Solubilization and Some Characteristics of the Follitropin Receptor from Calf Testis

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Immature calf testes were found to be an unusually rich source of follitropin (FSH) receptors. Particulate fractions derived from such testes bound 32% of added biologically active radiolabeled human FSH (125I-labeled hFSH) and had a binding capacity of 52 x 10^-14 mol/mg of protein. These values are considerably higher than those previously reported for FSH receptors in other types of beef or mature and immature rat testis. Solubilization of the receptor was achieved by extraction with Triton X-100. Its presence in detergent extracts of immature calf testes was demonstrated by gel filtration and sucrose density gradient centrifugation experiments, wherein the position of 125I-labeled hFSH-soluble receptor complex was determined after incubation of radioligand with soluble receptor in the absence and presence of 1000-fold excess unlabeled hormone. Separation of free 125I-labeled hFSH from that bound to soluble receptor was done by double precipitation with polyethylene glycol, with inclusion of appropriate controls to correct for co-precipitation of free 125I-labeled hFSH with the hormone-receptor complex. Binding of the 125I-labeled hFSH to soluble receptor was pH- and temperature-dependent, being maximal at pH 7.5 and 24°C after 3 h of incubation, and could be completely inhibited by excess unlabeled hormone. Large amounts (3000 ng) of other pituitary hormones did not inhibit binding of 125I-labeled hFSH to soluble receptor beyond that attributable to trace contamination with native hFSH. The characteristics of the 125I-labeled hFSH binding inhibition curve obtained with graded doses of unlabeled hormone were similar whether solubilized or particulate receptors were utilized.

Triton X-100 extracts of particulate fractions from such nongonadal tissue as liver, kidney, and spleen did not result in solubilization of factors capable of binding 125I-labeled hFSH, demonstrating the tissue specificity of the Triton X-100-solubilized testicular receptor.

The binding capacity of solubilized receptor derived from immature calf testes, 19 x 10^-11 mol/mg of protein, was significantly (64%) less than that seen with particulate receptor prior to detergent extraction, but at least as high as that seen for FSH particulate receptors in larger beef testis or testes from mature or immature rats. Various trinucleotides (1 mM) inhibited the binding of 125I-labeled hFSH and also promoted dissociation of specifically bound 125I-labeled hFSH from preformed hormone-soluble receptor complex, in a manner analogous to that previously reported for membrane-bound receptors of rat testis. Scatchard analysis of binding data indicated one class of high affinity binding sites with an affinity constant (K,) of 2.1 x 10^6 M^-1. This is 2-fold greater than the K, seen for interaction of radioligand with particulate receptors. Based on gel filtration experiments, the Stokes radius of the solubilized receptor was estimated to be 47 Å and that of the hormone receptor complex, 50 Å. The sedimentation coefficient of the free solubilized receptor was estimated to be 6.3, whereas that of the hormone-receptor complex was estimated at 7.4. These values allowed calculation of molecular masses of 146,000 daltons for the free receptor and 183,000 for the hormone-receptor complex. The difference between these estimates, 37,000, is consistent with current molecular weight estimates of hFSH and suggests a binding of 1 molecule of hFSH per molecule of solubilized receptor. The diffusion coefficient of the free receptor was estimated at 4.35 x 10^-7 cm^2 sec^-1 and that of the hormone-receptor complex, 1.06 x 10^-7 cm^2 sec^-1. The frictional ratio of the free receptor was estimated at 1.30 and that of the hormone receptor complex, 1.34.

These studies represent initial reports on successful solubilization of hormone and tissue-specific FSH receptors from testis and form the basis for further studies on purification and chemical characterization of the solubilized receptor.

In an earlier report in this journal, we characterized the interaction of biologically active radiolabeled human follitropin with hormone-specific receptors present in crude homogenates of testes from mature rats (1) and, recently, with receptors in highly purified tubule membranes derived from the same source (2). In general, however, fundamental questions regarding receptor chemistry can best be answered through studies with solubilized receptors. Although many studies have appeared on solubilization and purification of lutropin receptors from testes and ovary (3-6), little information has heretofore been available on solubilization of FSH receptors from gonadal tissue. The latter problem is complicated by the primary need for a reliable method to separate receptor-bound FSH from free hormone, thereby allowing accurate and specific binding data to be obtained.

The abbreviations used are: FSH, follitropin; hFSH, human follitropin; hLH, human lutropin; hCG, human choriogonadotropin; LH, lutropin.
Solubilized FSH Receptors

MATERIALS AND METHODS

Solubilization of FSH Receptor from Calf Testes

Six to eight frozen calf testes (each weighing from 3.5 to 6.5 g prior to removal of the tunica) were defrosted, the tunica was removed, and the remaining tissue (about 1.5 g) was minced and then homogenized in 0.05 M Tris/HCl buffer, pH 7.5, containing 0.25 M sucrose (5 ml of 10% homogenate) (Brinkmann type PT-10), at maximal speed for 30 s. The homogenate was filtered through two layers of cheesecloth, and the filtrate was centrifuged at 130 × g for 10 min to remove nuclei and tissue fragments. The supernatant solution was then centrifuged at 32,000 × g for 30 min. These testicular pellets were used either for solubilization or as the receptor source for binding assays. When used for the latter purpose, they will hereafter be referred to as the "particulate fraction." For the binding assay utilizing the testicular particulate fraction, various quantities of labeled hormone were incubated with 10 μg of unlabeled hormone for 2 h at 4°C. After incubation, 0.2 ml of 5 mg/ml bovine γ-globulin was added, followed by 1.0 ml of 25% polyethylene glycol (w/v) in 0.1 M NaCl, pH 7.5. The tubes were placed in an ice bath and centrifuged at 4°C for 20 min. The resulting precipitates were washed three times with 0.1 M NaCl, pH 7.5, and the supernatant solutions were used in the assay. The assay solution was made up of 0.2 M Tris/HCl buffer, pH 7.5, containing 10% calf serum, and 10 mM MgCl2, and was incubated for 3 h at 37°C. The resulting precipitates were washed two more times with 0.1 M NaCl, pH 7.5, and then dissolved in 0.1 N NaOH. The radioactivity incorporated into the precipitates was measured by liquid scintillation counting.

