Ovulation and Serum Luteinizing Hormone in the Cycling Rat Following Administration of Gonadotropin Releasing Hormone[1] (37251)

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In the rat, it is well established that ovulation is preceded by a serum luteinizing hormone (LH) surge on the afternoon of proestrus (1, 2), with maximal LH release lasting 1–3 hr. Additionally, the estrogen surge which precedes the cyclic peak of LH (3), appears to be an essential signal leading to the ovulatory events. The involvement of estrogen is apparent since 4 and 5-day cyclic rats ovulate one day early following a single injection of estradiol benzoate (4, 5).

We have recently reported details of purification and a complete chemical description of the synthetic decapeptide amide, pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 (6), which has been called the gonadotropin-releasing hormone by Schally *et al.* (7). Structural studies and the initial synthesis of this decapeptide amide were conducted by Matsuo *et al.* (8). Subsequently, these workers demonstrated equal *in vivo* LH-releasing activity of the synthetic decapeptide to the natural porcine-releasing hormone (9). Moreover, the releasing hormone has induced ovulation in the hamster (10) and the constant-estrous rabbit (6, 11), but was only effective in half the late-anestrous ewes tested (6, 12). In the present experiments we attempted to determine the incidence of ovulation and serum LH changes at various stages of the rat estrous cycle following an injection of synthetic porcine-releasing hormone.

Materials and Methods. The decapeptide amide (Gn-RH) corresponding to the amino acid sequence of the native porcine-releasing hormone, was synthesized by the solid-phase method and purified by silica-gel chromatography (6). Mature female Sprague–Dawley rats (Sprague–Dawley, Madison, Wisconsin) weighing 200–250 g were individually caged in a controlled 14-hr (5:30 AM–7:30 PM) light and 10-hr dark environment throughout the studies. Vaginal smears were obtained daily (except Sunday) before 9:00 AM for sufficient time to establish the length and regularity of the cycle. Only rats showing regular 5-day cycles were selected for experimentation. Experimental animals received an intravenous injection of 5 μg Gn-RH dissolved in 1 ml 0.9% saline at 9:00 AM. Control animals received 1 ml saline. Blood was collected from all rats by jugular puncture at various times after the injection, held at room temperature for 24 hr, centrifuged and the serum decanted and stored at −20°. Serum LH was assayed by radioimmunoassay as described by Niswender *et al.* (13) and expressed as ng NIH-LH-S16/ml serum. The rats were killed by cervical dislocation 26–28 hr after treatment and the reproductive organs removed and examined. The oviducts were compressed between two slides and the ova counted under a low-power microscope. Sodium pentobarbital [Nembutal®] was injected intraperitoneally, at a dose level of 25 mg/kg, into some of the rats to block the spontaneous proestrous LH surge. The significance of differences among treatments was determined using Duncan's new multiple range test (14).

Results. Table I presents the ovulation data and serum LH changes following Gn-RH injection at various stages of the estrous cycle. Ovulation was induced when the decapeptide was injected at 9:00 AM of proestrus, but not if injected at that time earlier in the cycle (Expt. 1.). Furthermore, pentobarbital injected at 1:00 PM of

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TABLE I. Effect of Gonadotropin Releasing Hormone[a] on Ovulation and Serum LH Levels in the Cycling Rat.

Stage of cycle	Treatment	No. ovulating	Mean no. ova recovered ± SE	Serum LH[b] (ng/ml ± SE) 0 min	30 min	60 min	120 min	240 min	5:00 PM
				Experiment 1					
Metestrus	Gn-RH	0/5	0.0	1.2 ± 0.2	33.5 ± 1.4	23.6 ± 2.2	5.5 ± 0.8	1.4 ± 0.2	
Diestrus-1	Gn-RH	0/5	0.0	1.8 ± 0.5	33.5 ± 3.9	24.1 ± 6.6	4.0 ± 0.9	1.6 ± 0.2	
Diestrus-2	Gn-RH	0/4	0.0	1.1 ± 0.1	11.1 ± 1.6	18.8 ± 1.4	5.0 ± 0.4	1.7 ± 0.4	
Proestrus	Gn-RH	5/5	10.4 ± 1.2	1.7 ± 0.3	54.1 ± 11.4	60.6 ± 10.1	15.8 ± 1.9	5.3 ± 3.0	25.2 ± 9.0
Proestrus	Saline	5/5	10.7 ± 0.7	1.3 ± 0.2	1.2 ± 0.3	1.3 ± 0.2	1.0 ± 0.1	2.0 ± 0.5	42.0 ± 12.9
					Experiment 2				
					0 min	45 min	180 min	5:00 PM	
Proestrus	Saline	5/5	10.8 ± 1.0		1.2 ± 0.1	0.9 ± 0.2	2.4 ± 0.6	66.2 ± 11.7	
Proestrus	Saline + Nembutal	0/8	0.0		1.3 ± 0.2	0.7 ± 0.2	0.7 ± 0.1	0.6 ± 0.1	
Proestrus	Gn-RH	4/4	9.0 ± 1.8		1.2 ± 0.2	47.4 ± 5.0	7.4 ± 2.3	13.8 ± 4.3	
Proestrus	Gn-RH + Nembutal	8/9	8.9 ± 1.1[c]		1.5 ± 0.3	52.2 ± 4.7	4.7 ± 1.3	3.5 ± 0.6	

