LH Release by LH-Releasing Hormone in Golden Hamsters at Various Stages of Estrous Cycle[1] (36514)

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It is well known that ovulation is preceded by a surge of luteinizing horomone (LH) secretion in rats (1), hamsters (2), pigs (3), sheep (4), monkeys (5) and human beings (6). In the normal rat, administration of LH-releasing hormone (LH-RH) induces a significant rise of serum LH, but the magnitude is usually considerably smaller than that observed at the time of preovulatory surge (7). We have demonstrated that the pituitary response to LH-RH is augmented by estrogen (8) and suppressed by progesterone or progesterone plus estrogen (9). Since the serum estrogen level is elevated when the preovulatory surge of LH occurs (10–13), and progesterone secretion increases after ovulation (5, 13), it has been considered that various levels of these steroid hormones in the circulation may influence the pituitary responsiveness to LH-RH (14). Since secretion rates of ovarian steroids vary during the estrous cycle, it is possible that the pituitary responsiveness to LH-RH may also vary at different stages of the cycle. Antunes-Rodrigues, Dhariwal and McCann (15) investigated LH release in cycling rats using crude sheep hypothalamic extracts followed by bioassay for LH, but they found little evidence for such cyclic fluctuations. On the other hand, we observed that, in the sheep, the greatest LH release was induced when LH-RH was injected on day 1 of estrus (16). The failure to observe significant fluctuations in the pituitary responsiveness to LH-RH in the study by Antunes-Rodrigues, Dhariwal and McCann (15) may be due to use of an insensitive assay method for measuring serum LH. Moreover, since LH-RH may be very quickly inactivated *in vivo*, it may be necessary to administer a relatively large dose of LH-RH in order to maintain a concentration which is sufficient to stimulate a sizeable release of LH. In the present study, we performed a similar study in the golden hamster, which shows a very regular 4-day cycle and whose stage of estrus is easily determined by checking the vaginal discharge.

Materials and Methods. Female golden hamsters (Con Olson Co., Inc.) were used throughout the experiment. All animals were housed in animal quarters equipped to provide controlled light (5 a.m. to 7 p.m. light; 7 p.m. to 5 a.m., dark) and temperature. Free access to Purina Chow biscuits and water was provided. The estrous cycle which occurred every 4 days in most of the animals (17) was investigated by checking the vaginal discharge. Experiments were started after the animals showed two regular 4-day cycles. At 1:00 p.m., all hamsters were injected intraperitoneally with 13 mg of phenobarbital/100 g body wt in order to block the spontaneous surge of LH release in proestrus (18) and to make the experimental conditions uniform for the whole group. Experimental animals received various iv doses of natural or synthetic LH-RH dissolved in 0.2 ml 0.9% saline between 3:00 to 4:00 p.m. Controls animals received 0.2 ml 0.9% saline. Animals were bled from the aorta 15 min after the injection. Serum was separated by centrifugation and kept at −20° until it was assayed for LH. Serum LH was determined by radioimmunoassay as described by Goldman and

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TABLE I. Serum LH Levels After iv Injection of Saline or LH-RH at Various Stages of Estrous Cycle in Golden Hamsters Pretreated with Phenobarbital.

Group	Stage of estrous cycle	Material injected	Dose of LH-RH(μg)	Mean LH $\pm$ SE (ng/ml)[a]	p[b]
A					
1	Diestrus 1	Saline	—	0.6 $\pm$ 0.20 (4)[c]	—
2		Natural LH-RH	0.1	15.7 $\pm$ 1.56 (4)	—
3	Diestrus 2	Saline	—	0.8 $\pm$ 0.29 (4)	
4		Natural LH-RH	0.1	23.4 $\pm$ 2.37 (4)	vs 2: .01
5	Proestrus	Saline	—	2.0 $\pm$ 0.28 (4)	vs 1: NS
6		Natural LH-RH	0.1	31.5 $\pm$ 4.18 (4)	vs 2: .01 vs 4: .01 vs 8: .05
7	Estrus	Saline	—	0.9 $\pm$ 0.05 (4)	
8		Natural LH-RH	0.1	24.0 $\pm$ 8.90 (3)	vs 2: .01 vs 4: NS
B					
1	Diestrus 1	Saline	—	0.7 $\pm$ 0.24 (4)[c]	—
2		Synthetic LH-RH	0.4	20.6 $\pm$ 3.12 (7)	
3	Diestrus 2	Saline	—	1.1 $\pm$ 0.18 (4)	
4		Synthetic LH-RH	0.4	41.3 $\pm$ 2.74 (7)	vs 2: .01
5	Proestrus	Saline	—	1.2 $\pm$ 0.23 (4)	
6		Synthetic LH-RH	0.4	108.3 $\pm$ 8.01 (7)	vs 2: 0.1 vs 4: .01 vs 8: .01
7	Estrus	Saline	—	1.4 $\pm$ 0.23 (4)	—
8		Synthetic LH-RH	0.4	17.7 $\pm$ 3.11 (7)	vs 2: NS vs 4: .01

