Purification of Follitropin Receptor from Bovine Calf Testes*

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Follitropin (FSH) receptors were solubilized from pure light membranes of bovine calf testis, using an optimum detergent to protein ratio of 0.01. The soluble FSH receptor fraction was gel filtered through Sepharose 6B to isolate an active fraction (6B-Fr-1) which behaved as a complex of FSH receptor and Gs protein. The 6B-Fr-1 was concentrated by ultrafiltration and further purified by sequential Sepharose 4B gel filtration, DEAE-cellulose chromatography (to separate the receptor from Gs protein), and wheat germ lectin affinity chromatography. The purified receptor had an FSH-binding capacity of \( \approx 3.47 \, \text{nmol/mg of protein} \) with a \( K_d \) of \( 1.9 \times 10^{-10} \, \text{M} \). Yield was 526 \( \mu \text{g/11.5 kg} \) tested. Radioiodinated, as well as unlabeled purified FSH receptor, migrated on sodium dodecyl sulfate-polyacrylamide gels as a single major band of \( M_w \approx 240,000 \). This band was not affected by \( 8 \, \text{M urea treatment prior to analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, but treatment with dithiothreitol induced the loss of the 240-kDa band, with appearance of an } M_w \approx 60,000 \, \text{band. The availability of highly purified, stable FSH receptor should allow direct studies on its structure-function relationships.}

There are only a few studies on the purification of follitropin receptor (11, 12). Although in earlier studies a significant amount of soluble receptor was recovered by using hormone-affinity chromatography, the isolated receptor from bovine testis was either inactive (12) or low in activity (11). The presence of low concentrations of follitropin receptors in crude membranes as well as poor stability and yield after solubilization by detergents, have been the main problems experienced in those studies. In this report, we described a new procedure that overcame these problems and allowed the preparation of substantial quantities of follitropin receptor in a highly purified and stable state.

Testis plasma membranes contain both high affinity and low capacity, as well as low affinity and high capacity FSH-binding sites (13, 30). The occupancy of high affinity FSH-binding sites leads to the activation of adenylate cyclase (1, 4). Bovine calf testis homogenate was, therefore, centrifuged to remove low affinity and high capacity FSH-binding sites into a \( 7000 \times g \) sediment fraction containing heavier plasma membranes. The \( 7000 \times g \) supernatant containing only high

MATERIALS AND METHODS AND RESULTS†

† Portions of this paper (including “Materials and Methods,” “Results,” and Figs. 1–13) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.

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DISCUSSION

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[1] Present address: Institute of Biophysics, Academy of Science, Beijing, China.
[2] The abbreviations used are: FSH, follitropin; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; Gpp(NH)p, guanosine 5'-imidodiphosphate; bLH, bovine lutropin; hCG, human chorionic gonadotropin; TSH, thyrotropin.
affinity and low capacity FSH-binding sites was further processed.

An important feature of follitropin receptor activity in the crude testicular tissue homogenate is its rapid decline due to proteolytic degradation and the presence of follitropin receptor-binding inhibitors (28, 29). These inhibitors, if not removed, co-solubilize and co-purify with the receptor and are responsible for decreasing the follitropin-binding activity of the detergent-solubilized receptor. An initial concentration of the 7000 x g supernatant by Amicon DC-10 ultrafiltration was carried out in the presence of a protease inhibitor, sodium p-hydroxymercuribenzoate. This rendered FSH receptor activity in the resulting concentrate more stable and also removed significant amounts of inactive proteins as well as FSH receptor-binding inhibitors.

A major limitation in using crude testicular plasma membranes for detergent solubilization has been the lower recovery of solubilized FSH receptors (31, 32), requiring purification of membrane-containing fractions prior to detergent extraction. Sucrose density gradient centrifugation offers the most straightforward and efficient method to purify crude plasma membranes. Use of a Ti-15 rotor of 1.6-liter capacity in the present study facilitated a preparative fractionation of concentrated 7000 x g supernatant by sucrose density gradient centrifugation. Significant amounts of lipid and inactive proteins (85%) were separated from FSH receptor-containing fractions by this procedure. Moreover, a side fraction (SDG Fraction III) isolated during centrifugation appeared to be a potential starting fraction for isolation of LH/hCG receptor from testis.

