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Effect of Intermittent Infusion of LH-Releasing Hormone on Serum LH and FSH Levels In Immature Male Rats[1] (38659)

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After the isolation, elucidation of the structure, and synthesis of LH-releasing hormone (LH-RH) (1–3), the effects of synthetic LH-RH on gonadotropin secretion have been extensively studied in rats. LH-RH has been shown to release LH as well as FSH from the pituitary gland (1, 3). Addition of LH-RH into the incubation medium stimulated the release of both LH and FSH from rat pituitaries *in vitro*, both LH and FSH being released simultaneously with superimposable time courses (4, 5). However, in most *in vivo* studies, the release of FSH after LH-RH administration was considerably smaller than that of LH (6). To obtain a greater stimulation of release of FSH, it is necessary to administer LH-RH by prolonged infusion (7, 8). It was postulated that LH-RH regulates secretion of both LH and FSH, and no convincing evidence for the existence of another FSH-RH has been presented so far (9). However, under some experimental conditions, FSH may be released without an apparent correlation with the LH release (10). On the other hand, pulsative patterns of the secretion of gonadotropins have been demonstrated in menopausal women and in castrated monkeys (11–13), which could be controlled by pulsative secretion of LH-RH. The present experiment was designed to investigate the effect of the intermittent infusion of LH-RH on LH and FSH release and to examine if such a condition would indeed result in dissociation of increase in serum LH and FSH levels. Since a prolonged infusion of LH-RH stimulates a sizeable release of FSH as well as LH, and since the half-life of FSH is considerably longer than that of LH (14), an intermittent-infusion of LH-RH might result in a significant rise of serum FSH without concomitant release of LH, a situation which is often observed during the early follicular stage of menstrual cycle. The effect of estrogen in combination with intermittent infusion of LH-RH on pituitary responsiveness was also investigated.

Materials and Methods. Since immature male rats are sensitive to FSH-RH activity as well as LH-RH activity (15), 25 day-old, male rats of Sprague–Dawley strain (ARS Sprague–Dawley, Madison, WI) were used throughout this experiment. Under urethane anesthesia (0.6 ml of a 25% solution/100 gbw), the animals were infused with synthetic LH-RH (50 ng/ml of saline solution) or with saline solution into the jugular vein using an infusion pump (Harvard Apparatus Co.). The infusion rate was 0.50 ml/1 hr.

In the first experiment, the rats were given three, 1-hr infusions with 1-hr intervals between infusion. In the second experiment, they were given two, 2-hr infusions with 1-hr interval. At/or 1 hr after the termination of the last infusion, four animals of each group were decapitated and blood was collected from the trunk. In the third experiment performed according to the same schedule as the first experiment (three 1-hr infusions with 1-hr intervals between infusion), four animals of each group were decapitated, and blood was collected from the trunk at 1-hr intervals from the start of the infusion. In the fourth experiment, the animals were injected sc with 10 μg estradiol benzoate (EB) in sesame oil or with 0.2 ml of sesame oil alone, 24 hr before the start of the infusion; otherwise the procedures were the same as those in experiment 1. Serum was separated and kept frozen until assayed for gonadotropins. Serum LH was determined by the double antibody radioimmunoassay

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of Niswender *et al.* (16), and serum FSH by the double antibody radioimmunoassay of Daane and Parlow (17). NIH-LH-S-17 and NIAMD-Rat FSH-RP 1 were used as standards for LH and FSH, respectively. One ng of NIH-LH-S-17 is equivalent to 34 ng of NIAMD-Rat-LH-RP_1. The significance of the differences among mean LH and FSH levels of each group was examined by Duncan's new multiple range test (18).

Results. In the first and second experiments, at the end of intermittent infusion, both serum LH and FSH levels significantly increased as compared with those in the animals infused with saline. In the animals given three, 1-hr infusions of LH-RH, serum LH and FSH levels rose by 147 and 130%, respectively (Fig. 1). But in the animals given 2-hr infusions, LH and FSH levels increased by 3358 and 216%, respectively (Fig. 2). On the other hand, 1 hr after the end of infusions of LH-RH, serum LH levels fell, reaching saline control values, but FSH levels remained higher (Fig. 1 and 2). Slight differences in control LH and FSH levels between these two experiments could reflect variation among different batches of animals, different times of experiments, and/or different assays.

The results from the third experiment are shown in Fig. 3. Serum LH levels were significantly higher than the control values at 1, 3, and 5 hr. FSH levels were significantly elevated at 3, 4, 5, and 6 hr. Thus, at 4 and 6 hr, serum FSH levels were elevated without a concomitant rise in LH levels. It is interesting that the maximum rise in LH was observed at 3 hr, but not at 5 hr.

