LH-RH–RECEPTOR INTERACTION IS INHIBITED BY DES-HIS–2-DES-GLY–10-LH-RH–ETHYLAMIDE

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1. Introduction

Luteinizing hormone - releasing hormone (LH-RH) is a hypothalamic hormone which stimulates the secretion of pituitary hormone(s) which regulate ovulation [1]. The elucidation of the amino acid sequence (pGlu–His–Trp–Ser–Tyr–Gly–Leu–Arg–Pro–Gly–NH_2) of LH-RH [1] has enabled a considerable number of related peptides to be synthesized and examined for biological activities. Much data has accumulated on the structure-activity relationship of this hormonal peptide. The results of these studies suggested that the active site of LH-RH was located in the N-terminal region of the molecule, that is, the pGlu and the His residues played important roles in the hormonal activity [2–5]. Furthermore, studies on analogs of LH-RH lacking the N-terminal pGlu ring structure suggested that the CO-NHCHCO- group was the minimum necessary part of the pGlu residue to exhibit biological activity. Recently several analogs of LH-RH with enhanced LH and FSH releasing activity were synthesized [6–10]. Interest in derivatives of LH-RH has arisen, primarily, because of the possibility of finding analogs of LH-RH which would inhibit the release of gonadotropins instead of stimulating them. This may form the basis of a new contraceptive method. Part of the mechanism of action of LH-RH involves the interaction of the hormone with pituitary cell receptors [11], and the binding of LH-RH to the adenohypophysis could be located at the level of the plasma membrane [12–13].

An important criterion for an inhibitor of LH-RH actions would be that it should effectively compete with the hormone for pituitary receptor sites. We were therefore prompted to report on data concerning our receptor studies with the peptide des-His–2-des-Gly–10-LH-RH–ethylamide, which was shown to act as antagonist of LH-RH in vivo [14].

2. Materials and methods

LH-RH, ^{125}I-labeled LH-RH and des-His–2-des-Gly–10-LH-RH–ethylamide were obtained from Farbwerke Hoechst AG, Frankfurt, GFR. The specific activity of [^{125}I] LH-RH used in the present experiments was 241 mCi/mg. Specific biological activity of ^{125}I-labeled LH-RH was indistinguishable from unlabeled material. Female rats of the Sprague Dawley strain (Mus Rattus AG, Brunnthal, GFR) weighing 175–200 g were used for all experiments. The animals were housed in a temperature-controlled room lighted for only 12 hr a day and unrestricted access was provided to food and water. The rats were killed by decapitation, the posterior lobes of the pituitaries were removed and the anterior pituitaries cut in halves. Five halves were transferred aseptically into 25 ml Erlenmeyer flasks, which contained 2 ml of sterile medium. The incubation medium consisted of 9 parts Medium 199 (Biocult Labs., Glasgow) 1 part fetal bovine serum (Reheis Chemical Comp., Chicago) and 50 U/ml each of penicillin and streptomycin were added to reduce bacterial contamination. The opposite halves of the anterior lobes of the pituitaries served as controls and were treated in the same manner. All incubations were carried out at 37°C under an atmosphere of 95% air and 5% CO_2. Pre-incubation of the glands was performed for 1 hr. The medium was changed and incubation was continued for 4 hr. New medium contained LH-RH

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(10 ng/ml) and/or des-His–2-des-Gly–10-LH-RH–ethylamide at concentrations of 0.1, 5, 10, 100 and 1000 ng/ml medium. The controls were incubated in the absence of peptides. Five incubation flasks were set up for the experimental and control groups. After incubation the media were snap frozen and stored at – 25°C. The pituitaries were washed twice with buffer (0.01 M phosphate, 0.15 M NaCl, 0.1% NaN_3) and snap frozen in 2 ml of the same buffer. Pituitary protein was determined according to Lowry et al. [15]. Media were assayed for LH and FSH by radioimmunoassay as described by Niswender et al. [16]. The separation of bound from free tracer was modified by using a solid-phase second antibody (Dasp, anti-rabbit, N.V. Organon, Oss, Holland).

Data were analysed by Student's paired t-test.

Plasma membranes of adenohypophyses were prepared according to previously described procedures [17]. Plasma membranes were suspended in Hepes buffer (mM: NaCl, 137; KCl, 5; Na_2HPO_4, 0.7; *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, 25; $MgCl_2$, 5; sucrose, 300; pH 7.2). Concentrations of plasma membrane fractions are reported as protein content [15].

The [^{125}I] LH-RH binding assay was carried out at 4°C. All solutions were made up in Hepes buffer. Aliquots of plasma membranes (111 μg protein) were mixed with various concentrations of LH-RH and des-His–2-des-Gly–10-LH-RH–ethylamide, and incubated for 30 min. [^{125}I] LH-RH (323.2 pmoles) was added and incubation continued for another 30 min. Final incubation volume was 80 μl. The reaction was stopped by adding a 10-fold excess of ice cold phosphate buffer (0.01 M phosphate, pH 7.2), and the incubation mixtures were immediately filtered through a cellulose acetate filter (EHWP 02500, Millipore Corp., Bedford, Mass. 01730, USA). The filters were washed three times with 2 ml of the same phosphate buffer. Binding assays were carried out in triplicates.

