LH-RH INTERACTION WITH THE PITUITARY PLASMA MEMBRANE

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1. Introduction

Recent experiments showed that synthetic luteinizing hormone-releasing hormone (LH-RH) stimulated the release of luteinizing hormone (LH) *in vivo* [1] as well as *in vitro* [2]. Clinically LH-RH has been used to test the reserve capacity of the pituitary [3–5] and to provoke ovulations [6, 7] in sterility patients. Several papers indicate that sex steroids may modulate the response of the pituitary to LH-RH *in vivo* [8, 9]. Similar interactions with sex steroids were observed *in vitro* (unpublished data). Recently, we postulated that the *in vitro* responsiveness to LH-RH of male and female rat pituitaries of different ages may be influenced by steroid hormones [10, 11].

Despite the accumulation of data concerning the LH-RH stimulated release of LH, little is known about the mechanisms controlling this process. The present experiments were designed to get some information on the primary steps involved in the LH-RH action. A very recent paper deals with pituitary binding sites for LH-RH of normal rat anterior pituitary cells in culture [12]. We were therefore prompted to report on our own data concerning the interaction of LH-RH with the pituitary plasma membrane.

2. Materials and methods

Female rats of the Sprague Dawley strain (Mus Rattus AG, Brunnthal, GFR) were decapitated and the pituitaries placed in ice cold 0.3 M sucrose. The posterior lobes were removed and discarded. The adenohypophyses were homogenized and plasma membranes isolated according to previously described procedures [13]. Membrane concentrations are reported as protein contents [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 9.03 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 0°C, and incubation time was 30 min except for kinetic experiments. 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). Aliquots of membranes were mixed with various concentrations and specific activities of labeled LH-RH depending upon the experiment. Final incubation volume was 70 µl unless otherwise stated. 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), immediately filtered through a cellulose acetate filter (EHWP 02500, Millipore Corp., Bedford, Mass. 01730, USA), and washed 3 times with 2 ml of BSA diluent. Hydrophobic peptides are known to adsorb to derivatives of cellulose. Several types of filters were tested and the present cellulose acetate filters exhibited the lowest non-specific adsorption of [^{125}I]LH-RH, which we could further suppress by equilibrating the filters in BSA diluent prior to their use. For this reason a Millipore filter blank was included for each incubation reaction. This control was done by incubating labeled LH-RH without plasma membranes, but filtration and washing procedures were the same as for samples containing the plasma membranes.

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3. Results and discussion

Binding of LH-RH to the plasma membrane of the anterior pituitary is directly proportional to the protein concentration of the membrane preparation over a wide range (fig. 1). The only limitation being the capacity of the cellulose acetate filters and the time required to filter and wash the assay samples. [^{125}I]-LH-RH bound to the cell membrane is rapidly removed when the washing time of the filters is extended. This is due to a rapid back reaction (fig. 2). After 2 min 42% dissociation was recorded, and dissociation of the LH-RH receptor complex levels off thereafter. The dissociation does not follow simple first order kinetics and is stimulated by unlabeled LH-RH (unpublished data). This suggests two modes of attachment of LH-RH, one of them is firm binding and the other a loose attachment. Such a conclusion may be also drawn from experiments on the binding of [^{125}I]LH-RH to the pituitary plasma membrane as a function of labeled LH-RH (our own unpublished data). Similar results were obtained for binding of [^{3}H]LH-RH to whole pituitary cells in culture [12].

A high degree of specificity of the LH-RH receptor is suggested by the absence of competition for LH-RH binding by three peptide hormones (fig. 3). On the other hand, unlabeled LH-RH displaces the labeled hormone in proportion to its relative concentration. Virtually complete inhibition of [^{125}I]LH-RH binding was recorded in the presence of 10 μg unlabeled LH-RH (fig. 3). This offers a possibility to assay LH-RH by competitive protein binding, although the present system cannot be used to estimate physiological serum levels of LH-RH because of the bad sensitivity. Further-

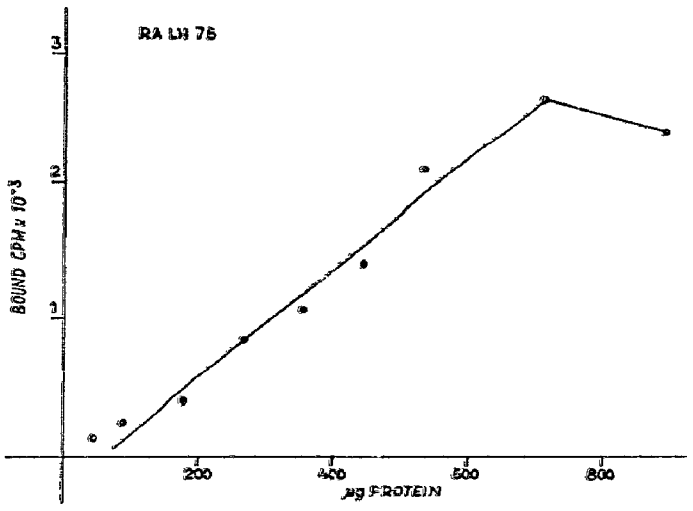

Fig. 1. Effect of protein concentration of anterior pituitary plasma membrane fraction on [^{125}I]LH-RH (68 pmoles/ml) binding. Final incubation volume was 60 μl.

