LH-RELEASING ACTIVITY OF POTENT LH-RH ANALOGS IN VITRO

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SUMMARY

The LH-releasing activity of eight superactive analogs of LH-RH was measured in pituitary cells in primary culture. Introduction of the C-terminal ethylamide modification into [D-Ala^6]- and [D-Leu^6]-LH-RH (two peptides already 3 times more active than LH-RH) increases their activities 10-fold. [D-Phe^6]- and [D-Trp^6]-LH-RH are 90 and 100 times more active than LH-RH, respectively. The ethylamide derivatives of these two compounds are however approximately six times less active than the parent peptides.

INTRODUCTION

Numerous studies have already clearly indicated the clinical importance of LH-RH for both diagnostic and therapeutic use in man. Since LH-RH has such a short half-life in man (2, 3) and other mammalian species (4), it is however imperative to devise compounds with prolonged biological activity. Useful agonists will thus be those which: 1) will have high affinity for the pituitary LH-RH receptor and 2) will be resistant to degradation by plasma and tissue peptidases.

The present study performed with primary cultures of anterior pituitary cells describes the LH-releasing activity of eight superactive analogs of LH-RH possessing a D-amino acid a position 6 in the presence or absence of the C-terminal modification of Fujino et al. (5).

MATERIALS AND METHODS

Cell preparation

Primary cultures of enzymatically dispersed cells from anterior pituitaries of adult female Sprague-Dawley rats were prepared and incubated as described (6) except that the HEPES buffer was free of Ca^{2+} and that the cell suspension was washed by centrifugation through a layer of 4% bovine serum albumin after the Viokase treatment. 5-7 x 10^5 cells in 1.5 ml of Dulbecco's modified Eagle's medium (DMEM) containing 10% horse serum and 2.5% calf serum were plated in 35 x 10 mm Falcon petri dishes. Cells were usually used four days after plating.

Incubation procedure

Cells were washed four times with DMEM without sera before addition of fresh DMEM and incubation for 5 hours in the presence or absence of the indicated concentrations of LH-RH or of its analogs prepared by solid-phase synthesis and purified as described previously (7, 8). Triplicate dishes were used in all groups. At the end of incubations, media were spun at 100 xg for 5 min at 4°C and the supernatants frozen at -20°C until hormone assays.

LH measurements

LH release was measured by double-antibody radioimmunoassay (9, 10) using rat hormones (NIAMDD rat LH-1-3 and LH-RP-1) and rabbit antiserum (NIAMD anti-rat serum 1) kindly provided by Dr. A.F. Parlow for the National Institute of Arthritis and Metabolic Diseases, Rat Pituitary Hormone Program. Goat anti-rabbit γ-globulin was a product of Endocrinolab, Quebec.

Calculations

Radioimmunoassay data were analyzed with a Hewlett-Packard desk-top calculator using a program written in this laboratory and based on model II of Rodbard and Lewald (11). Dose-response curves and 50% effective doses (ED_{50}) were calculated using a weighted iterative non-linear least squares regression (12). Data on Figures are expressed as mean ± S.E.M. of triplicate incubations.

RESULTS AND DISCUSSION

The *in vitro* dose-response curves obtained for the eight peptides, together with LH-RH, are shown in Figures 1, 2 and 3. Activities, calculated and compared with LH-RH on the basis of the 50% effective doses, appear in Table I. [D-Ala^6]- and [D-Leu^6]-LH-RH were both about 3 times more potent than LH-RH, in rough agreement with results obtained previously

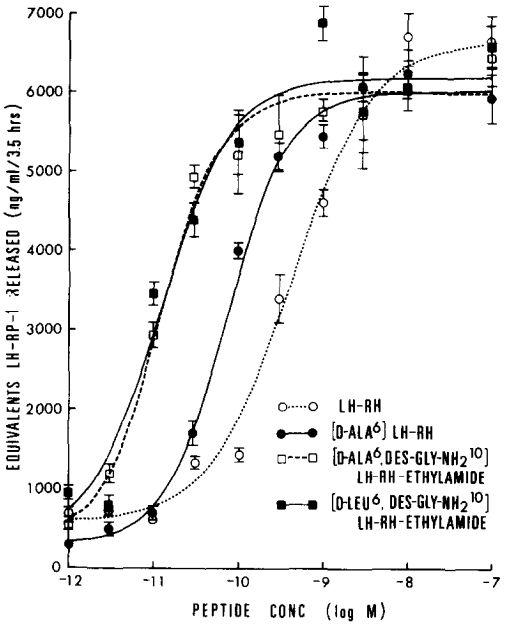

Figure 1: LH dose-response curves of LH-RH, [D-Ala^6] LH-RH,[D-Ala^6, desGly-NH_2^{10}] LH-RH ethylamide and [D-Leu^6, des-Gly-NH_2^{10}] LH-RH ethylamide in rat anterior pituitary cells in primary culture.

