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The Effect of Subcutaneous Administration of Synthetic Luteinizing Hormone Releasing Factor on Plasma Gonadotropins and Prolactin in the Rat (37822)

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A decapeptide thought to correspond to natural luteinizing hormone releasing factor (LRF) has recently been isolated and synthesized (1, 2) and is capable of releasing LH, and to a lesser extent, FSH, both *in vitro* (3) and *in vivo* (4, 5). In nearly all *in vivo* work, the peptide has been administered by quick intravenous injection (6); however, in two studies, the effects of infusion of the peptide were evaluated (4, 5). Following infusions, the release of FSH was greater than that which followed intravenous injection. It appeared of interest to determine if a similarly altered pattern of release would follow the subcutaneous injection of the peptide, which would be a more convenient means for providing relatively sustained concentrations of the hormone in body fluids. The present results demonstrate that subcutaneous administration of the decapeptide stimulates LH and, to a lesser extent, FSH release, and that relatively low doses of the peptide provoke rapid and relatively long lasting effects.

Material and Methods. Adult male rats from Simonsen Laboratories (Gilroy, CA) were used either intact or 3 weeks following orchidectomy. The animals were housed in a room maintained at 24° with constant lighting (lights on 5 AM–7 PM). They were given free access to Purina chow and water and were used for experiments when they weighed 260–280 g.

Prior to the experiment, all animals were anesthetized with tribromo ethanol (TBE) (Winthrop Labs). Tribromo ethanol at a concentration of 2.5% in physiological saline was injected intraperitoneally (1 ml/100 g body wt). The animals were anesthetized within 2–3 min, and an initial heparinized blood sample was obtained from the external jugular vein. Immediately thereafter, synthetic LRF or an equivalent volume of physiological saline was injected. Additional blood samples were taken from the jugular at 10, 20, 60, 120, and 240 min after injection of LRF or diluent. All doses of LRF were dissolved in 0.2 ml of physiological saline.

Plasma gonadotropins and prolactin were assayed by radioimmunoassay (RIA). LH was measured by the method of Niswender *et al.* (7), and the results were expressed in terms of the NIH-LH-S-1 reference preparation. FSH and prolactin were assayed utilizing the kits supplied by NIAMD, and the results were expressed in terms of the RP-1 rat FSH and prolactin, respectively.[4] Significance of differences between groups was determined by Student's *t* test.

Results. Effect of bleeding and anesthesia on plasma gonadotropins and prolactin. Tribromo ethanol induced general anesthesia which lasted for approximately 20 min. Thereafter, the animals remained quiet if not disturbed. Because of the short duration of the anesthesia, it was necessary to reanesthetize the animals with supplemental doses of TBE for blood samples taken after 20 min. There was a slight, but not significant, decrease in plasma LH concentration during the experiment in intact male rats (Fig. 1). On the other hand, FSH levels remained unchanged until they began to rise at 2 hr

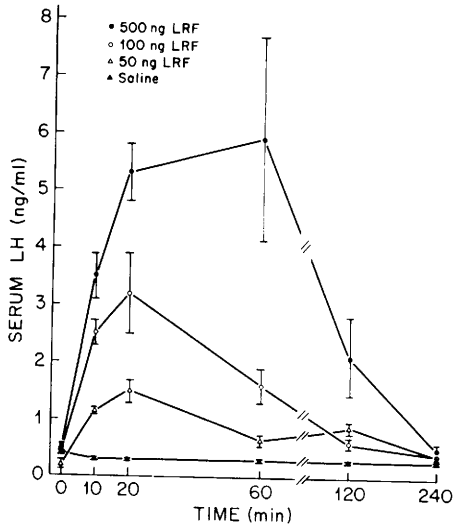

FIG. 1. The effect of various doses of sc synthetic LRF on serum LH in intact TBE-anesthetized male rats. In this and subsequent figures, vertical bars represent the SEM.

after injection (Fig. 2). By 4 hr after injection, FSH concentration had increased significantly above initial values ($P < .005$). Plasma prolactin concentration was significantly lowered within 10 min of the injection of saline (Table I) and remained low throughout the duration of the experiment.

