HIGH-AFFINITY BINDING SITES IN DETERGENT-SOLUBILIZED GONADOTROPIN RECEPTORS

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1. Introduction

Solubilization and characterization of particulate high-affinity gonadotropin receptors derived from the interstitial cell fraction of the rat testis [1–3], and homogenates of the pseudopregnant rat ovary [4,5], has been performed by extraction with the non-ionic detergents Triton X-100, Lubrol PX and Lubrol WX. Binding studies with ^{125}I-labeled hCG have shown that such soluble preparations contain a single class of gonadotropin binding sites, with $Ka = 0.6 \times 10^{10}$ M^{-1} and binding capacity of $0.02 - 0.13 \times 10^{-12}$ mol/mg protein [1–4]. The mean affinity constant of the solubilized receptors is significantly lower than that observed in the original particulate receptors ($Ka = 2–4 \times 10^{10}$ M^{-1}) from which the Triton-extracted fraction was prepared. During incubation at 4°C and 34°C, the free or uncharged soluble receptors were found to be relatively unstable, probably due to enzymatic degradation of the gonadotropin binding site. By contrast the formed hormone–receptor complex was much less susceptible to degradation after solubilization, suggesting that the binding sites were stabilized by association with the trophic hormone [2,3,6]. More recently, serial measurement of the binding capacity of solubilized free receptors during storage have revealed the presence of a small proportion of high-affinity receptors with association constant similar to that of the parent preparation. The demonstration and possible significance of these high-affinity sites are described in the present report.

2. Materials and methods

For binding studies, the 120–27 000 *g* fraction of fragmented rat interstitial cells was prepared by physically teasing apart the decapsulated rat testis, followed by mixing the mass of dispersed tubules for 10 min with a magnetic stirrer at room temperature [2]. All succeeding steps were performed at 0–4°C. After filtration through cotton wool, the particulate suspension was centrifuged at 120 *g* for 20 min; the supernatant solution was then centrifuged at 27 000 *g* for 20 min to sediment membrane fragments with high binding affinity for hCG [7]. Soluble gonadotropin receptors were prepared by extraction of the particulate binding fraction (20 mg protein) with 1 ml of 1% Triton X-100 for 30 min [1,2]. After dilution to 10 ml with 0.15 M phosphate-buffered saline (pH 7.4), the preparation was centrifuged at 360 000 *g* (average) for 1 hr. The supernatant solution containing the solubilized gonadotropin receptors was used for binding studies when freshly prepared and after storage at 4°C for 16 hr or 48 hr.

Binding studies were carried out as previously described [1,2] by equilibration of 500 μl of receptor preparation (1.93 mg protein/ml) with 50 000 cpm (10^{-11} M) of ^{125}I-labeled hCG (50 μCi/μg). The biological activity of the labelled hormone was approximately 10 000 IU/mg, as determined by in vitro radioligand-receptor assay [8] and stimulation of steroidogenic activity in the isolated rat testis [9]. Competitive binding studies were performed with added unlabeled gonadotropin under equilibrium

conditions at 4°C for 16 hr. Separation of receptor-bound and free tracer was performed by double precipitation with polyethylene glycol as previously described [1,2]. In all experiments, the non-specific bound radioactivity was determined by incubation with ^{125}I-hCG in the presence of an excess of unlabeled hCG (10^{-7} M), and did not exceed 1–2% of the added tracer. All determinations of bound and free radioactivity were performed by counting in an automatic gamma spectrometer for a sufficient time to reduce the counting error to 1%. Association constants and concentrations of binding sites were derived both graphically [10] and by computer analysis [11] of Scatchard plots of the binding data [12]; similar results were obtained by each method of analysis.

