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Prolonged Release of LH and FSH and Depletion of Pituitary Gonadotropin Content after Administration of [D-LEU^6, desGly-NH$_2$^{10}]-LH-RH Ethylamide[1] (39686)

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The incorporation of D-amino acids in position six of the LH-RH decapeptide chain produces analog more potent and longer-acting than the natural hormone (1). The incorporation of D-Leucine in position 6 as well as the substitution of the glycine amide at the *c*-terminus with an ethylamide group, increases the LH and FSH releasing activities even more. Thus, [D-Leu^6, desGly-NH$_2$^{10}]-LH-RH ethylamide is 20-60 times more potent than LH-RH (2). Recently, Rippel *et al.* (3) have claimed that the superactive analogues are merely more potent than their parent hormone and lack prolonged activity. In order to verify this assumption, several doses of [D-Leu^6, desGly-NH$_2$^{10}]-LH-RH ethylamide were injected and the patterns of release of gonadotropins compared with those produced by different amounts of LH-RH, using a sensitive immature male rat assay (2, 4, 5). In addition, since it has been demonstrated that injection of large doses of purified porcine LH-RH produces a depletion of pituitary LH content (6) and since there is also some indirect evidence that a depletion of pituitary gonadotropin content may occur either after a prolonged stimulation (7) or several injections with synthetic LH-RH (8), the effects of [D-Leu^6, desGly-NH$_2$^{10}]-LH-RH ethylamide on the pituitary content of gonadotropin were investigated as well.

Materials and methods. Twenty-five-day-old male rats of the ARS Sprague-Dawley strain were used for all experiments. They were housed in quarters with controlled temperature (about 22°) and light (14 hr light and 10 hr darkness), and had free access to Purina laboratory chow, fresh vegetables, and water. For the immature male rat assay, groups of four animals each were injected with either synthetic LH-RH (50 and 150 ng/rat) or [D-Leu^6, desGly-NH$_2$^{10}]-LH-RH ethylamide (3, 9, and 50 ng/rat) or vehicle alone (0.2 ml of 0.1% gelatine/0.9% NaCl solution). Blood was collected by decapitation at different times after injections and the sera obtained by centrifugation was frozen until assayed for gonadotropins. Means of serum LH and FSH levels in every group were calculated, analyzed by Duncan's new multiple range test (9) and plotted on an arithmetic graph against time; the areas under the curves were arbitrarily taken to represent the integrated serum hormone levels (2, 4, 5). For the depletion test, the animals were injected with either 1 or 10 μg of [D-Leu^6, desGly-NH$_2$^{10}]-LH-RH ethylamide and blood and pituitaries were collected after 2, 4, 6 and 8 hr. The sera were separated by centrifugation. Anterior pituitaries were homogenized in 1 ml of 0.9% NaCl and frozen until assayed for gonadotropin content. In all experiments, LH and FSH were measured by radioimmunoassay using the methods described by Niswender *et al.* (10) and Daane and Parlow (11), respectively. NIH-LH-S17 and NIAMD-FSH-RPI were used as standard preparations. The details of these radioimmunoassays were described previously (7, 12). Hormone levels in pituitary depletion test were compared by Duncan's new multiple range test (9).

The LH-RH analog was prepared by the solid phase method as described previously (2).

Results. Figure 1 shows the patterns of gonadotropin release induced by various doses of LH-RH and [D-Leu^6, desGly-NH$_2$^{10}]-LH-RH ethylamide. It can be seen that, when LH-RH was given, serum levels

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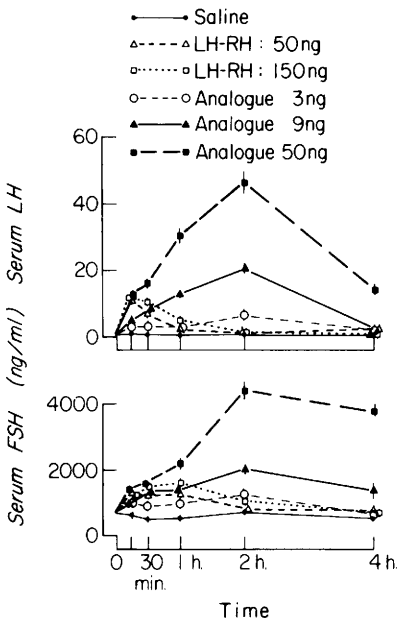

