

Effect of Estradiol on the Response to LH-RH in Male Rats at Different Times After Castration¹ (37492)

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Pretreatment with estradiol benzoate will augment the LH release induced by LH-RH in normal diestrous rats (1, 2). In male rats, however, estradiol benzoate inhibited rather than stimulated LH and FSH release from the pituitary in response to LH-RH (3). Estradiol benzoate given together with testosterone propionate caused greater inhibition of the pituitary response to LH-RH in intact male rats (3). In view of these results, it was thought that the inhibition by estradiol benzoate of LH-RH induced gonadotropin release in male rats could be the result of the combined effects of exogenous estradiol plus endogenous testosterone prompted a study of castrated male rats.

Materials and Methods. Male rats of the Sprague-Dawley strain were used and kept under controlled constant temperature, with free access to Purina laboratory chow and tap water. The illumination schedule was 14 hr light and 10 hr darkness. The rats were castrated and injected sc with either 20 μ g estradiol benzoate (EB) or sesame oil (SO) at the following intervals after the operation: 1 day, 7 days, 1 month and 2 months. Forty-eight hours after the injection of sesame oil or estradiol benzoate, the rats were anesthetized with urethane (0.6 ml of a 25% solution/100 g body weight), and a blood sample was taken from the jugular vein of each rat. Both the control group treated with sesame oil, and the estradiol-treated group were divided into 2 subgroups, each of them injected iv with saline or pure synthetic LH-RH. In most of the groups, the dose of LH-RH was

0.1 μ g/rat, but in the group pretreated with sesame oil or estradiol 1 month after castration, the dose of LH-RH was 0.02 μ g/rat. Twenty minutes after the injection, the rats were bled from the jugular vein. The blood samples were kept under refrigeration overnight, centrifuged, and the sera were separated and kept frozen until assayed for gonadotropins. In each sample FSH and LH were assayed using the double-antibody radioimmunoassay as described by Daane and Parlow (4), and Niswender *et al.* (5), respectively. Serum FSH values were expressed as ng NIAMD-Rat FSH-RP1/ml and serum LH as ng NIH-LH-S₁₇/ml. The significance of the differences was tested by means of the Student's *t* test or the Duncan's new multiple range test (6).

Results. Serum LH and FSH levels progressively increased with time and after castration in the SO-treated control rats (Tables I and II). A single injection of 20 μ g EB significantly lowered the serum FSH and LH concentrations 48 hr later (Tables I and II) whereas the iv injection of saline did not significantly modify the FSH and LH levels in any of the groups studied. On the other hand, the injection of LH-RH induced a significant increase in serum LH in all the groups regardless of their pretreatment (Table I) and the intervals after castration. However, in the rats pretreated with EB 1 day after castration, the magnitude of the rise in serum LH after LH-RH injection was significantly lower than in the SO group. The pituitary response to LH-RH in terms of LH release in rats treated with EB 7 days after castration, was significantly higher than in the oil-treated rats. In the rats treated with EB 30 and 60 days after castration, the LH release after LH-RH injection was

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TABLE I. Effect of the Pretreatment with Estradiol Benzoate on the LH Release Induced by LH-RH in Castrated Male Rats.

Time after castration (day(s))	Pretreatment ^a	Injection	Dose	Serum LH levels		
				Before injection	After injection ^{b,c}	Increase ^d
1	Oil (6)	Saline	—	6.5 ± 1.9	3.8 ± 0.7	-2.5 ± 1.3
1	Oil (7)	LH-RH	0.1 µg/rat	3.4 ± 0.7	34.2 ± 5.0 (e)	30.7 ± 5.1
1	Estradiol (6)	Saline	—	0.10 ± 0.01	0.2 ± 0.1	0.10 ± 0.07
1	Estradiol (7)	LH-RH	0.1 µg/rat	0.1 ± 0.01	13.7 ± 2.2 (e)	13.6 ± 2.2 (d)
7	Oil (6)	Saline	—	26.3 ± 3.3	18.3 ± 3.7	-7.9 ± 2.8
7	Oil (6)	LH-RH	0.1 µg/rat	27.2 ± 3.9	97.6 ± 15.6 (b)	70.3 ± 12.1
7	Estradiol (6)	Saline	—	5.3 ± 0.4	4.6 ± 0.8	-0.7 ± 0.5
7	Estradiol (6)	LH-RH	0.1 µg/rat	3.8 ± 0.6	114.4 ± 13.1 (e)	110.2 ± 13.5 (e)
30	Oil (4)	Saline	—	36.4 ± 3.0	35.6 ± 4.2	-0.8 ± 2.1
30	Oil (5)	LH-RH	0.02 µg/rat	37.5 ± 6.5	94.1 ± 4.7 (e)	56.5 ± 6.2
30	Estradiol (4)	Saline	—	10.6 ± 0.4	10.2 ± 0.2	-0.3 ± 0.2
30	Estradiol (6)	LH-RH	0.02 µg/rat	9.9 ± 1.0	147.7 ± 15.9 (e)	138.7 ± 15.6 (e)
60	Oil (4)	Saline	—	49.2 ± 9.1	70.0 ± 17.5	20.7 ± 9.0
60	Oil (5)	LH-RH	0.1 µg/rat	47.4 ± 3.1	119.1 ± 29.5 (a)	73.9 ± 27.9
60	Estradiol (6)	Saline	—	15.2 ± 4.0	10.8 ± 1.8	-4.1 ± 2.2
60	Estradiol (5)	LH-RH	0.1 µg/rat	20.0 ± 6.7	181.0 ± 19.0 (e)	160.9 ± 17.6 (e)

^a Number of animals in parentheses.