[a] Single intravenous dose of 5 μg Gn-RH.
[b] Expressed as NIH-LH-S16.
[c] Based on eight ovulating rats.

proestrus (Expt. 2) did not prevent ovulation in those rats which received Gn-RH 4 hr earlier. Additionally, the mean number of ova recovered from rats which received Gn-RH was similar to the ovulation rate of control proestrous rats.

Intravenous administration of 5 μg Gn-RH significantly increased serum LH at all stages of the cycle. Nevertheless, the serum LH response appeared related to the stage of the cycle, since diestrus-2 rats were the least and proestrous animals the most responsive. The peak serum LH concentration occurred at the 30-min bleeding at metestrus and diestrus-1, but 30 min later at proestrus. Serum LH concentration in proestrous rats at 30, 60, and 120 min following Gn-RH was significantly ($p < 0.01$) greater than the serum LH levels in all other groups. In addition, the serum LH increase in diestrus-2 rats at 30 min after Gn-RH was significantly ($p < 0.05$) less than in rats at other stages of the cycle. At 240 min after Gn-RH injection serum LH had returned to baseline levels in all groups except proestrous rats ($p > 0.05$).

Discussion. The early studies of Arimura *et al.* (15) demonstrated ovulation of the pentobarbital-blocked proestrous rat using highly purified porcine LH-releasing factor. A dose of 10 μg of releasing hormone, injected over a 30-min period, induced full ovulation in all rats; whereas, 1 or 2 μg occasionally caused ovulation. Recently, Arimura *et al.* (17) reported the successful induction of ovulation in the pentobarbital-blocked proestrous hamster after two subcutaneous injections of synthetic Gn-RH. Their studies also indicated the direct involvement of the pituitary, since animals hypophysectomized just prior to treatment did not ovulate following Gn-RH, but did ovulate if given LH. Our data in the rat, in which Gn-RH injection was at 9:00 AM, illustrate the absence of an ovulatory response except on the day of proestrus. The dose of Gn-RH we used was large and capable of releasing sufficient LH to induce ovulation, since we have recently observed 100% ovulation in the proestrous rat following a single intravenous dose of 0.32 μg synthetic Gn-RH (unpublished). Also, it is apparent that serum LH elevations induced by Gn-RH during metestrus and diestrus in this study approach ovulatory levels, assuming ovarian competence.

That the ovary is incompetent during the early stages of the cycle was suggested by Holsinger and Everett (16) who found maximal follicle responsiveness to LH at and after 10:00 PM on diestrus-2. Thus, our inability to ovulate the cycling rat, except at proestrus, is not surprising. Others have induced ovulation in the rat with synthetic Gn-RH prior to 10:00 PM of diestrus-2 (Yamazaki *et al.*, personal communication). They observed ovulation following the subcutaneous injection of Gn-RH at 2:30 PM of diestrus-2. Furthermore, they also reported that proestrous rats were more responsive than diestrous animals, since 60 and 400 ng Gn-RH/100 g body weight was necessary to ovulate 90% of the rats, respectively.

In the present study, the pituitary responsiveness to synthetic Gn-RH was greater in the proestrous rat than at other times of the cycle, as was also reported in the hamster (18). It has been suggested that progressive increases in estrogen secretion from the ovaries, detectable the morning of proestrus in the rat (19), play a role in increasing the pituitary responsiveness. Others (20), using crude sheep hypothalamic extracts and bioassay techniques, found no evidence of responsiveness changes in the rat. Interestingly, Debeljuk *et al.* (21) observed that the pituitary responsiveness in the rat to highly purified porcine-releasing hormone in terms of LH release progressively decreased up to 35 days of age and was also low in mature cycling animals. Since no attempt was made by these workers to separate the mature rats into stage of the estrous cycle, differences in pituitary responsiveness may have been obscured. In contrast, studies in the sheep have indicated that the pituitary responsiveness to porcine LH-releasing hormone was increased at estrus, presumably due to an augmentation effect of estrogens (22, 23).

