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Effects of Castration, Steroid Replacement, and Hypophysectomy on Hypothalamic LHRH and Serum LH[1,2] (39890)

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Introduction. Serum LH and FSH levels have been shown to increase after castration in rats, and gonadal steroid replacement to decrease secretion of hypophyseal gonadotrophins (1, 2). However, the interactions between hypothalamic LHRH and gonadal steroids are not completely understood. Chowers and McCann (3) found no change in content of hypothalamic LH-releasing factor (LRF) as measured by bioassay after castration or injections of gonadal steroids, whereas Piacsek and Meites (4) reported that gonadal steroids reduced hypothalamic LRF content in castrated rats. Shin *et al.* (5) observed that immunoassayable LHRH content in the hypothalamus of male rats was decreased after castration, and testosterone replacement resulted in an increase in LHRH content. We considered it of interest to reexamine the effects of castration, gonadal steroids, and also of hypophysectomy on immunoassayable LHRH in the hypothalamus and on serum LH in rats of both sexes.

Materials and methods. Sprague-Dawley male and female rats (Spartan Research Animals, Haslett, Mich.) weighing 225-250 g each were used in experiments I and III. In experiment II, both intact and hypophysectomized Sprague-Dawley male rats weighing 250-275 g each were purchased from Hormone Research Labs, Chicago, Illinois. Rats were housed in an air-conditioned (24 ± 1°) and light-controlled (lights on from 0600-2000 hr daily) room and were given Purina Rat Chow (Ralston Purina Co., St. Louis, Mo.) and water ad libitum. The hypophysectomized rats were supplied with oranges and sugar cubes in addition to their regular diet.

Experiment I. Male rats were castrated under ether anesthesia and killed by decapitation 1, 7, and 14 days later. Intact control rats were decapitated at the same time. The trunk blood and brain were rapidly removed after decapitation.

Experiment II. Both hypophysectomized and nonhypophysectomized male rats were either maintained as controls or castrated under ether anesthesia and treated daily with or without 0.5 mg of testosterone propionate (TP)/300 g body weight in corn oil for 2 weeks. Rats not given TP received corn oil only. At the end of treatment, the rats were decapitated and trunk blood and brains were removed.

Experiment III. Female rats were intact or ovariectomized. Two weeks after ovariectomy, they were injected sc with 1 μg of estradiol benzoate (EB), 2.5 mg of progesterone (PRG), or with a combination of EB and PRG. Control intact and ovariectomized rats received corn oil only. One week after treatment, all rats were decapitated at about 1000 hr, and trunk blood and brain were immediately removed. A block of hypothalamic tissue constituting the region lying between the rostral borders of the optic chiasm and mammillary bodies and medial from the optic tracts were dissected to a depth of about 2 mm. The average weights of the hypothalamus in experiments II and III were 40.25 ± 0.85 and 35.13 ± 0.58 mg, respectively. Hypothalamic tissue was homogenized in 1 ml of 0.1 *N* HCl and neutralized with 1 ml of 0.1 *N* NaOH. After centrifugation at 7000 rpm for 20 min in a Sorvall refrigerated centrifuge, the supernatant was diluted with 0.1% gelatin in phos-

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phate-buffered saline (PBS) to an appropriate concentration. Both serum and hypothalamic extracts were stored at −20° until assayed for LH and LHRH, respectively.

Radioimmunoassays (RIA). Hypothalamic LHRH was measured by the double-antibody RIA described by Nett *et al.* (6). Anti-Gn-RH serum, R-42 pool, was kindly provided by Dr. G. D. Niswender of Colorado State University, and was used at a final dilution of 1:280,000. Synthetic LHRH (Lot α, CN-79, 479-11K, TM 10455x151-2, Parke Davis Co., Detroit, Mich.) was used as a reference standard and for radioiodination by the chloramine T method. Samples were assayed in duplicate at two dilutions.

Serum LH also was assayed in duplicate at two dilutions by a double-antibody RIA using the ovine-ovine system (7). Values were expressed in terms of the reference standard, NIAMDD rat LH-RP-1. Data were analyzed statistically by analysis of variance and Student-Newman Keuls multiple-range test (8). The level of significance chosen was $P < 0.05$. Standard errors are used in the text and figures.

