LH-RH INTERACTION WITH THE PITUITARY PLASMA MEMBRANE IS AFFECTED BY SEX STEROIDS

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1. Introduction

Variation of pituitary responsiveness to synthetic LH-RH was observed during different phases of the menstrual cycle [1–3]. Recently changes of in vitro response to LH-RH were reported for pituitaries of male and female rats, respectively, at different ages [4, 5]. Furthermore, LH-RH stimulated release of gonadotropins could be provoked by estrogen pretreatment in females suffering from amenorrhoea who did not respond to LH-RH prior to the steroid application [6]. Additionally, LH-RH stimulated-LH release was strikingly higher in normal pubertal children than in children of prepubertal ages [7]. These findings suggested that changes in hypophyseal responsiveness to LH-RH were affected by estradiol and/or progesterone. Additional evidence for this suggestion was obtained by recent in vitro experiments, which showed that 17 β-estradiol and progesterone could modulate responses of adenohypophyses to LH-RH [8].

Very recently LH-RH was shown to be bound to isolated plasma membranes of rat anterior pituitaries [9]. LH-RH–receptor interaction with the anterior pituitary plasma membrane was characterized and found to be a temperature and pH dependent process [10]. Data currently available strongly suggest that two distinct LH-RH binding sites are present in the pituitary receptor system of intact rats. But, one binding site of the receptor system could no longer be detected if binding experiments were performed with plasma membranes obtained from adenohypophyses of chronically ovariectomized rats [11]. This observation and data on in vitro stimulation of gonadotropin release by LH-RH in adenohypophyses of intact and chronically ovariectomized rats led us to assume that LH and FSH release respectively, was mediated by distinct and specific receptors for LH-RH [11]. In addition, the effector system which consequent to LH-RH–receptor interaction mediates alterations in cellular function was found to be the adenylate cyclase system [12].

The present experiments were carried out to study the effects of sex steroids on LH-RH–receptor interaction. Furthermore, we wish to report on data, which suggest that the LH-RH effector system is modulated by steroid hormones also.

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. The animals were housed in a temperature-controlled room lighted for only 12 hr a day. Unrestricted access was provided to food and water. The animals were bilaterally ovariectomized, and divided into 4 groups of 60 animals each. Daily subcutaneous injections of 5 μg 17β-estradiol plus 3 mg progesterone, in 0.1 ml sesame oil were begun 34 days later. The control group received only sesame oil. Injections were continued through 6 days; All animals were killed by decapitation 41 days after ovariectomy. Pituitaries were placed into ice cold 0.3 M sucrose, posterior lobes were removed and the adenohypophyses were homogenized by 10 strokes of a Teflon-glass homogenizer. Plasma membranes were isolated according to previously described procedures

[13]. 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 cell membranes are reported as protein content [14]. 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 542.93 Ci/mmole. The specific biological activity of [^{125}I]LH-RH was indistinguishable from that of unlabeled LH-RH.

The [^{125}I]LH-RH binding assay was carried out at 6°C. Aliquots of membranes were mixed with various concentrations of labeled LH-RH. Final incubation volume was 70 μl. After 30 min incubation time 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) and immediately filtered through a cellulose acetate filter (EHWP 02500, Millipore Corp., Bedford, Mass. 01730, USA). The filters were washed 3 times with 2 ml of BSA diluent. This procedure was described in detail recently [9].

Incubation of adenylate cyclase was carried out in solutions containing 3.2 mM ATP, an ATP-regenerating system (20 mM creatine phosphate, 50 I.U. creatine phosphokinase/ml), 2.5 mM theophylline, 5 mM $MgCl_2$, 20 mM Tris–HCl (pH 7.5), 4 mg bovine serum albumin/ml, 1 mM EDTA and 200 μg bacitracin/ml. Reactions were started with the addition of a 10 μl suspension of membrane protein. Incubations were carried out at 30°C for 15 min. Reactions were stopped by rapidly placing the incubation tubes into an ice-bath and quickly adding 50 μl aliquots of these solutions to pre-cooled incubation mixtures, which were used for subsequent estimation of cAMP by a competitive protein binding assay [15] with material obtained from The Radiochemical Center, Amersham, UK. In table 1 and text adenylate cyclase activity refers to pmoles cAMP produced per mg plasma membrane protein after 15 min incubation.