3. Results and discussion

Equilibrium binding studies performed on freshly-prepared soluble gonadotropin receptors with labeled hCG showed a single order of binding sites, with association constant of 0.7 ± 0.1 (SD) × 10^{10} M^{-1} (n = 8) and binding capacity of 20–60 femtomol/mg protein (fig.1). When the soluble free receptor preparation was stored at 4°C for 16 hr, by standing or by dialysis against buffer containing 0.1% Triton, the Scatchard plots of binding data obtained in each case showed two orders of binding sites (fig.2). The major component with *K*a of 0.66 ± 0.07 (SD) × 10^{10} M^{-1} (n = 4) was of similar affinity to that observed in the freshly prepared soluble receptor. A marked reduction in the number of gonadotropin binding sites to less than 10% of the original value had occurred at this time. In addition, the presence of a small number (1.7 femtomol/mg protein) of high affinity receptors sites with *K*a of 3.8 ± 0.5 × 10^{10} M^{-1} (n = 4) as found in the original particulate receptor preparation, was apparent in the preparation maintained at 4°C for 24 hr.

When storage of the preparation was extended for a further period of time, up to 48 hr, the lower affinity site had completely disappeared, leaving only a small quantity of the high-affinity site. This change is

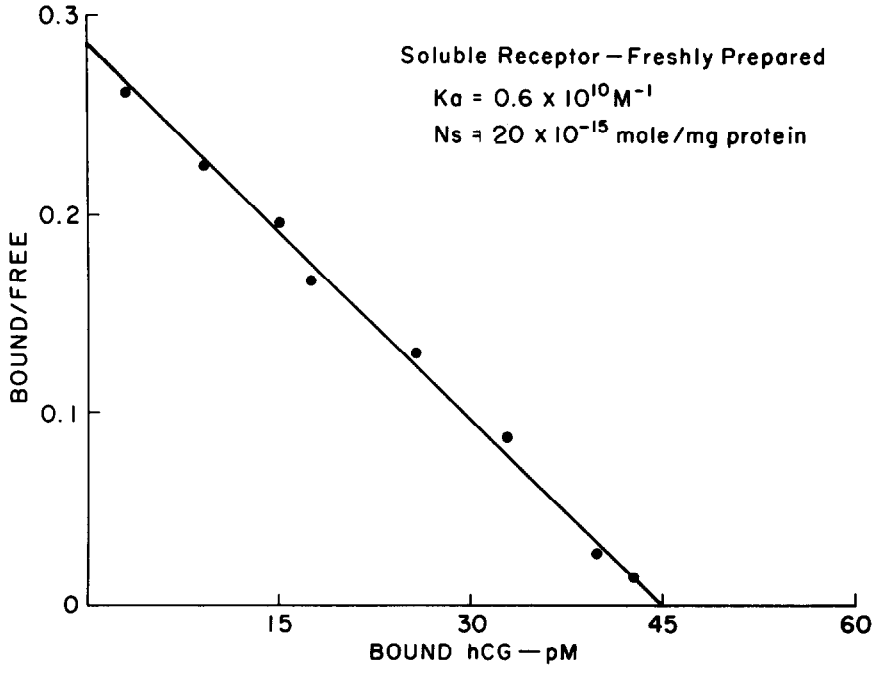

Fig.1. Scatchard plot derived from binding-inhibition data obtained with ^{125}I-hCG and freshly prepared Triton X-100 solubilized receptors, indicating the presence of a single order of binding sites.