FIG. 1. Serum LH and FSH levels after sc administration of several doses of H-LH and [D-Leu^6, desGly-$NH_2{}^{10}$]-LH-RH ethylamide in immature male rats. Vertical lines indicate the standard error of the mean. Each point represents a mean of four rats.

of gonadotropin started to increase immediately after injections, reaching a peak at 15 min for LH and between 30 to 60 min for FSH. They then declined, reaching control levels at 2 hr. In comparison, the LH-RH analog, at all doses used, produced a completely different pattern of gonadotropin release, reaching a peak at 2 hr for both LH and FSH. At 4 hr, serum LH of the group treated with 50 ng of the analog remained elevated ($P < 0.05$ as compared to LH-RH treated group) as did the FSH serum levels of the groups treated with 50 ($P < 0.01$) and 9 ng ($P < 0.05$) of the analog.

Potencies of [D-Leu^6, desGly-$NH_2{}^{10}$]-LH-RH ethylamide calculated from the curves of Fig. 1 are presented in Tables I and II. The potencies varied according to the doses of the analog H and LH-RH used. The LH-releasing activity of the analog was 20 to 60 times greater (Table I) and the FSH-releasing activity was 10 to 30 times higher than that of LH-RH (Table II).

The effect of administration of 1 or 10 μg of [D-Leu^6, desGly-$NH_2{}^{10}$]-LH-RH ethylamide on serum LH and FSH and pituitary content of gonadotropin is shown in Fig. 2. Serum LH after injection of both doses of analog remained elevated for up to 6 hr and FSH levels were still higher at 8 hr when

TABLE I. LH-RELEASING ACTIVITIES OF [D-LEU^6, desGly-$NH_2{}^{10}$]-LH-RH ETHYLAMIDE (ANALOG) BASED ON THE COMPARISON OF INTEGRATED SERUM LH LEVELS RELEASED BY THE ANALOG VS THOSE RELEASED BY LH-RH

Dose Analog (ng/rat)	LH-RH (ng/rat) LH-RH (ng/rat)	
	50	150
3	25.9	51.1
9	32.2	63.5
50	20.9	35.7

TABLE II. FSH-RELEASING ACTIVITIES OF [D-Leu^6, desGly-$NH_2{}^{10}$]-LH-RH ETHYLAMIDE (ANALOG) BASED ON THE COMPARISON OF INTEGRATED SERUM FSH LEVELS RELEASED BY THE ANALOG VS THOSE RELEASED BY LH-RH.

Dose Analog (ng/rat)	LH-RH (ng/rat)	
	50	150
3	17.3	33.6
9	15.0	31.3
50	9.5	18.6

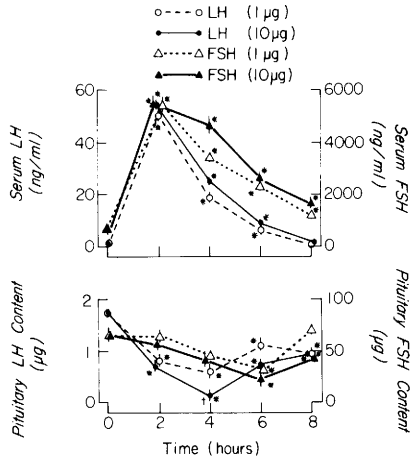

FIG. 2. Serum LH and FSH levels and gonadotropin pituitary content after sc injection of 1 or 10 μg of [D-Leu^6, desGly-$NH_2{}^{10}$]-LH-RH ethylamide in immature male rats. The analogue was injected at 0 time. Each point represents a mean of four rats. Vertical lines indicate the SEM. Duncan's new multiple range test: * Significantly different from the value of respective gonadotropin level at 0 time. +Significantly different from the value of the respective group injected with 1 μg of the analog.