Results. Effect of castration on hypothalamic LHRH content in male rats. Hypothalamic LHRH content in the male rat 1 day after castration was not different from that in intact controls. However, hypothalamic LHRH content was significantly decreased 7 days after castration and further decreased by 14 days after castration (Fig. 1). The hypothalamic LHRH content of castrated male rats 1 and 2 weeks later was 77 and 55% of that in intact controls.

Effects of castration (CAS), hypophysectomy (HYPOX), and TP on hypothalamic LHRH content and serum LH levels in male rats. Figure 2 shows that CAS, HYPOX, or both, each significantly decreased hypothalamic LHRH content. Replacement with TP brought the LHRH content back to normal or even higher than in intact controls. Serum LH levels in the intact group averaged 24 ± 5 ng/ml, but 2 weeks after castration it had risen to 944 ± 166 ng/ml (Fig. 3). TP replacement reduced serum LH down to 4 ± 1 ng/nl, which was significantly lower than that in the intact controls.

Effects of ovariectomy and steroid replacement on hypothalamic LHRH content and serum LH levels in female rats. Figure 4 shows that, 3 weeks after ovariectomy, hypothalamic LHRH content was decreased significantly from 6.4 ± 0.3 to 4.5 ± 0.2 ng. EB treatment daily for 1 week resulted in a return of LHRH content in ovariectomized rats to intact levels. Daily injection of progesterone for 1 week did not change hypothalamic LHRH content in the ovariectomized rats. Hypothalamic LHRH content in rats given the combination of EB and PRG was slightly greater than that in intact con-

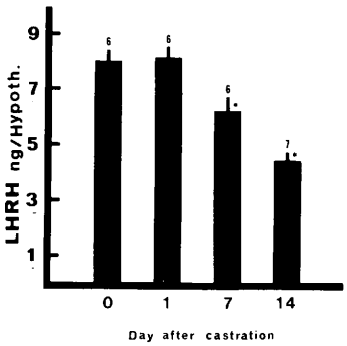

FIG. 1. Hypothalamic LHRH content in intact male rats, and in rats after castration for 1, 7, and 14 days. The number above the standard error bar indicates the number of rats per group. *Values are significantly different from intact controls.

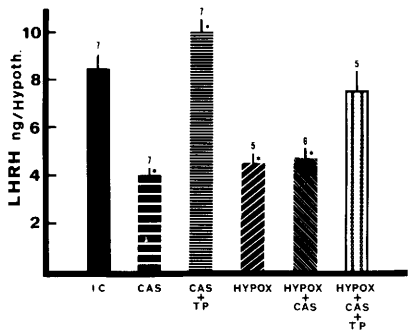

FIG. 2. Hypothalamic LHRH content in intact controls (IC), in castrated (CAS) rats, in CAS rats treated with testosterone propionate (CAS-TP), in hypophysectomized controls (HYPOX), in HYPOX-CAS rats, and in HYPOX-CAS-TP-treated male rats. Rats were CAS or HYPOX and then given 0.5 mg of TP/300 g body weight/day for 2 weeks. The number above the standard error bar indicates the number of rats per group. *Values are significantly different from intact controls.

