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Effect of Short-Term Castration and Starvation upon Hypothalamic Content of Luteinizing Hormone-Releasing Hormone in Adult Male Rats[1] (39088)

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Hypothalamic regulation of the secretion of pituitary luteinizing hormone (LH) and follicle stimulating hormone (FSH) is mediated by the decapeptide, luteinizing hormone-releasing hormone (LH-RH) (1), which until recently was measured by bioassay or bioimmunoassay (2). Synthesis of LH-RH has permitted generation of specific antisera and development of radioimmunoassays capable of quantitating picogram amounts of this hormone (3–7). We report a radioimmunoassay for LH-RH developed in our laboratory and its application to the measurement of this hormone in hypothalamic extracts from normal adult male rats and from rats subjected to castration and/or starvation.

Materials and methods. Rabbit antiserum to Wyeth synthetic LH-RH conjugated to bovine serum albumin (BSA) (8) was prepared in rabbits (9) and utilized in a non-equilibrium, double antibody radioimmunoassay at a final concentration of 1:17,500. Structure-specificity analyses suggest that the antiserum to LH-RH is directed against amino acids 5 through 9 of the decapeptide sequence (-Try-Gly-Leu-Arg-Pro-).[2] Beckman synthetic LH-RH (Lot #01701) was employed as standard for dose interpolation and as tracer after labeling with ^{125}I and purification on a column of Sephadex G-25 (fine) (3). Unreacted, free ^{125}I eluted from this column before ^{125}I-LH-RH (6). The assay buffer, 0.01 *M* phosphate–0.15 *M* NaCl, pH 7.5, containing 1% BSA, was used to dilute standards and hypothalamic extracts. Buffer, standard, or hypothalamic extract and anti-LH-RH serum were incubated at 4°C for 48 hr prior to addition of ^{125}I-LH-RH (7500 cpm = 8 pg). The incubation was continued for 48 hr, and antiserum to rabbit gamma globulin was added. Seventy-two hours later the tubes were centrifuged (30 min, 3000 rpm, 4°C), the supernatant decanted, and the radioactivity in the precipitates counted in an automatic gamma counter. Serum concentrations of LH and FSH were determined by double antibody radioimmunoassays (10). The logit-log method of Rodbard *et al.* (11) was employed for analysis of radioimmunoassay data. The unpaired *t* test was utilized to compare different groups of animals.

The experimental design of this study and the serum LH and FSH data have been reported previously in a study which described the effects of exogenous LH-RH upon pituitary secretion of LH and FSH *in vitro* (12). The hypothalamic content of LH-RH of the animals whose pituitaries were utilized in the foregoing report has now been quantitated. Adult male rats, maintained at 23°C, 14 hr light:10 hr darkness, were orchiectomized or maintained intact. After surgery they were divided into groups permitted to eat and drink *ad lib.* or deprived of chow but permitted free access to tap water. Seven days after castration the animals were sacrificed by decapitation. The mid-portion of the hypothalamus bounded by the posterior border of the optic chiasm anteriorly, the lateral hypothalamic sulci, the anterior border of the mammillary bodies posteriorly, and the third ventricle superiorly was dissected, immediately frozen and stored at −20°C. This section of hypothalamus contained the median eminence, ventromedial and arcuate nuclei where the majority of hypothalamic LH-RH is found (7). Shortly before assay the hypothalamic fragment was thawed and extracted in absolute methanol. After centrif-

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[2] S. Kaplan, M. Aubert, and M. Grumbach, personal communication.

ugation the methanol extract was decanted and dried under an air stream. The residue was reconstituted in buffer immediately before introduction of graded doses into the radioimmunoassay.