Precipitation with Polyethylene Glycol

An aliquot of the supernatant solution was added to an equal volume of 25% polyethylene glycol (w/v) in 0.1 M NaCl, pH 7.5. The tubes were placed in an ice bath and centrifuged at 4°C for 20 min. The resulting precipitates were washed three more times with 0.1 M NaCl, pH 7.5, and the supernatant solutions were used in the assay. The assay solution was made up of 0.2 M Tris/HCl buffer, pH 7.5, containing 10% calf serum, and 10 mM MgCl2, and was incubated for 3 h at 37°C. The resulting precipitates were washed two more times with 0.1 M NaCl, pH 7.5, and then dissolved in 0.1 N NaOH. The radioactivity incorporated into the precipitates was measured by liquid scintillation counting.

Detection of Receptor Activity

For solubilization, the pellets were suspended in 1.0 ml of 1% Triton X-100 in 0.05 M Tris/HCl buffer, pH 7.5, at 4°C and then incubated with gentle agitation for 30 min at the same temperature. The suspension was then diluted 1:10 with Tris/HCl buffer and centrifuged at 400,000 × g for 90 min. When a more concentrated solution of the solubilized receptor was needed, detergent was removed from the extract by agitation with Bio-Beads SM-2 for 2 h at 4°C as described by Holloway (13) prior to concentration by ultrafiltration or similar techniques. The resulting supernatant solution was used as the source of detergent. The assay tubes were placed in an ice bath and centrifuged at 4°C for 20 min. The resulting precipitates were washed three more times with 0.1 M NaCl, pH 7.5, and the supernatant solutions were used in the assay. The assay solution was made up of 0.2 M Tris/HCl buffer, pH 7.5, containing 10% calf serum, and 10 mM MgCl2, and was incubated for 3 h at 37°C. The resulting precipitates were washed two more times with 0.1 M NaCl, pH 7.5, and then dissolved in 0.1 N NaOH. The radioactivity incorporated into the precipitates was measured by liquid scintillation counting.

For precipitation with polyethylene glycol, the solubilized receptor preparation was incubated for 6 h or sometimes overnight at 4°C with 10 ng of 125I-labeled hFSH in a final volume of 0.8 ml. The buffer used for incubation was 0.05 M Tris/HCl buffer, pH 7.5, containing 0.1% (w/v) egg albumin and 5 mM MgCl2. At the end of the incubation period, 0.5 ml of cold buffer containing 0.1% Triton X-100 was added, and the mixture was applied on a column (1 × 5 cm) of Sephadex G-200 previously equilibrated and developed with the solvent buffer containing 0.1% Triton X-100. The radioactivity of the eluted fractions was counted in an Auto-Gamma counter.

Extraction of Preformed Calf Testis FSH-Receptor Complex

In some experiments, calf testis particulate fractions were incubated with 125I-labeled hFSH under conditions known to favor formation of the hormone-receptor complex. The reaction was terminated after 30 min by the addition of a 25 mM Tris/HCl buffer, pH 7.5, containing 0.25 M sucrose. The resulting precipitates were washed three more times with 0.1 M NaCl, pH 7.5, and then dissolved in 0.1 N NaOH. The radioactivity incorporated into the precipitates was measured by liquid scintillation counting.

Detection of Receptor Activity

For solubilization, the pellets were suspended in 1.0 ml of 1% Triton X-100 in 0.05 M Tris/HCl buffer, pH 7.5, at 4°C and then incubated with gentle agitation for 30 min at the same temperature. The suspension was then diluted 1:10 with Tris/HCl buffer and centrifuged at 300,000 × g for 90 min. When a more concentrated solution of the solubilized receptor was needed, detergent was removed from the extract by agitation with Bio-Beads SM-2 for 2 h at 4°C as described by Holloway (13) prior to concentration by ultrafiltration or similar techniques. The resulting supernatant solution was used as the source of detergent. The assay tubes were placed in an ice bath and centrifuged at 4°C for 20 min. The resulting precipitates were washed three more times with 0.1 M NaCl, pH 7.5, and the supernatant solutions were used in the assay. The assay solution was made up of 0.2 M Tris/HCl buffer, pH 7.5, containing 10% calf serum, and 10 mM MgCl2, and was incubated for 3 h at 37°C. The resulting precipitates were washed two more times with 0.1 M NaCl, pH 7.5, and then dissolved in 0.1 N NaOH. The radioactivity incorporated into the precipitates was measured by liquid scintillation counting.

