PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 146, 17–20 (1974)

In Vivo Comparison of LH-RH and FSH-RH Activities of [DES-GLY^{10}] [PRO^{9}-Ethylamide]–LH-RH, [DES-GLY^{10}][PRO^{9}-Propylamide]–LH-RH, and LH-RH Using Immature Male Rats[1] (38034)

AKIRA ARIMURA, JESUS A. VILCHEZ-MARTINEZ, AND ANDREW V. SCHALLY

Department of Medicine, Tulane University School of Medicine, and Endocrine and Polypeptide Laboratories, Veterans Administration Hospital, New Orleans, Louisiana

Soon after synthetic LH-releasing hormone (LH-RH) became available, clinicians attempted to use it for induction of ovulation in sterile women. Although several reports on successful induction of ovulation following administration of synthetic LH-RH in sterile women have appeared (1–4), the percentage of women ovulating was only 15%–30%. This low rate of ovulation could be in part due to the short action of LH-RH resulting from a rapid metabolic breakdown (5). The total amount of LH and FSH released as well as the duration of rise in serum gonadotropin levels after injection of LH-RH may not be sufficient to induce ovulation even if the ovaries contain mature follicles. We have observed in rats that the magnitude of elevation of serum LH and FSH levels was considerably larger when LH-RH was infused iv over a prolonged period of time rather than given as a quick injection (6). Fujino *et al.* (7, 8) reported two LH-RH analogues, [Des-Gly^{10}] [Pro^{9}-ethylamide]–LH-RH and [Des-Gly^{10}] [Pro^{9}-propylamide]–LH-RH, which showed greater ovulation inducing activity than LH-RH when tested in rabbits and in rats. The present study has been designed to examine the release patterns of LH and FSH following the administration of these LH-RH analogues.

Materials and Methods. Twenty-five day old male Sprague-Dawley rats were used as recipients for LH-RH and its analogues. They were kept under a constant temperature of 22°C and controlled light (14 hr light and 10 hr darkness), and fed Purina Chow rat diet. The animals were divided into 4 groups, each group consisting of 24 rats. Group 1 was injected with 0.2 ml 0.9% saline sc in the back. Groups 2, 3, and 4 received LH-RH, [Des-Gly^{10}] [Pro^{9}-ethylamide]–LH-RH (EA-LH-RH) and [Des-Gly^{10}] [Pro^{9}-propylamide]–LH-RH (PA-LH-RH), respectively. LH-RH was supplied by Dr. Y. Baba, Sankyo Co., Ltd, Tokyo. EA-LH-RH was synthetized by Dr. D. Coy in our laboratories (9) PA-LH-RH was a gift from Dr. M. Fujino, Takeda Chemical, Ind., Ltd., Osaka. All of these materials were dissolved on 0.9% saline and injected sc in a volume of 0.2 ml.

Four rats from each group were decapitated and blood was collected from the trunk at 0.5, 1, 2, 3, 4, and 6 hr after injection. The sera were separated by centrifugation and kept at −20°C until assayed for LH and FSH. Serum LH was determined by radioimmunoassay as described by Niswender *et al.* (10). NIH-LH-S-17 was used as the standard reference preparation. Serum FSH was also determined by radioimmunoassay as described by Daane and Parlow using NIAMD-Rat-FSH-RIA Kit (11). NIAMD-Rat-FSH-RP-1 was used as the standard reference preparation. The mean serum LH and FSH levels were compared by Duncan's new multiple range test (12).

Results. Results in 2 similar experiments

[1] This study was supported in part by USPHS research grant AM-09094, AM-07476, Veterans Administration and Population Council, New York, N. Y.

