SOLUBLE FSH RECEPTORS FROM THE RAT TESTIS

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1. Introduction

Isolation and analysis of peptide hormone receptors is an obvious requirement for the complete understanding of hormone–receptor interactions. The location of such receptors in the hydrophobic environment of the cell membrane has led to the use of non-ionic detergents for their extraction and fractionation in soluble forms. Detergents such as Triton X-100 and Lubrol have been employed to solubilize cholinergic receptors [1], and peptide hormone receptors, for insulin [2] LH/hCG [3–5] and prolactin [6]. Extraction and fractionation of FSH receptors has been rendered difficult by the relatively low binding of labeled FSH by particulate and soluble FSH receptor preparations. This report describes the extraction and initial characterization of detergent-solubilized FSH receptors from rat testis, and the spontaneous release of water-soluble sites from particulate testis fractions.

2. Materials and methods

Testes from 20-day old male rats were decapsulated and homogenized in conical glass tissue grinders in Dulbecco's phosphate-buffered saline (PBS), 5 ml/2.5 g tissue. The homogenates were centrifuged at 27 000 × *g* for 30 min and the pellets were resuspended in 1% Triton X-100 for 30 min at 4°C. Treatment of the particulate binding fraction from 15 immature rat testes with 1 ml 1% Triton was found to extract to 20–40% of the FSH binding sites. The soluble fraction was then diluted with PBS to 0.1% Triton prior to centrifugation for binding studies. The solubilized FSH receptor sites remained in the supernatant solution after centrifugation at 360 000 × *g* for 3 h. In some experiments, testis particles were preincubated with ^{125}I-labelled FSH at 22°C for 15 h, then washed extensively with PBS prior to solubilization of the receptor–hormone complex.

Binding studies were performed with soluble preparations containing 0.1% Triton (0.5 ml, mg protein) by incubation under equilibrium conditions with increasing concentrations of unlabeled FSH in the presence of a constant quantity (30 000 dpm) of ^{125}I-labelled FSH (0.2 ml), followed by precipitation of the hormone–receptor complex with polyethylene glycol (PEG) as described for soluble LH receptors [3,4]. Binding-inhibition data were converted to saturation curves and Scatchard plots for analysis of binding constants and receptor concentrations [7]. The non-specific binding present in tubes containing excess unlabeled FSH (Pergonal, 1 U/tube) was about 10% of the specific binding value. Binding studies on particulate fractions were carried out by incubating the homogenate or 27 000 × *g* fraction with tracer for 16 h at 22°C, followed by Millipore filtration.

Binding analysis was also performed on a water-soluble testicular fraction observed during preparation of testis particles for detergent solubilization. For these studies, the 27 000 × *g* supernatant was centrifuged at 360 000 × *g* for 60 min and equilibrium binding-inhibition studies were performed on the high speed supernatant fraction.

Tracer ^{125}I-labelled FSH used for binding assay was prepared by radioiodination of highly purified FSH (LER 1535, 3050 IU/mg) by a lactoperoxidase method [8] followed by purification on Sepharose–concanavalin A [8]. The tracer preparation was kept

frozen until just prior to use, when it was purified by binding to testicular FSH receptors, followed by acid elution to obtain biologically active tracer hormone.

The specific activity of the ^{125}I-labelled FSH tracer was measured by self displacement in the FSH radioligand–receptor assay [9] and the maximal binding activity of each tracer preparation (40–50%) was determined as previously described [9]. Partition chromatography of soluble receptors was carried out at 4°C on 0.9 × 100 cm columns of Sepharose equilibrated with 50 mM Tris–Cl buffer, pH 7.4, containing 0.1% Triton X-100 and 0.01% bovine serum albumin.

3. Results

The solubilization of testicular FSH receptors was most efficiently performed by detergent extraction of the 27 000 × g fraction of the testis homogenates from 21-day old rats, with 1% Triton X-100. This detergent extracted 20–40% of the prelabeled FSH receptor sites, while other non-ionic detergents such as Lubrol PX or WX, and ionic detergents such as sodium deoxycholate, were less effective.