Detection of Receptor Activity

For solubilization, the pellets were suspended in 1.0 ml of 1% Triton X-100 in 0.05 M Tris/HCl buffer, pH 7.5, at 4°C and then incubated with gentle agitation for 30 min at the same temperature. The suspension was then diluted 1:10 with Tris/HCl buffer and centrifuged at 300,000 × g for 90 min. When a more concentrated solution of the solubilized receptor was needed, detergent was re-
solubilized FSH Receptors

Measurement of Physical Properties of the Solubilized Receptor

Values for the sedimentation coefficient ($s$) were obtained by ultracentrifugation through sucrose gradients as described by Martin and Ameo (16). Centrifugation was through a 5 to 20% ($w/v$) sucrose gradient in 0.05 m Tris/HCl buffer at pH 7.5 in 0.1% Triton X-100 using a Beckman model L-55 ultracentrifuge operated at 40,000 rpm for 16 h at 4°C. The relative positions of marker enzymes or proteins were determined by measurement of enzyme activity or ultraviolet absorbance. The location of the free solubilized receptor was determined through use of the polyethylene glycol assay, and the location of solubilized $^{125}$I-labeled hFSH receptor complex was determined by counting of radioactivity. The value for Stokes radius ($a$) was obtained from gel filtration data as recommended by Siegel and Monty (17). Gel filtration was through a column (1 x 92 cm) of Sepharose 6B in 0.05 m Tris/HCl buffer, pH 7.5, containing 0.1% Triton X-100 at 4°C. The location of various preparations utilized in such studies was done as described earlier for the ultracentrifugation studies. The average distribution coefficient ($K_a$), for various reference proteins was experimentally determined, and the $K_a$ was plotted against the Stokes radius values as obtained from the literature (17). The Stokes radius of the soluble receptor was then determined from this plot after experimental measurement of its $K_a$. The values for Stokes radius ($a$) and sedimentation coefficient ($s$) were then utilized to calculate the molecular mass ($M$) by the relationship:

$$M = 6\pi n N a S / (1 - (n a / p))$$

In equation 1, $n$ is the system viscosity in ergs/cm$^2$, $N$ is Avagadro's number, $a$ is the partial specific volume assumed to be 0.72, and $p$ is the density of the medium in g/cm$^3$. The diffusion coefficient ($D$) was also calculated from knowledge of the Stokes radius and diffusion coefficient by the relationship:

$$D = k T / 6 \pi N a$$

In this equation, $k$ is the Boltzman constant, and the other symbols have already been defined.

Finally, the frictional ratio, $f/f_o$, was calculated from knowledge of the sedimentation coefficient, Stokes radius, and molecular weight by the relationship:

$$f/f_o = a / (36 M / 4 \pi N a)^{1/3}$$

RESULTS

Source of FSH Receptors for Solubilization - The low concentration of FSH receptors in rat testis (1, 2, 11, 19, 20), together with their relatively small size, rendered testes from that species a somewhat impractical receptor source for large scale solubilization and eventual purification studies. It was necessary, therefore, to identify a more convenient source of testicular FSH receptors. Particulate fractions were prepared from bovine testes of different sizes representing various stages of glandular development and were examined for ability to bind $^{125}$I-labeled hFSH under equilibrium conditions. Binding of $^{125}$I-hFSH was evident in particulate fractions from all sizes of testes, but the greatest binding was to immature (3.5 to 6.5 g) calf testis (Table I). Approximately 32% of total added radioactivity was specifically bound to the immature calf testis compared with 17% to medium sized (15 to 25 g) and 13% to mature (100 to 120 g) boar testis. Therefore, the highest reported specific binding of radiolabeled FSH to excess receptors in crude testicular membrane fractions from any species was 12 to 15% (11, 21). Although greater binding to highly purified rat testicular membrane fractions has been reported (2, 19), this high binding capacity was a reproducible observation seen in numerous experiments (Table I). The amount of hormone binding to immature calf testis was significantly greater than to testis from immature or mature rats (Table I). Analysis of binding data shown in Fig. 1 by Scatchard analysis (22) confirmed that the number of high affinity binding sites in small calf testis ($52 \times 10^{-14}$ mol/mg of protein) was significantly greater than that seen in medium sized calf testis ($16 \times 10^{-14}$ mol/mg of protein) or large testis ($12 \times 10^{-14}$ mol/mg of protein), or in testis from mature rats ($3.49 \times 10^{-13}$ mol/mg of protein) or immature rats ($8.5 \times 10^{-14}$ mol/mg of protein) (Table I). Testes from immature calves (gland weight, 3.5 to 6.5 g prior to decapsulation) were therefore chosen as the source of receptor in subsequent solubilization studies.

Solubilization of the FSH Testicular Receptor from Small Calf Testes - To establish conditions suitable for extraction of
FSH receptors from immature calf testes, preliminary studies were carried out using preformed FSH particulate-receptor complexes. Solubilization of the preformed hormone-receptor complex, prepared by prelabeling the 32,000 g sedimented calf testicular receptor particles with 125I-labeled hFSH, showed that 80 to 90% of the bound radioactivity could be extracted with 1% Triton X-100 in 0.05 M Tris/HCl buffer, pH 7.5. Binding of 125I-labeled hFSH in the detergent-extracted and buffer-washed particulate pellet was negligible. To establish the presence of receptor in Triton X-100 tissue extracts, calf testicular particulate fractions were extracted with 1% Triton X-100. The detergent-solubilized extract was separated from glandular residue by centrifugation at 300,000 g, diluted to 0.1% Triton X-100 with assay buffer, incubated with 125I-labeled hFSH under conditions favoring association of the hormone-receptor complex, and then passed through a column of Sephadex G-200 (Fig. 1). A parallel experiment was carried out wherein the 125I-labeled hFSH was incubated with the detergent extract in the presence of a 1000-fold excess of cold hormone. As can be seen in Fig. 1, in the absence of excess cold hormone, a large molecular weight peak (Curve A) of radioactivity emerged near the void volume of the column (determined with blue dextran), followed by a second peak of radioactivity (Curve B), which emerged in the position of free 125I-labeled hFSH (determined in a separate filtration). In the presence of excess cold hormone, however, no radioactivity appeared near the void volume but only in the position of free 125I-labeled hFSH (Curve C). These results were interpreted as confirmation that the Triton X-100 extraction procedure had solubilized a large molecular weight testicular tissue component capable of complexing free 125I-labeled hFSH and presumably representing the solubilized FSH receptor.

