Peptide–receptor protein relationships: importance in estradiol feedback

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1. INTRODUCTION

The gonadotropin releasing hormone (GnRH) acts to release bovine luteinizing hormone (LH) [1] as well as follicle-stimulating hormone (FSH) [2] in vivo and stimulates the release of LH from bovine anterior pituitary tissue slices during superfusion in vitro in a dose-dependent manner [1]. Fully biologically active ^{125}I-labeled GnRH binds to isolated anterior pituitary cells in a dose-dependent and saturable manner and the concentration dependence curve (physiological concentrations of GnRH) exhibits positive cooperativity [3,4]. Similarly, GnRH binding in isolated anterior pituitary plasma membranes, solubilized plasma membranes and purified GnRH receptor protein (GnRH.RP) is to multiple binding sites [5]. GnRH.RP purified to homogeneity aggregates in solution upon binding of GnRH present in physiological concentrations [5].

The concentration-dependence binding curves of labeled GnRH to isolated anterior pituitary cells of different origin suggested involvement of sex steroids [3,4]. The apparent effect of a predominantly estrogenic environment was inducible by the pretreatment of steer anterior pituitary plasma membranes in vitro with physiological concentrations of estradiol 17-β [3,4]. Here, the modulation of GnRH binding by estradiol 17-β at the level of GnRH.RP is investigated since steroids were implicated in normal LH response to GnRH in the bovine [1,6] as well as other species [7,8] and in the feedback control of preovulatory LH surge, in general.

2. MATERIALS AND METHODS

Details of the methodology used here may be found in [4,5,9]. Briefly, the GnRH receptor protein was coupled to a nylon fiber through an acyl-azide intermediate at a rate of 23 fmol/fiber. Binding kinetics were then studied with labeled GnRH in solution. The affinity fiber was first pretreated for 10 min in buffer (Tris–HCl, 0.1 M, pH 9.0) then incubated for 30 min with ^{125}I-GnRH. Washing, in excess of plain buffer, was for 3 min. In experiments with estradiol pretreatment, the fibers were incubated with the specified concentration of steroid in buffer for 10 min, then washed slightly prior to the incubation with ^{125}I-GnRH (7×10^{-10} M).

Separation of bound and free GnRH was by removal of the solid phase from the solution and thoroughly washing it in excess buffer. The solid phase was then counted in the γ-counter for retained ^{125}I-radioactivity. For all assays, non-specific (non-saturable) binding was determined by incubating the test material in the presence of 10^{-7} M GnRH. This was subtracted from total binding and, unless otherwise stated, the results represent saturable or specific binding. Non-specific absorption of labeled protein was also subtracted.

3. RESULTS

The concentration-dependence binding curve of ^{125}I-GnRH to purified GnRH receptor protein is shown in fig.1. This binding had the characteristics described earlier but $\geq$ 3 binding sites participating within $7 \times 10^{-11} - 7 \times 10^{-10}$ M GnRH. Half-maximum binding occurred at $(1.7 \pm 0.2) \times 10^{-10}$ M, $(3.0 \pm 0.3) \times 10^{-10}$ M and $(5.9 \pm 0.3) \times 10^{-10}$ M GnRH, respectively. The binding curve resembles that of the intact anterior pituitary membranes and solubilized anterior pituitary

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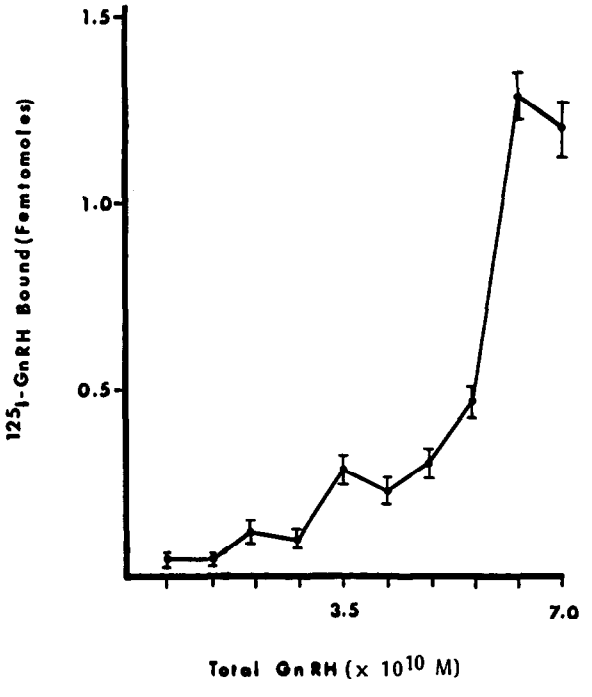

Fig.1. Concentration dependence of specific ^{125}I-GnRH binding to purified GnRH receptor protein. The values represent mean ± SE of 6 parallel measurements. The result is typical of 6 similar expt obtained with different batches of the receptor protein.

