Effect of Castration or Testosterone Treatment on Hypothalamic FSH-RF and Pituitary FSH of Male Rats* (33723)

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Hypothalamic extracts from several mammalian species stimulate the release of follicle stimulating hormone (FSH) from the pituitary gland both *in vivo* and *in vitro* (1). The active principle has been purified and separated from the other hypothalamic factors which stimulate or inhibit the release of other pituitary hormones. This principle was named the FSH-releasing factor (FSH-RF). In several of the hypothalamic releasing factors it was possible to demonstrate an alternation in the content of stored releasing factor in the hypothalamus in situations associated with an altered rate of secretion of the pituitary hormone affected (1). These findings suggest that altered rates of release of these hypothalamic factors mediate changes in secretion rate of various pituitary hormones. In the present communication, experiments are described in which the content of hypothalamic FSH-RF was studied in two conditions associated with altered FSH secretion. The two conditions were castration which is followed by enhanced FSH release and treatment with testosterone which inhibits FSH release.

Methods. In most experiments adult male rats of the Sherman strain (body wt. 204.8 ± 2.6 g) were used. They were housed under conditions of constant temperature and lighting (14 hr of light and 10 hr of darkness) and fed Purina chow diet. Groups of animals were castrated while anesthetized with ether. Normal rats served as controls for these experiments. Other groups were injected s.c. daily with testosterone (2 mg of testosterone propionate[3] in 0.1 ml of sesame oil) or with the oil vehicle. In a few experiments rats of the Sprague-Dawley strain were employed.

At sacrifice, organ weights were determined after removal of the anterior pituitary for FSH assay and of the stalk-median eminence (SME) for determination of FSH-releasing activity.

The FSH concentration in the pituitaries of the experimental animals was determined by the method of Steelman and Pohley (2) as modified by Parlow and Reichert (3) with a three-point design and total doses of 50 and 150 μg of the NIH FSH-S-3 reference preparation[4] and a single dose of pituitary extract prepared as described earlier (4).

The FSH-releasing activity of the hypothalamus of the experimental animals was determined by *in vitro* assay as previously described (4, 5). Results were expressed as micrograms of FSH released per milligram of anterior pituitary per hour. In each experiment the effect of SME extract from control rats was compared with that of SME extract from experimental animals. The FSH in the incubation media was assayed as described above.

Results. The effect of castration on hypothalamic content of FSH-RF. The FSH-releasing potency of SME extracts was examined at varying time intervals after castration in a total of 12 experiments (Table I, Fig. 1). One day after the operation there was a suggestion of increase in FSH-releasing activity in a single experiment as indicated by enhanced FSH release from the incubated

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TABLE I. Effect of Castration on Hypothalamic FSH-RF Activity.

Expt. no.	Time post-castration (days)	Dose of SME[a] (fraction/pit.)	Type of donor rat	FSH release (μg/mg of pit./6 hr)	λ[b]	% of control
1		1/16	Control	8.84 (7.24–10.80)[c]		
	1	1/16	Castrate	11.06 (8.52–14.46)	.095	125
2		1/8	Control	13.22 (10.14–17.22)		
	8	1/8	Castrate	11.74 (9.28–14.84)	.102	88
3		1/16	Control	9.64 (6.54–14.18)		
	8	1/16	Castrate	4.34 (3.10– 6.08)	.149	45[e]
4		1/16	Control	10.38 (8.58–12.56)		
	8	1/16	Castrate	10.64 (8.76–12.94)	.078	103
5		1/16	Control[d]	14.36 (11.31–18.26)		
	14	1/16	Castrate[d]	9.20 (7.03–12.05)	.100	64.2[f]
6		1/20	Control[d]	13.06 (9.29–18.36)		
	14	1/20	Castrate[d]	6.52 (5.28– 8.06)	.113	49.9[f]
7		1/16	Control	12.42 (10.44–21.00)		
	15	1/16	Castrate	11.66 (7.40–18.40)	.161	94
8		1/16	Control	9.12 (7.64–10.90)		
	15	1/16	Castrate	7.32 (6.26– 8.56)	.068	80[e]
9		1/20	Control	10.96 (8.64–13.94)		
	15	1/20	Castrate	8.60 (7.08–10.46)	.089	78[e]
10		1/16	Control	7.72 (5.18–11.48)		
	22	1/16	Castrate	6.58 (4.58– 9.44)	.156	87.6
11		1/16	Control	21.09 (16.34–27.17)		
	24	1/16	Castrate	15.45 (12.53–19.14)	.104	73.2[e]
12		1/16	Control	3.34 (2.40– 4.64)		
	29	1/16	Castrate	2.72 (1.96– 3.78)	.139	82

[a] SME = stalk-median eminence.
[b] λ = index of precision.
[c] Mean (95% confidence limits).
[d] SME from rats of Sprague-Dawley strain.
[e] $p < .05$ when compared with control.
[f] $p < .01$ when compared with control.

pituitaries, but this increase was not significant statistically. FSH-releasing activity was decreased in 10 of 11 experiments when estimated 8–29 days after castration. In the only case where a decrease was not observed, essentially no change was obtained in one of the 3 experiments at 8 days. This decrease observed in the other cases was significant in only 6 of the 10, but the overall mean decrease in all 11 experiments of 22 $\pm$ 4.8% in the quantity of FSH released was highly significant, even though small quantitatively. The greatest decrease appeared to occur at 14–15 days postcastration. At this time interval 4 of 5 decreases in FSH release were significant; however, the mean decrease of 27 $\pm$ 7% was not significantly different from that recorded at other times.