Separation of 125I-labeled hFSH bound to detergent-solubilized receptor from free hormone through use of the gel filtration technique was not suited for routine analysis of receptor concentration. A more convenient separation of bound hormone from free 125I-labeled hFSH was accomplished by double precipitation with polyethylene glycol (Carbowax 6000) essentially as described by Dufau et al. (3). Fig. 2 shows the effect of increasing concentrations of polyethylene glycol on precipitation of receptor-bound 125I-labeled hFSH obtained by Triton X-100 solubilization of the fulltrropin receptor. A final concentration of 12.5% polyethylene glycol was sufficient to precipitate the solubilized hormone-receptor complex (Fig. 2, Curve A). As discussed under "Materials and Methods," it is necessary to include a control tube in the assay to account for precipitation by polyethylene glycol of small amounts of free 125I-labeled hFSH in order to obtain a correct assessment of the extent of nonspecific binding (Fig. 2, Curves B and C).

**Tissue and Hormone Specificity of Triton X-100-solubilized Testicular FSH Receptor** — Lee and Ryan (5) reported that Triton X-100 extracts of rat liver, after purification by affinity chromatography, bound 125I-labeled hCG with the same affinity as did detergent extracts of rat ovaries and concluded that hCG receptors in liver might have been unmasked after solubilization. Bhalla et al. (23) have recently reported extraction from rat testes of ethanol-soluble "high affinity gonadotropin binding sites" having "partial tissue specificity." Dufau et al. (24), however, have reported solubilization from rat testes with Triton X-100 of hCG receptors that show a high degree of tissue and hormone specificity. For study of the tissue and hormone specificity of Triton X-100-solubilized putative testicular FSH receptors, particulate tissue fractions and detergent extracts of such nongonadal tissue as calf liver, kidney, and spleen were similarly prepared and examined for their ability to bind 125I-labeled hFSH. As can be seen in Table II, 125I-labeled hFSH binding to particulate tissue fractions and to detergent extracts of liver, kidney, and spleen was negligible. These results indicate that FSH receptors are probably neither present nor masked in nongonadal tissue, and the results strengthen the significance of FSH binding to components of detergent-solubilized testicular extracts.

Retention of hormone specificity by the solubilized testicular FSH receptor was indicated by the absence of competition for 125I-labeled hFSH binding sites by high concentrations (5000 ng) of other peptide hormones, such as prolactin, adrenocorticotropic hormone, thyrotropin, growth hormone, and, significantly, hCG. The slight inhibition (25%) seen by high concentrations (5000 ng) of LH is probably due to the usual contamination of this hormone with small amounts of native hFSH. Only unlabeled hFSH inhibited the binding of 125I-labeled hFSH to the soluble receptor in a dose-related fashion and at low hormone concentrations consistent with the amount of radioligand added to the system (Fig. 3). The characteristics of the binding inhibition obtained with the solubilized receptor (Fig. 3, Curve B) were similar to those seen for inhibition of 125I-labeled hFSH binding to particulate receptors by unlabeled hFSH (Fig. 3, Curve A).

**Some Characteristics of the 125I-labeled hFSH-soluble Receptor Interaction** — Specific binding of 125I-labeled hFSH to the soluble receptor was studied after incubation at 4, 24, and 37° for various periods of time up to 15 h (Fig. 4). Binding was maximal at 24°, reaching equilibrium after about 4 h of incubation; whereas at 4° binding was slow and did not reach equilibrium.

![Fig. 2. Precipitation of soluble receptor-bound 125I-labeled hFSH by increasing concentrations of polyethylene glycol (Carbowax 6000). Curve A, total 125I-labeled hFSH bound to the receptor; Curve B, binding that occurs in the presence of excess cold hormone, that is, the nonspecific binding; Curve C, nonspecific binding that has been corrected for co-precipitation of free 125I-labeled hFSH. For details of the assay and explanation of Curve C, see "Materials and Methods."](image-url)
described in the text. No significant binding of human growth hormone, adrenocorticotropic hormone, human thyrotropin, ovine prolactin, or human chorionic gonadotropin to soluble receptor was seen at total doses of 5000 ng. hLH (5000 ng) resulted in an approximately 25% inhibition of hormone binding compared to appropriate controls, presumably due to contamination with small amounts of native hFSH.

In the latter instances, maximal binding at 37° was reached after about 2 h and was maintained at that level for up to 4 h, the longest interval tested.

The specific binding of ¹²⁵I-labeled hFSH to solubilized receptor increased linearly to a concentration of 1.0 mg of soluble receptor protein and plateaued thereafter (Fig. 5). Similar results were obtained in experiments utilizing highly purified rat tubule membrane receptors (2). The explanation for this phenomenon is not readily apparent, but it could be due to the effect of an FSH binding inhibitor present in low concentrations and noticeable only at relatively high levels of soluble receptor. The presence of such an inhibitor has been suggested on the basis of earlier studies on the interaction of ¹²⁵I-labeled hFSH with membrane-bound testicular receptors (2).

