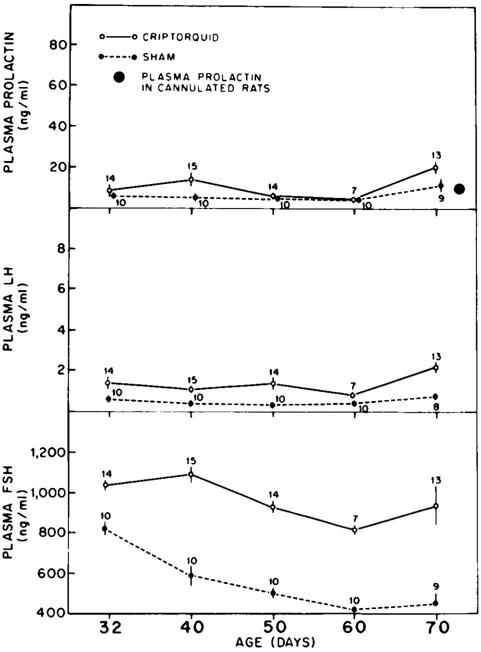

FIG. 1. Plasma prolactin, LH, and FSH concentrations in intact and cryptorchid male rats decapitated at different ages. In this and subsequent figures, vertical lines represent the standard error of the mean and numbers next to the circles indicate number of animals used.

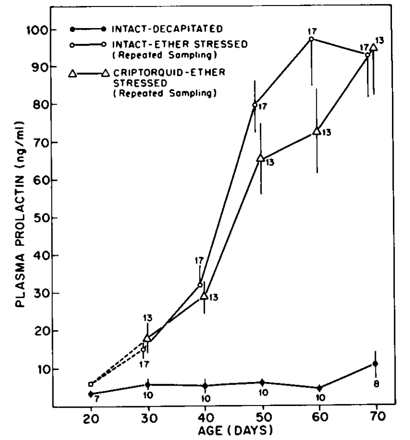

FIG. 2. Effect of repeated stress by ether anesthesia on plasma prolactin levels of intact and cryptorchid developing male rats. Animals were bled at 10-day intervals from Day 30 to Day 70 following a 3-min period of exposure to ether fumes. Prolactin values at Day 20 are from a different group of animals.

determining the FSH fall might also bring about a gradual, age-dependent elevation in prolactin titers. The decline in FSH levels appears to be related, at least in part, to germinal epithelium maturation since in maturing cryptorchid rats, FSH failed to fall, even though Leydig cell function remained unaltered (2).

The present results show that as early as Day 32, i.e., 10 days after surgery, plasma FSH in cryptorchid rats was significantly elevated and remained so throughout the entire period studied. Interestingly, plasma LH was also somewhat elevated in cryptorchid rats, an observation suggesting that androgen secretion by the Leydig cells was also impaired to some extent, or, alterna-

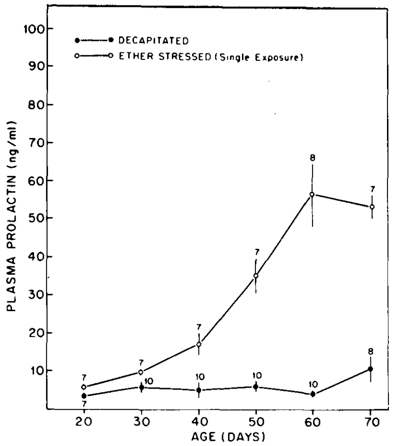

FIG. 3. Effect of a single exposure to ether fumes on plasma prolactin concentration of intact developing male rats. Different groups of animals were sacrificed at 10-day intervals from Day 20 to Day 70, following a 3-min period of exposure to ether fumes. Plasma prolactin titers in decapitated rats from Fig. 2 are also represented.

tively, that the seminiferous tubules are also able to exert a modulatory action on LH secretion. Since no consistent differences in weight of the accessory sex organs were found between experimental and control animals, it would appear that the increased LH levels could effectively maintain the weight of these organs through stimulation of nondamaged Leydig cells.

The observations reported here are in partial agreement with those previously reported by Swerdloff *et al.* (2), in that plasma FSH declined with age in the intact animals; however, in their experiments, the elevation of FSH was observed in cryptorchid animals only at 49 days of age rather than as early as 32 days as found in the present study. Furthermore, LH increased gradually with age in their study, and there was only a suggestion of this in the present work between 60 and 70 days. Also, elevation in LH in cryptorchids was only observed after 63 days in their study rather than as early as 32 days in the present experiments. These discrepancies may be related to the different strain of rats utilized or to the different times at which the animals were sacrificed. The patterns of LH and FSH observed in the present study are in agreement with those previously reported in intact animals by Negro-Vilar *et al.* (1).