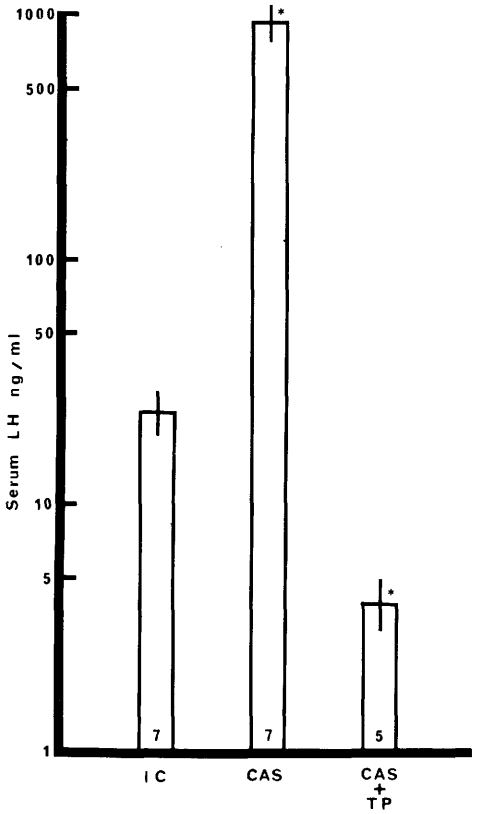

FIG. 3. Serum LH levels in IC, CAS, and CAS-TP-treated male rats. The number inside each column indicates the number of rats per group. *Values are significantly different from intact controls. Ordinate is given in log scale.

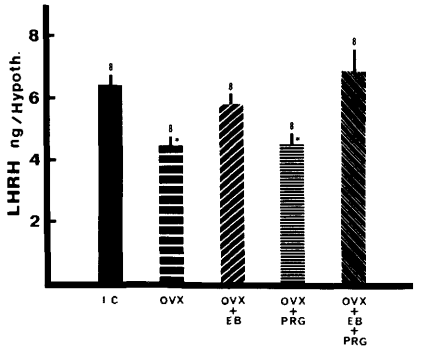

FIG. 4. Hypothalamic LHRH content in IC, OVX rats, OVX-EB-treated rats, OVX-PRG-treated rats, and OVX-EB-PRG-treated rats. Rats were ovariectomized for 2 weeks, followed by treatment for 1 week with EB (1 μg/day) or PRG (2.5 mg/day) or both. The number above the standard error bar indicates the number of rats per group. *Values are significantly different from intact controls.

trols, but this difference was not significant.

Serum LH levels in the female rats are shown in Fig. 5. In the intact controls, serum LH concentration was 24 ± 4 ng/ml. Three weeks after ovariectomy, serum LH rose to 1136 ± 70 ng/ml. Injection of EB for 1 week reduced the postcastration LH rise to 243 ± 11 ng/ml. Administration of PRG alone to ovariectomized rats decreased serum LH slightly but significantly to 800 ± 78 ng/ml, whereas the combination of EB and PRG decreased serum LH almost to intact control levels (36 ± 2 ng/ml).

Discussion. Hypothalamic LHRH content in castrated male rats decreased significantly by 1 week, which agrees with the report by Root *et al.* (9). Furthermore, hypothalamic LHRH content declined even further by 2 weeks after castration. Concentrations of LHRH in the portal blood of castrated rats and of LHRH in the serum of castrated ewes were reported to be increased (10, 11). The decreased content of hypothalamic LHRH after castration in the present study could reflect increased turnover rate. This also is indicated by the ob-

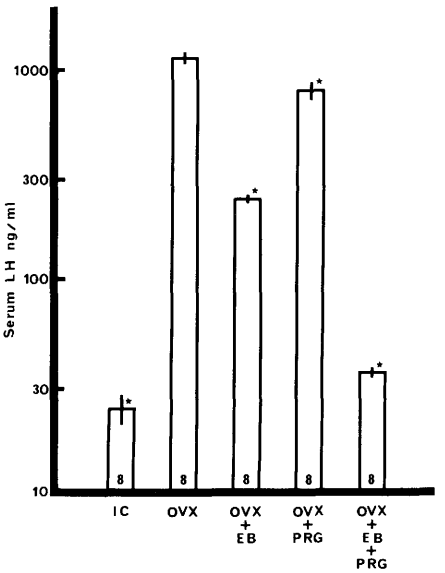

FIG. 5. Serum LH levels in the same female rats described in Fig. 4. The number inside each column indicates the number of rats per group. *Values are significantly different from the OVX group. Ordinate is given in log scales.

servation that castration decreased hypothalamic LHRH content but increased serum LH levels. After replacement with TP, hypothalamic LHRH content rose significantly over intact control values and serum LH was reduced to a level significantly lower than that in the intact controls. This reciprocal relationship suggests that TP might act on the hypothalamus to block LHRH release.