Analysis of binding data was performed by Scatchard plot analysis [18] by a computer program

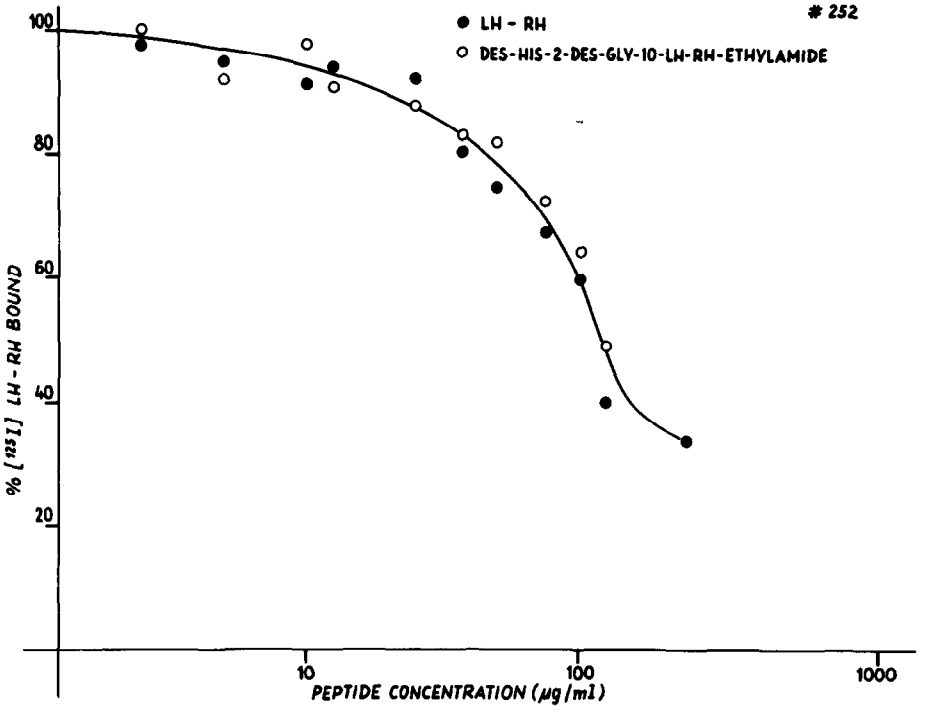

Fig. 1. Binding of [^{125}I] LH-RH to the pituitary plasma membrane as function of the concentration of unlabeled LH-RH and octapeptide antagonist. Means ± SD of three determinations.

(unpublished) and was run on a PDP-8/e (Digital Equipment Corp., Maynard, Mass., USA).

3. Results and discussion

Recently LH-RH interaction with the pituitary plasma membrane was described [12–13] and found to be a temperature, cation and pH dependent process [19]. Furthermore, LH-RH binding to its pituitary receptors was shown to be a rapid and reversible reaction [12,20]. In addition, two populations of binding sites with an apparent affinity constant of approx. 2×10^{-8} M and 2×10^{-7} M were observed [13]. LH-RH interaction was found to be a highly specific process [12,20] and analysis of binding data obtained from experiments with analogs suggested proportionality between releasing ability and receptor affinity [21]. Similarily, TRH binding sites were reported to have affinities for TRH analogs which are proportional to their biological potencies [22].

Des-His–2-des-Gly–10-LH-RH–ethylamide was found to compete with [^{125}I] LH-RH for LH-RH binding stes (fig. 1). Relative binding at all competing concentrations of the LH-RH analog studied was parallel to that obtained at equivalent LH-RH concentrations. Similarity in the kinetics of binding

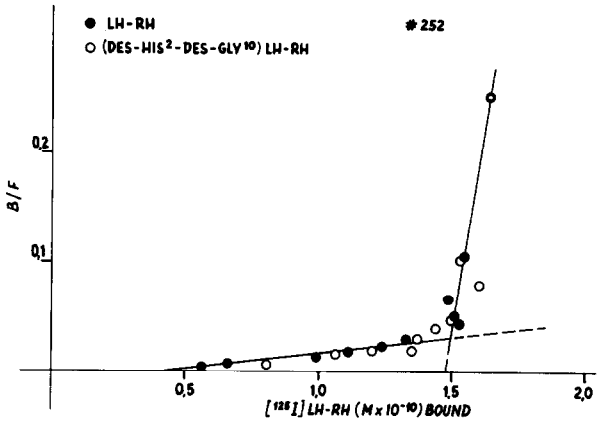

Fig. 2. Analysis of competition data of fig. 1 by Scatchard plot.

data are exemplified by Scatchard and reciprocal plots (figs. 2 and 3). In such analysis it is seen that des-His–2-des-Gly–10-LH-RH–ethylamide is truly competitive for the LH-RH receptor. The affinity of the analog for the receptor is the same as that of LH-RH as evidenced (fig. 1) by the concentrations of LH-RH and its analog required to 50% compete for saturating amounts of bound [^{125}I] LH-RH [23,24]. Because of these data previous assumptions [21,25] no longer