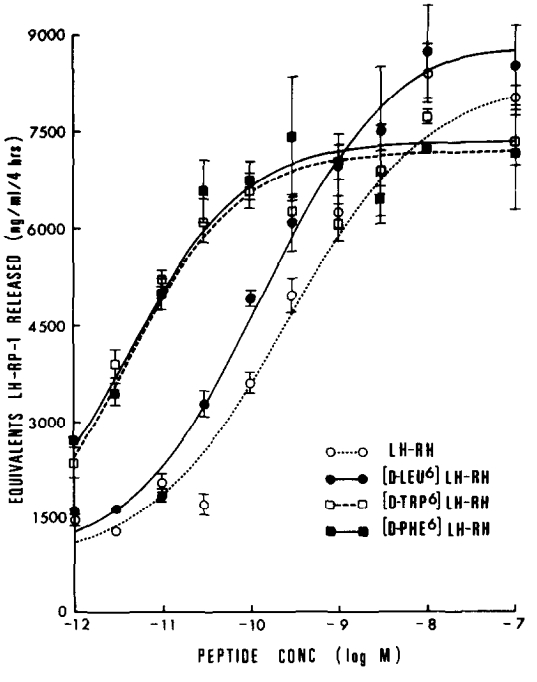

Figure 2: LH dose-response curves of LH-RH, [D-Leu^6] LH-RH, [D-Trp^6] LH-RH and [D-Phe^6] LH-RH ethylamide in rat anterior pituitary cells in primary culture.

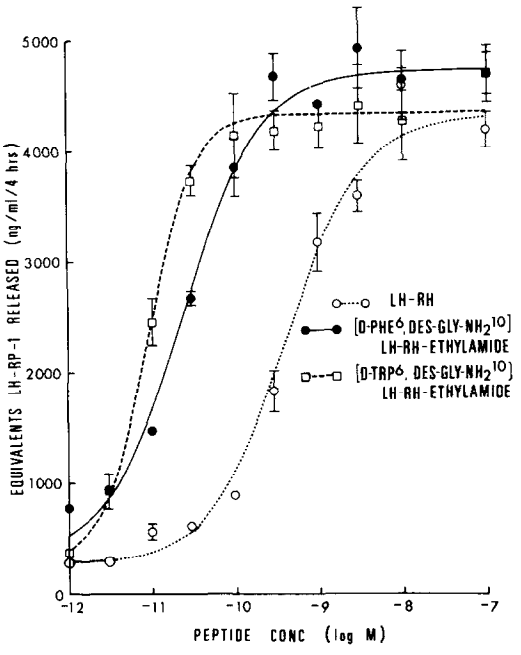

Figure 3: LH dose-response curves of LH-RH, [D-Phe^6, desGly-$NH_2{}^{10}$] LH-RH ethylamide and [D-Trp^6, desGly-$NH_2{}^{10}$] LH-RH ethylamide in rat anterior pituitary cells in primary culture.

(7, 8, 13) *in vivo* in the rat. Introduction of the C-terminal ethylamide modification into the two peptides increases their activities ten fold. A similar potentiating effect of the C-terminal modification has been observed in *in vivo* experiments (7, 8, 14), although somewhat higher potencies (up to 40 times the activity of LH-RH) were reported.

When bulkier and more lipophilic amino acids, D-phenylalanine or D-tryptophan, were placed in position 6 of the parent decapeptide, a very dramatic effect on LH-releasing properties took place. [D-Phe^6]- and [D-Trp^6] LH-RH were 90 and 100 times more active than LH-RH, respectively, by far the highest activities yet reported for any LH-RH analog. Surprisingly, the ethylamide derivatives of these two compounds were

TABLE I

LH-releasing activity of LH-RH analogs *in vitro*. LH release was measured after 5 hours of incubation with increasing concentrations of the indicated analogs in primary cultures of rat anterior pituitary cells. Relative activities of the analogs were compared with LH-RH at the 50% effective doses.

PEPTIDE	LH-releasing activity relative to LH-RH
LH-RH	1
[D-Leu^6]-LH-RH	3
[D-Leu^6, des-Gly-$NH_2{}^{10}$]-LH-RH ethylamide	30
[D-Ala^6]-LH-RH	3
[D-Ala^6, des-Gly-$NH_2{}^{10}$]-LH-RH ethylamide	30
[D-Phe^6]-LH-RH	90
[D-Phe^6, des-Gly-$NH_2{}^{10}$]-LH-RH ethylamide	15
[D-Trp^6]-LH-RH	100
[D-Trp^6, des-Gly-$NH_2{}^{10}$]-LH-RH ethylamide	15

approximately six times less active than the parent peptides and only half as active as [D-Ala^6, desGly-$NH_2{}^{10}$]- or [D-Leu^6, desGly-$NH_2{}^{10}$]-LH-RH ethylamide. Apparently, the interaction, probably conformational in character, between the position 6 and C-terminal alterations becomes unfavourable when D-amino acids with large and/or hydrophobic side-chains occur in position 6.

A comparison of these results with those reported *in vivo*

(15) reveals an interesting divergence in activities calculated from the two methods. In the rat, in vivo, [D-Phe^6]- and [D-Trp^6]-LH-RH are only 10 and 13 times more active than LH-RH, respectively, and hence not quite as effective as [D-Ala^6, desGly-NH$_2$^{10}]- and [D-Leu^6, desGly-NH$_2$^{10}]-LH-RH ethylamide. Therefore, although [D-Phe^6]- and [D-Trp^6]-LH-RH probably have much higher affinities for the pituitary receptor sites than any of the ethylamide analogs, several of the latter are more active in vivo. The most likely explanation for this appears to be that the ethylamide peptides are generally more resistant to physiological degradation and inactivation.

These results illustrate the value of conducting dual bioassays as a tool in designing LH-RH analogs with certain desirable features. It should be mentioned that the [D-Phe^6]- and [D-Trp^6]-peptides show great promise as structures on which to base the development of LH-RH antagonists. Several such compounds have been synthesized which are very effective in inhibiting gonadotropin release and ovulation in animals (16, 17).

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