Results. Fig. 1 depicts a logit-log plot of the standard curve for LH-RH and demonstrates that graded doses of hypothalamic extracts from intact, castrated, and starved animals inhibit the binding of tracer LH-RH to anti-LH-RH serum in a manner parallel to that of the synthetic standard. Introduction of increasing amounts of tracer LH-RH into the assay also gives a response that is parallel to that obtained with increasing amounts of cold standard, indicating that labeling of LH-RH did not alter its immunologic characteristics. Ninety percent of synthetic LH-RH added to cerebral cortex was recovered by the extraction procedure. LH-RH was not detected by radioimmunoassay in methanol extracts of rat cerebral cortex or pituitary, nor did the anti-LH-RH serum bind rat LH, FSH, growth hormone, prolactin or thyrotropin, adrenocorticotropin 1–24, synthetic somatostatin, or thyrotropin-releasing hormone. The least detectable quantity of LH-RH was 5–10 pg per tube; the 50% binding point (B_{50}) of the assay was 92 pg. The intraassay coefficient of variation of the assay was 10%. All samples in this experiment were determined in the same assay in order to avoid interassay variability.

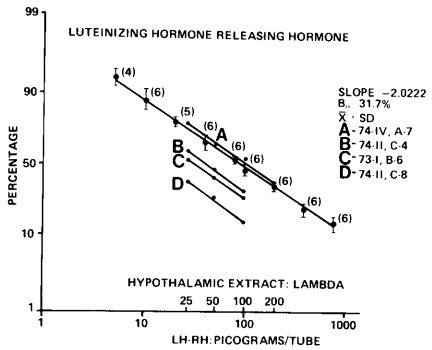

FIG. 1. Standard curve for synthetic LH-RH. Methanol extracts of hypothalami from adult male rats cross-react in the radioimmunoassay in a manner parallel to that of the Beckman standard.

The hypothalamic content of LH-RH was significantly lower in castrated animals, whether fed or starved, than in intact rats (Table I). There was no difference between the hypothalamic content of LH-RH in *intact* fed or starved animals, nor between *castrated* fed or starved rats. Serum concentrations of LH and FSH were significantly higher in castrated animals regardless of their nutritional status.

Discussion. Hypothalamic content of immunoreactive LH-RH declined within 7 days after castration in adult male rats. Other investigators (5, 6, 13) have reported that the hypothalamic levels of immunoreactive and bioassayable LH-RH in adult male rats were significantly reduced 21 and 60 days

TABLE I. EFFECTS OF CASTRATION AND STARVATION UPON HYPOTHALAMIC CONTENT OF LH-RH

	Body weight[a] (g)	Serum LH[a] (ng/ml)	Serum FSH[a] (ng/ml)	Hypothalamic LH-RH (ng/ hypothalamus)
Ad lib. fed				
Intact (12)	353.2[b]	25.3	379.7	1.68
	5.3	5.1	39.0	0.19
		$P < 0.01$	$P < 0.01$	$P < 0.01$
Castrated (14)	300.4	94.9	893.9	0.67
	4.6	10.0	64.2	0.07
Starved				
Intact (12)	208.0	27.6	194.4	1.82
	4.4	4.8	24.2	0.17
		$P < 0.01$	$P < 0.01$	$P < 0.01$
Castrated (12)	205.7	167.6	1173.4	0.69
	3.8	27.4	110.4	0.09

[a] Data previously reported (12).
[b] $\bar{x} \pm 1$ SEM.

after castration, and that hypothalamic content of LH-RH declined within 7 days after ovariectomy of adult female rats. Previous studies in castrated rats have demonstrated that the rate of incorporation of ^{3}H-tyrosine into bioassayable LH-RH in hypothalamic extracts *in vitro* was increased (14), and that the concentration of LH-RH was elevated in hypophysial portal blood and in the serum of castrated ewes (15, 16). These observations suggest that the turnover and secretion of LH-RH are increased in castrated animals and that the decreased content of hypothalamic LH-RH reflects greater utilization rather than diminished synthesis. Moguilevsky *et al.* (13) observed an increase in bioassayable LH-RH after incubation of hypothalami from orchiectomized but not intact rats and suggested that this represented increased synthesis of LH-RH. Kuhl and Taubert (17, 18) have reported recently that the activity of hypothalamic L-cystine arylamidase, which degrades LH-RH, was increased by administration of LH. The lower hypothalamic content of LH-RH in castrated animals may also reflect accelerated degradation of this hormone.

