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The Effect of Oral and Vaginal Administration of Synthetic LH-RH and [D-ALA^6, DES GLY^{10}-NH$_2$]-LH-RH Ethylamide on Serum LH Levels in Ovariectomized, Steroid Blocked Rats[1] (38678)

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Recently, Amoss *et al.* (1) and Humphrey *et al.* (2) reported that a sizeable increase of serum LH could be obtained following oral and vaginal administration of high doses of synthetic LH-RH. [D-Ala^6, DesGly^{10}-NH$_2$]-LH-RH ethylamide which was recently prepared in our laboratory was found to be considerably more potent than LH-RH in stimulating the release of LH and FSH in rats (3, 4) and in human beings (5) when it was injected sc or iv. The present paper deals with the effect of oral and vaginal administration of this potent analog on serum LH levels in comparison with the effect of LH-RH in rats.

Materials and methods. Female rats of the Sprague–Dawley strain with body weights of 250 g were housed in quarters equipped to provide controlled temperature and illumination, and had free access to Purina Lab Chow and tap water. They were ovariectomized, and 3 wk later, injected with 25 mg progesterone and 50 μg estradiol benzoate in oil. The animals were used for experiment 48 hr after injection of steroids. Under anesthesia with urethane (0.15 g/100 g body wt, sc), the rats were given test samples either orally or through the vaginal route. For oral administration, the test preparation was dissolved in 0.1% gelatin/0.9% saline so that 0.5 ml of the solution contained 1000, 100, and 10 μg of LH-RH (Farbwerke Hoechst AG) or 10, 1, or 0.1 μg of [D-Ala^6, DesGly^{10}-NH$_2$]-LH-RH ethylamide (D-Ala^6-LH-RH-EA). For the vaginal suppository, LH-RH or the analog was suspended in Carbowax 1000 (Matheson Coleman and Bell Co.) (MW 950–1050) at 48° so that each 0.05 ml contained similar doses to those used for oral administration. These peptides were then applied into the vagina as described by Humphrey *et al.* (2). Control rats received 0.05 ml of solid Carbowax 1000.

Blood samples were collected through the jugular vein before and 15, 30, 60, and 120 min after administration of the test samples. Sera were separated by centrifugation, and stored at −20° until assayed for LH content. Serum LH levels were determined in duplicate by radioimmunoassay as reported by Niswender *et al.* (6) and expressed in terms of NIH-LH-S-17. The data were subjected to statistical analysis using Duncan's new multiple range test.

Results. Oral Administration. Synthetic LH-RH was found active in raising serum LH levels by the oral route at all doses tested. As shown in Fig. 1, LH levels reached a peak 15 min after administration. The mean peak level after 1000 μg LH-RH (48 ng/ml) was the same as that after 100 μg (50 ng/ml) but higher than that after 10 μg (19 ng/ml). LH levels declined thereafter, and reached levels which were not different from control values at 60 min.

Oral administration of 0.1 μg of D-Ala^6-LH-RH-EA failed to raise serum LH levels. However, 1 and 10 μg of the analog induced progressive rise of LH levels, the highest level being 28 ng/ml and 131 ng/ml, respectively.

Vaginal administration. Vaginal application of LH-RH induced a greater and more sustained elevation of serum LH levels as compared to the oral administration at the same doses. Vaginal application of 100 μg of LH-RH in Carbowax caused progressive rise of serum LH throughout the period of