Perhaps the greatest obstacle to the isolation of follitropin receptors is the identification of procedures which disrupt the membrane lipid matrix and extract receptors without causing denaturation. A series of systematic studies indicated that an optimum ratio of protein to Triton X-100 is of critical importance for the extraction of stable, active FSH binding activity (13). The optimum ratio of protein to Triton X-100 was found to be 20 mg of protein to 1 ml of 0.2% Triton X-100. An interesting feature of bovine testis FSH receptor solubilized by Triton X-100 under these conditions was its slight, but reproducibly higher, FSH-binding activity (1.4-fold) than that of membrane-bound receptor. Increased hormone-binding activity following membrane solubilization has also been reported for prolactin and thyrotropin receptors (33, 34).

The exposure of membranes to organic solvents under conditions which extract lipids or perturb the internal arrangements of lipids can facilitate the extraction of membrane proteins (35, 36). Petroleum ether was previously shown to be effective to facilitate the solubilization of LH/hCG receptors from bovine (22) as well as rat (37) ovarian membranes. In the present study, a simultaneous treatment of the Triton X-100-solubilized fraction from DC-2 concentrate with 0.2 volume (v/v) of petroleum ether removed free lipids in the organic phase and resulted in a 40% increase in the FSH-binding activity compared to untreated membranes. The detergent-soluble fraction from DC-2 concentrate after centrifugation at 145,000 x g retained full specific FSH-binding activity, while hCG-binding activity was only 47% of that originally present. Approximately 88% of the follitropin receptors originally present in the light membranes were extracted by detergent under the above optimum conditions, and this is a considerable improvement over the 20–40% recovery obtained in the previous reports (31, 32). Importantly, the soluble follitropin receptors in the detergent extract were stable and functional as reflected by FSH stimulation of adenylate cyclase (13) suggesting that the fraction could be an ideal precursor for further purification of receptor.

Since follitropin receptor after chromatography on Sepharose 6B was a complex of receptor and G-protein (3), we examined the conditions under which the complex could be dissociated. Detergents at sufficiently elevated concentrations are known to uncouple the functional units of adenylate cyclase (38, 39). We, therefore, exposed 6B-Fr-1 to a higher concentration of Triton X-100, while maintaining the optimum ratio between sample protein and Triton previously shown to preserve FSH receptor activity (13). This was achieved through a 2.5-fold concentration of 6B-Fr-1 by ultracentrifugation using an Amicon TCF-2 unit fitted with a Diaphragm YM-30 membrane (exclusion limit M<sub>c</sub> < 30,000), without loss of receptor activity. Excessive concentration of active material should be avoided as this leads to sample turbidity with loss of FSH-binding activity.

Through an application of ion exchange chromatography on DEAE-cellulose, an excellent separation between FSH receptor and Gpp(NH)p binding activities was achieved. One of the common features of the Gpp(NH)p-binding protein, LH/hCG receptors, is that they are effectively adsorbed by anion exchange resins (22, 40, 41). Clearly, the Gpp(NH)p binding activity and low (but significant) LH/hCG receptors present in 4B-Fr-2 as contaminants were adsorbed by DEAE-cellulose and eluted, which is consistent with the findings from previous reports (22, 40, 41). Most FSH-binding activity of 4B-Fr-2 was unadsorbed by DEAE-cellulose as described under "Materials and Methods," resulting in a significant purification of 4B-Fr-2 fraction.

Like most cell surface proteins, membrane receptors generally contain covalently bound carbohydrate chains (42-45). Most FSH receptor activity present in the DEAE unadsorbed fraction was adsorbed by wheat germ lectin and eluted by the specific sugar, N-acetylgalactosamine, indicating a glycoprotein nature of FSH receptor. A common feature of receptors for FSH, LH/hCG (44, 45), and TSH (43) appears to be their glycoprotein nature. Another advantage of lectin affinity chromatography was the separation of trace amounts of Gpp(NH)p binding activity and inactive proteins of nonglycoprotein nature from FSH receptor. The final receptor preparation was free of "125I-hFSH and Gpp(NH)p binding activities, and was stable when stored in buffer containing 0.02% Triton X-100 and 30% glycerol at 1-4 °C for 1 week or several months at -80 °C.