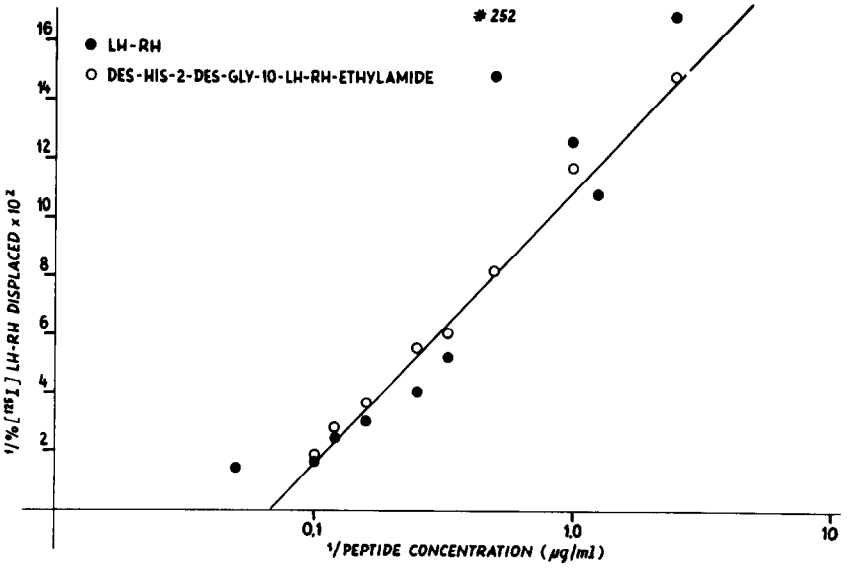

Fig. 3. Analysis of binding data of fig. 1 by double reciprocal plot.

Table 1
LH and FSH release of adenohypophyses in vitro in the absence and presence of LH-RH and for des-His–2-des-Gly–10-LH-RH–ethylamide. Data are means ± SD of five determinations and are expressed in terms of NIAMD-Rat LH-RP-1 and NIAMD-Rat FSH-RP-1

LH-RH (ng/ml)	analog (ng/ml)	μg LH/ml ± SD	p vs. control	μg FSH/ml ± SD	p vs. control
–	–	12.3 ± 0.8		1.2 ± 0.2	
10	–	24.7 ± 1.8	< 0.005	3.3 ± 0.4	< 0.005
–	1000	15.5 ± 1.7	NS	1.5 ± 0.3	NS
–	100	13.2 ± 1.1	NS	1.8 ± 0.1	NS
–	10	12.9 ± 1.2	NS	2.0 ± 0.2	NS
–	5	15.5 ± 2.0	NS	1.7 ± 0.2	NS
–	0.1	12.7 ± 1.3	NS	1.2 ± 0.1	NS
10	1000	16.3 ± 1.5	NS	1.5 ± 0.1	NS
10	100	18.0 ± 0.3	NS	2.5 ± 0.4	< 0.05
10	10	17.4 ± 1.3	NS	2.8 ± 0.1	< 0.025
10	5	28.2 ± 1.7	< 0.005	4.0 ± 0.3	< 0.005

hold that the histidyl residue in the LH-RH molecule is critical for the recognition of the hormone by its receptor.

Des-His–2-des-Gly–10-LH-RH–ethylamide possessed only negligible releasing activity, and blocked LH-RH stimulated LH and FSH release (table 1). The competitive antagonism of the octapeptide to LH-RH and the lack of gonadotropin releasing activity of the analog at concentrations tenfold higher than that required to suppress the response to LH-RH indicate dissociation of the binding and secretory process. Our data are in agreement with the previous observation that intracarotid infusion of the octapeptide antagonist effectively blocked responses to subsequent intravenous injections of LH-RH [14]. Also, des-His–2-LH-RH was reported to inhibit secretion of LH by rat anterior pituitary cells in vitro [25]. It is noteworthy that another des-histidine peptide, des-His-l-glucagon, is a competitive antagonist of glucagon [26].

At present no definite explanation can be given for the observed dissociation of ligand–receptor interactions and subsequent biological responses. In the absence of data on conformation of either LH-RH or the structural analog, we cannot ascertain whether the properties of the octapeptide are a result of alteration of functional groups or are secondary to conformational changes. A possible interpretation of our data may be that the octapeptide, for reasons not known, blocks the processes which transmit the binding of LH-RH to gonadotropin release. An alternative explanation invokes the concept of cooperative interactions [27] between LH-RH binding sites. According to a model reported previously [28] our results could mean that the LH-RH receptor site and the elements mediating the secretory process can exist in two forms in equilibrium. A secretion-triggering and a resting state. LH-RH would then have preferential affinity for the secretory state. The antagonism of the analog could be a consequence of interaction and stabilization of the LH-RH receptor in the inactive resting configuration. But, further work is necessary to obtain more information on LH-RH-receptor interactions.

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