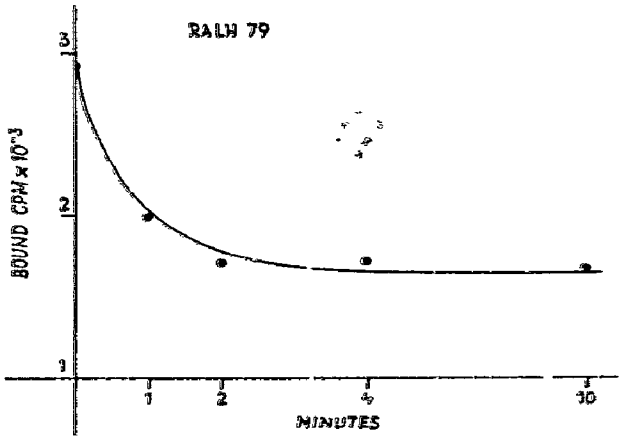

Fig. 2. Dissociation rate of LH-RH receptor complex at 0°C. [^{125}I]LH-RH (136 pmoles/ml) was incubated with 355 μg protein of plasma membrane in a final volume of 30 μl for 30 min before being diluted with 0.8 ml BSA diluent.

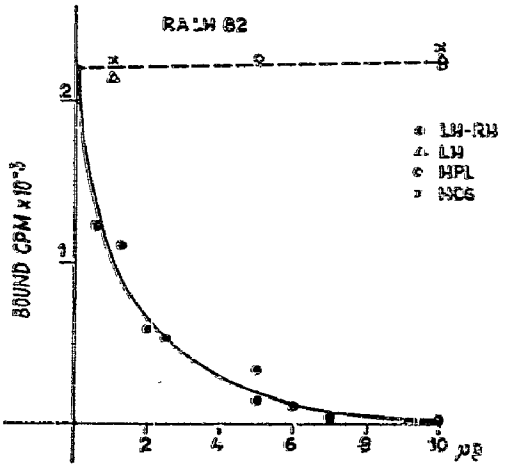

Fig. 3. Competition between [^{125}I]LH-RH and unlabeled LH-RH for binding to plasma membrane fraction. 3.5 μg Protein was incubated with varying amounts of unlabeled LH-RH, LH, human placental lactogen (HPL) and human chorionic gonadotropin (HCG) for 10 min. [^{125}I]LH-RH (87 pmoles/ml) was added and incubation continued for 30 min.

more, fig. 3 suggests the release of firmly attached LH-RH, which is similar to data on the displacement of thyrotropin releasing hormone [18]. The complexity of this reaction merits further examination. No binding of LH-RH is recorded for plasma membranes isolated from hearts of rats (unpublished data). This adds further support to the specificity of the LH-RH receptor in the pituitary.

A rapid association of LH-RH with the plasma membrane was observed. At 10 min, 80% association was recorded and a plateau is reached at 30 min (unpublished data). This rapid association of LH-RH with the plasma membrane may explain the fast increase of LH serum levels *in vivo* after LH-RH application [3]. Furthermore, fast association and dissociation of LH-RH and plasma membrane is responsible for the regulation of acutely increased LH serum levels at midcycle of the female.

Binding of LH-RH to whole rat anterior pituitary cells in culture was described very recently [12]. Binding of LH-RH to the adenohypophysis could be located at the level of the plasma membrane by the present experiments. There are striking similarities of the binding characteristics at the target organs of larger peptides like ACTH [15], insulin [16], and glucagon [17] with those of the decapeptide LH-RH and with those of the tripeptide thyrotropin releasing factor [18]. Among the similarities are the high specificity and the location of the receptor in the plasma membrane. The latter is also the main site of adenylate cyclase in mammalian tissues [19]. We are currently studying various factors influencing the binding of LH-RH to the plasma membrane.

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References

[1] Arimura, A., Debeljuk, L., Matsuo, H. and Schally, A.V. (1972) Proc. Soc. Exptl. Biol. Med. 139, 851.
[2] Reuding, T.W., Schally, A.V., Arimura, A. and Matsuo, H. (1972) Endocrinology 90, 764.
[3] Schneider, W. and Spona, J. (1972) Wien. Klin. Woch. 84, 581.
[4] Lundberg, P.O. and Wide, L. (1973) Europ. J. Clin. Invest. 3, 49.
[5] Rebar, R., Yen, S.S.C., Vandenberg, G., Naftolin, F., Ehara, Y., Engblom, S., Ryan, K.J., Rivier, J., Amoss, M. and Guillemin, R. (1973) J. Clin. Endocrinol. Metab. 36, 10.
[6] Schneider, W., Spona, J. and Matt, K. (1973) Wien. Klin. Woch. in press.
[7] Zarate, A., Canales, E.S., Schally, A.V., Valdes, L.A. and Kastin, A.J. (1972) Fertility Sterility 23, 672.
[8] Debeljuk, L., Arimura, A. and Schally, A.V. (1972) Endocrinology 90, 1578.
[9] Debeljuk, L., Arimura, A. and Schally, A.V. (1972) Proc. Soc. Exptl. Biol. Med. 139, 774.
[10] Spona, J. and Luger, O. (1973) FEBS Letters 32, 49.
[11] Spona, J. and Luger, O. (1973) FEBS Letters 32, 52.
[12] Grant, G., Vale, W. and Rivier, J. (1973) Biochem. Biophys. Res. Commun. 50, 771.
[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] Lefkowitz, R.J., Roth, J., Pricer, W. and Pastan, I. (1970) Proc. Natl. Acad. Sci. U.S. 65, 745.
[16] Robinson, Jr., C.A., Boshell, B.R. and Reddy, W.J. (1972) Biochim. Biophys. Acta 290, 84.
[17] Rodbell, M., Krans, H.M.J., Pohl, S.L. and Birnbaumer, L. (1971) J. Biol. Chem. 246 (1971) 1861.
[18] Grant, G., Vale, W. and Guillemin, R. (1972) Biochem. Biophys. Res. Commun. 46, 28.
[19] Robinson, G.A., Butcher, R.W. and Sutherland, E.W. (1969) Ann. Rev. Biochem. 37, 149.