PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 149, 576–579 (1975)

Blockade of the Pre-ovulatory LH Surge in Hamsters by an Inhibitory Analog of LH-RH[1] (38855)

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The search continues for inhibitory analogs of LH-RH which have a high affinity for pituitary receptors and better resistance to degradation by plasma and tissue peptidases. Des-His^2-desGly^{10}-LH-RH ethylamide was the first analog reported to be a potent inhibitor of LH release induced by LH-RH both *in vivo* with immature male rats (1) or ovariectomized steroid-blocked rats (2) and *in vitro* (3). Subsequently, other analogs of LH-RH having an enhanced ability to inhibit the LH and FSH released by LH-RH have been reported (4, 5). Recently, D-Phe^2-D-Leu^6-LH-RH was synthesized in our laboratories based on the inhibitory analog D-Phe^2-LH-RH (6) and the powerful gonadotropin-releasing analog D-Leu^6-LH-RH (7). It showed a greatly prolonged suppression of LH release in response to LH-RH in immature male rats (8). The surge of circulating LH on the afternoon of proestrus or of mid-cycle has been associated with an increase in pituitary sensitivity to LH-RH (9–11) and also with an increase of LH-RH activity as determined by bioassay (12) or radioimmunoassay in plasma (13). Since the administration of an antiserum to synthetic LH-RH suppressed the cyclic surge of LH and FSH in proestrous rats and prevented ovulation (14), we studied the ability of D-Phe^2-D-Leu^6-LH-RH to suppress the cyclic surge of peripheral LH on the day of proestrus and observed its effects on ovulation in cycling hamsters.

Material and Methods. D-Phe^2-D-Leu^6-LH-RH was synthesized by the solid phase method in our laboratories. Sexually mature female golden hamsters (Lakeview Hamsters Colony, Newfield, NJ) weighing 100–130 g were used in this experiment. They were maintained in groups of eight animals per cage in an environmentally controlled room illuminated daily from 0700–1800 hr and had free access to tapwater and Purina Lab Chow. The 4-day estrous cycle was monitored by the presence of a characteristic vaginal discharge on the morning of the day of ovulation. After three consecutive 4-day cycles, the animals were divided into three groups. The first group of 52 untreated animals served as the control. Every 30–60 min between 1 PM and 10 PM, four randomly selected animals within this group had their blood taken from the jugular vein under light ether anesthesia. On the next morning the oviducts were examined for ova in a random group of 12 animals. A second group of 40 animals was subjected to treatment with D-Phe^2-D-Leu^6-LH-RH. The analog was first dissolved in 20 μl of propylene glycol and then diluted with 0.9% saline solution so that 0.2 ml of the diluent contained 750 μg of peptide which was injected subcutaneously in the back. All animals within this group received four sc injections containing 750 μg each of D-Phe^2-D-Leu^6-LH-RH at 3, 4, 5, and 6 PM on the day of proestrus. Thirty minutes after the first sc injection of the analog, and subsequently every 30–60 min throughout the afternoon, four different animals were selected to have blood taken from the jugular vein under anesthesia. Each hamster was bled only once. On the next morning, ovulation was checked in 30 random animals. A third group of 44 hamsters was treated with diluent alone. Blood was collected in the usual fashion and ovulation was checked in 24 random animals.

Sera samples were separated after centrifugation and stored at −20° until assayed

[1] This study was supported in part by USPH Research Grants CH-0555, AM-07467, Veterans Administration, NIH Contract 72-2741 and The Population Council.

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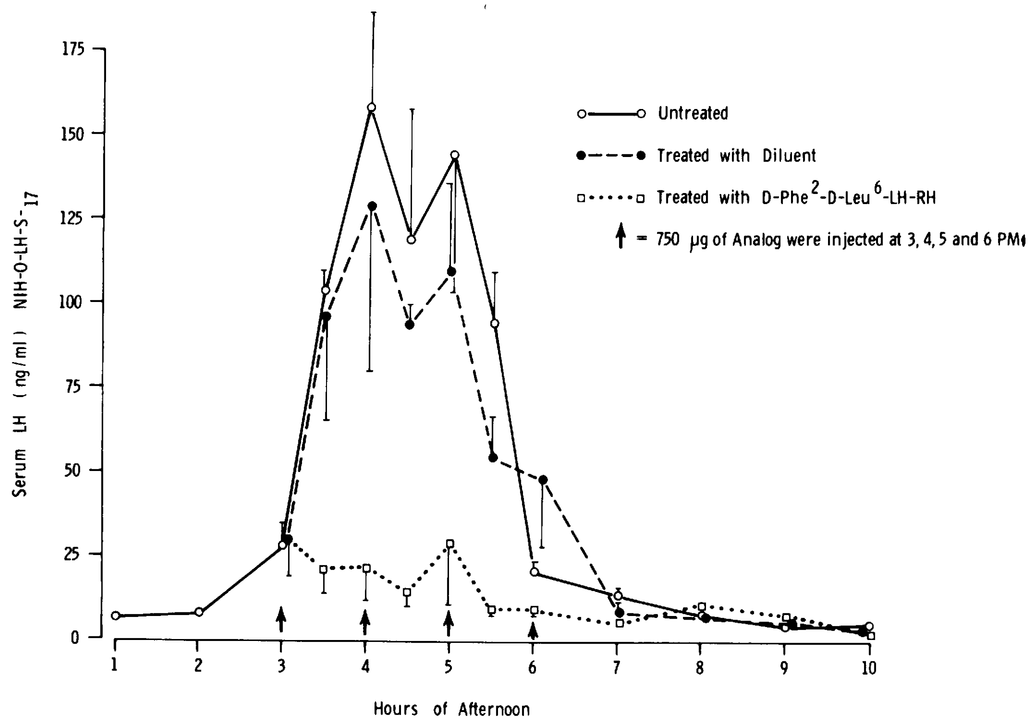

