In Vivo and *in Vitro* Effects of Synthetic Luteinizing Hormone-Releasing Hormone (LH-RH) Upon the Secretion of Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) in Intact and Castrated, Fed and Starved Adult Male Rats[1] (37520)

ALLEN W. ROOT AND GREGORY E. DUCKETT,
with the technical assistance of HOMA KAMALI

Pediatric Research Laboratory, Division of Pediatrics, Albert Einstein Medical Center, and the Department of Pediatrics, Temple University School of Medicine, Philadelphia, Pennsylvania 19141

The secretion of LH and FSH is altered by starvation in humans and in laboratory animals (1–3). In man, starvation inhibits gonadotropin secretion (2), while increasing the secretion of growth hormone (4). We have previously demonstrated that starvation depresses serum concentrations of FSH in intact male rats and may suppress serum LH values in castrated animals (3). Other investigators have demonstrated that hypothalamic follicle stimulating hormone releasing factor activity and growth hormone-releasing factor activity are suppressed by inanition (5, 6). In order to determine whether there may also be altered pituitary responsiveness to hypothalamic stimulation during starvation, we have studied the effects of synthetic hypothalamic LH-RH upon the secretion of LH and FSH *in vivo* and *in vitro* in adult male rats starved for seven days. These observations form the basis of the present report.

Materials and Methods. Adult male rats (Charles River) were maintained in controlled environmental conditions of temperature (73°F) and light (14 hr of light, 10 hr of darkness daily) and permitted free access to standard laboratory rat chow (Purina) and tap water. After acclimatization the animals were either maintained intact or orchiectomized under light ether anesthesia. Thereafter, the animals were divided randomly into "fed" and "starved" groups. The former were permitted to feed and drink *ad libitum* while the latter were deprived of all chow for 168 hr while permitted free access to tap water. At the completion of this period, in *in vivo* experiments, the animals were anesthetized with urethane ip (150 mg/100 g B.W.). After cardiac puncture, in order to obtain baseline specimens, the internal jugular vein was exposed and synthetic LH-RH,[2] 0.1 μg/100 g B.W. diluted to 0.5 ml with normal saline, was injected into the jugular vein over one minute. Animals were decapitated either 15 or 45 min after injection of LH-RH and blood was collected from the trunk. Serum was stored at —20° until assayed.

In *in vitro* experiments at the completion of the period of starvation the animals were decapitated without anesthesia; trunk blood was collected. The pituitary was immediately removed and the anterior pituitary separated from the posterior. The former was incubated in capped Ehrlenmeyer flasks in 1 ml KRBG (7) for 1 hr; the medium was then changed—alternate pituitaries received 1 ml of KRBG or 1 ml of KRBG containing synthetic LH-RH[3] 0.1 μg/ml. Incubation was then continued for 5 hr. Incubations were carried out at 37° in a Dubnoff metabolic shaker; each flask was gassed with 5%

[1] Supported by U.S. Public Health Service Grant HD 04840.

[2] Synthetic LH-RH (C1-785, Lot Q) was kindly provided by Dr. J. R. Reel, Parke, Davis & Co., Ann Arbor, Michigan 48106. It was dissolved in normal saline and stored in small aliquots at —20° until employed in the studies cited.

[3] See footnote 2.

TABLE I. Effect of Synthetic LH-RH Upon Secretion of LH and FSH in Intact and Castrated, Fed and Starved Adult Male Rats *in Vivo*.

		Luteinizing hormone (ng/ml)			Follicle stimulating hormone (ng/ml)		
	Body wt (g)	Base	+15 min	+45 min	Base	+15 min	+45 min
Intact							
Fed	342.0 (11)[a,d]	17.3 (4)	73.2 (5)	63.4 (5)	—	313.2 (5)	387.9 (5)
	3.1	1.7	23.4	19.2		17.3	30.0
			> [b]				> [b]
Starved	191.5 (13)	—	55.7 (6)	22.5 (6)	—	241.3 (6)	210.0 (6)
	3.0		13.5	5.2		26.5	36.2
Castrated							
Fed	318.6 (16)	146.8 (8)	397.8 (7)	359.2 (7)	883.8 (11)	858.6 (7)	958.5 (7)
	4.7	32.1	28.6	38.8	28.2	48.9	54.6
			> [b]		> [c]	> [b]	> [b]
Starved	212.2 (16)	235.2 (2)	713.9 (7)	458.8 (8)	1315.0 (2)	1759.6 (7)	1463.2 (8)
	3.3	54.0	68.4	59.3	535.5	118.9	75.8

[a] Mean ± SEM.
[b] $p < 0.01$.
[c] $p < 0.05$.
[d] Number of animals.