3. Results and discussion

Previously it was reported that two distinct pituitary binding sites for LH-RH could be observed with apparent affinity constants of approx. 2×10^{-8} M and approx. 1×10^{-7} M, respectively, and it was found that the high affinity binding site was lost, when anterior pituitary plasma membranes were obtained from chronically ovariectomized rats [11]. Furthermore, data were published which showed that elevated serum LH levels of chronically ovariectomized rats could be suppressed by steroid treatment to levels found in intact animals [16]. These observations led us to investigate whether a second binding site of the LH-RH–receptor system, which was lost after ovariectomy, may be restored by treatment of chronically ovariectomized rats with sex steroids. Binding of [^{125}I]LH-RH as function of labeled LH-RH shows that application of 17β-estradiol (E_2), progesterone (P) and 17β-estradiol plus progesterone (E_2+P), respectively, to ovariectomized rats leads to regeneration of a second binding site (fig. 1). This can also be derived from Scatchard plots (not shown), from which apparent affinity constants could be calculated of approx. 1×10^{-9} M and approx. 1×10^{-8} M for the groups treated with E_2 and P, respectively, and approx. 2×10^{-9} M and approx. 1×10^{-8} M for the group treated with E_2 + P. It is noteworthy that apparent affinity constants are somewhat different from those found previously for intact animals [11], whereas the control group (data not shown) which did not receive any steroid treatment exhibited an apparent affinity constant of approx. 2×10^{-7} M which is consistent with data reported

Table 1
Influence of treatment of chronically ovariectomized rats with sex steroids on adenylate cyclase activity of isolated plasma membranes of adenohypophyses.

	Adenylate cyclase activity in the presence of	
Treatment of animals	Basal	100 ng LH-RH
17β-Estradiol (E_2)	106.60 ± 13.90	109.33 ± 9.30
Progesterone (P)	77.60 ± 3.00	82.00 ± 1.40
17β-Estradiol and progesterone (E_2 + P)	82.06 ± 5.72	196.56 ± 28.62

Reactions were started with the addition of enzyme at concentrations of plasma membrane protein of 182.5 μg (E_2), 167.5 μg (P) and 262.0 μg (E_2+P), respectively. Other conditions were as described in the text. Activity of adenylate cyclase is expressed as pmoles cAMP produced per mg plasma membrane protein after 15 min incubation. Data are reported as mean ± SD of four determinations.

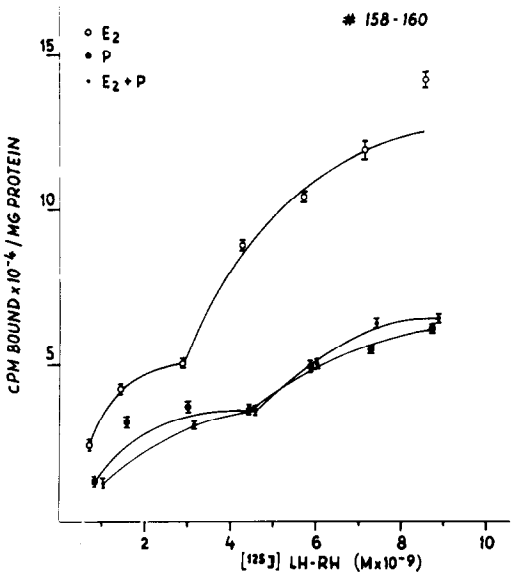

Fig. 1. Binding of LH-RH as function of [^{125}I]LH-RH concentration to anterior pituitary plasma membranes of chronically ovariectomized rats, which were treated with 17β-estradiol (E_2), progesterone (P), and a combination of the two steroid (E_2+P). 182.5, 167.5 and 262.0 μg respectively, plasma membrane protein of E_2, P and E_2+P, respectively, treated animals was used. All other conditions were as described in the text. Data shown are mean ± SD of four determinations.