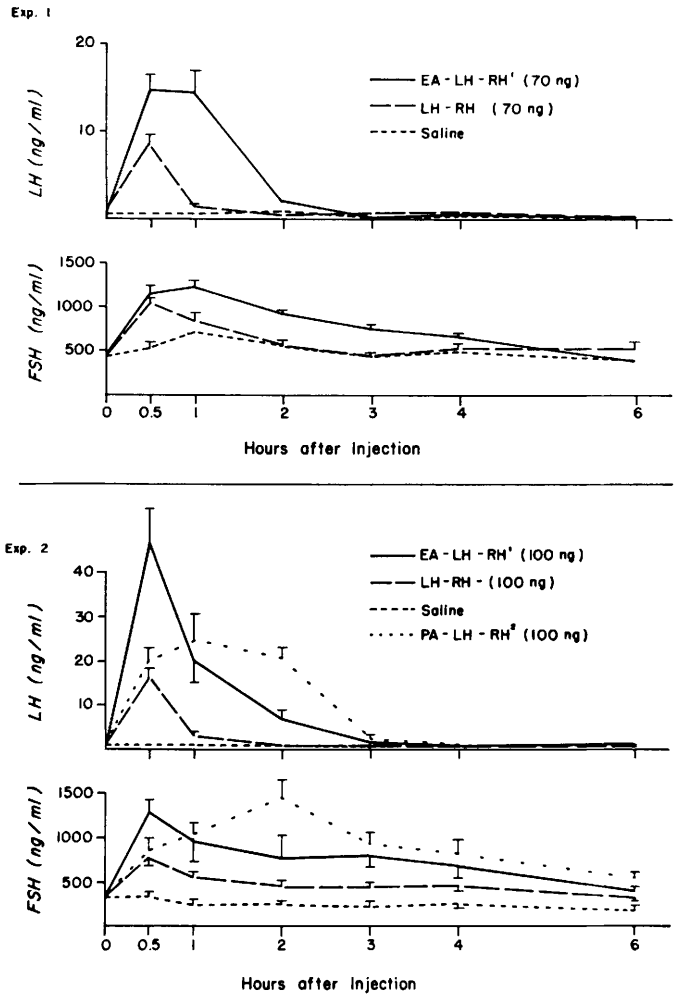

FIG. 1. Mean serum LH and FSH levels at various time intervals after sc injection of LH-RH, [Des-Gly^{10}] [Pro^{9}-ethylamide]–LH-RH (1) and [Des-Gly^{10}] [Pro^{9}-propylamide]–LH-RH (2) in 25 day old male rats. LH expressed as NIH-LH-S17. FSH expressed as NIAMD-Rat-FSH-RP-1. The vertical line on each point indicates standard error of mean. Duncan's new multiple range test. Exp. 1. LH levels significantly higher than corresponding control values 30 min after LH-RH and 30 min and 1 hr after EA-LH-RH. EA-LH-RH induced greater rise of LH than did LH-RH 30 min after injection. FSH levels. Significantly higher FSH levels after EA-LH-RH were higher than those after LH-RH at 1–3 hr. Exp. 2. LH levels significantly higher than corresponding control values 30 min after LH-RH, and 30 min, and 1 hr after EA-LH-RH, and 30 min, 1 and 2 hr after PA-LH-RH. LH levels 30 min after EA-LH-RH were considerably higher than those after PA-LH-RH or LH-RH. FSH levels. Significantly higher than corresponding control values 30 min after LH-RH and 30 min, 1–4 hr after EA-LH-RH and PA-LH-RH. At 30 min, FSH levels after EA-LH-RH were higher than those after LH-RH and PA-LH-RH. At 1 hr, the values were the same for EA-LH-RH and PA-LH-RH. At 2 hr, the levels after PA-LH-RH were considerably higher than those after EA-LH-RH.

are illustrated in Fig. 1. In the first series of experiments, the effects of administration of 70 ng of LH-RH and EA-LH-RH on serum LH and FSH levels were compared at various intervals after injection. In the second experiment, 100 ng of LH-RH, EA-LH-RH, and PA-LH-RH were used. Both serum LH and FSH levels increased 30 min after sc injection of 70 ng of either LH-RH or EA-LH-RH. LH levels after EA-LH-RH were significantly higher than after LH-RH whereas FSH levels were similar. One hour after injection, LH and FSH levels remained elevated in EA-LH-RH injected rats, whereas the levels were as low as control values in LH-RH treated rats. In EA-LH-RH treated rats, FSH levels were still significantly higher than those in control rats 2 and 3 hr after injection.

When doses of 100 ng of LH-RH and EA-LH-RH were used, essentially similar results to those in the first experiment were obtained except for higher FSH levels at 30 min in EA-LH-RH treated rats than in LH-RH injected animals. LH and FSH levels in PA-LH-RH injected rats were similar to those in LH-RH treated rats at 30 min. At 1 hr, LH and FSH levels in the rats injected with either EA-LH-RH or PA-LH-RH were both comparable and significantly higher than those in the control and LH-RH treated rats. Two hours after injection, LH levels remained high only in rats injected with PA-LH-RH. FSH levels in both EA-LH-RH and PA-LH-RH treated rats remained high, but they were considerably higher in the latter group than those in the former. Three hours after injection, in both PA-LH-RH and EA-LH-RH treated rats, LH levels were as low as those in the control animals, but FSH levels remained high up to 4 hr after injection.