Analysis of equilibrium binding studies on particulate and detergent-solubilized FSH receptors, by saturation curves or Scatchard plots, revealed a single order of binding sites. The equilibrium association constant (K_a) of the particulate receptors was $1.6 \pm 0.5 \times 10^9$ M^{-1} (mean ± SD, $n = 3$) and the concentration of sites was 17 fmol/mg protein (fig.1). The K_a of the detergent-solubilized receptors was $8.5 \pm 1.6 \times 10^9$ M^{-1} ($n = 3$), and the concentration of sites was 8.2 fmol/mg protein (fig.2).

In addition to the FSH receptors solubilized by detergent treatment, spontaneously water-soluble sites with high affinity for ^{125}I-labelled FSH were demonstrated in immature testis homogenates. Significant binding activity was recovered when the 27 000 × g supernate (from particles used for detergent solubilization) was further centrifuged at 360 000 × g for 2 h and then equilibrated with ^{125}I-labelled hFSH. The K_a of the water-soluble receptor sites was 1.17×10^9 M^{-1} and their concentration was 4.6 fmol/mg protein. In relation to the total receptor concentration, such water soluble-receptors represented about 20% of the FSH receptors in the testis. The water-soluble receptors were not retained during filtration through 0.45 μm millipore membranes, and were not associated with a detectable level of lipid material in the high-speed supernatant fraction.

The elution profile of the Triton-solubilized testicular FSH receptors during gel filtration on Sepharose 6B is shown in fig.3. The mean K_{av} of the soluble hormone–receptor complex was 0.30 in two experiments, corresponding to a Stokes radius of 65 Å. Partial dissociation of labeled hormone from the FSH–receptor complex was observed during column fractionation, and an additional peak of free ^{125}I-labelled FSH was always resolved by gel filtration, with K_{av} of 0.59 (fig.3).

4. Discussion

These studies have demonstrated that the FSH receptors of the immature rat testis can be recovered in a soluble form by detergent extraction with Triton X-100. The detection and analysis of soluble FSH binding sites was found to require the use of optimal tracer preparations of ^{125}I-labelled hFSH. Such tracer should exhibit high specific binding to excess particulate receptors (40–50% of the added tracer) and should give low non-specific binding in the PEG precipitation assay. The most suitable tracer was prepared by enzymatic radioiodination of hFSH, followed by purification on Sepharose-Concanavalin A and enrichment by 'affinity chromatography' on testicular receptor sites. The use of less optimal tracer is attended by considerable difficulty in the demonstration of soluble FSH receptors.

The association constant of the detergent-solubilized FSH receptors was somewhat higher than that of the particulate receptors. This finding was in contrast to the fall in binding affinity observed during solubilization of testicular LH receptors [3–5]. A comparable rise in binding affinity was reported to occur after solubilization of prolactin receptors from the liver [5]. The detergent-extracted FSH receptors could not be analyzed by gel filtration in the free form, but were readily observed as the hormone–receptor complex when extracted from particles previously labeled with ^{125}I-labelled hFSH.