FIG. 1. Effect of sc administration of D-Phe^2-D-Leu^6-LH-RH on the surge of LH in the afternoon of proestrus in cycling hamsters.

for LH. Serum LH was determined in duplicate by the procedure described by Goldman and Porter (15) and expressed as nanograms per milliliter in terms of NIH-Ovine-LH-S_{17}. Mean serum LH levels of the three groups at each time interval were calculated and compared with each other using Duncan's new multiple range test (16). The integrated serum LH levels for each group were also determined over the length of the LH surge by a procedure described elsewhere (17). Ovulation was checked at 0800–1000 of the day of estrus by dissection of the oviducts under a microscope, inspection of the cumulus, and counting of the ova.

Results. Measurement of serum LH levels by radioimmunoassay throughout the afternoon of proestrus in the untreated animals indicated that the surge of LH occurred between 1400 and 1900 hr, and on the next morning the ovulation rate was 12.8 ± 0.6 ova per animal ($n = 12$).

Figure 1 shows that four sc injections of 750 μg of D-Phe^2-D-Leu^6-LH-RH at 3, 4, 5, and 6 PM on the day of proestrus in cycling hamsters led to 82.8% suppression of the surge of peripheral LH as compared with diluent-treated animals. The differences in LH levels between animals treated with diluent and analog were significant at 3:30, 4, 4:30, 5, 5:30, and 6 PM ($P < 0.05$). Serum LH levels of the untreated animals were also recorded and plotted in Fig. 1.

Table I shows the mean numbers of ova recovered in animals from each group on the morning of the day of estrus. Treatment with D-Phe^2-D-Leu^6-LH-RH led to 29.6% inhibition of ovulation ($P < 0.05$) as compared with diluent-treated animals.

Discussion. Several *in vivo* systems have been developed to measure the anti-LH-RH activity of LH-RH analogs. In preliminary experiments, the higher anti-LH-RH activity of D-Phe^2-D-Leu^6-LH-RH after sc administration appeared to occur at 60–90 min after sc injection into proestrous hamsters. We, therefore, selected 3, 4, 5, and 6 PM on the afternoon of proestrus as appropriate for administrtaion of the analog on the basis that the physiological surge of circulating

TABLE I. SUPPRESSION OF OVULATION IN CYCLING HAMSTERS BY D-Phe[2]-D-Leu[6]-LH-RH.

Treatment[a]	Number of animals	Number of ova (Mean ± SE)	Percentage of inhibition
Diluent treated	$n = 24$	11.4 ± 0.3	
Analog treated	$n = 30$	8.0 ± 0.5	29.6 ($P < 0.05$)[b]

[a] Treatment began in the afternoon of proestrus. 750 μg of analog or diluent were injected at 3, 4, 5, and 6 PM.

[b] Statistically different from diluent-treated animals.

LH in our strain of hamsters took place between 1400 and 1900 hr. This is in agreement with the report of Bast and Greenwald (18). The results reported here indicated that, although treatment with D-Phe[2]-D-Leu[6]-LH-RH led to almost complete suppression of the preovulatory surge of LH in cycling hamsters, only partial inhibition of ovulation was obtained. It has been shown that FSH alone can induce ovulation in pharmacologically blocked hamsters (19, 20). In our previous studies in rats, the release of FSH was inhibited less than that of LH by analogs of this type (5). We have not measured FSH release in this study but the possibility exists that the FSH release was affected to a lesser degree than LH by the inhibitor. In such a case, it could not be excluded that this FSH synergized the remaining LH to produce a high level of ovulation as seen in Table I. The small peaks of peripheral LH still present at the time of the surge of LH in animals treated with the analog may be a consequence of the intrinsic gonadotropin-releasing activity of the peptide and/or the schedule of treatment used. In view of the strong inhibition of LH release by the analog, its failure to produce better blockade of ovulation was disappointing. It is possible, however, that prolonged administration before and after the surge, over the whole cycle or over several cycles might block ovulation better.