Maximal specific binding of ¹²⁵I-labeled hFSH to the solubilized receptor occurred at about pH 7.5, decreasing at values away from this pH optimum. This is similar to the pH optimum seen for binding of ¹²⁵I-labeled hFSH to membrane-bound receptors (2). The lesser binding to solubilized receptors seen at pH 5 and pH 9 could be returned to maximal values by readjustment of the pH to 7.5, indicating that the pH effect may be a reversible phenomenon.

Effect of Nucleotides on Specific Binding of ¹²⁵I-labeled FSH to Soluble Receptor—We have previously reported that various nucleotides reduce the binding of ¹²⁵I-labeled hFSH to testicular receptors and enhance the dissociation of bound hormone from preformed hormone-membrane receptor complex (2). In this study, we tested the effects of nucleotides on ¹²⁵I-labeled hFSH binding to and dissociation from detergent-solubilized testicular receptor. As can be seen in Table III, 1 mM concentrations of ATP, GTP, UTP, CTP, and ITP inhibited the binding of ¹²⁵I-labeled hFSH to testicular receptors and enhanced the dissociation of bound hormone from preformed hormone-membrane receptor complex (2). In other experiments, ¹²⁵I-labeled hFSH was allowed to incubate with solubilized receptors under conditions known to favor formation of the hormone-receptor complex. The ¹²⁵I-labeled hFSH-soluble receptor complex was separated from unbound hormone by polyethylene glycol precipitation and then resuspended in the buffered solution of the respective nucleotides. The ability of the nucleotides to enhance dissociation of bound hormone was then assessed by a second polyethylene glycol precipitation of ¹²⁵I-labeled hFSH that remained bound to the soluble receptor after incubation with the nucleotide. As can be seen in Table IV, nucleotides that inhibited binding of ¹²⁵I-labeled hFSH to the solubilized receptor also promoted dissociation of the hormone-receptor complex in a manner similar to that seen with nucleotide effects on pre-
Effect of various nucleotides on uptake and dissociation of \(^{125}\text{I}\)-labeled hFSH by soluble testicular receptor

Aliquots of Triton X-100-solubilized testis particulate fraction were incubated with 10 ng of \(^{125}\text{I}\)-labeled hFSH for 6 h at 24° in Tris/HCl assay buffer, pH 7.3. Nonspecific binding was estimated in the presence of 1000-fold molar excess of cold hormone. Separation of bound from free hormone was by polyethylene glycol precipitation. Incubation of \(^{125}\text{I}\)-labeled hFSH with receptor was in the presence of 1 mM concentrations of the respective nucleotides in order to determine the effect on hormone binding. For the dissociation experiment, the \(^{125}\text{I}\)-labeled hFSH-receptor complex was precipitated by polyethylene glycol and resuspended in assay buffer in the presence of the respective nucleotides (1 mM), and the amount of radioactivity remaining bound to the receptor after 1 h of incubation at 24° was determined by a second polyethylene glycol precipitation.

**TABLE III**

| Nucleotide | \(^{125}\text{I}\)-labeled hFSH specific uptake | Dissociation of \(^{125}\text{I}\)-labeled hFSH from preformed hormone-receptor complex |
|------------|---------------------------------|--------------------------------------------------|
|            | \(\text{mol} \times 10^{-14} \text{ mol/mg protein}\) | \% Inhibition |
| No addition | 16.0 | 49.4 |
| ATP        | 0.1  | 49.4 | 02  |
| GTP        | 7.7  | 52.0 | 69  |
| UTP        | 9.8  | 38.7 | 44  |
| CTP        | 8.1  | 49.4 | 52  |
| ITP        | 8.7  | 45.6 | 55  |

**TABLE IV**

Some characteristics of the free and hormone-complexed soluble receptor

Numbers are ± S.E. of at least replicate determinations.

| Property                      | Free receptor | \(^{125}\text{I}\)-Labeled hFSH receptor complex |
|-------------------------------|---------------|----------------------------------------------|
| Molecular weight (\(M\))      | 146,000       | 183,000                                      |
| Diffusion coefficient (\(D\)) | 4.35 x 10^{-7} cm² s⁻¹ | 4.06 x 10^{-7} cm² s⁻¹ |
| Frictional ratio (\(f/f_o\))  | 1.30          | 1.34                                         |
| Stokes radius (\(\bar{a}\))   | 47 ± 1.0 Å    | 50 ± 1.2 Å                                  |
| Sedimentation coefficient (\(s\)) | 6.3 ± 0.1 | 7.4 ± 0.3                                   |
| Affinity constant (\(K_a\))   | 1.2 ± 0.04 x 10¹⁵ M⁻¹ (particulate) | 2.1 ± 0.1 x 10¹⁵ M⁻¹ (solubilized) |
| Binding capacity (\(n\))      | 52 ± 3.2 x 10⁻¹⁴ mol/mg (particulate) | 19 ± 0.6 x 10⁻¹⁴ mol/mg (solubilized) |

formed hormone particulate-receptor complexes (2). In the earlier study with tubule membranes, it was not possible to conclude that binding of the nucleotide was to the receptor itself. However, the results described here more clearly suggest that the \(^{125}\text{I}\)-labeled hFSH receptor may possess a nucleotide binding site that, when occupied, results in conformational or other changes in the receptor, causing decreased binding or enhanced dissociation of bound hormone.