In experiment 4 in the EB treated group, the mean serum LH level was the same as that in the oil-treated control group, but serum FSH level was significantly smaller than the control group after the saline infusion (Table I). At the end of the last infusion with LH-RH, LH levels in the EB pretreated group were significantly greater than the oil pretreated animals, but FSH levels in the EB group were smaller than the control group.

Discussion. Although infusion of synthetic LH-RH usually increased both serum LH and FSH levels, appropriate experimental conditions as used in the present study can lead to an increase in FSH levels

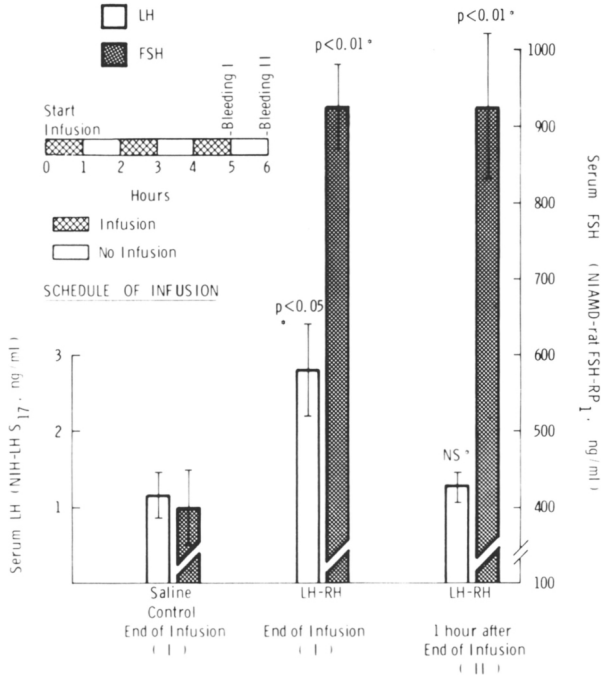

FIG. 1. Effect of intermittent infusion (1 hr each infusion period) of LH-RH on pituitary responsiveness in immature male rats. Dose of LH-RH: 75 ng/rat. Four animals per group were used. SE of the mean on the top of each bar. Significant differences of the values vs control saline value are also shown.

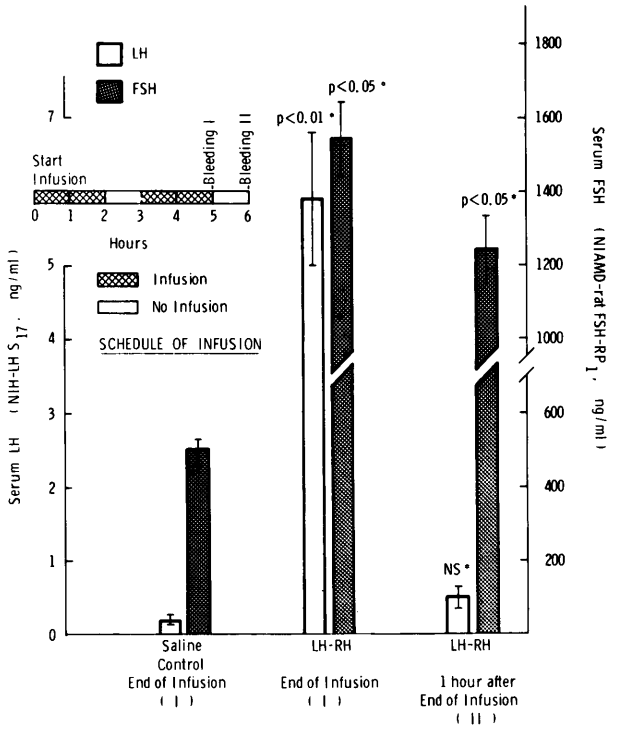

FIG. 2. Effect of intermittent infusion (2 hr each infusion period) of LH-RH on pituitary responsiveness in immature male rats. Dose of LH-RH: 100 ng/rat. Four animals per group were used. SE of the mean on the top of each bar. Significant differences of the values vs. control saline value are also shown.