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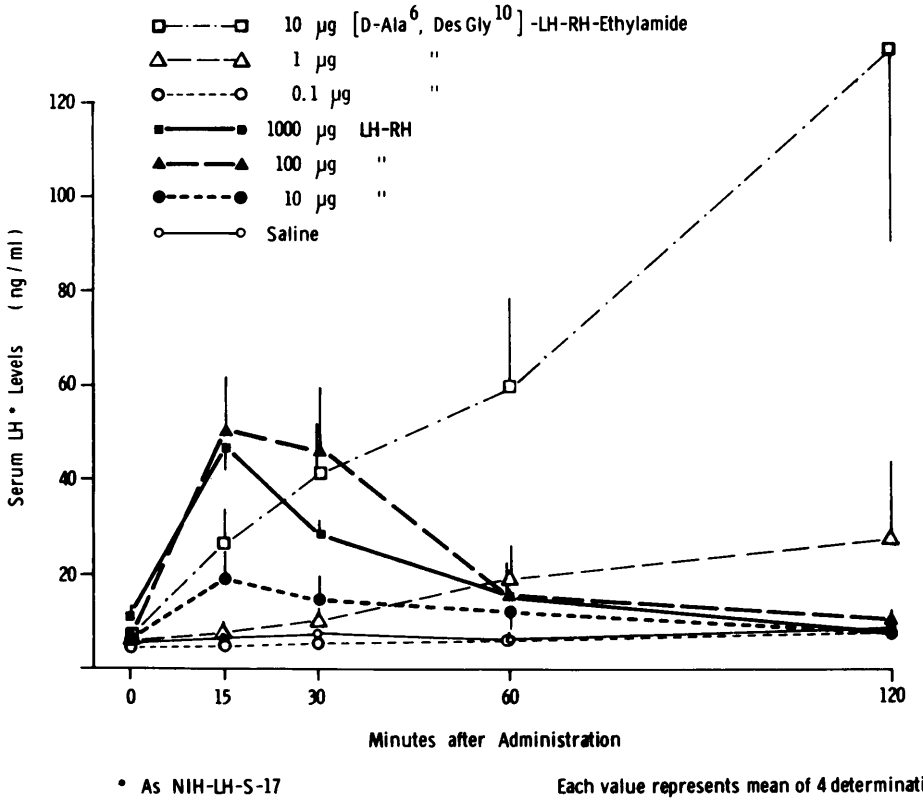

FIG. 1. Serum LH levels following oral administration of 0.1% gelatin/0.9% saline, synthetic LH-RH or [D-Ala^6, DesGly^{10}-NH$_2$]-LH-RH ethylamide in ovariectomized, estrogen and progesterone treated rats. Each value represents mean of four determinants. Vertical lines indicate standard errors of the means for each group of determinations. Administration of the analog induced a progressive rise of LH, whereas the elevation of LH after LH-RH was transient.

observation, resulting in a level as high as 246 ng/ml at 120 min. On the other hand, 10 μg of LH-RH elicited a peak LH increase at 15 min (55 ng/ml) and serum LH levels declined gradually toward the baseline thereafter (Fig. 2).

Vaginal administration of 10 and 1 μg of the analog, but not 0.1 μg induced a significant and progressive rise of serum LH levels with the highest value of 232 and 103 ng/ml, respectively. The response induced by 10 μg of the analog was comparable to that for 100 μg synthetic LH-RH (Fig. 2).

Discussion. Our previous observation showed that D-Ala^6-LH-RH-EA was about 31 times more potent than LH-RH when the integrated serum LH levels of the time course response to this analog were compared with those produced by the same dose of the latter for a period of 6 hr after sc injection. Fifteen to 12 times greater LH/FSH releasing potencies were also observed for a period of 2 hr following iv infusion. In the present study, oral administration of D-Ala^6-LH-RH-EA also elicited a greater LH release than did the same dose of LH-RH. The patterns of serum LH levels resulting from oral administration of these two peptides were quite different. After administration of either 10 or 1 μg D-Ala^6-LH-RH-EA, serum LH levels increased progressively for at least 2 hr, whereas LH-RH induced a peak of LH at 15 min which was followed by a gradual decrease. This could be due to the slower absorption of the analog than LH-RH. LH-RH which escapes enzymatic destruction in the alimentary canal may be rapidly transferred into the general