Comparison of \(^{125}\text{I}\)-hFSH Binding to Particulate and Solubilized Calf Testicular Receptors — The kinetics of uptake of \(^{125}\text{I}\)-labeled hFSH by the particulate-binding fractions of immature calf testis, as well by Triton X-100-solubilized receptor from the same source, is shown in Fig. 6. Saturation of the soluble (Curve B) and particulate (Curve A) receptor sites was seen at about 100 ng of \(^{125}\text{I}\)-labeled hFSH (3 x 10⁻⁹ M) per ml.

![Fig. 6. Binding of increasing concentrations of \(^{125}\text{I}\)-labeled hFSH to particulate and soluble receptors from immature calf testes. Aliquots of each type of receptor were incubated with varying concentrations of \(^{125}\text{I}\)-labeled hFSH for 16 h at 24°. Nonspecific binding was determined in the presence of 1000-fold molar excess of unlabeled hFSH. Separation of bound from free radioligand was accomplished by centrifugation in the case of the particulate receptor and by the polyethylene glycol assay for the soluble receptor. Each point is the mean of results from duplicate experiments.](http://www.jbc.org/)

![Fig. 7. Scatchard analysis of binding of \(^{125}\text{I}\)-labeled hFSH to particulate and soluble testicular receptors. The plot is of data shown in Fig. 6. Particulate or soluble receptor protein (0.58 mg) was present in each assay tube. When discussed in the text, results are described in terms of binding per mg of each type of receptor.](http://www.jbc.org/)

Analysis of binding data by Scatchard technique (22) indicated one class of high affinity binding sites in both particulate and solubilized receptor (Fig. 7). There was a marked reduction in binding capacity but concomitant increased binding affinity of the receptor after solubilization with detergent (Table IV). The binding capacity decreased from 52 x 10⁻¹⁴ mol/mg of protein in the particulate fraction to 19 x 10⁻¹⁴ mol/mg of protein for the soluble receptor. The association constant of the solubilized receptor was greater than that of the particulate receptor fraction (Table IV). An increase in affinity of receptor for hormone after solubilization has been seen in other systems, such as with rabbit mammary gland receptors for prolactin (25).

Some Physical Properties of Solubilized Receptors — The sedimentation coefficient of the solubilized receptor, either free or associated with \(^{125}\text{I}\)-labeled hFSH, was estimated by ultracentrifugation through sucrose density gradients as recommended by Martin and Ames (16). The results are summarized in Fig. 8. The sedimentation coefficient of the free recep-
Solubilized FSH Receptors

Fig. 8. Sedimentation of free solubilized receptor and hormone·receptor complex in sucrose density gradients containing 0.1% Triton X-100. Centrifugation was performed with a Beckman L5-50 ultracentrifuge at 40,000 rpm for 18 h at 4°C, after which the gradient was fractionated by collection of 0.4-ml samples. The position of the reference proteins was determined by ultraviolet absorbance. The position of the 125I-labeled hFSH·receptor complex was determined by the counting of radioactivity, and that of the free receptor by the polyethylene glycol assay.

Fig. 9. Gel filtration of free solubilized receptor and hormone·receptor complex through Sepharose 6B. The $K_{av}$ (average distribution coefficient) = elution volume of protein ($V_e$) - outer volume of column ($V_o$)/(total volume of the column ($V_t$) - $V_o$). The Sepharose 6B column (1 x 92 cm) was equilibrated and developed with 0.05 M Tris/HCl buffer, pH 7.7 (43), containing 0.1% Triton X-100. The soluble receptor, however, appears less stable than the membrane-bound receptor and more susceptible to loss of hormone after detachment from the membrane or loss of an essential stabilizing component such as a phospholipid (2) or a ganglioside (28). Nonspecific binding to a control tube to account for co-precipitation of free Y-labeled hFSH from receptor-bound hormone. One modification in our use of the polyethylene glycol assay was the addition of 20% polyethylene glycol. This was found to be important in allowing correct assessment of nonspecific binding that, if uncorrected for this variable, becomes spuriously high (Fig. 2).

The binding characteristics of the soluble receptor appear very similar to those previously reported for FSH receptors in highly purified membranes derived from mature rat testes (2). The soluble receptor, however, appears less stable than the membrane-bound receptor and more susceptible to loss of binding activity on standing, especially at 37°C. This could be due to intrinsic instability of the receptor after detachment from the membrane or loss of an essential stabilizing component such as a phospholipid (2) or a ganglioside (28). Nonspecific enzymatic degradation at the higher temperature is also a possibility.

Hormone specificity of the FSH receptor was retained after solubilization. Only unlabeled FSH inhibited 125I-labeled hFSH binding in a dose-related manner. Very high concentrations of a variety of protein and peptide hormones, such as hCG, prolactin, thyrotrophin, adrenocorticotropic hormone, and hLH did not inhibit receptor binding of 125I-labeled hFSH. The inhibition seen at high concentrations (5000 ng) of hLH is attributed to contamination of such fractions with small amounts of intact FSH. The tissue specificity of the FSH receptor solubilized from calf testes by Triton X-100 was demonstrated by the fact that similarly prepared detergent extracts of such bovine nongonadal tissue as liver, kidney, and spleen did not bind 125I-labeled hFSH to any significant degree (Table II).