In castrated animals, the initial plasma LH values were markedly elevated above those found in intact rats as a result of the removal of the inhibitory effects of gonadal steroids (Fig. 3). Plasma LH levels were significantly lowered by the procedure within

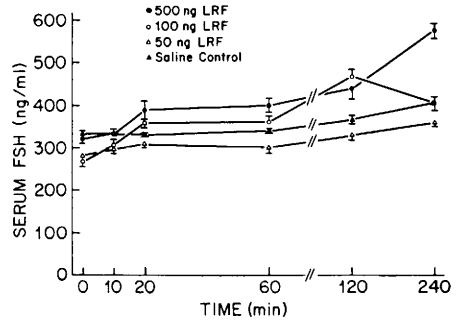

FIG. 2. Serum FSH following sc injection of synthetic LRF in intact TBE-anesthetized male rats.

10 min of the initial bleeding. The values then rebounded to initial levels within 60 min. FSH was also elevated in the castrates (Fig. 4) and was similarly suppressed within 10 min after the initial blood sampling. In contrast to LH, plasma FSH had returned to normal levels only by 4 hr. As in intact animals, prolactin was quickly suppressed within 10 min (Table II) and remained low throughout the experiment, although this lowering was no longer significant by 2 hr.

Effect of LRF on plasma gonadotropins and prolactin in intact rats. A highly significant increase in plasma LH concentration appeared within 10 min after injection of all doses of LRF tested (Fig. 1). Peak levels were obtained at 20 min with both the 50- and 100-ng doses, but the maximum level was maintained at 1 hr only after the highest dose of 500 ng. There was a clear dose–response relationship, and the higher doses also caused a more prolonged elevation of LH. The values were still slightly but significantly elevated above the value in saline-injected controls at 4 hr after the injection of all doses.

TABLE I. Serum Prolactin in Normal Rats After sc Injection of Synthetic LRF.

Time after injection (min)	Dose of LRF (ng)			
	0	50	100	500
0	76 ± 13[a] (18)	113 ± 23 (6)	148 ± 51 (6)	99 ± 31 (6)
10	28 ± 5 (18)	32 ± 10 (6)	28 ± 6 (6)	57 ± 33 (6)
20	22 ± 5 (18)	32 ± 10 (6)	32 ± 7 (6)	25 ± 8 (6)
60	19 ± 3 (18)	21 ± 9 (6)	26 ± 8 (6)	18 ± 5 (6)
120	27 ± 6 (18)	46 ± 29 (6)	24 ± 4 (6)	16 ± 3 (6)
240	39 ± 9 (18)	47 ± 16 (6)	113 ± 23 (6)	28 ± 4 (6)

[a] Mean ± SEM (number of rats in parentheses).

Results with plasma FSH concentration contrasted sharply with those just described for LH in that there was very little effect of the decapeptide on plasma FSH (Fig. 2). Since plasma FSH rose during the experiment, the pertinent comparison is with the

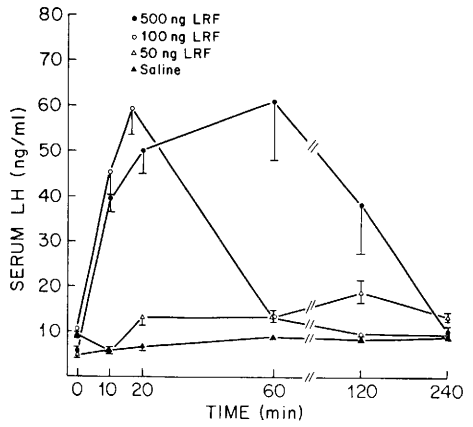

FIG. 3. Serum LH following sc injection of synthetic LRF in TBE-anesthetized castrated rats.

saline-injected controls. Using this criterion, there was no effect on plasma FSH with the 50-ng dose and only a small effect significant at 2 hr after injection with the 100-ng dose of the peptide. Using the highest dose of 500 ng of synthetic LRF, a highly significant increase of small magnitude was observed only at 4 hr.

Since prolactin declined as a result of the procedure of bleeding, injection, and anesthesia, results after injection of LRF should be compared to those of the saline-injected controls (Table I). Using this comparison, there was no significant effect of LRF except for an elevation at 4 hr with the 100-ng dose.