earlier [11]. The apparent affinity constants of the present experiments agree well with data recorded for LH-RH binding studies with pituitary cells of intact rats in culture [17] but no definite explanation for these different observations is presently available. One possible explanation for the discrepancy may be that in addition to the regeneration of a second binding site which is lost after ovariectomy, sex steroids may also alter the affinity of binding sites. Experiments are in progress to investigate this problem in detail. But, the present data add further evidence to the suggestion that steroid hormones may play an important role in the primary action of LH-RH, and it is intriguing that sex steroids may regulate LH-RH-provoked LH and FSH release, respectively, by influencing LH-RH–receptor interaction.

Since adenylate cyclase was shown to be a possible LH-RH effector system [12] it was of interest to note that treatment of chronically ovariectomized animals with various sex steroids resulted in marked differences in basal and LH-RH-stimulated formation of cAMP (table 1). Plasma membranes of adenohypophyses of animals treated with E_2, P and E_2+P, respectively, exhibited similar basal activity of adenylate cyclase, but no stimulation by LH-RH was observed for the E_2 and P group, respectively. Production of cAMP could be stimulated by LH-RH approximately 2-fold over the basal levels in pituitary plasma membranes isolated from E_2+P treated animals (table 1). The degree of enzyme stimulation is of the same order as found previously for plasma membrane fractions prepared from intact rats [12]. There is no definite explanation available at present for the discrepancy between binding data and results of adenylate cyclase activation. But experiments are in progress to investigate whether there is a difference in the in vitro response to LH-RH between anterior pituitaries of chronically ovariectomized rats treated with various steroids.

The present experiments support our previous assumption that LH-RH stimulated gonadotropin release could be modulated by steroid hormones at the level of the pituitary [8]. In addition, data reported here provide evidence that steroid modulation of LH-RH actions may at least in part occur at the level of the pituitary plasma membrane. Although the present experiments provide some insight into the molecular basis of events involved in the initiation of cellular response to LH-RH, further work is necessary to understand the coupling of receptor interaction with the effector system.

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References

[1] Yen, S.S.C., Vandenberg, G., Rebar, R. and Ehara, Y. (1972) J. Clin. Endocrinol. Metab. 35, 931.
[2] Nillius, S.J. and Wide, L. (1972) J. Obstet. Gynaec. Brit. Commonw. 79, 865.
[3] Thomas, K., Cardon, M., Donnez, J. and Ferin, J. (1973) Contraception 7, 289.

[4] Spona, J. and Luger, O. (1973) FEBS Letters 32, 49.
[5] Spona, J. and Luger, O. (1973) FEBS Letters 32, 52.
[6] Schneider, W., Spona, J. and Matt, K. (1973) Wien. klin. Wschr. 85, 360.
[7] Roth, J.C., Kelch, R.P., Kaplan, S.L. and Grumbach, M.M. (1972) J. Clin. Endocrinol. Metab. 35, 926.
[8] Spona, J., Endocr. exp., in press.
[9] Spona, J. (1973) FEBS Letters 34, 24.
[10] Spona, J. (1973) Endocr. exp., submitted for publication.
[11] Spona, J. (1973) FEBS Letters 35, 59.
[12] Spona, J. (1973) unpublished data.
[13] Meldolesi, J., Jamieson, J.D. and Palade, G.E. (1971) J. Cell. Biol. 49, 109.
[14] Lowry, O.H., Rosebrough, N.J., Farr, A.R. and Randall, R.J. (1951) J. Biol. Chem. 193, 265.
[15] Gilman, A.G. (1970) Proc. Natl. Acad. Sci. U.S. 67, 305.
[16] Saksena, S.K., Steele, R. and Harrer, M.J.K. (1973) J. Reprod. Fertil. 32, 495.