CO_2 in air at −1, 0, +1 and +3 hr. Upon termination of the second incubation, the incubate was stored at −20° until assayed. The pituitary halves were weighed to 0.1 mg.

Serum and incubate concentrations of LH and FSH were determined by double antibody radioimmunoassays employing reagents provided by the Hormone Distribution Officer, NIAMDD, NIH. Characteristics of these assays have been described (3). Statistical analysis was performed employing the unpaired Student's *t* test and Monroe Programmable Calculator 1785 protocol 3011-S.

Results. Intrajugular administration of synthetic LH-RH to intact–fed rats was followed by a 4.2-fold increase in serum LH concentrations (Table I). There were insufficient specimens for baseline analysis of serum LH values in intact–starved animals; however, serum LH concentrations at +15 and +45 min after LH-RH injection were not significantly different from control values. Previous data (3) and the data in Table II indicate that resting serum levels of LH in intact–fed and –starved male rats are similar; therefore, one may conclude that synthetic LH-RH stimulated release of LH in both intact–fed and intact–starved adult male rats. In these animals, baseline data for FSH concentrations were not available due to paucity of specimens. However, previous data (3) and data in Table II indicate that serum FSH values are reduced in intact–starved male rats. LH-RH did not appear to stimulate the secretion of FSH in either fed or starved intact animals *in vivo*.

In castrated animals synthetic LH-RH stimulated a 2.7-fold increase in serum LH concentrations in fed animals and a 3-fold increase in starved rats. Serum LH levels +15 min after administration of LH-RH were significantly higher in castrated–starved animals than in castrated–fed rats. Baseline serum FSH concentrations were slightly higher in castrated, starved animals than in control animals and did not change appreciably after administration of synthetic LH-RH.

In vitro synthetic LH-RH significantly enhanced the secretion of both LH and FSH from pituitaries of intact and castrated, fed and starved animals (Table II). In intact animals, the basal release of LH and FSH expressed per milligram of pituitary weight was similar for fed and starved rats; how-

TABLE II. Effect of Synthetic LH-RH upon Release of LH and FSH from Pituitaries of Intact and Castrated, Fed and Starved Adult Male Rats *in Vitro*.

		Luteinizing hormone			Follicle stimulating hormone		
			Pituitary			Pituitary	
	Body weight (g)	Serum (ng/ml)	μg/5 hr	μg/mg /5 hr	Serum	μg/5 hr	μg/mg /5 hr
Intact							
Fed	353.2 (12)[a]	25.3 (12)	13.2 (6)	1.74	379.7 (12)	16.5 (6)	2.20
	5.3	5.1	1.3	0.34	39.0	1.9	0.48
			>[b]	>[c]		>[b]	
			29.1 (6)[e]	*3.25*		*25.8 (6)*	*2.86*
			2.9	*0.39*		*1.3*	*0.19*
Starved	208.0 (12)	27.6 (12)	12.8 (6)	2.15	194.4 (12)	13.7 (6)	2.25
	4.4	4.8	0.8	0.24	24.2	1.8	0.26
			>[b]	>[b]		>[b]	>[b]
			29.4 (6)	*4.72*		*26.8 (6)*	*4.45*
			2.2	*0.33*		*3.0*	*0.56*
Castrated							
Fed	300.4 (14)	94.9 (14)	9.3 (7)	1.23	893.9 (14)	14.6 (7)	1.93
	4.6	10.0	1.5	0.21	64.2	1.4	0.22
			>[b]	>[b]		>[b]	>[b]
			31.0 (7)	*4.00*		*24.9 (7)*	*3.19*
			4.5	*0.50*		*2.2*	*0.20*
Starved	205.7 (12)	167.6 (11)	10.9 (6)	2.12	1173.4 (11)	13.2 (6)	2.57
	3.8	27.4	1.0	0.14	110.4	2.1	0.40
			>[b]	>[b]		>[b]	>[c]
			36.9 (5)	*6.80*		*25.8 (5)*	*4.74*
			4.8	*1.0*		*2.9*	*0.63*