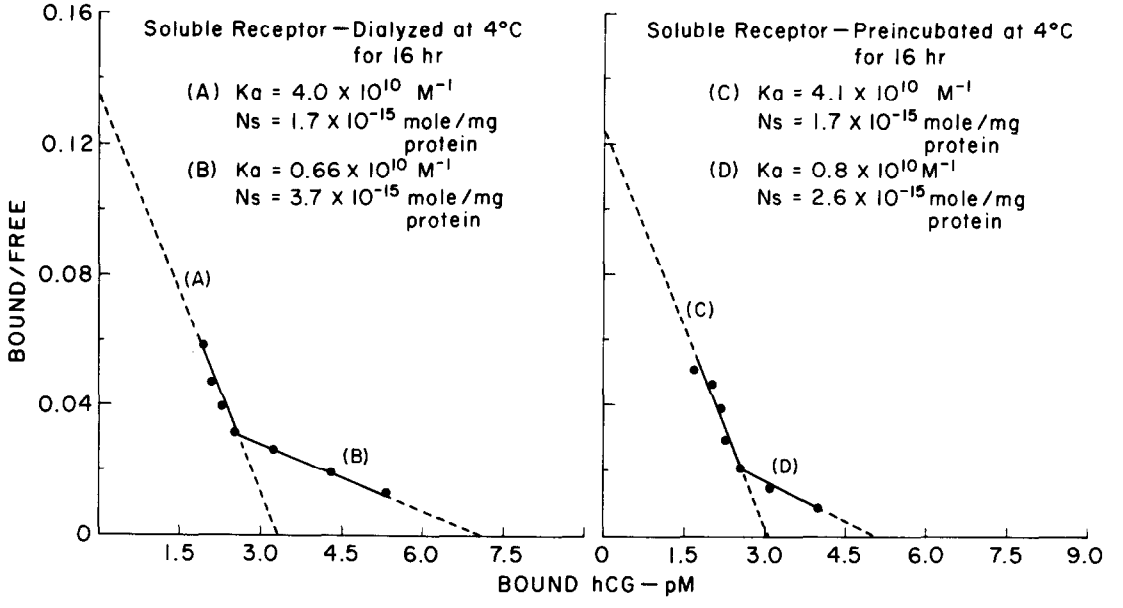

Fig.2. (Left) Scatchard plot of binding-inhibition data obtained with solubilized testis receptors aged by storage at 4°C for 16 hr. (Right) Scatchard plot of binding data derived from receptors aged by dialysis at 4°C for 16 hr. Both analyses show the presence of two binding sites.

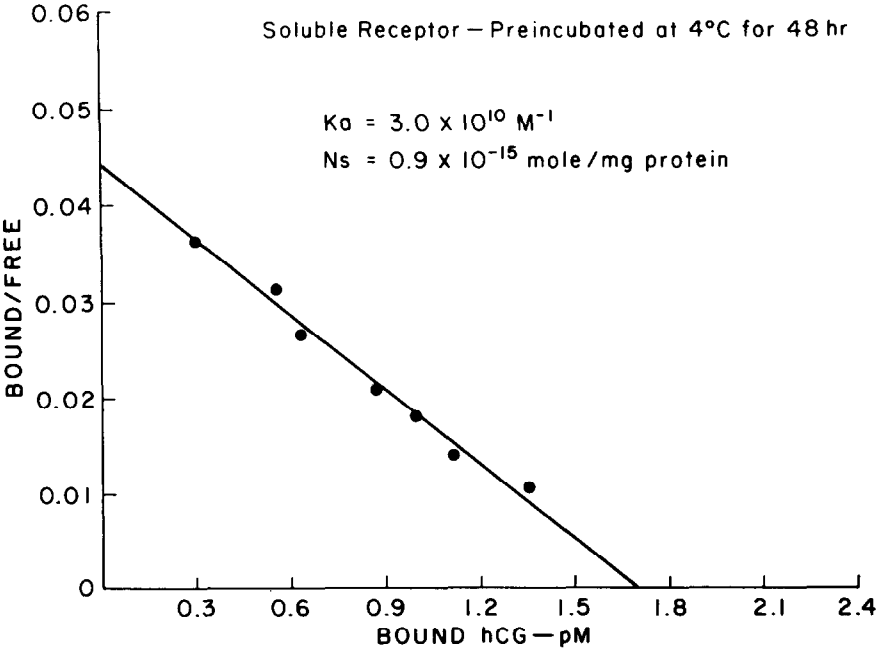

Fig.3. Scatchard plot of binding data obtained after more prolonged storage of the soluble receptors (48 hr at 4°C) showing only the higher affinity site, with complete disappearance of the lower affinity component.

demonstrated by the result of binding studies performed on a receptor preparation aged for 48 hr (fig.3), showing only a single order of sites with Ka = 3.0 × 10^{10} M^{-1} (n = 2) in amount (0.9 femtomol/mg protein) equivalent to only 1.5% of the original concentration of binding sites.