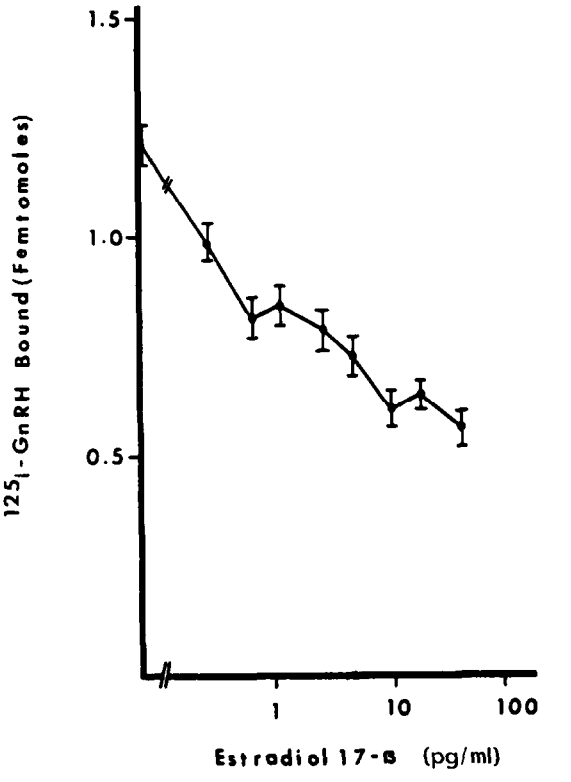

Fig.2. Specific binding of ^{125}I-GnRH to its receptor protein pretreated with specified concentrations of estradiol 17-β. The dots represent means ± SE of 6 parallel measurements. The result is typical of 6 similar expt obtained with different batches of the receptor protein.

membranes. A detailed description of similar preparations appeared in [5,7].

The effect of estradiol 17-β, which is the main estrogen in cattle, has been investigated at physiological levels (0.3–40 pg/ml).

Estradiol 17-β in the buffer, prior to the exposure of immobilized receptor protein to its hormone, decreased subsequent binding of labeled GnRH to the immobilized receptor protein (fig.2). This decrease was dose dependent but plateaus were observed at 0.6–2.5 pg/ml and 10–40 pg/ml estradiol. This phenomenon was observed in 6 expt run with 4 batches of purified GnRH receptor protein. Statistical analysis by Student's t-test was done for the sextuplets for each of 0.6, 1.2 and 2.5 pg estradiol/ml as one group and 10, 20 and 40 pg estradiol/ml as the other group, respectively. The difference between the group means was highly significant ($P < 0.001$) while the difference within the means was not ($P > 0.5$). This suggests hindrance of some binding sites for ^{125}I-GnRH depending on the estradiol 17-β level in the preincubation medium. Whether there is an additional plateau at < 0.3 pg estradiol/ml is under investigation and of theoretical value only. The physiological level is rarely < 1 pg/ml; it is on av 5.1 pg/ml on day 15 of the cycle; 10.8 pg/ml on the day prior to ovulation; and higher when stimulated [6].

4. DISCUSSION

The evidence that estradiol 17-β at physiological levels modulates GnRH binding to immobilized purified GnRH receptor protein is the first demonstration that its effect on isolated anterior pituitary plasma membrane, and possibly on the secretion of LH from anterior pituitary tissue in vitro [4] and in vivo [6], is mediated directly by the GnRH receptor. Similarly, these findings suggest that the concentration of GnRH need not vary greatly to induce the

ovulatory stimulus [6]. Experiments designed to provide details of molecular events underlying the feedback mechanism involved in the release of gonadotropins at the pituitary level are underway.

Since GnRH binding under these conditions is mediated by a single molecular entity (receptor protein) the different capacity of ^{125}I-GnRH which is estradiol 17-β concentration-dependent indicates the effect of estradiol 17-β on the hindrance of some binding sites, depending on the estradiol level. Considering that only one concentration of ^{125}I-GnRH was used (10^{-9} M) here, the receptor protein, even though immobilized, may have bound estradiol 17-β to the same or similar sites, which are responsible for the stepwise or sigmoid concentration-dependence binding curves of a fully biologically active ^{125}I-GnRH [3–5,9]. Thus these data support the structural model of the gonadotropin hormone receptor.

Whether the effect of estradiol 17-β described here is of any importance in explaining the contraceptive effect of 'the pill' is of interest. Under continuously elevated estradiol 17-β concentration the binding of the gonadotropin releasing hormone is low and thus the effect of the releasing hormone on luteinizing hormone release could be abolished, at least in part. Since the estradiol 17-β levels used in these experiments are physiological, the question could be of much clinical interest.

These observations for gonadotropin releasing hormone may be relevant for the definition of the biological function of the receptor in general and warn that the concentrations of the individual peptide hormones might not be physiologically as relevant as earlier suspected. For example, under varying concentrations of steroids extracellularly the receptor may be modified and the physiological importance of hormone concentration measurement will vary accordingly. Receptor protein binding as described here may be a useful parameter of biological function of the hormone (in this case the gonadotropin releasing hormone) because it estimates the extent of the hormone–receptor interaction and, therefore, true biological outcome, largely independently of widely fluctuating (precisely measured) hormone concentrations.

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