Regarding the pattern of plasma prolactin during sexual development, the results clearly indicate that impaired function of seminiferous tubules does not affect prolactin levels at any age studied. An earlier report (1) had indicated that plasma prolactin levels increased as the animal approached maturity and that the rate of ventral prostate growth could be, in fact, related to the increasing circulating prolactin levels. The ability of prolactin to synergize with testicular androgens in promoting growth of the accessory sex organs is well established (6–10). Moreover, it appears that prolactin may act to elicit the growth of these organs even in the absence of androgens, since anterior pituitary grafts evoked an increase in accessory organ weight in castrated-adrenalectomized or hypophysectomized rats (11).

While the present results do not controvert the concept that prolactin is a physiological participant in the maintenance of functional integrity of accessory sex organs, they indicate that the rate of growth of these organs cannot be readily correlated with corresponding changes in basal plasma prolactin levels since decapitated rats did not show any increase in prolactin throughout the period studied in spite of dramatic changes in accessory sex organ weight. By contrast, animals subjected to a nonspecific stress (ether anesthesia plus bleeding) responded with an increase in prolactin levels, a response that became greater as the animal approached maturity. The increasing prolactin levels previously observed between Day 20 and adulthood may, therefore, reflect an enhanced age-dependent responsiveness of the prolactin-releasing apparatus to stress stimuli.

The developmental increase in prolactin titers reported previously (1) may have been the result of caging the animals in larger groups than in the present study, which resulted in some stress as the animals were removed from the cage and decapitated. It is still possible that prolactin may play a physiological role in the development of the sex accessories in view of its extreme responsiveness to stress. The environmental stimuli which the animals are exposed to may produce intermittent stress-related rises in prolactin which could be involved in stimulating sex accessory growth.

In the present study, animals subjected to an ether-bleeding stress appeared to be extremely sensitive to subsequent exposures to ether as judged by the larger prolactin responses observed in that group than in rats stressed only once. Although interesting, no definitive conclusions can be drawn from these data since plasma samples from these two groups were analyzed in different assays.

The increase in stress-induced prolactin release during development may be attributed to an augmented pituitary capacity to release prolactin as the animal matures, or, alternatively, to a gradual development of the hypothalamic control system which regulates prolactin secretion. In other experiments (unpublished results), it has been found that the pituitary response to blockade of the hypothalamic dopaminergic inhibitory system that controls prolactin secretion is fully developed by Day 35 in the male rat. At this juncture, it is tempting to speculate that the pattern of prolactin response to stress during development is associated with maturational changes in the content and/or availability of the hypothalamic prolactin releasing factor (PRF). Rapid changes in plasma prolactin induced by stress stimuli are thought to be caused by an enhanced release of PRF rather than by an inhibition of PIF release (12, 13).

Finally, the observation that plasma prolactin concentrations barely increased during development in the absence of stress reinforces the concept that it is important to minimize stress when *in vivo* studies on prolactin release are performed (14).

Summary. In order to determine the possible role of the seminiferous tubules in the regulation of prolactin secretion during sexual development, male rats were rendered cryptorchid at 22 days of age, and thereafter different groups of animals were decapitated at 8–10 day intervals between Day 32 and Day 70. Cryptorchid rats showed destruction of the germinal epithelium accompanied by increased plasma FSH and, to a much lesser extent, increased plasma LH titers. Nevertheless, plasma prolactin levels were similar to those of intact controls throughout the entire period studied. Plasma prolactin titers in intact controls remained uniformly low from Day 20 to Day 70, contrasting with previous reports in which increasing prolactin levels have been observed during sexual development. To determine the reason for this apparent discrepancy, a longitudinal experiment was conducted in which intact and cryptorchid male rats were bled every 10 days from Day 30 to Day 70, following a 3-min period of exposure to ether fumes. The prolactin response to this stress increased markedly with age. A similar pattern of prolactin was observed in a cross-sectional study in which different groups of intact animals were bled following a 3-min period of ether exposure, at ages ranging from 20 to 70 days. The results indicate that unlike FSH secretion, prolactin secretion is not controlled by the seminiferous tubules. In addition, they suggest that the pattern of increasing plasma prolactin previously described in the developing male rat is at least in part caused by an age-dependent increase in responsiveness of prolactin to stress.

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