[a] Mean ± SEM.
[b] $p < 0.01$.
[c] $p < 0.02$.
[d] $p < 0.05$.
[e] Italics indicate pituitaries were incubated in the presence of synthetic LH-RH (see text).

ever, synthetic LH-RH released significantly more LH and FSH per milligram of pituitary weight from pituitaries of starved animals than from those of fed rats. The basal secretion of LH (per milligram of pituitary weight) but not of FSH was significantly greater from pituitaries of castrated–starved rats than from pituitaries of castrated–fed animals. Synthetic LH-RH stimulated the release of significantly more LH and FSH per mg of pituitary weight from pituitaries of castrated-starved rats than from pituitaries of castrated–fed animals.

Discussion. Present data indicate that *in vivo* the pituitary of the starved rat whether intact or castrated, is as sensitive to the LH releasing activity of synthetic LH-RH as is the pituitary of the normally fed animal. Failure of this preparation of synthetic LH-RH to release FSH *in vivo* either in intact or castrated–fed or –starved animals is unexplained. However, Arimura *et al.* (8) demonstrated that FSH-releasing activity of LH-RH (employing even larger quantities than used herein) was significantly greater when it was infused over a period of several hours than when it was administered as a bolus injection. The preparation of synthetic LH-RH employed in these experiments clearly stimulated the release of FSH *in vitro*. The enhanced secretion of LH and FSH during incubation with LH-RH when the data are ex-

pressed per milligram of pituitary weight reflects the smaller size of the pituitary in the starved animal. Weigelmann and Solbach (9) reported that there was normal LH and FSH secretion following administration of synthetic LH-RH in 4 out of 5 females with anorexia nervosa and secondary amenorrhea.

These data support the concept that the hypothalamus is more vulnerable to the affects of acute starvation than is the hypophysis and indicate that the aberrations of gonadotropin secretion recorded during inanition do not primarily reflect abnormal pituitary responsiveness to hypothalamic stimulation.

Summary. Serum LH concentrations increased significantly following the administration of synthetic LH-RH *in vivo* in both intact and castrated, normally fed and starved adult male rats. Synthetic LH-RH failed to affect the serum concentration of FSH in the same animals. *In vitro,* basal and LH-RH-induced secretion of LH and FSH from pituitaries donated by intact and castrated, fed and starved rats were similar. The data indicate that the pituitary of the starved adult male rat is as sensitive to the gonadotropin-releasing effects of synthetic LH-RH as is the pituitary of the normally fed rat both *in vivo* and *in vitro.*

1. Perloff, W. H., Lasche, E. M., Nodine, J. H., Schneeberg, N. G., and Viellard, C. B., J.A.M.A. **155**, 1307 (1954).

2. Leathem, J. H., J. Anim. Sci. Suppl. **25**, 68 (1966).

3. Root, A. W., and Russ, R. D., Acta Endocrinol. **70**, 665 (1972).

4. Pimstone, B. L., Barbezat, G., Hansen, J. D. L., and Murray, P., Amer. J. Clin. Nutr. **21**, 482 (1968).

5. Negro-Vilar, A., Dickerman, E., and Meites, J., Fed. Proc. **27**, 269 (1968).

6. Dickerman, E., Negro-Vilar, A., and Meites, J., Endocrinology **84**, 814 (1969).

7. Borgett, P., Chavancy, C., DuPont, A., Labrie, F., Arimura, A., and Schally, A. V., Proc. Nat. Acad. Sci USA **69**, 2677 (1972).

8. Arimura, A., Debeljuk, L., and Schally, A. V., Endocrinology **91**, 529 (1972).

9. Wiegelmann, W., and Solbach, H. G., Horm. Metab. Res. **4**, 404 (1972).

Received April 25, 1973. P.S.E.B.M., 1973, Vol. 144.