Further inhibitory analogs of LH-RH with an increased affinity for the receptor site and prolonged biological half-life have been prepared and could lead to complete inhibition of ovulation.

Summary. Cycling hamsters were injected sc with 750 μg of D-Phe[2]-D-Leu[6]-LH-RH, an inhibitory analog of LH-RH at 3, 4, 5, and 6 PM on the day of proestrus. Serum LH levels were determined by radioimmunoassay in samples collected throughout the afternoon of proestrus. Treatment with D-Phe[2]-D-Leu[6]-LH-RH led to 82.8% suppression of the physiological surge of LH as compared with the integrated LH levels of diluent-treated animals. When ova were counted, treatment with D-Phe[2]-D-Leu[6]-LH-RH was shown to produce 29.6% ($P < 0.05$) inhibition of ovulation as compared with diluent-treated animals. No rebound phenomenon in serum LH was observed in the evening of proestrus in animals treated with the analog. Incomplete inhibition of ovulation may be due to the possibility that the small LH surge was well in excess of that needed to produce ovulation or could be synergized with an incomplete FSH surge which was not measured in this study. The small peaks of LH still present in the analog-treated animals may be explained by the intrinsic gonadotropin-releasing activity of the analog and/or the schedule of treatment used.

The authors are grateful to Mrs. D. Pierson, Mrs. J. Gauthier, and Mr W. Carter for their enthusiastic technical assistance and to Dr G. Niswender, Dr D. Ward, and NIH for the gift of material used in radioimmunoassay.

1. Coy, D. H., Vilchez-Martinez, J. A., Coy, E. J., Arimura, A., and Schally, A. V., J. Clin. Endocrinol. Metab. **37**, 331 (1973).
2. Coy, D. H., Coy, E. J., Schally, A. V., Vilchez-Martinez, J. A., Debeljuk, L., Carter, W. H., and Arimura, A., Biochemistry **13**, 323 (1974).
3. Spona, J., Fed. Eur. Biochem. Soc. Lett. **48**, 89 (1974).
4. Monahan, M. W., Amoss, M. S., Anderson, H. A., and Vale, W., Biochemistry **12**, 4616 (1973).
5. Vilchez-Martinez, J. A., Coy, D. H., Coy, E. J., Schally, A. V., and Arimura, A. Fert. Steril. (in press 1975).
6. Rees, R. W. A., Foell, T. J., Chai, S. Y., and Grant, N., J. Med. Chem. **17**, 1016 (1974).
7. Vilchez-Martinez, J. A., Coy, D. H., Arimura, A., Coy, E. J., Hirotsu, Y., and Schally, A. V., Biochem. Biophys. Res. Commun. **59**, 1226 (1974).
8. Vilchez-Martinez, J. A., Coy, D. H., Coy, E. J., Schally, A. V., de la Cruz, A., and Nishi, N.,

Abstracts 57th Annual Meeting of the Endocrine Society, New York, NY (1975).
9. Ferland, M. W., Borgeat, P., Labrie, F. Bernard, J., DeLean, A., and Raynaoud, J. P., J. Mol. Cell. Endocrinol. (in press, 1975).
10. Arimura, A., Debeljuk, L., and Schally, A. V., Proc. Soc. Exp. Biol. Med. **140**, 609 (1972).
11. Yen, S. S. C., Vanderberg, G., Rebar, R., and Ehara, Y., J. Clin. Endocrinol. Metab. **35**, 931 (1972).
12. Malacara, J., Seyler, L. E., Jr., and Reichlin, S., J. Clin. Endocrinol. Metab. **34**, 271 (1972).
13. Arimura, A., Kastin, A. J., Schally, A. V., Saito, M. Kumasaka, T., Yaoi, Y., Nishi, N., and Ohkura, K., J. Clin. Endocrinol. Metab. **38**, 510 (1974).
14. Koch, Y. P., Chobsieng, V., Zor, M., Friedkin, M., and Lindner, H. R., Biochem. Biophys. Res. Commun. **55**, 623 (1973).
15. Goldman, D., and Porter, J. C., Endocrinology **87**, 676 (1970).
16. Steel, R. G., and Torrie, J. H., "Principles and Procedures of Statistics," p. 107. McGraw-Hill, New York (1960).
17. Arimura, A., Vilchez-Martinez, J. A., Coy, D. H., Coy, E. J., Hirotsu, Y., and Schally, A. V., Endocrinology **95**, 1174 (1974).
18. Bast, J. D., and Greenwald, G. S., Endocrinology **94**, 1295 (1974).
19. Greenwald, G. S., Endocrinology **88**, 671 (1971).
20. Goldman, B. D., and Mahesh, V. B., Endocrinology **84**, 236 (1969).

Received Jan. 7, 1975, P.S.E.B.M., 1975, Vol. 149.