The "short-loop" feedback of LH on pituitary LH secretion was first proposed by Sawyer and Kawakami (12). In our study, hypophysectomy reduced the hypothalamic LHRH content to the same extent as castration or the combination of castration and hypophysectomy. The present data suggest that the reduction of hypothalamic LHRH after hypophysectomy is due mainly to atrophy of the testes and to loss of testosterone secretion.

Dahlén *et al.* (13) reported that hypothalamic LHRH content was correlated with body weight, and they suggested that the lower content of LHRH in hypophysectomized rats was due to loss of body weight. However, our results do not support their view, since TP treatment of hypophysectomized-castrated male rats raised LHRH content to that of intact rats, but did not return body weight to that of intact rats (213 ± 6 vs 336 ± 5 g). Although TP given to hypophysectomized-castrated male rats brought LHRH content back to intact levels, the values were significantly lower than in non-hypophysectomized-castrated rats treated with TP. This may indicate either that hypothalamic tissue was damaged by hypophysectomy or that pituitary hormones are involved in regulation of LHRH secretion.

Hypothalamic LHRH content in cycling female rats has been shown to exhibit changes during different stages of the estrous cycle. Araki *et al.* (14) found a significant reduction of LHRH in midhypothalamic areas at 1100 hr on proestrus. We therefore killed the female rats in our study at about 1000 hr in order to eliminate any variation in LHRH content. Three weeks after ovariectomy, LHRH content in the whole hypothalamus was decreased significantly, which agrees with the findings in the midhypothalamic region by Araki *et al.* (14). Daily treatment with EB alone for 1 week returned LHRH content back to the normal level, but PRG alone did not change hypothalamic LHRH content in long-term ovariectomized rats, which confirms earlier results obtained by bioassay in our laboratory. With a combination of EB and PRG, increased hypothalamic LHRH content was even higher than in the intact controls, although this difference was not significant. These observations strongly suggest that EB plays a dominant role in regulating LHRH secretion, and that PRG, if it exerts any effect at all, works synergistically with EB on LHRH secretion.

The postcastration rise in serum LH was inhibited by daily injection of either EB or 2.5 mg of PRG given for 1 week, even though the PRG effect was slight. The results on PRG do not agree with those reported by McPherson *et al.* (15), McCann (16), or Nallar *et al.* (17). They gave only PRG for 5 days or less, and this may account for the difference in results. The present studies provide further evidence that gonadal steroids can regulate LH secretion by acting on the hypothalamus to modulate LHRH levels. However, our data do not exclude the possibility that steroids also can act on the pituitary directly to modulate LH secretion, as reported by Ferland *et al.* (18).

Summary. The effect of castration (CAS), hypophysectomy (HYPOX), and gonadal steroids on hypothalamic luteinizing hormone-releasing hormone (LHRH) content was studied in male and female rats. Hypothalamic LHRH content was significantly reduced by 1 week after castration in male rats and was further reduced by 2 weeks. HYPOX decreased LHRH content in male rats to the same extent as in CAS rats alone, suggesting that loss of gonadal function was mainly responsible for the fall in hypothalamic LHRH in these rats. In castrated male rats testosterone propionate (TP) at a dose of 0.5 mg/300 g body weight raised hypothalamic LHRH content above that of intact rats and reduced serum LH below the intact level. However, in castrated-hypophysectomized rats, TP treatment only returned hypothalamic LHRH content to the intact level.

Ovariectomy (OVX) for 3 weeks decreased hypothalamic LHRH content significantly, whereas estradiol benzoate (EB, 1

μg) replacement for 1 week returned LHRH content to intact levels and reduced serum LH significantly. Progesterone (PRG) (2.5 mg) alone had no effect on hypothalamic LHRH content, but decreased serum LH levels slightly in ovariectomized rats. Treatment with a combination of EB and PRG raised hypothalamic LHRH content to levels even higher than those in intact rats, but this difference was not significant. These observations indicate that gonadal steroids can regulate LH secretion by acting on the hypothalamus to modulate LHRH secretion, and suggest that the effect of hypophysectomy in reducing LHRH content is due mainly to loss of testosterone secretion.

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