The effect of LRF on plasma gonadotropins and prolactin in orchidectomized rats. Because of the significant decline in plasma LH which occurred in castrate control animals, the most rigid comparison is between values in this group of animals and in the animals injected with LRF. Using this criterion, it is apparent that, as in the intact animals, all doses of LRF provoked LH release in the castrates (Fig. 3). The 50-ng dose prevented the significant decline which took place in the saline-injected controls at 10 min, and the values were significantly elevated above the initial values and those obtained in the saline-injected controls by 20 min. This elevation persisted for 2 hr after injection. Much greater effects were observed with the two higher doses of LRF. In these instances, highly significant increases in LH were apparent at 10 min, and there was no difference in the response to the two doses at 10 or 20 min. The principal difference in response to the highest dose was a much more prolonged elevation in LH which reached a peak at 1 hr. The relative increase above initial values was quite similar to that obtained in intact animals. For example, there was a 12-fold elevation above basal values at 1 hr after the 500-ng dose in both normals and castrates. Since the initial plasma levels were much higher in the castrates, the absolute magnitude of the increment in plasma LH in the castrates was some tenfold greater.

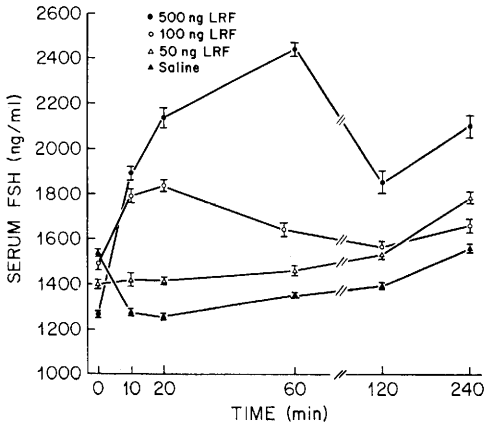

FIG. 4. Serum FSH following sc injection of synthetic LRF in TBE-anesthetized castrated rats.

Since the procedure resulted in a precipitous decline in FSH levels in the castrates and these levels gradually returned to the control values at 4 hr (Fig. 4), again, it is pertinent to compare the results in the LRF-treated animals with those in the saline-injected controls. Even the lowest dose of 50 ng of LRF prevented the decline in FSH that occurred in control rats and evoked an increase in FSH release in the castrates which was apparent only at 4 hr after injection. Using higher doses of 100 and 500 ng, an increase in plasma FSH occurred within 10 min which was similar with both doses at this time, but the response to the higher dose persisted longer and did not peak until 1 hr after injection instead of at 20 min for the 100-ng dose. Levels were still high at 2 and 4 hr after injection of both of these higher doses. The response in terms

TABLE II. Serum Prolactin in Castrated Rats After sc Injection of Synthetic LRF.

Time after injection (min)	Dose of LRF (ng)			
	0	50	100	500
0	85 ± 14[a] (18)	106 ± 33 (6)	114 ± 24 (6)	131 ± 11 (6)
10	29 ± 6 (18)	34 ± 13 (6)	34 ± 12 (6)	67 ± 17 (6)
20	28 ± 9 (18)	36 ± 12 (6)	28 ± 12 (6)	48 ± 9 (6)
60	31 ± 14 (18)	25 ± 11 (6)	45 ± 17 (6)	18 ± 5 (6)
120	39 ± 16 (18)	11 ± 2 (6)	53 ± 28 (6)	13 ± 3 (6)
240	48 ± 12 (18)	24 ± 8 (6)	74 ± 30 (6)	70 ± 40 (6)

[a] Mean ± SEM (number of rats in parentheses).

of FSH was clearly enhanced both on a relative and absolute basis in the castrates as compared to the response in the intact animals.

In the case of prolactin, none of the doses of synthetic LRF altered the pattern of declining concentration of plasma prolactin observed in the saline-injected controls (Table II).

Discussion. It is important to comment on the effect on hormone release of the procedure of injection, bleeding, and anesthetization with TBE. Although these procedures failed to alter plasma LH in intact animals, they did result in a transient decline in the levels in castrates. By contrast, there was a delayed rise in FSH in intact animals and a fall followed by a rise back to control levels in castrates. In both normals and castrates, plasma prolactin levels declined. The decline in hormone levels may be the result of the anesthesia which depressed the CNS centers which normally regulate release of gonadotropin-releasing factors. The decline in prolactin release is consistent with a suppression of prolactin-releasing factor release and with a continued discharge of prolactin-inhibiting factor. Declines in levels of gonadotropins and prolactin following anesthesia have been previously noted after ether (6) or Nembutal (8, 9). Because of its ease of administration, TBE appears to be a convenient anesthetic for use in hormonal studies in the rat.