It is of interest to compare the duration of the endogenous LH secretion on the afternoon of proestrus to the LH release induced by Gn-RH the morning of proestrus. Monroe *et al.* (1) observed periods of 1–3 hr of maximal LH release with greatly different

serum concentrations at any given time between animals. Gn-RH induced a 30-fold increase in serum LH within 30 min and an apparent peak serum LH between 30 and 60 min following injection. Serum levels were 10 and 4 times greater than basal levels at 2 and 4 hr after injection of Gn-RH, respectively, similar to the observations made the afternoon of proestrus (1, 2).

Summary. The synthetic decapeptide, pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 (Gn-RH) was evaluated for induction of ovulation in the cycling rat. All injections of the decapeptide were made at 9:00 AM. At the intravenous dose of 5 μg, Gn-RH induced ovulation only during proestrus in both the normal cycling and pentobarbital-blocked rat. Serum LH elevation following Gn-RH was greater at proestrus than at metestrus or diestrus-1, and least at diestrus-2. Peak serum levels of LH were, respectively 60.6, 33.5, 33.5, and 18.8 ng/ml NIH-LH-S16. In the ovulating rat, serum LH elevations after Gn-RH were similar in magnitude and duration to the normal proestrous levels.

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1. Monroe, S. E., Rebar, R. W., Gay, V. L., and Midgley, A. R., Endocrinology **85**, 720 (1969).

2. Piacsek, B. E., Schneider, T. C., and Gay, V. L., Endocrinology **89**, 39 (1971).

3. Neill, J. D., Freeman, M. E., and Tillson, S. A., Endocrinology **89**, 1448 (1971).

4. Ying, S. Y., and Greep, R. O., Proc. Soc. Exp. Biol. Med. **139**, 741 (1972).

5. Everett, J. W., Endocrinology **43**, 389 (1948).

6. White, W. F., Hedlund, M. T., Rippel, R. H., Arnold, W., and Flouret, G. R., Endocrinology, in press.

7. Schally, A. V., Arimura, A., Kastin, A. J., Matsuo, H., Baba, Y., Redding, T. W., Nair, R. M. G., Debeljuk, L., and White, W. F., Science **173**, 1036 (1971).

8. Matsuo, H., Baba, Y., Nair, R. M. G., Arimura, A., and Schally, A. V., Biochem. Biophys. Res. Commun. **43**, 1334 (1971).

9. Arimura, A., Matsuo, H., Baba, Y., Debeljuk, L., Sandow, J., and Schally, A. V., Endocrinology **90**, 163 (1972).

10. Arimura, A., Matsuo, H., Baba, Y., and Schally, A. V., Science **174**, 511 (1971).

11. Amoss, M., Blackwell, R., and Guillemin, R., J. Clin. Endocr. **34**, 434 (1972).

12. Reeves, J. J., Arimura, A., Schally, A. V., Kragt, C. L., Beck, I. W., and Casey, J. M., J. Anim. Sci. **35**, 84 (1972).

13. Niswender, G. D., Midgley, A. R., Monroe, S. E., and Reichert, L. E., Jr., Proc. Soc. Exp. Biol. Med. **128**, 807 (1968).

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

15. Arimura, A., Schally, A. V., Saito, J., Müller, E. E., and Bowers, C. Y., Endocrinology **80**, 515 (1967).

16. Holsinger, J. W., Jr., and Everett, J. W., Endocrinology **86**, 257 (1970).

17. Arimura, A., Matsuo, H., Baba, Y., and Schally, A. V., Science **174**, 511 (1972).

18. Arimura, A., Debeljuk, L., and Schally, A. V., Proc. Soc. Exp. Biol. Med. **140**, 609 (1972).

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

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

21. Debeljuk, L., Arimura, A., and Schally, A. V., Endocrinology **90**, 1499 (1972).

22. Reeves, J. J., Arimura, A., and Schally, A. V., J. Anim. Sci. **32**, 123 (1970).

23. Reeves, J. J., Arimura, A., and Schally, A. V., Biol. Reprod. **4**, 88 (1971).

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