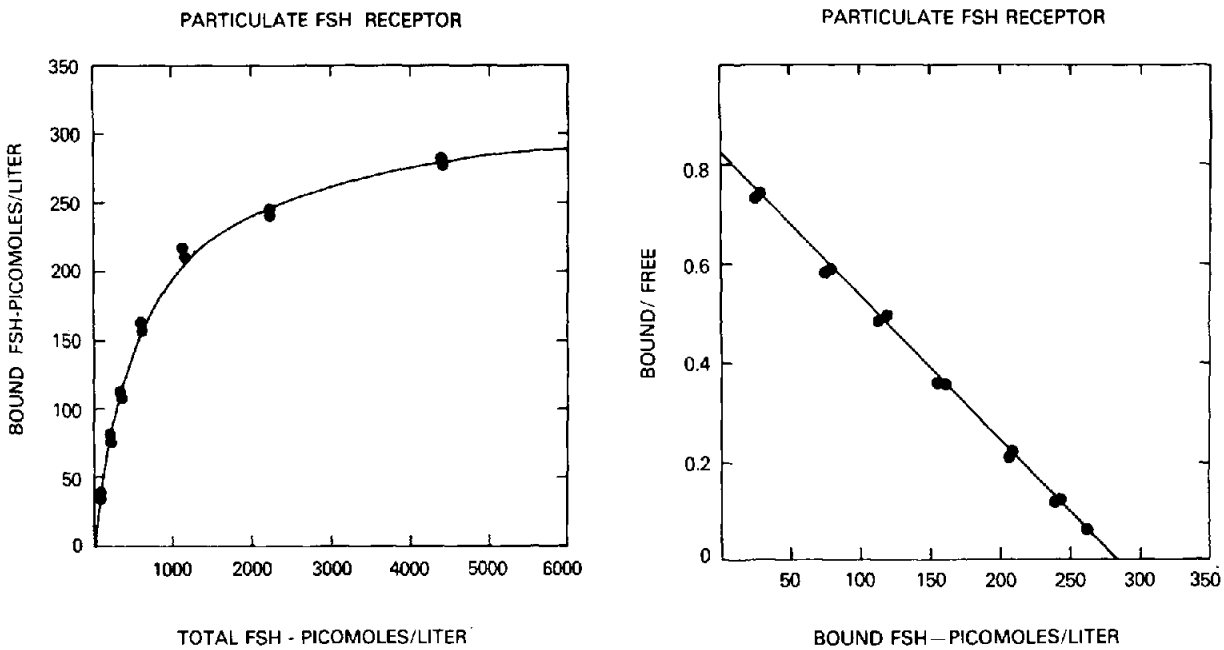

Fig.1. Left: Saturation curve for specific binding of hFSH to particulate receptors, obtained by computer fitting of total and specifically bound ^{125}I-labelled hCG. Right: Scatchard plot of binding data obtained by incubation of particulate receptor with ^{125}I-labelled FSH (2 ng) and increasing concentrations of hFSH for 16 h at 4°C.