The results indicate that subcutaneous injection of LRF is a convenient way to administer the hormone and that the response of the pituitary within 10 min after administration is almost as great as that observed following intravenous administration of comparable doses (unpublished data). The results further indicate that much more prolonged effects are obtained following the subcutaneous route of administration, probably because of the relatively slow absorption from the subcutaneous depot as a result of minimal inactivation in the depot.

In intact animals, there was only a very minimal response in terms of FSH release which was delayed and occurred after the higher doses of 100 and 500 ng of the peptide. These doses produced very large increments in LH release.

The relative magnitude of release of LH in normal and castrate animals was similar, but because of the much higher basal levels of the hormone in the castrates as the result of the removal of steroid feedback, the absolute increments in plasma LH were much greater. It is clear, therefore, that the total amount of LH released from the castrate pituitary in response to LRF is much greater than in intact animals. Such glands contain increased stores of gonadotropins (10), and this is the probable reason for the increased output when stimulated. Even in this situation there appeared to be a maximal acute release which was reached with the 100-ng dose. The higher dose of 500 ng produced a more long-lasting effect.

The FSH response to the decapeptide was clearly enhanced in the castrates such that really marked increases in hormone levels followed injection of the higher doses of 100 and 500 ng of the peptide. Nevertheless, it is apparent from these results, as from results from intravenous injection (6), that the predominant effect produced by the decapeptide is a release of LH. The subcutaneous route probably produces a situation quite similar to that which follows infusion of the peptide intravenously (4, 5), and this accounts for the greater FSH release than is seen following intravenous injection.

Another difference between the response of the LH- and FSH-secreting cells was the rather delayed response in terms of FSH release in most circumstances.

The selective release of LH observed in the intact animals certainly argues that this decapeptide is not the single gonadotropin-releasing factor which produces release of both FSH and LH from the gland. An additional argument for a separate FSH-releasing factor is provided in the results of saline-injected controls in which a delayed increase in FSH took place in the intact animals. There was no delayed increase in LH in these animals. It is almost inconceivable that this delayed increase in FSH could be caused by endogenous release of the decapeptide, since these animals were so much less sensitive to the decapeptide in terms of FSH than LH release. Dissociations in FSH and LH release following hypothalamic lesions (11) and stimulation (8) and partial separations of the LH- and FSH-releasing activity of hypothalamic extracts (12–14) also support the existence of a distinct FSH-releasing factor.

In striking contrast to the effects on LH and FSH release was the apparent lack of effect of the decapeptide on prolactin release. There was a significant elevation following the 100-ng dose at 4 hr in intact animals, but since this was not reproduced even by a higher dose and was not observed in the castrates, it probably represents an artifact. The decapeptide is not the prolactin-inhibiting factor (PIF). In early studies, evidence for a separation of PIF from LRF was obtained (15).

Summary. The effect of subcutaneous injection of synthetic LRF was evaluated in normal and castrated rats under tribromoethanol (TBE) anesthesia. The procedure of anesthetization with TBE followed by injection and bleeding produced no change in plasma LH in intact males, but a decline followed by a rebound to initial levels in castrates. FSH levels showed a delayed rise in the intact animals and a decline followed by return to initial values in the castrates. In both intact and castrate animals, prolactin levels declined acutely and remained low throughout the experiment. Subcutaneous administration of LRF produced a dose-related acute release of LH in intact and castrate males. The response was prolonged compared to that observed in previous studies following intravenous administration of the peptide. Minimal and delayed FSH release occurred following high doses of 100 or 500 ng of the peptide in intact animals, but dose-related increases of considerable magnitude took place in the castrates. In general, responses in terms of FSH release were more sluggish than those obtained with LH. The peptide had no clear effect on the release of prolactin in these animals as judged by its failure to alter plasma prolactin concentration. It is concluded that subcutaneous administration of LRF is a convenient way to provoke gonadotropin release and that the responses are quite prolonged, mimicking the effects observed following infusion of the peptide.

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