Bhalla et al. (23) have recently studied the interaction of
ethanol-soluble extracts of rat testes with radiolabeled hLH and hFSH using dextran-coated charcoal, viewing the solubilized factors as representing "high affinity gonadotropin binding sites," with "partial tissue specificity." This is in contrast to the apparent tissue and hormone specificity seen with the detergent-solubilized calf testicular FSH receptor. Also, although the binding of radiolabeled gonadotropins to solubilized receptors utilizing the charcoal procedure is described by Bhalla et al. (23) as being a saturable process, studies on whether such binding could be inhibited by excess cold hormone, essential criteria in the usual assessment of specific binding, were not reported. All calculations, including Scatchard analysis, Hill plots, and estimates of $K_m$ apparently utilized total counts bound, which were not corrected for non-specific binding (23). We have shown, however, that $^{125}$I-labeled hFSH binding to detergent-solubilized calf testicular receptor can be inhibited in a dose-related manner by concentration of unlabelled hormone consistent with the amount of radioligand added to the system and that the characteristics of the inhibition curve are similar to those seen when tubule membranes are utilized as the receptor source.

Significant reduction of $^{125}$I-labeled hFSH binding to soluble receptor was observed in the presence of a 1 mM concentration of several trinucleotides. These nucleotides also enhanced dissociation of labeled hormone from soluble receptor. Such effects are similar to those observed in studies of nucleotide effects on the binding of $^{125}$I-labeled hFSH to, and its dissociation from, purified tubule membranes from rat testes (2) but are different from the results of Cheng (29), who observed no effect of nucleotides on $^{125}$I-labeled hFSH-specific uptake by crude membrane fractions derived from mature bovine testes. Our results with both solubilized and membrane-bound receptor suggest that the FSH testicular receptor may possess a nucleotide-binding site that, when occupied, induces conformational changes in the receptor leading to enhanced dissociation of the specifically bound hormone. A possible role for nucleotides in local regulation of hormone binding has, of course, been suggested for a number of other hormones, including glucagon (37) and luteinizing hormone (30), hCG (31), and thyroid hormone (32).

Binding inhibition curves obtained when using the soluble receptor were similar to those observed with the particulate binding fraction of calf testes (Fig. 3). Scatchard analysis of binding data to each type of receptor indicated the presence of a single order of high affinity binding sites, in contrast to the situation with receptors in highly purified tubule membranes from mature rats, where two classes of binding sites were detected (2). A marked reduction in binding capacity of the FSH receptor was seen after solubilization, together with increased binding affinity (Table IV). The latter is similar to the observations of Shiu and Friesen (25), who showed that prolactin receptors from rabbit mammary glands exhibited higher affinity for the hormone after solubilization with Triton X-100. In contrast, Dufau et al. (3, 33) and Lee and Ryan (5) observed that hCG receptor solubilized from rat gonads had a lower affinity for $^{125}$I-labeled hCG than did membrane-bound receptors. The decreased binding capacity of solubilized receptor for $^{125}$I-labeled hFSH could be due to removal of the FSH receptor from its normal conformational state as organized in the intact cell membrane. Preliminary studies suggest that stability of the solubilized receptor can be enhanced by its complexing with $^{125}$I-labeled hFSH. The explanation for the increased binding affinity of the receptor after solubilization is as yet uncertain.

The values obtained for the molecular mass of the solubilized FSH testicular receptor (146,000) and the $^{125}$I-labeled hFSH-receptor complex (160,000) are somewhat less than those reported by Dufau et al. (24) for solubilized rat testicular LH and hCG receptors, either free (184,000) or complexed with hCG (224,000). The difference in daltons between the free solubilized and hormone-complexed FSH receptor, 37,000, is consistent with recent estimates of the molecular mass of human FSH (26) and suggests binding of 1 molecule of hormone per molecule of receptor. The values calculated for the Stokes radius, frictional ratio, and diffusion coefficient of the FSH testicular receptor (Table IV) are less than, whereas the sedimentation coefficients seen with free (6.2 S) and FSH complexed solubilized receptor (7.4) and determined in 0.1% Triton X-100, are similar to those reported for hCG receptors solubilized from rat ovarian and testicular membranes by Dufau et al. (3). $^{125}$I-labeled hCG receptors solubilized from plasma membranes of bovine corpus lutea, however, were reported to have a sedimentation coefficient of 5.1, when measured in the presence of Triton X-100 (6).

Tissue factors of relatively low molecular weight have also been reported to interact with gonadotropins. Lee and Ryan (5) utilized Triton X-100 to solubilize a factor from rat corpus lutea, thought to represent an LH receptor, which had a minimal molecular weight of 70,000. Hanner and Savena (34) used 6 M guanidine to solubilize several components from bovine corpus luteum with molecular weights ranging from 30,000 to 70,000, which they speculated may represent subunits of the gonadotropin receptor. Earlier studies from this laboratory (35) described preparation of a rat testicular ethanol-soluble factor capable of interacting with $^{125}$I-labeled hLH and $^{125}$I-labeled hFSH by several criteria and which, on the basis of gel filtration experiments, corresponded to molecular weights of 22,500 to 67,000.

Molecular weights considerably in excess of that reported here for the solubilized testicular receptor have been proposed for solubilized receptors of various other peptide hormones. Cuatrecasas (27) solubilized insulin receptors from rat liver and fat cell membranes through use of Triton X-100 and estimated its molecular weight to be 300,000, with a Stokes radius of 70 Å and a sedimentation coefficient of 11.0. Giorgio et al. (36) solubilized glucagon receptors from rat liver membranes using Lubrol-PX and reported it to have a molecular mass of 190,000 daltons with a Stokes radius of 42 Å. Shiu and Friesen (25) utilized Triton X-100 to solubilize prolactin receptors from rabbit mammary glands, estimating its molecular weight to be 220,000. Our earlier studies with the ethanol-soluble factor (35), as well as the latter two estimates of molecular weight (25, 36), are based on filtration behavior of the solubilized receptor to that of marker proteins of known molecular weight and are subject to the reservations usually associated with that technique (17, 18). Clearly, definitive assessments of the physical properties of solubilized peptide hormone receptors must await their final purification, something yet to be achieved for each example cited.