Short-term starvation did not alter hypothalamic levels of LH-RH in either intact or castrated animals, an unexpected finding inasmuch as hypogonadotropic hypogonadism is well documented in states of chronic nutritional deprivation (19). Other studies have demonstrated that bioassayable hypothalamic FSH-releasing factor activity and serum concentrations of FSH were decreased in intact starved animals (10, 12, 20) and that short-term starvation did not inhibit pituitary gonadotrope secretory responsiveness to synthetic LH-RH *in vivo* or *in vitro* (12). Failure of the present study to demonstrate an effect of starvation upon hypothalamic content of LH-RH may reflect a too-brief period of starvation, selective inhibition of LH-RH release from the median eminence, or decrease in its rate of degradation. Alternatively, starvation may alter pituitary gonadotrope sensitivity to LH-RH, or there may be an FSH releasing factor distinct from LH-RH (21), which is specifically affected by starvation. Lastly, inanition may affect pituitary hormone secretion directly at the pituitary level.

Summary. A sensitive and specific radioimmunoassay for hypothalamic LH-RH has been described. Within 7 days after castration there is a significant decline in hypothalamic content of LH-RH in adult male rats. Total starvation for 7 days does not affect hypothalamic content of LH-RH in either intact or castrated rats.

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1. Schally, A. V., Arimura, A., and Kastin, A. J., *Science* **179**, 341 (1973).
2. Malacara, J., Seyler, L. E., Jr., and Reichlin, S., *J. Clin. Endocrinol. Metab.* **34**, 271 (1972).
3. Nett, T. M., Akbar, A. M., Niswender, G. D., Hedlund, M. J., and White, W. F., *J. Clin. Endocrinol. Metab.* **36**, 880 (1973).
4. Jones, H. A., Burger, H. G., Cumming, I. A., Findlay, J. K., and deKretser, D. M., *J. Clin. Endocrinol. Metab.* **96**, 384 (1974).
5. Shin, S. H., Howitt, C., and Milligan, J. V., *Life Sci.* **14**, 2491 (1974).
6. Araki, S., Ferin, M., Zimmerman, E. A., and Vande Wiele, R. L., *Endocrinology* **96**, 644 (1975).
7. Palkovits, M., Arimura, A., Brownstein, M., Schally, A. V., and Saavedra, J. M., *Endocrinology* **95**, 554 (1974).
8. Goodfriend, T. L., Levine, L., and Fasman, G. D., *Science* **144**, 1344 (1964).
9. Vaitukaitis, J., Robbins, J. B., Neischlag, E., and Ross, G. T., *J. Clin. Endocrinol. Metab.* **33**, 988 (1971).
10. Root, A. W., and Russ, R. D., *Acta Endocrinol. (Kbh)* **70**, 665 (1972).
11. Rodbard, D., Bridson, W., and Rayford, P. L., *J. Lab. Clin. Med.* **74**, 770 (1969).
12. Root, A. W., and Duckett, G. E., *Proc. Soc. Exp. Biol. Med.* **144**, 30 (1973).
13. Moguilevsky, J. A., Scarchi, P., Debeljuk, L., and Faigon, M. R., *Neuroendocrinology* **17**, 189 (1975).
14. Moguilevsky, J. A., Enero, M. A., and Szwarefarb, B., *Proc. Soc. Exp. Biol. Med.* **147**, 434 (1974).
15. Ben-Jonathan, N., Mical, R. S., and Porter, J. C., *Endocrinology* **93**, 497 (1973).

16. Nett, T. M., Akbar, A. M., and Niswender, G. D., *Endocrinology* **94**, 713 (1974).
17. Kuhl, H., and Taubert, H. D., *Acta Endocrinol. (Kbh)* **78**, 634 (1975).
18. Kuhl, H., and Taubert, H. D., *Acta Endocrinol. (Kbh)* **78**, 649 (1975).
19. Root, A. W., *J. Pediat.* **83**, 187 (1973).
20. Negro-Vilar, A., Dickerman, E., and Meites, J., *Fed. Proc.* **27**, 269 (1968).
21. Igarashi, M., Taya, K., and Ishikawa, J., *in* "Psychoneuroendocrinology" (N. Hatotani ed.), p. 178. Karger, Basel (1974).

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