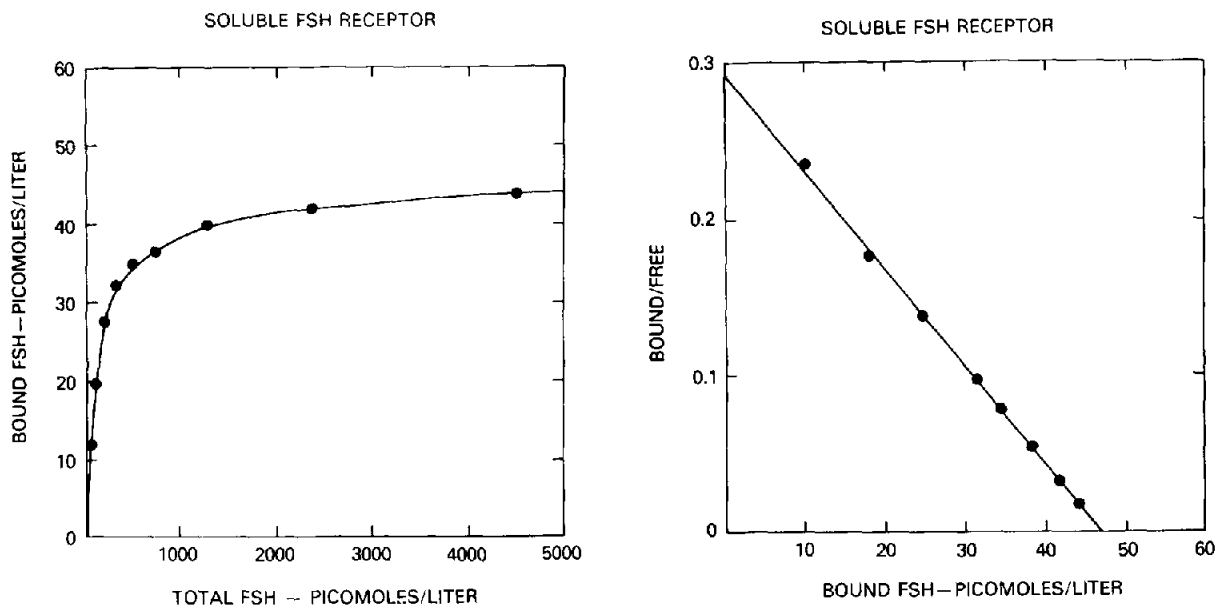

Fig.2. Left: Saturation curve for specific binding of hFSH to detergent-solubilized receptors. Right: Scatchard plot analysis of the equilibrium binding data.

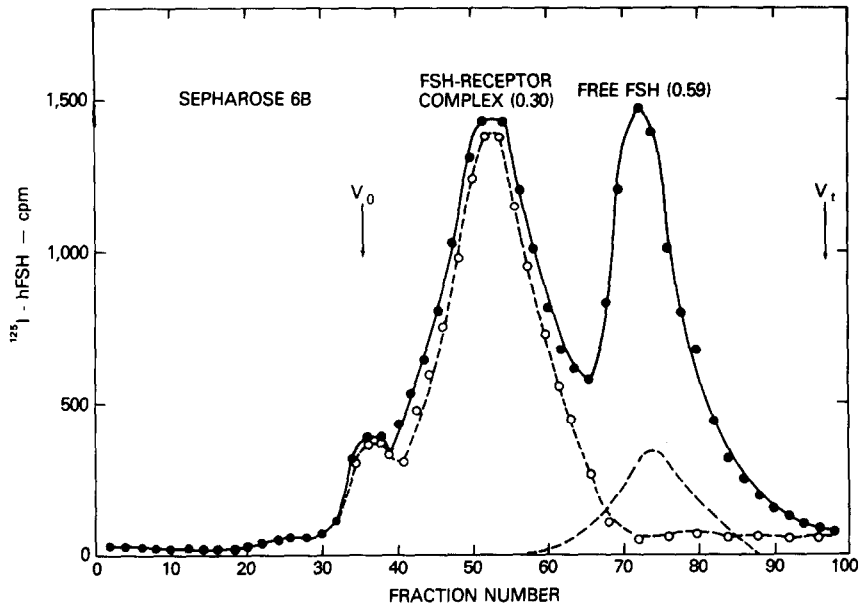

Fig.3. Gel filtration on Sepharose 6B of the hormone–receptor complex extracted with Triton X-100 from testis particles labeled with ^{125}I-labelled FSH. Two discrete peaks of radioactivity were resolved (•) corresponding to free FSH (K_{av} 0.59) and the hormone–receptor complex (K_{av} 0.30), which was precipitable by polyethylene glycol (○). The hormone–receptor complex was not present when preceding incubation with ^{125}I-labelled FSH was performed in the presence of excess FSH (–).

The partition coefficient of the Triton-solubilized FSH–receptor complex on 5% agarose beads was similar to that of the detergent-soluble LH–receptor complexes from testis and ovary, with Stokes radius of about 65 Å. The dissociation of free ^{125}I-labelled hFSH from the hormone–receptor complex during gel filtration is in contrast with the tight binding observed during analytical studies of the testicular and ovarian LH receptors [3–5]. Since the equilibrium binding constants of the two solubilized receptors are quite similar, the more marked dissociation of the FSH–receptor complex implies a significantly higher dissociation rate constant for the FSH receptor site. The nature of the water-soluble FSH receptors has yet to be determined. This minor fraction of the receptor population could represent newly-synthetized molecules in the process of being incorporated into the plasma membrane, or membrane receptors that have undergone internalization as a consequence of membrane turnover. It is also possible that these receptors are loosely attached to the membrane, and are rendered soluble during the initial homogenization procedure. Further analysis of these receptors could be complicated by their relatively low association constant and small concentration in the testis. However, the detergent-solubilized FSH receptors are more amenable to further characterization, and should be of value for more detailed studies on the molecular interaction between FSH and specific receptor sites.

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