^b Expressed as ng NIH-LH-S₁₇/ml ± SE.

^c The letters in parentheses indicate the following: (a) $p < 0.05$. (b) $p < 0.01$. (c) $p < 0.001$. (d) The LH increase after LH-RH was significantly smaller ($p < 0.01$) in the group treated with estradiol 1 day after castration than in the correspondent oil-treated group. (e) The LH increase after LH-RH was significantly greater ($p < 0.01$) in the groups treated with estradiol 7 days, 30 days, and 60 days after castration than in the respective oil-treated groups.

TABLE II. Effect of the Pretreatment with Estradiol Benzoate on the FSH Release Induced by LH-RH in Castrated Male Rats.

Time after castration (days)	Pretreatment ^a	Injection	Dose	Serum FSH level ^b		
				Before injection	After injection	Increase
1	Oil (6)	Saline	—	1.82 ± 0.15	2.00 ± 0.10	0.19 ± 0.01
1	Oil (7)	LH-RH	0.1 µg/rat	1.57 ± 0.12	2.27 ± 0.15 ^c	0.68 ± 0.15
1	Estradiol (6)	Saline	—	ND ^d	ND	—
1	Estradiol (7)	LH-RH	0.1 µg/rat	ND	0.40 ± 0.08	0.40 ± 0.08
7	Oil (6)	Saline	—	2.76 ± 0.37	2.47 ± 0.29	-0.29 ± 0.12
7	Oil (6)	LH-RH	0.1 µg/rat	1.94 ± 0.10	2.13 ± 0.20	0.25 ± 0.09
7	Estradiol (6)	Saline	—	1.15 ± 0.10	1.10 ± 0.09	-0.04 ± 0.06
7	Estradiol (6)	LH-RH	0.1 µg/rat	1.08 ± 0.07	1.78 ± 0.22	0.70 ± 0.21
30	Oil (4)	Saline	—	3.00 ± 0.37	2.82 ± 0.34	-0.46 ± 0.07
30	Oil (5)	LH-RH	0.02 µg/rat	3.50 ± 0.28	3.47 ± 0.1	-0.16 ± 0.06
30	Estradiol (4)	Saline	—	1.83 ± 0.07	1.83 ± 0.22	—
30	Estradiol (6)	LH-RH	0.02 µg/rat	2.20 ± 2.79	2.79 ± 0.15	0.62 ± 0.19
60	Oil (4)	Saline	—	2.87 ± 0.48	2.39 ± 0.32	-0.47 ± 0.23
60	Oil (5)	LH-RH	0.1 µg/rat	2.45 ± 0.18	2.97 ± 0.27	0.52 ± 0.22
60	Estradiol (4)	Saline	—	1.74 ± 0.14	1.89 ± 0.27	0.23 ± 0.22
60	Estradiol (5)	LH-RH	0.1 µg/rat	1.75 ± 0.21	2.69 ± 0.34	0.94 ± 0.17

^a Number of animals in parentheses.^b Expressed as µg NIAMD-FSH-RP1/ml ± SE.^c $p < 0.01$.^d ND = not done.

significantly greater than in the respective control groups treated with SO. On the other hand, LH-RH induced a significant release of FSH only in the group treated with SO 1 day after castration. In all other groups, although serum FSH levels tended to rise after the injection of LH-RH, they were not statistically significant as compared with those in the respective controls.

Discussion. In a previous investigation, estradiol benzoate was shown to inhibit the pituitary response to LH-RH in intact male rats (3). The present investigation indicates that administration of estrogen also inhibited the pituitary response to LH-RH in these rats shortly after castration. One week after castration or later, however, pretreatment with estradiol benzoate augmented the LH release induced by LH-RH as observed in female rats (2). The results suggest that the inhibitory effect of estradiol benzoate on the pituitary response to LH-RH in male rats is probably due to the interaction with endogenous testosterone and exogenously administered estrogen. If this is the case, the influence of testosterone appears to persist at least for a few days after castration. It was demonstrated by others that serum testosterone levels decreased rapidly after castration (7). However, it is possible that some of this steroid remains bound to the receptors or, at any rate, that the biochemical changes induced in the tissue persist for a certain period after testosterone had disappeared from the circulation (8, 9). It was demonstrated that the pituitary of castrated rats is able to take up considerable amounts of ^3H -testosterone and ^3H -5-dihydrotestosterone (10). The pituitary of intact immature rats also shows binding affinity for ^3H -testosterone (11). At longer intervals after the castration, when the influence of testosterone had completely disappeared, the pretreatment with estradiol may augment the pituitary response to LH-RH, as demonstrated in intact diestrous female rats (1).

Increments in serum FSH after LH-RH injection were insignificant, except in SO groups 1 day after castration. Insignificant stimulation of the release of FSH by LH-RH in castrated rats suggests that testosterone may facilitate FSH secretion under certain condi-

tions (12).

Summary. The effects of pretreatment with estradiol benzoate on the pituitary release of LH and FSH in response to LH-RH were investigated in male rats at various time intervals after castration. In oil-treated control rats, LH-RH brought about a significant increase in serum LH at all the periods after castration. One day after castration, pretreatment with estradiol significantly inhibited the response to LH-RH, as observed in normal male rats. One week after castration estradiol induced a greater response to LH-RH than observed in oil-treated control rats, although the difference was not significant. One and two months after castration, estradiol significantly increased the response to LH-RH.

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