In order to determine if strain differences in rats would affect the result, 2 experiments with animals of the Sprague-Dawley strain were performed. These showed an even greater loss in hypothalamic FSH-RF activity 14 days after castration than was recorded in the rats of the Sherman strain. The quantity of FSH released by pituitaries incubated with hypothalamic extract from Sprague-Dawley castrates was significantly less than with control hypothalami in both instances; the mean decrease was 57%.

TABLE II. Effect of Castration on Pituitary FSH-Concentration and Content.

Expt. no.	Time post-castration (days)	Type of pituitary	Anterior pituitary wt. (mg)	Pituitary FSH	
				Concentration (μg/mg)	Content (μg/gland)
1		Control	5.82 ± 0.36[a]	49.09 (39.99–60.26)[b]	285.7 (232.7–350.7)[b]
	1	Castrate	6.12 ± 0.14	65.01 (51.88–81.47)[c]	397.8 (317.5–498.6)[c]
2		Control	6.32 ± 0.08	52.48 (42.27–65.16)	331.7 (267.1–411.8)
	8	Castrate	6.82 ± 0.41	40.83 (33.42–50.00)[c]	278.5 (227.9–341.0)
3		Control	7.25 ± 0.30	30.23 (24.62–37.10)	219.2 (178.5–269.0)
	14	Castrate	8.14 ± 0.28	23.92 (19.19–29.84)[c]	194.8 (156.2–242.9)
4		Control	7.60 ± 0.28	42.27 (34.75–51.40)	321.3 (264.1–390.6)
	15	Castrate	8.49 ± 0.30	40.55 (33.19–49.55)	344.3 (281.8–420.7)
5		Control	7.72 ± 0.30	43.85 (35.42–54.45)	338.5 (272.7–420.4)
	22	Castrate	8.86 ± 0.24	43.55 (36.54–53.21)	385.9 (315.9–471.4)

[a] Mean ± SEM.
[b] Mean (95% confidence limits).
[c] $p < .05$ versus control.

Pituitary FSH after castration. Castration was associated with a transient elevation ($p<.05$) in pituitary FSH concentration observable 1 day after operation (Table II). This was superceded by a fall ($p<.05$) in concentration at 8 days and a return to normal on measurement 15 and 22 days after removal of the testes. The increase in FSH at 1 day was also significant ($p<.05$) when calculated as content, but since pituitary weight increased after castration, the decrease in content at 8 days was not significant.

Effect of testosterone on hypothalamic FSH-RF. In contrast to the decrease in FSH-RF observed 8–29 days after castration, treatment of intact rats with 2 mg of testosterone propionate daily for 2 weeks, resulted in either no changes (3 experiments) or an increase ($p<.05$) (1 experiment) in the stored FSH-RF on comparison with the oil-injected controls (Table III). The mean FSH release in the 4 experiments was only 9% above that observed in the controls.

Effect of testosterone on pituitary FSH. Treatment with this steroid had no consistent significant effect on pituitary FSH, although a significant increase in both pituitary FSH concentration and content was obtained in 1 of the 3 cases (Table IV).

Effect of testosterone on sex accessory organ weight. As expected, a highly significant increase in weight of both seminal vesicles and ventral prostates was observed in each of the 3 experiments with testosterone in which these organs were weighted.

Discussion. It has been well documented that castration leads to an increase in FSH

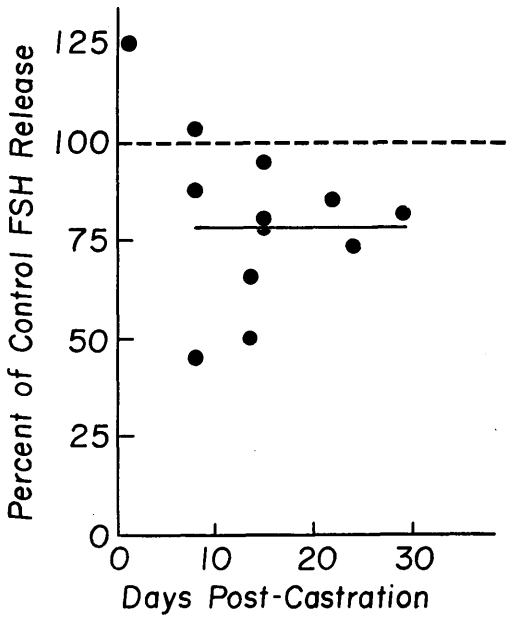

FIG. 1. Effect of castration on FSH-releasing activity of rat hypothalamus *in vitro*: solid horizontal line is the mean decrease in FSH-releasing activity of hypothalami from rats castrated for 8–29 days; each point represents a separate assay.