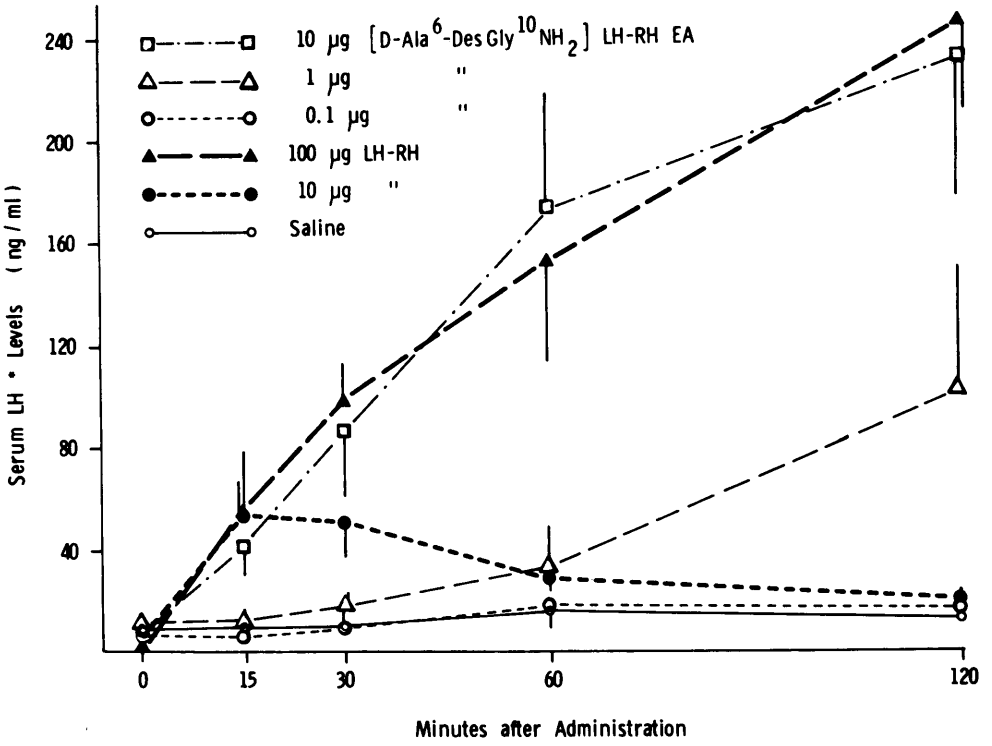

FIG. 2. Serum LH levels following vaginal administration of Carbowax 1000 (0.05 ml), synthetic LH-RH or [D-Ala^6, DesGly^{10}-NH$_2$]-LH-RH ethylamide in Carbowax in ovariectomized EP blocked rats. Each value represents mean of four determinants. Vertical lines indicate standard errors of the means for each group of determinations. LH patterns were similar for 100 μg of LH-RH and 10 μg of the analog.

circulation, resulting in an immediate rise of serum LH, but the stimulation is only of short duration, suggesting rapid metabolic breakdown. D-Ala^6-LH-RH-EA however could be more resistant to *in vivo* breakdown either before or after absorption by the mucosa of the alimentary tract. It is interesting that the maximum LH levels induced by the analog (at 120 min) were significantly greater than the peak levels after LH-RH which occurred at 15 min, and that the integrated serum LH levels after 10 μg of the analog were greater than those after 1000 μg LH-RH.

The different patterns of serum LH levels after vaginal application of 10 μg LH-RH and 1 μg of the analog again suggest a difference between LH-RH and the analog in their resistance to metabolic or enzymatic destruction. However, the similar LH patterns after larger doses of LH-RH and the analog could reflect a smaller enzymatic destruction of LH-RH in the vagina than in the alimentary canal.

Although an accurate quantitative comparison of the results in two different experiments is difficult, the same doses of either LH-RH or D-Ala^6-LH-RH-EA elicited greater responses after vaginal application than oral administration. This might be also explained by a lesser extent of destruction of these peptides in the vagina than in the alimentary tract.

In any case, D-Ala^6-LH-RH-EA induced considerably greater and more prolonged elevation of serum LH levels than did the same dose of LH-RH. This tendency is particularly evident in the case of oral administration, suggesting a possible oral use of this analog in clinical applications.

Summary. Effects of oral and vaginal administration of LH-RH and [D-Ala^6, DesGly^{10}-NH$_2$]-LH-RH ethylamide (D-Ala^6-LH-RH-EA) on serum LH levels in ovariectomized, estrogen, progesterone treated rats were investigated. Oral administration of synthetic LH-RH induced a quick rise of serum LH levels with the greatest elevation at 15 min at any dose levels tested. On the other hand, oral administration of D-Ala^6-LH-RH-EA resulted in a slow but progressive rise of LH during 120 min of observation. The total amount of LH released by 10 μg of the analog was much greater than the total released by 1000 μg of LH-RH. Vaginal administration of 100 μg of LH-RH mixed with Carbowax induced a progressive rise of LH which was indistinguishable from that following 10 μg of the analogue, suggesting that the potency of the analogue is 10 times greater than that of LH-RH for vaginal administration. Ten μg of LH-RH given through the vagina induced a rapid rise of LH with the peak at 15 min, whereas 1 μg of the analog induced a slow but progressive rise. Greater resistance of D-Ala^6-LH-RH-EA than LH-RH against *in vivo* breakdown is postulated as one of the causes of greater and prolonged LH release by the former.

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