Discussion. The results show that the magnitude of elevation of serum LH and FSH levels after injection of EA-LH-RH was greater than after the same dose of LH-RH, though the patterns of changes in LH and FSH levels in time were similar. This pattern, however, was different in the rats treated with PA-LH-RH. With the dose of 100 ng, LH levels remained elevated at nearly the same levels at 30 min, 1 hr, and 2 hr in PA-LH-RH treated rats, but sharply decreased after they reached the peak levels (30 min) in both LH-RH and EA-LH-RH injected animals. Although LH levels 30 min after injection of PA-LH-RH were smaller than those after EA-LH-RH and not different from those after LH-RH, the integrated amount of released LH in the former, expressed as the total area above levels for saline injected control rats (Fig. 1), was comparable to that for EA-LH-RH and approximately 5.5 times greater than that for LH-RH. This relative activity is in agreement with that estimated by ovulation inducing activity as tested in rabbits and rats (7, 8). Similarly, the integrated amounts of FSH in the rats treated with 100 ng of EA-LH-RH and PA-LH-RH were approximately 7 and 9.4 times greater than those in LH-RH treated rats, respectively. This suggests that PA-LH-RH is a better releaser of FSH than EA-LH-RH. The finding, however, may not necessarily indicate that PA-LH-RH has greater intrinsic FSH-RH activity than EA-LH-RH or LH-RH. Because slight but protracted elevation of serum LH-RH caused by prolonged iv infusion induced greater release of gonadotropins especially FSH (6), and peak LH and FSH levels after PA-LH-RH injection in the present study occurred later than those after LH-RH or EA-LH-RH, the greater activity of this analogue may be due to other factors such as slower breakdown in the circulation.

On the other hand, the similar patterns of both LH and FSH release by EA-LH-RH, and LH-RH suggest that the greater LH-RH/FSH-RH activities of the former is not due to the prolonged action, but factors such as greater affinity with the receptor, and/or greater intrinsic activity. The ovulation inducing activity of EA-LH-RH appeared to be greater than that of PA-LH-RH (7). An early burst of LH release after EA-LH-RH might account for this difference. In any case, these data suggest that EA-LH-RH is more efficient for inducing ovulation and PA-LH-RH is better for follicular stimulation.

Summary. The LH-RH and FSH-RH activities of the synthetic LH-RH decapep-

tide, [Des-Gly^{10}] [Pro^{9}-ethylamide]–LH-RH (EA-LH-RH) and [Des-Gly^{10}] [Pro^{9}-propylamide]–LH-RH (PA-LH-RH) were evaluated using immature male rats. A sc injection of 70 ng or 100 ng of EA-LH-RH induced a greater rise in serum LH and FSH levels than did these doses of LH-RH, but the pattern of changes in serum LH and FSH levels was similar. Elevation of serum LH and FSH levels after injection of 100 ng of PA-LH-RH was more prolonged than after the same dose of EA-LH-RH or LH-RH. The highest levels of LH were observed after EA-LH-RH. Peak FSH levels in response to EA-LH-RH and PA-LH-RH were similar and considerably higher than those after LH-RH. The intergrated amounts of LH released after 100 ng of EA-LH-RH and PA-LH-RH were similar and approximately 5.5 times greater than those after 100 ng of LH-RH. The integrated amounts of FSH released after 100 ng of EA-LH-RH and PA-LH-RH were approximately 7 and 9.4 times greater than those after LH-RH, respectively. Thus, EA-LH-RH and PA-LH-RH have been shown to release more gonadotropins than does LH-RH, and, in addition, administration of PA-LH-RH exhibits a more prolonged effect.

The authors wish to express thanks to Dr. Abba J. Kastin for his help in preparing the manuscript, and to Mr. L. Garcia, Mrs. J. Gauthier and Mrs. M. Essinger for their excellent technical assistance.

1. Zarate, A., Canales, E. S., Schally, A. V., Ayala-Valdex, L., and Kastin, A. J., Fert. and Steril. **23**, 672 (1972).

2. Akande, E. O., Bonnar, J., Carr, R. J., Corker, C. S., Dutton, A., MacKinnon, P. C. B., and Robinson, D., The Lancet **15**, 112 (1972).

3. Breckwoldt, M., Czygan, P. J., Lehmann, D., and Bettendorf, G., Acta Endocrinol., in press.

4. Zarate, J., Dabancens, A., Rodriguez-Bravo, R., Barsby, F., and Schally, A. V., Fert. and Steril. in press (1974).

5. Redding, T. W., Kastin, A. J., Gonzalez-Barcena, D., Coy, D. H., Coy, E. J., Schalch, D. S., and Schally, A. V., J. Clin. Endocrinol. Metab. **37**, 626 (1973).

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

7. Fujino, M., Shinagawa, S., Yamazaki, I., Kobayashi, S., Obayashi, M., Fukuda, T., Nakayama, R., White, W. F., Rippel, R. H., Arch. Biochem. Biophys. **154**, 488 (1973).

8. Fujino, M., Shinagawa, S., Obayashi, M., Kobayashi, S., Fukuda, T., Yamazaki, I., Nakayama, R., White, W. F., and Rippel, R. H., J. Med. Chem. **16**, 1144 (1973).

9. Coy, D. H., Coy, E. J., Schally, A. V., Vilchez-Martinez, J. A., Debeljuk, L., Carter, W. H., and Arimura, A., Biochemistry, in press (1973).

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

11. Daane, T., and Parlow, A. F., Endocrinology **88**, 653 (1971).

12. Steel, R. G., and Torrie, J. H., "Principles and Procedures of Statistics," p. 107. McGraw-Hill Book Co., Inc., New York (1960).

Received Nov. 21, 1973. P.S.E.B.M., 1974, Vol. 146.