TABLE III. Effect of Testosterone on FSH-RF *in Vitro*.

Expt. no.	Dose of SME[a] (fraction/pit.)	Type of donor rat	FSH release (μg/mg of pit./6 hr)	λ[b]	% of control
1	1/8	Control	11.58 (8.76–16.10)[c]		
	1/8	Testosterone	10.84 (8.38–14.04)	.115	93.9
2	1/8	Control	12.78 (9.64–16.98)		
	1/8	Testosterone	11.44 (8.88–14.74)	.110	89.7
3	1/16	Control	14.50 (10.09–20.81)		
	1/16	Testosterone	12.44 (9.09–17.00)	.135	85.8
4	1/5	Control	17.62 (12.94–24.00)		
	1/5	Testosterone	29.37 (20.65–41.78)[d]	.139	166.7[d]

[a] SME = stalk-median eminence.
[b] λ = index of precision.
[c] Mean (95% confidence limits).
[d] $p < .05$ on comparison with control.

release as evidenced by increased plasma levels of this hormone, whereas treatment with testosterone causes an inhibition of its release (6). The altered level of testicular steroids presumably results in the altered rates of FSH release either by altering the rate of release of the hypothalamic FSH-RF or by affecting the pituitary FSH-secreting cells directly. The decreased content of hypothalamic FSH-RF in the castrate animal is consistent with the hypothesis that increased release of FSH-RF causes augmented FSH secretion in this condition. Apparently, resynthesis of FSH-RF does not keep up with enhanced release and a fall in content of stored factor results.

When FSH release is inhibited by testosterone, there was the converse tendency for FSH-RF to accumulate in the hypothalamus, presumably because of decreased release, but this effect was significant in only one of the 4 experiments.

Although the above interpretation of the data is the one favored by the authors, alternative interpretations are possible. Conclusive proof requires the demonstration that the rate of release of the FSH-RF is enhanced in castrates and lowered by testosterone. It may prove possible to obtain such evidence since recent data indicates that circulating releasing factors can be detected in peripheral blood of hypophysectomized rats (1, 7, 8).

The present data on FSHœRF content are at variance with those of Mittler and Meites (4), who recently reported a rise in hy-

TABLE IV. Effect of Testosterone on Pituitary FSH.

Expt. no.	Type of pituitary	Anterior pituitary wt. (mg)	Pituitary FSH	
			Concentration (μg/mg)	Content (μg/gland)
1	Control	7.68 ± 0.59[a]	36.73 (27.61–48.87)[b]	280.9 (212.2–375.9)[b]
	Testosterone	7.17 ± 0.24	48.87 (33.04–72.28)	351.0 (236.8–517.2)
2	Control	7.14 ± 0.29	47.42 (38.02–59.16)	338.2 (271.9–421.8)
	Testosterone	7.01 ± 0.23	64.12 (55.46–74.13)[c]	450.0 (388.7–519.9)[c]
3	Control	7.67 ± 0.23	50.14 (37.60–66.86)	384.6 (288.4–512.8)
	Testosterone	7.61 ± 0.32	50.00 (36.90–67.76)	380.5 (280.8–514.9)

[a] Mean ± SEM.
[b] Mean (95% confidence limits).
[c] $p < .05$ versus control.

pothalamic FSH-RF after castration and a fall with testosterone treatment. They used animals of the Sprague-Dawley strain as donors of hypothalami, and we first thought that a strain difference might account for the discrepancy between the results of the 2 groups. When rats of this strain were employed in 2 experiments, the decrease in FSH-RF observed after castration was, if anything, more pronounced. A different supplier was used by us than that employed by Mittler and Mietes and so it is still possible that a substrain difference may account for the difference in results observed. In any case it is clear that alterations in stored FSH-RF can be evoked by altering the level of androgens in the rat.

The tendency towards an elevated content of FSH in the pituitaries of the androgen-treated rats is in agreement with data from other laboratories (6). Apparently although release of FSH is suppressed in this situation, synthesis is less affected leading to a rise in FSH content. The elevation in pituitary FSH observed transiently after castration requires confirmation.

Castration produced a transient elevation of FSH concentration, followed by a decrease in concentration with return to base line in 15 days. These changes are in good agreement with the data of Steinberger and Duckett (9).

Summary. Following castration of adult, male rats there was a decline in hypothalamic FSH-RF, when assayed by an *in vitro* method, which was apparent within 8 days and persisted for at least 29 days postoperatively. When intact rats were treated with a large dose of testosterone propionate (2 mg/day) for 2 weeks, there was no consistent alternation in the level of stored FSH-RF. Castration and testosterone treatment were associated with alteration in weights of sex accessories, anterior pituitary weight, and FSH concentration. The decline in hypothalamic FSH-RF in castrates, at a time when FSH release is increased above normal levels, was considered to be consistent with the view that enhanced release of FSH-RF may play a role in augmenting FSH release in this condition.

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