[a] Expressed as NIH-LH-S-17.
[b] Duncan's new multiple range test.
[c] Numbers of animals in the group.

Porter (2) using anti-ovine LH, No. 15, prepared by Dr. G. Niswender. Each serum sample was assayed in duplicate and the mean LH concentration, expressed in terms of NIH-LH-S-17, was calculated. Mean LH concentration in each group was compared with that of other groups by Duncan's new multiple range test (19). Natural pure LH-RH (AVS 77-33 No. 219-269) was prepared from pig hypothalamic tissue as described elsewhere (20). This preparation was active in releasing LH at 0.25 ng in ovariectomized rats pretreated with estrogen and progesterone. Synthetic LH-RH (lot R3) was prepared by Dr. R. Geiger (Farbwerke Hoechst, Frankfurt/M., W. Germany) (21). The LH-RH potency of this synthetic LH-RH is essentially the same as that of the natural pure LH-RH (AVS 77-33 No. 219-269) (22).

Results. Serum LH levels 15 min after injection of 0.9% saline were the same in the phenobarbital-treated hamsters throughout the estrous cycle, although they tended to rise on the day of proestrus in one of the experiments (Table I). This indicates a nearly complete block of preovulatory surge of LH by phenobarbital. Intravenous administration of 0.1 μg natural LH-RH increased serum LH significantly at all stages of the cycle, as compared with the values after saline injection (Table IA). The LH levels after LH-RH were lowest on the first day of diestrus and highest on the day of proestrus. Mean LH levels after LH-RH on the day of estrus was higher than that on the first day of diestrus, but individual LH values in estrus varied a great deal as indicated by a large standard error of the mean (Table IA). In an attempt to ob-

serve clearer trends in response to LH-RH, a larger dose, 0.4 μg of synthetic LH-RH and a larger number of animals per group were utilized in the next experiment. The results are shown in Table IB. Again serum LH levels after 0.9% saline injection remained at low levels throughout the cycle. The highest response to LH-RH was observed in proestrus. LH levels after LH-RH at this time were significantly higher than those at any other stage of the cycle. The second highest response was observed on the second day of diestrus. The lowest response was seen on diestrus day 1 and at estrus.

Discussion. The results clearly demonstrate that pituitary response to LH-RH differed at various stages of the estrus cycle. As expected, the greatest response to LH-RH was observed in proestrus, indicating that the pituitary LH cells are most sensitive to LH-RH at that time. We have previously demonstrated that pretreatment with estrogen augmented the response to LH-RH in the rat (8). Progressive increase in estrogen secretion from the ovaries, detectable in the morning of proestrus in the rat (10, 11), which probably occurs in the hamster as well, may possibly play a role in increasing the pituitary responsiveness to LH-RH in the afternoon of proestrus. The second greatest LH release after LH-RH was seen on the second day of diestrus. In late diestrus, the estrogen secretion may be increasing, although the rise is too small to be measured by currently used methods. In the experiment with natural LH-RH (Table IA), some of the hamsters in estrus showed a good response to LH-RH, but some showed poor responses. Such variation of pituitary responsiveness in estrus could be related to differences in levels of ovarian steroids in the circulation. It should be noted that combined administration of estrogen and progesterone showed the most powerful suppression on LH-RH-induced LH release in normal rats (9). The lowest response observed on diestrus day 1 could be explained by the suppressive effect of estrogens plus progestins (9, 14) which are secreted by the newly formed corpus luteum. Recently a similar type of pituitary response to exogenously injected LH-RH was observed in women who showed the greatest responses near midcycle and the lowest during the midluteal stage (Arimura, unpublished data). More accurate information about the interrelationship between steroid secretion and the pituitary responsiveness to LH-RH could be obtained by simultaneous determination by sensitive and specific methods of levels of endogenous ovarian steroids and LH-RH-induced LH release.