In previous studies, solubilization of gonadotropin binding particles with Triton X-100 has been found to cause a modest but consistent reduction of the association constant of the gonadotropin receptors. The rapid decay of free soluble receptors during storage is probably due to enzymatic degradation, since treatment of solubilized binding fractions with sulfhydryl reagents has been shown to reduce the rate of receptor inactivation [6]. The conditions employed in the present studies led to the detection of residual higher-affinity sites with association constant similar to that of the original particulate preparation. Aging of the soluble receptor preparation resulted in drastic reduction or disappearance of the lower affinity sites, which appear to be more susceptible to degradation than the high-affinity site. Such differential degradation of gonadotropin receptors in Triton-solubilized testis particles has revealed the presence of a small population of high-affinity receptor sites, which were previously undetectable against the background of the major lower-affinity component consequent upon detergent extraction.

The high-affinity sites demonstrable in soluble gonadotropin receptors stored at 4°C for 24 and 48 hr could originate from two sources in the original particulate receptor population. The more obvious origin is the well-characterized, apparently single-order, set of testicular sites with Ka of 2–4 × 10^{10} M^{-1} for hCG demonstrated by previous binding studies. In this case, the small proportion (<2%) of sites remaining after 48 hr would represent a minor residual fraction of the soluble receptors which retained the Ka of the original particulate receptors. Such a small fraction of high-affinity sites would not have been detectable against the predominant receptor species with Ka of 0.7 × 10^{10} M^{-1} in the freshly-solubilized preparation.

An alternative explanation for the high-affinity receptors demonstrable in the soluble testis preparation is that they result from detergent modification of a group of extremely high affinity receptors present in the original particulate fraction. The predominant receptor species in testis particles has been shown to behave as a single order of binding sites with Ka of 2–4 × 10^{10} M^{-1} [2,7], and careful analysis of both equilibrium and kinetic binding curves for ^{125}I-hCG has never shown evidence of the presence of sites with affinity higher than 4 × 10^{10} M^{-1}. However, recent studies on the biological responsiveness of dispersed Leydig cells in vitro have shown extremely high sensitivity to LH and hCG, with half-maximal stimulation of testosterone production in the presence of hCG concentrations as low as 25 picog/ml, equivalent to about 5 × 10^{-13} M [13]. Such a response implies the existence of receptor sites with association constant in the region of 2 × 10^{12} M^{-1}, a value considerably higher than that obtained by binding studies performed in testis homogenates and intact Leydig cells. However, the testis has been shown to possess a high proportion of 'spare' gonadotropin receptors for LH or hCG, of which only a small proportion (<1%) need be occupied to evoke a maximum steroidogenic response [14].

The presence of such large excess of specific binding sites could significantly influence the sensitivity of the Leydig cell to gonadotropin, by producing a full steroidogenic response with relatively low receptor occupancy induced by low concentrations of gonadotropin. Such a situation has also been described for the insulin binding sites of fat cells, and the concomitant stimulation of lipogenesis from glucose [15]. Alternatively, it is also conceivable that the presence of excess receptor sites could conceal the existence of a small proportion of high-affinity sites with binding constant commensurate with the high sensitivity of steroidogenesis to hCG. Taken together, these earlier observations are consistent with the presence of a small number of functionally significant gonadotropin receptor sites of higher affinity than those hitherto demonstrated by binding studies with radioiodinated gonadotropins. Such receptors could be the source of the soluble high-affinity sites demonstrated during the present studies, after moderate reduction of binding affinity consequent upon detergent extraction from particulate membrane preparations, and exposure by preferential degradation of the lower-affinity binding sites.

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