Luteinizing Hormone Releasing Activity of an Analogue of Luteinizing Hormone Releasing Hormone, [des-Gly-NH₂¹⁰, Pro-ethylamide⁹]-LH-RH in Cattle

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Abstract A single injection of 50 or 200 μ g of a luteinizing hormone releasing hormone (LH-RH) analogue [des-Gly-NH₂¹⁰, Pro-ethylamide⁹]-LH-RH (EA-LH-RH) was given intramuscularly to male calves and changes in plasma levels of luteinizing hormone (LH) were assayed before and after the administration. The peak levels after administration of 50 and 200 μ g of EA-LH-RH were 6.5 and 1.4 times higher than those at corresponding doses of synthetic LH-RH, respectively. Total amounts of LH released (areas under the curve) at the two doses of EA-LH-RH were 9.0 and 1.7 times larger than those at the corresponding doses of synthetic LH-RH, respectively. Time attaining to the peak level in the plasma LH concentration was longer in the EA-LH-RH group than in the synthetic LH-RH group at each dose. These results demonstrate that EA-LH-RH possesses greater and longer LH releasing activity than the synthetic LH-RH in cattle.

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It has been reported that luteinizing hormone releasing hormone (LH-RH) stimulates both synthesis and release of pituitary gonadotropin¹⁾. After the structure of porcine and ovine LH-RH was elucidated^{2,3)}, many analogues have been synthesized⁴⁻⁷⁾. Some of them have been found to possess greater LH-RH activity than the LH-RH itself. An analogue, [des-Gly-NH₂¹⁰, Pro-ethylamide⁹]-LH-RH (EA-LH-RH) is evaluated to be approximately 5 times active as compared to LH-RH by ovulation inducing assay in the rat⁸⁾. For future application of this agent for controlling of bovine reproduction and the therapy of bovine reproductive dysfunction, LH releasing activity of this agent was examined in cattle in comparison with synthetic LH-RH.

Materials and Methods

Four male calves of the Japanese Brown breed were used. They were 6-month-old, and between 169 and 226 kg.

Single intramuscular injection of 50 or 200 μ g of EA-LH-RH or the synthetic LH-RH dissolved in 5 ml of physiological saline was given to each calf. All animals were subjected to 4 separate injections under a 2 $\times$ 2 factorial experimental design. The interval between two successive injections was one week.

Blood samples were collected from the jugular vein *via* polyethylene catheter at 5-min-intervals from -5 min to 15 min and at 15-min-intervals from 30 min to 6 hr after injection of EA-LH-RH or the synthetic LH-RH. Blood was icechilled until plasma was separated by centrifugation and all samples were stored at -20°C until assayed.

Plasma levels of LH were determined by the double antibody radioimmunoassay described by NISWENDER *et al.*⁹⁾. Purified LH (LER-1056-C2) was labeled with ¹²⁵I (New England Nuclear) and the procedure was essentially the same as described by HUNTER and GREENWOOD¹⁰⁾. Antiserum to ovine LH (GDN #15) was used at a dilution of 1:40,000. Triplicated standard curves were run with NIH-LH-B7 in each assay, and the results were expressed in terms of ng NIH-LH-B7 per ml of plasma. All assays of plasma samples were run in duplicate.

The response to EA-LH-RH and synthetic LH-RH was estimated both by plasma LH concentrations and by total amounts of LH released in terms of areas under the LH response curves.

Results and Discussion

Changes in plasma LH concentrations after administration of 50 µg of EA-LH-RH and the synthetic LH-RH are shown in Fig. 1-A. Injection of EA-LH-RH resulted in a rapid and marked elevation of plasma LH concentrations with the peak observed at 120 min post-injection. Plasma LH concentrations after the injection of synthetic LH-RH reached a peak at 15 min post-injection and gradually declined thereafter to the basal level. The magnitude of elevation of plasma LH concentration induced by EA-LH-RH was much greater than that by synthetic LH-RH. The peak LH concentration in the EA-LH-RH group was 6.5 times as high as that in the synthetic LH-RH group. The amount of LH released in the EA-LH-RH group was 9 times larger than that in synthetic LH-RH group.

Changes in plasma LH concentration after administration of 200 µg of EA-LH-RH and synthetic LH-RH are illustrated in Fig. 1-B. After administration of EA-LH-RH, the concentrations rose promptly, reached almost the maximal level at 45 min, peaked at 135 min post-injection and then declined gradually. Injection of the synthetic LH-RH also induced the similar changes and the peak concentration was observed at 90 min post-injection. The peak concentration in the EA-LH-RH group was 1.4 times greater than that in the synthetic LH-RH group. The amount of LH released in the EA-LH-RH group was 1.7 times larger than that of the synthetic LH-RH group.

The LH response to EA-LH-RH was greater than that to the parent peptide at both doses and is agreeable with previous reports on EA-LH-RH studied in rats¹¹⁾ and human¹²⁾. The increased biological activity may be due to either greater affinity to binding sites on target cells, greater efficiency to promote secretion after binding to receptor(s) at the target organ, a slower enzymic breakdown, or a combination of these factors¹³⁾.

Fig. 1. Changes in mean plasma LH concentrations after intramuscular injection of 50 (A) and 200 (B) µg of EA-LH-RH (solid line) or synthetic LH-RH (dotted line) in cattle. (Mean±S.E.M.: four samples for each points)

The pattern of response to EA-LH-RH was essentially similar to that to the synthetic LH-RH. However, time attaining to the peak level was longer in the EA-LH-RH group than in the synthetic LH-RH group at each dose level which may principally be ascribed to the longer half-life of the analogue as reported that the half life of EA-LH-RH is 2 times longer than that of the synthetic LH-RH in human¹²).

All findings indicate that EA-LH-RH would be an efficacious agent for controlling gonadal function and the therapy of gonadal dysfunction in cattle.

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牛における黄体形成ホルモン放出ホルモン類縁物質
[des-Gly-NH₂¹⁰, Pro-Ethylamide⁹]-LH-RH
の黄体形成ホルモン放出効果

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黄体形成ホルモン放出ホルモンの類縁物質 [des-Gly-NH₂¹⁰, Pro-ethylamide⁹]-LH-RH (EA-LH-RH) はラットの排卵誘起反応で，合成 LH-RH の約 5 倍の力価を有することが報告されている。しかし，牛における同ホルモンの黄体形成ホルモン (LH) 放出効果についてのこのような報告は見当らない。本実験は牛に同ホルモンを投与して，投与後の血中 LH 濃度を経時的に測定し，合成 LH-RH 投与時の反応と比較検討したものである。供試動物には褐毛和種の雄子牛 4 頭を用い，EA-LH-RH あるいは合成 LH-RH の 50 あるいは 200 μ g を筋肉注射によって投与した。EA-LH-RH の 50 ある

いは 200 μ g 投与後の血中 LH 濃度の上昇曲線のピーク値は同量の合成 LH-RH 投与後のものに比べて高く，それぞれ 6.5 および 1.4 倍であった。また，LH 濃度の上昇曲線に囲まれた面積の比較では，EA-LH-RH は合成 LH-RH に比べて広く，それぞれ 8.4 および 1.7 倍であった。これらの結果から，EA-LH-RH は合成 LH-RH に比較して大きな LH 放出効果をもつことが明らかにされた。また，LH 濃度の上昇曲線がピーク値に達するまでには，いずれの投与量においても，EA-LH-RH の方が LH-RH より長時間を要した。

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