Summary. Stimulation of LH release by administration of natural or synthetic luteinizing hormone-releasing hormone (LH-RH) was studied at different stages of the estrous cycle in the golden hamster. The animals were pretreated with phenobarbital to prevent a spontaneous preovulatory surge of LH. In these animals serum LH levels after saline injection remained low throughout the estrous cycle. Intravenous injection of 0.1 or 0.4 μg LH-RH significantly increased serum LH levels compared to appropriate controls. The greatest response to LH-RH was observed during proestrus. The smallest response was observed on the day 1 of diestrus, and in most of the animals, at estrus. The pituitary response to LH-RH on day 2 of diestrus was smaller than during proestrus, and greater than that observed during day 1 of diestrus and estrus. The results indicate that pituitary responsiveness to LH-RH varies at different stages of estrus cycle in the golden hamster.

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1. Gay, V. L., Midgley, A. R., Jr., and Niswender, G. D., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **29**, 1880 (1970).

2. Goldman, D., and Porter, J. C., Endocrinology **87**, 676 (1970).

3. Niswender, G. D., Reichert, L. E., Jr., and Zimmerman, D. R., Endocrinology **87**, 576 (1970).

4. Niswender, G. D., Roche, J. F., Foster, D. L., and Midgley, A. R., Jr., Proc. Soc. Exp. Biol. Med. **129**, 903 (1968).

5. Kirton, K. T., Niswender, G. D., Midgley, A. R.,

Jr., Jaffee, A. B., and Forbes, A. D., J. Clin. Endocrinol. Metab. **30**, 107 (1970).

6. Midgley, A. R., Jr., and Jaffee, R. B., J. Clin. Endocrinol. Metab. **28**, 1669 (1968).

7. Arimura, A., and Schally, A. V., Endocrinology **87**, 653 (1970).

8. Arimura, A., and Schally, A. V., Proc. Soc. Exp. Biol. Med. **136**, 290 (1971).

9. Debeljuk, L., Arimura, A., and Schally, A. V., Proc. Soc. Exp. Biol. Med. **139**, 774 (1972).

10. Miyake, T., Endocrinol. Jap., Suppl. **1**, 83 (1969).

11. Hori, T., Ide, M., and Miyake, T., Endocrinol. Jap. **16**, 351 (1969).

12. Hotchkiss, J., Atkinson, L. E., and Knobil, E., Endocrinology **89**, 177 (1971).

13. Harris, G. W., and Naftolin, F., Brit. Med. Bull. **26**, 3 (1970).

14. Arimura, A., Kastin, A. J., and Schally, A. V., *in* "Gonadotropins, 1971" (B. B. Saxena, ed.) New York (1972), in press.

15. Antunes-Rodrigues, J., Dhariwal, A. P. S., and McCann, S. M., Proc. Soc. Exp. Biol. Med. **122**, 1001 (1966).

16. Reeves, J. J., Arimura, A., and Schally, A. V., J. Anim. Sci. **32**, 123 (1971).

17. Ward, M. C., Anat. Rec. **94**, 139 (1946).

18. Greenwald, G. S., Endocrinology **88**, 671 (1971).

19. Steel, R. G. D., and Torrie, J. H., "Principles and Procedures of Statistics," McGraw-Hill, New York (1960).

20. Schally, A. V., Nair, R. M. G., Redding, T. W., and Arimura, A., J. Biol. Chem. **246**, 7230 (1971).

21. Geiger, R., Konig, W., Wissman, H., Geisen, K., and Enzmann, F., Biochem. Biophys. Res. Commun. **45**, 767 (1971).

22. Schally, A. V., Kastin, A. J., and Arimura, A., *in* "Vitamins and Hormones" (P. Munson, E. Diczfalusy, R. S. Harris, and J. L. Lorraine, eds.), Vol. 30. Academic Press, New York, in press.

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