LH-RH STIMULATED GONADOTROPIN RELEASE MEDIATED BY TWO DISTINCT PITUITARY RECEPTORS

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1. Introduction

It was postulated that one hypothalamic hormone controls stimulation of the release of both luteinizing hormone (LH) as well as follicle stimulating hormone (FSH) [1]. Recently a hypothalamic decapeptide was isolated and termed luteinizing hormone-releasing hormone (LH-RH) [2]. Subsequently LH-RH was synthesized [3, 4] and exhibited physiological and biological properties indistinguishable from those of natural releasing hormone isolated from porcine hypothalami. The compound was shown to stimulate the release of LH as well as FSH [5]. Synthetic LH-RH was reported to stimulate LH and FSH secretion of rat anterior pituitaries *in vitro* in amounts directly related to the dose of the releasing hormone [6]. Normal rat anterior pituitary cells in culture were shown to respond to LH-RH stimulation by gonadotropin release [7] and to bind labeled LH-RH [8].

Very recently we could locate the binding of LH-RH to the adenohypophysis at the level of the plasma membrane [9]. Our previous data suggested two modes of attachment of LH-RH to the cell membrane, one of them was postulated to be firm binding and the other a loose attachment [9]. The present experiments were carried out to substantiate our deductions about the binding data and to characterize the two binding sites for LH-RH. We wish to report on data suggesting two specific pituitary binding sites for LH-RH, which mediate the release of LH and FSH, respectively.

2. Materials and methods

Female rats of the Sprague Dawley strain (Mus Rattus AG, Brunnthal, GFR) weighing 175–200 g were used throughout this study. LH-RH and ^{125}I-labeled LH-RH were obtained from Farbwerke Hoechst AG, Frankfurt, GFR. The specific activity of [^{125}I]LH-RH was 266.32 Ci/mmole. The specific biological activity of [^{125}I]LH-RH was indistinguishable from that of unlabeled LH-RH. All other materials were of highest purity and obtained from local suppliers.

For the incubation of pituitary pieces of intact and ovariectomized rats the posterior lobes were removed, the adenohypophyses cut in quarters and randomized. Four quarters each were incubated in 25 ml Erlenmeyer flasks containing 2 ml of sterile medium which consisted of 9 parts Medium 199 (Biocult Labs., Glasgow) and 1 part fetal bovine serum (Reheis Chemical Comp., Chicago). Incubation was carried out at 37°C under an atmosphere of 95% air and 5% CO_2. Stimulation of gonadotropin release was tested by adding 0.5 μg LH-RH per ml medium. No LH-RH was added to the media of the control flasks. The procedure was described in detail previously [10, 11]. Media were assayed for LH and FSH by a minor modification [10] of a radioimmunoassay method, described by Niswender et al. [12].

The isolation of pituitary plasma membrane fractions of intact and ovariectomized rats was carried out according to procedures described previously [13]. Membrane concentrations are reported as protein contents [14];

Table 1

Days after ovariectomy	μg LH/ml Medium ± SD		μg FSH/ml Medium ± SD	
	Control	Experiment	Control	Experiment
0	2.27 ± 0.126	4.30 ± 0.190	0.70 ± 0.001	1.84 ± 0.21
7	7.25 ± 1.09	16.7 ± 1.90	1.18 ± 0.27	3.76 ± 0.50
30	20.5 ± 2.1	69.2 ± 1.43	0.42 ± 0.09	0.56 ± 0.06

Basal and LH-RH stimulated LH and FSH release of adenohypophyses of intact rats (0 days) and of animals 7 and 30 days, respectively, after ovariectomy incubated *in vitro*.

The [^{125}I]LH-RH binding assay was carried out at 0°C for 30 min. All solutions were made up in a HEPES buffer (mM: NaCl, 137; KCL, 5; Na_2HPO_4, 0.7; N-2-hydroxyethylpiperazin-*N'*-2-ethanesulfonic acid (HEPES), 25; $MgCl_2$, 5; sucrose, 300; pH 7.2). Plasma membranes (2695 ± 66 μg protein/ml) were mixed with various concentrations of labeled LH-RH. Final incubation volume was 70 μl. The incubation mixtures were diluted with a 10-fold excess of ice cold BSA diluent (0.01 M phosphate buffer, 5% bovine serum albumin, pH 7.2) filtered through a cellulose acetate filter (EHWP 02500, Millipore Corp., Bedford, Mass. 01730, USA) and washed 3 times with 2 ml of BSA diluent. This procedure was described in detail recently [9].

Affinity constants and binding capacities were calculated from data of the binding experiments by means of Scatchard plots.

3. Results and discussion

The progressive increase of basal as well as LH-RH stimulated LH secretion of anterior pituitaries of ovariectomized rats incubated *in vitro* is a function of the time after ovariectomy (table 1). Thirty days after ovariectomy a plateau of LH secretion is reached [6]. On the other hand, FSH release of control groups and of LH-RH stimulated pituitaries, respectively, increases (table 1) until 15 days after ovariectomy when a drop in FSH release is observed, which levels off at 30 days [6]. Thirty days after ovariectomy FSH release is lower than at 7 days and 0 days (intact animals), respectively (table 1).