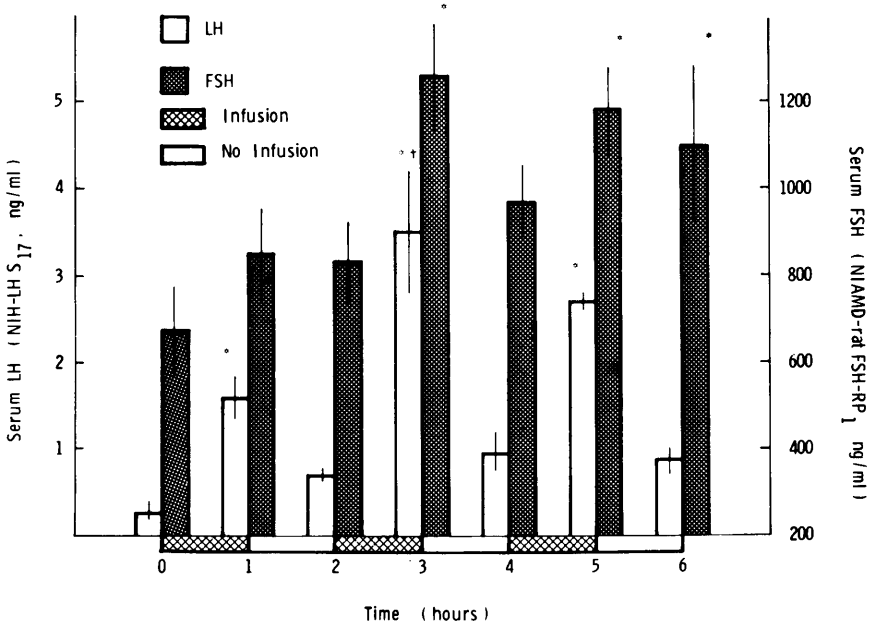

FIG. 3. Time course of LH and FSH release by intermittent infusion (1 hr each infusion period) of LH-RH on pituitary responsiveness in immature male rats. Dose of LH-RH: 75 ng/rat. Four animals per group were used. SE of the mean on the top of each bar. * Significantly different from the respective value at time 0. † Significantly different from the respective value at time 1 hr.

TABLE I. EFFECT OF 10 μg OF ESTRADIOL BENZOATE (EB) ON THE PITUITARY RESPONSIVENESS TO IV INTERMITTENT INFUSION OF LH-RH (100 ng/RAT) IN IMMATURE MALE RATS.

Group	Pretreatment	Intermittent infusion[a]	Serum LH (ng/ml)		Serum FSH (ng/ml)	
			At end of[a] last infusion	One hour after end of last infusion	At end of last infusion	One hour after end of last infusion
1	Oil	Saline	0.66 ± 0.20	—	663.6 ± 28.9	—
2	Oil	LH-RH[b]	3.32 ± 0.6[d]	0.89 ± 0.1	1450.0 ± 148.3	921.1 ± 58.2
3	Estradiol benzoate[c]	Saline	0.67 ± 0.16	—	202.6 ± 25.6[e]	—
4	Estradiol benzoate[c]	LH-RH[b]	4.95 ± 0.40	1.01 ± 0.2	1056.7 ± 93.1	807.6 ± 90.0

The values are expressed in Means ± SE. Four rats were used.

[a] The intermittent infusion was performed for three 1-hr infusion periods, 1-hr intervals between each infusion period.

[b] Synthetic LH-RH Hoechst R-4'.

[c] EB was administered sc 24 hr before infusion.

[d] Significantly different ($P < 0.05$) from the respective values of group 1 and 4. Duncan's new multiple range test.

[e] Significantly different ($P < 0.05$) from the respective value of group 1.

without an elevation of LH levels. The increase of FSH can be explained by the considerably longer biological half-life of FSH (14). This suggests that the rise of serum FSH without a concomitant LH rise, which is occassionally observed in some physiological conditions, does not necessarily indicate the presence of another FSH-RH other than LH-RH. Pulsatile secretion of LH (11–13) could reflect pulsatile release of LH-RH. Crighton *et al.* (19) reported pulsative rises of immunoreactive LH-RH levels in the sheep plasma at 1.5- to 6-hr intervals, although their values of LH-RH were too high to be acceptable as indicating true LH-RH. A situation similar to that used in the present experiment may possibly exist under physiological conditions.

As shown in Fig. 3, it is possible under special experimental conditions to find a pulsatile pattern of LH release after LH-RH administration. This is in agreement with the findings in human beings (11–13), other species (12), and with a recent report from Mortimer *et al.* (20) who found asynchronous changes in serum LH and FSH after 1 hr infusion with synthetic LH-RH in man.

The maximum rise in serum LH level was observed at 3 hr, but not at 5 hr. The same was observed in constant iv infusion of LH-RH in immature male rats for a long period of time (8, 21). In those experiments, LH reached a peak at 3 hr, and decreased thereafter in spite of the continued infusion of LH-RH. Libertum *et al.* (22) observed a similar phenomenon in ovariectomized adult rats. Apparently, there is some exhaustion of the LH synthesizing machinery.

In an earlier report from our laboratory, it was shown that estradiol benzoate slightly inhibited LH release after a quick injection of LH-RH in adult male rats and in newly castrated rats (23, 24). On the other hand, this steroid augmented the LH response in female rats and chronically castrated male rats (24). The suppressive effect of estradiol in adult male rats could be explained by the interaction of androgenic steroid with estrogen since the combination of testosterone and estradiol significantly depressed the pituitary response to LH-RH. The results obtained in Table I using intact immature male rats show that EB depressed the basal level of serum FSH, but augmented the LH release after LH-RH administration. This is in agreement with our previous experiment (unpublished observation) using immature male rats, in which pretreatment with EB augmented pituitary LH response to quick injection of LH-RH. The endogenous testosterone level in these rats may not be high enough to depress LH response by a combined action with exogenous EB (23, 24).