These are the first studies describing solubilization and characterization of FSH receptors from gonadal tissue. The detection of FSH receptors in immature calf testes in relatively high concentration suggests that testicular tissue from farm animals may prove a practical source of membrane-bound receptor and thereby facilitate attempts at purification and chemical characterization of the solubilized receptor.
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REFERENCES
1. Bhalla, V. K., and Reichert, L. E., Jr. (1974) J. Biol. Chem. 249, 43-51
2. Abou-Issa, H., and Reichert, L. E., Jr. (1976) J. Biol. Chem. 251, 3232-3237
3. Dufau, M. L., Charreau, E. H., and Catt, K. J. (1973) J. Biol. Chem. 248, 6973-6982
4. Dufau, M. L., Ryan, D. W., Baukal, A. J., and Catt, K. J. (1975) J. Biol. Chem. 250, 4822-4824
5. Lee, C. Y., and Ryan, R. J. (1974) in Gonadotropins and Gonadal Function (Moudgal, N. R., ed) pp. 444-459. Academic Press, New York
6. Thambryrajah, V., Azhar, S., and Menon, K. M. J. (1976) Biochim. Biophys. Acta 428, 35-44
7. Desbuquois, B., and Aurbach, G. D. (1971) J. Clin. Endocrinol. Metab. 33, 722-738
8. Steelman, S., and Pohley, F. M. (1953) Endocrinology 53, 604-610
9. Parlow, A. F. (1961) in Human Pituitary Gonadotropins (Albert, A., ed) pp. 300-303. Charles C Thomas, Springfield, Ill.
10. Reichert, L. E., Jr., and Bhalla, V. K. (1974) Endocrinology 94, 483-491
11. Ketelslegers, J. M., and Catt, K. J. (1974) J. Clin Endocrinol Metab 29, 1159-1162
12. Greenwood, F. C., Hunter, W. M., and Glover, J. S. (1963) Biochem. J. 89, 114-123
13. Holloway, P. W. (1973) Anal. Biochem. 53, 304-306
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275
15. Chandrarajan, J., and Klein, L. (1975) Anal. Biochem. 69, 622-636
16. Martin, R. G., and Amee, B. N. (1961) J. Biol. Chem. 236, 1372-1379
17. Siegel, L., and Monty, K. J. (1966) Biochim. Biophys. Acta 112, 346-362
18. Laurent, T. C., and Killander, J. (1964) J. Chromatogr. 14, 317-330
19. Means, A. R., and Vaittukaitis, J. L. (1972) Endocrinology 90, 39-46
20. Steinberger, A., Thanki, K. H., and Siegel, B. (1974) in Hormone Binding and Activation in the Testis (Dufau, M. L., and Means, A. R., eds) pp. 177-199. Plenum Press, New York
21. Cheng, K. W. (1975) J. Clin. Endocrinol. Metab. 41, 581-589
22. Scatchard, G. (1949) Ann. N. Y. Acad. Sci. 51, 660-672
23. Bhalla, V. K., Haskell, J., Grier, H., and Mahesh, V. B. (1976) J. Biol. Chem. 251, 4947-4957
24. Dufau, M. L., Charreau, E., Ryan, D., and Catt, K. J. (1974) Curr. Top. Mol. Endocrinol. 1, 47-77
25. Shiu, R. P. C., and Friesen, H. G. (1974) J. Biol. Chem. 249, 7905-7911
26. Ryan, R. J., Jiang, N. S., and Hanlon, S. (1970) Recent Prog. Horm. Res. 26, 105-130
27. Cuatrecasas, P. (1972) J. Biol. Chem. 247, 1980-1991
28. Mullin, B. R., Fishman, P., Lee, G., Aloj, S. M., Ledley, F., Winand, R. J., Kohn, L. D., and Brady, R. O. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 842-846.
29. Cheng, K. W. (1975) Biochem. J. 149, 123-132
30. Lee, C. Y., and Ryan, R. J. (1973) Biochemistry 12, 4609-4615
31. Rao, C. V. (1975) Mol. Cell. Endocrinol. 3, 255-271
32. Moore, W. V., and Wolfe, J. (1974) J. Biol. Chem. 249, 6255-6263
33. Dufau, M. L., and Catt, K. J. (1975) Nature New Biol. 242, 246-248
34. Haour, F., and Saxena, B. B. (1974) J. Biol. Chem. 249, 2195-2205
35. Bhalla, V. K., and Reichert, L. E., Jr. (1974) J. Biol. Chem. 249, 7996-8004
36. Giorgio, N. A., Johnson, C. R., and Blecher, M. (1974) J. Biol. Chem. 249, 428-437
37. Rodbell, M., Birnbaumer, L., Pohl, S. L., and Krans, H. M. J. (1971) J. Biol. Chem. 246, 1877-1882
Solubilization and some characteristics of the follitropin receptor from calf testis.
H Abou-Issa and L E Reichert, Jr

J. Biol. Chem. 1977, 252:4166-4174.

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