compared with basal values. The injection of 1 or 10 μg of [D-Leu^6, desGly-NH$_2$^{10}]-LH-RH ethylamide produced a significant depletion of the pituitary content of gonadotropin. LH content was lower than the preinjection value at 2 hr ($P < 0.05$) fell to the lowest value at 4 hr ($P < 0.05$), and was still below basal levels at 8 hr ($P < 0.05$) after injection of both doses. FSH content was decreased to a lesser extent, reached a nadir at 6 hr ($P < 0.05$) and was similar to preinjection values at 8 hr in the group treated with 1 μg of analog.

Discussion. Rippel *et al.* (3) have suggested that the superactive analog of LH-RH substituted in positions 6 and 10 are merely more active than their parent hormone without having prolonged activity. They showed that [D-Leu^6, desGly-NH$_2$^{10}]-LH-RH ethylamide produced a peak of LH later than LH-RH in diestrous rats, but the decline of the gonadotropin levels was similar for both peptides. They argued that this reflects an unexplained delay in LH increase after the injection of the analogue rather than a protracted effect. In contrast, the results presented here show that in immature male rats even a small dose of [D-Leu^6, desGly-NH$_2$^{10}]-LH-RH ethylamide (3 ng/rat) elicits a peak at 2 hr (compared to 15 min for each dose of LH-RH) and produces a completely different pattern of gonadotropin release than that brought about by any dose of LH-RH. Furthermore, we have observed the same profile of LH release when [D-Ala^6, desGly-NH$_2$^{10}]-LH-RH ethylamide, which is equal in potency to [D-Leu^6, desGly-NH$_2$^{10}]-LH-RH ethylamide[2] was compared to LH-RH using doses adjusted for differences in potencies in diestrous I day and diestrous II day rats (unpublished data). This supports our previous contention that the superactive analogues of LH-RH exhibit prolonged effects (2).

The LH and FSH releasing activities of [D-Leu^6, desGly-NH$_2$^{10}]-LH-RH ethylamide varied depending on the doses of either analogue or LH-RH given (Tables I and II). This would be expected since nonparallel exponential relationships exist between the doses of these peptides and their responses. Although the administration of relatively low doses of either LH-RH (100 ng/rat) or [D-Leu^6, desGly-NH$_2$^{10}]-LH-RH ethylamide (10 ng/rat) did not alter the pituitary content of gonadotropin (results not shown), the LH-RH analog given in doses of 1 or 10 μg/rat produced a depletion of the pituitary LH and FSH content (Fig. 2). The pituitary LH content fell to its lowest value at 4 hr after injection whereas the FSH content reached a nadir at 6 hr. The differences between the profiles are presumably due to different patterns of secretion of LH and FSH by the gonadotrophs (6). These results demonstrate that the gonadotropin pituitary content can be depleted by LH-RH analog. We reported previously that large doses of purified porcine LH-RH also induced some depletion of pituitary LH-content in rats (6). These observations should be taken into account when administering repeatedly or infusing continuously LH-RH or its analog to humans (8) and rats (7).

Summary. LH and FSH releasing activities of [D-Leu^6, desGly-NH$_2$^{10}]-LH-RH ethylamide were examined using immature male rats. The gonadotropin release induced by various doses of [D-Leu^6, desGly-NH$_2$^{10}]-LH-RH ethylamide followed a different pattern than when LH-RH was given, reaching a peak at 2 hr after injections, serum LH and FSH being still elevated at 4 hr. In contrast the LH and FSH release induced by LH-RH reached a peak in 15 min and declined at 1 hr. This provides further evidence that this analog is indeed long-acting. The LH-RH analog had a potency 20–60 times greater for LH release and 10–30 times higher for FSH release than LH-RH. [D-Leu^6, desGly-NH$_2$^{10}]-LH-RH ethylamide also caused a significant depletion of the pituitary gonadotropin content.

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