Summary. The effect of intermittent intravenous infusions of LH-RH on serum LH and FSH levels was studied in immature male rats. At the end of the infusion, serum LH and FSH levels were higher than those after saline infusion. One hr after the end of infusion of LH-RH, serum LH levels fell, reaching saline control values, but FSH levels remained higher. Moreover, a pulsative pattern of LH release but not of FSH, was obtained during the intermittent infusion with LH-RH. These findings indicate that under appropriate conditions after the administra-

tion of LH-RH, it is possible to find high FSH levels without a concomitant rise of LH levels. The continued elevation of FSH may be explained by the longer biological half-life of FSH. Estradiol benzoate depressed the basal serum FSH level and augmented LH release after intermittent infusion with LH-RH, indicating that estrogen modified FSH/LH ratio. The results suggest that discordance of plasma LH and FSH levels which is occassionally observed in some physiological conditions does not necessarily indicate the presence of another FSH-RH which is distinct from LH-RH decapeptide.

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1. Schally, A. V., Arimura, A., Baba, Y., Nair, R. M. G., Matsuo, H., Redding, T., Debeljuk, L., and White, W. F., Biochem. Biophys. Res. Commun. **43,** 393 (1971).
2. Matsuo, H., Baba, Y., Nair, R. M. G., Arimura, A., and Schally, A. V., Biochem. Biophys. Res. Commun. **43,** 1334 (1971).
3. Schally, A. V., Kastin, A. J., and Arimura, A., Fertil. Steril. **22,** 703 (1971).
4. Schally, A. V., Redding, T. W., Matsuo, H., and Arimura, A., Endocrinology **90,** 1561 (1972).
5. 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, p. 83. Academic Press, New York, (1972).
6. Arimura, A., Matsuo, H., Baba, Y., Debeljuk, L., Sandow, J., and Schally, A. V., Endocrinology **90,** 163 (1972).
7. Arimura, A., Debeljuk, L., and Schally, A. V., Endocrinology **91,** 529 (1972).
8. Debeljuk, L., Arimura, A., and Schally, A. V., Neuroendocrinology **11,** 130 (1973).
9. Schally, A. V., Arimura, A., Kastin, A. J., Matsuo, H., Baba, Y., Nair, R. M. G., Redding, T. W., Debeljuk, L., and White, A. F., Science **173,** 1036 (1971).
10. Bishop, W., Gsevry, C. P., Krulich, L., and McCann, S. M., Endocrinology **91,** 643, (1972).
11. Yen, S. S., Tsai, C. C., Naftolin, F., Vandenberg, G., and Ajabor, L., J. Clin. Endocrinol. Metabol. **34,** 671 (1972).
12. Dierschke, D. J., Bhattacharya, A. N., Atkinson, L. E., and Knobil, D., Endocrinology **87,** 850 (1970).
13. Vande Wiele, R. L., and Dyrenfurth, I., Pharmacol. Rev. **25,** 189 (1973).
14. Bogdanove, E. M., and Gay, V. L., Endocrinology **84,** 1118 (1969).
15. Debeljuk, L., Arimura, A., and Schally, A. V., Endocrinology **90,** 585 (1972).
16. Niswender, G. D., Midgley, Jr., A. R., Monroe, S. E., and Reichert, Jr., L. E., Proc. Soc. Exp. Biol. Med. **128,** 807 (1968).
17. Daane, T. W., and Parlow, A. P., Endocrinology **88,** 653 (1971).
18. Steel, R. G. D., and Torrie, J. H., *in* "Principles and Procedures of Statistics" McGraw-Hill, New York (1960).
19. Crighton, D. B., Foster, J. P., Holland, D. T., and Jeffcoate, S. L., J. Endocrinol. **59,** 373 (1973).
20. Mortimer, C. H., Besser, G. M., Goldie, D. J., Hook, J., and McNeilly, A. S., Nature (London) **246,** 22 (1973).
21. Vilchez-Martinez, J. A., Arimura, A., and Schally, A. V., Acta Endocrinol. Submitted (1974).
22. Libertun, C., Orias, R., and McCann, S. M., Endocrinology **94,** 1094 (1974).
23. Debeljuk, L., Arimura, A., and Schally, A. V., Endocrinology **90,** 1578 (1972).
24. Debeljuk, L., Arimura, A., and Schally, A. V., Proc. Soc. Exp. Biol. Med. **143,** 1164 (1973).

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