The different patterns of LH and FSH release after ovariectomy led us to investigate the binding characteristics of pituitary plasma membrane fractions of intact animals (0 days) and of rats 7 and 30 days, respectively, after ovariectomy. Pituitary cell membranes of intact animals used in the binding experiments possess two distinct binding sites for [^{125}I]LH-RH (fig. 1). One of the detected sites has an apparent affinity constant of ca. 2.10^{-8} M, and a lower capacity, whereas the second site has a lower affinity (ca. 2.10^{-7} M) but about six times higher capacity to bind LH-RH. Our data are somewhat different from those recorded for LH-RH binding studies with pituitary cells in culture, for which apparent affinity constants of ca. 2×10^{-9} M and ca. 2×10^{-8} M, respectively were reported [8]. This discrepancy may be explained by the fact that binding sites of the receptor system may get lost or modified during the preparation of plasma membrane fractions [15].

Seven days after ovariectomy a similar pattern of [^{125}I]LH-RH binding to the plasma membrane is recorded (fig. 2). Again, two distinct binding sites for LH-RH can be observed with apparent affinity constants of ca. 2×10^{-8} M and ca. 1×10^{-7} M respectively, and similar binding capacities were found as calculated for pituitary cell membranes of intact animals. But, one binding site of the receptor system for LH-RH is lost when binding experiments are performed with plasma membranes obtained from adenohypophyses of rats 30 days after ovariectomy. The apparent affinity constant of the remaining binding site (ca. 2×10^{-7} M) is similar to the one with the higher binding capacity of plasma membranes obtained from intact animals and rats 7 days after ovariectomy, respectively. The data of these binding experiments (fig. 2) are consistent with the finding that pituitaries of rats lose their ability to respond to LH-RH stimulation with respect to FSH release after long-term

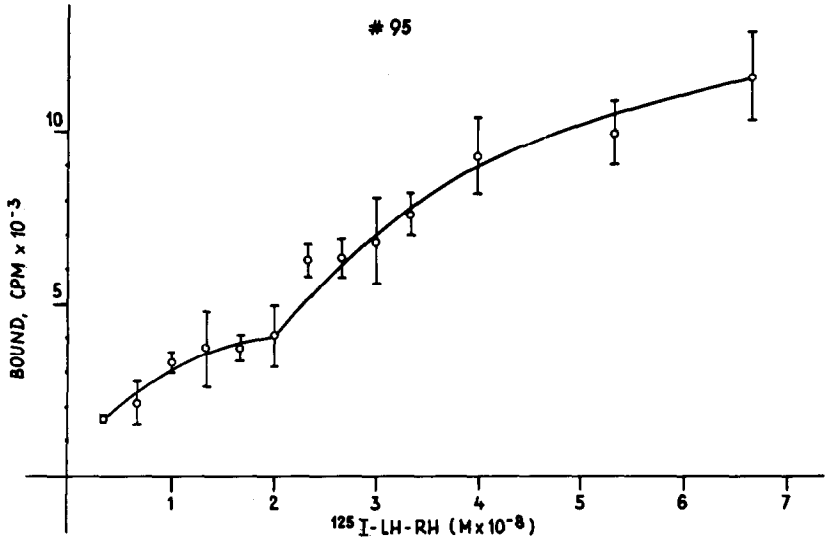

Fig. 1. Binding of [^{125}I]LH-RH to anterior pituitary plasma membrane fractions of intact female rats as function of LH-RH concentration.

ovariectomy (table 1). The present data strongly suggest that two distinct LH-RH binding sites are present in the pituitary receptor system of intact rats. One with higher affinity mediates the stimulation of LH release, the other possessing a lower affinity for LH-RH promotes stimulation of FSH release. The present data are also consistent with findings that the pituitary responds to LH-RH with a much lower FSH than LH release both *in vitro* [6] as well as *in vivo* [16]. Our results are also of interest in the light of recent experiments, which yield evidence for a distinct releasing hormone for FSH [17, 18, 19]. The present system

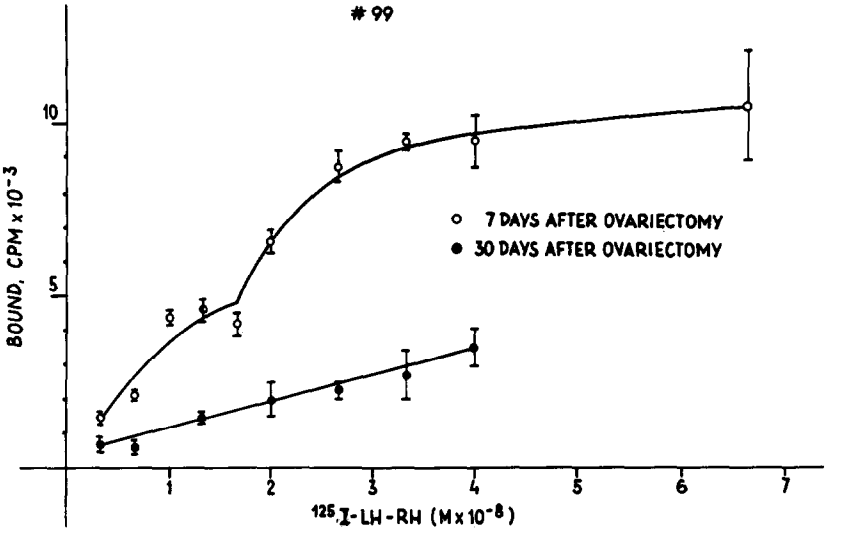

Fig. 2. Effect of increasing concentrations of [^{125}I]LH-RH to anterior pituitary plasma membranes of rats 7 and 30 days respectively, after ovariectomy.

offers a new approach to the study of mechanisms of action of releasing hormones for gonadotropins. Some of our recent data on different *in vitro* responsiveness of neonatal male and female rats [10, 11] may be explained by different binding characteristics of anterior pituitary plasma membranes. But, more work is needed for further elucidation of LH-RH actions.

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