Due to limited amounts of starting material, and decreased or loss of activity of soluble FSH receptor reported in earlier studies (11, 12), the yields in those studies cannot be compared with those from the current study. From the 24,750 pmol of receptor activity present in 25 liters of 7,000 x g supernatant, 1,823 pmol of receptor activity was recovered in 526 pg of protein of receptor, representing a 7.4% yield from 7,000 x g supernatant and 65% yield from detergent-soluble petroleum ether-treated fractions (Table I). Several lines of evidence established the purity of the final receptor preparation. Upon SDS-polyacrylamide gel electrophoresis, the preparation gave a single band in the region of M<sub>c</sub> ~ 240,000 under nonreduced and M<sub>c</sub> ~ 60,000 under reduced conditions. The receptor preparation could be effectively adsorbed to the ovine FSH Affi-Gel 10 column. An elution of bound follitropin receptor activity yielded an active preparation upon SDS-PAGE analysis and under reduced conditions gave a major band in the region of M<sub>c</sub> 60,000 (not shown). The affinity cross-linking of the purified receptor to 125I-hFSH produced a complex which, upon SDS-PAGE, revealed a band in the region of 300,000 under nonreducing conditions. The band, however, was abolished after incubation
of the receptor with \( ^{125} \)-labeled FSH in the presence of excess unlabeled FSH. A polyclonal antibody raised against the receptor preparation specifically inhibited the binding of \( ^{125} \)-labeled FSH to membrane-bound FSH receptors (46). Also the antibody behaved as an agonist causing increased conversion of androstenedione to estradiol in rat Sertoli cells (46). These findings provide immunochemical evidence for receptor specificity of the purified preparations. The fact that FSH preparations from different species effectively competed with radioiodinated hFSH for binding to purified receptor, while heterogenous hormones such as hLH, hCG, or bovine TSH preparations from different species effectively competed with radioiodinated hFSH for binding to purified receptor, while heterogenous hormones such as hLH, hCG, or bovine TSH failed to do so, indicated a high degree of hormone specificity, but lack of species specificity among the follitropin interactions with the purified receptor.

Our results on the structure of the purified FSH receptor, as well as those from previous studies on photoaffinity (7-10) and chemical cross-linking (7) of hormone to membrane-bound receptor, revealed an oligomeric nature of the follitropin receptor. The photoaffinity cross-linking studies by Smith et al. (8, 9) have suggested that FSH receptor of bovine calf testis consists of at least three subunits of Mr 32,000, 48,000, and 66,000, respectively. In contrast, the FSH receptor of porcine granulosa cells was shown to contain three subunits of Mr 32,000, 48,000, and 66,000, respectively. Moreover, the chemical cross-linking studies have shown that cation-FSH receptor contains subunits of Mr 45,000 or 49,000 (7). It may be emphasized that endogenous proteolysis could produce receptor-derived proteolytic fragments and increased multiplicity that could lead to erroneous interpretation of receptor structure. Moreover, varying amounts of detergent binding, hormone binding, and covalent coupling of the hormone to the receptor could significantly affect the conformation and thereby the Stokes radii of the molecules (38). In the present study, the purified FSH receptor behaved as a single entity of Mr \( \sim 240,000 \) under nonreducing conditions, and was not affected by treatment with urea (8 M), suggesting that the receptor may not contain noncovalently associated subunits. Treatment with the reducing agent di-thiothreitol induced loss of the 240,000 band, with the appearance of a band with Mr \( \sim 60,000 \). Since the 60-kDa band did not dissociate further in the presence of higher concentrations of di-thiothreitol (200 mM) and did not further resolve upon SDS-PAGE in a higher percent acrylamide gel, the possibility that this band (~60 kDa) is a receptor subunit seems plausible. Moreover, when the receptor-hormone complex was chemically cross-linked and analyzed by SDS-PAGE under reduced conditions, it showed a radioactive band of Mr \( \sim 88,000 \). The fact that the 60,000 component was specifically labeled by \( ^{125} \)-labeled FSH (50). A polyclonal antireceptor antibody raised in rabbits immunoprecipitated the two forms of FSH receptor under nonreduced and reduced conditions (51). Furthermore, both 240- and 60-kDa bands gave identical patterns of peptides after partial proteolytic digestion.

The oligomeric nature of the receptors for glycoprotein hormones such as LH/hCG and TSH is noteworthy (22, 43, 47, 48) and has raised conflicting opinions. It has been suggested that the formation of oligomeric form during purification seems to be unique to LH/hCG receptors and is probably caused by the intermolecular oxidation of the free-SH groups present in the receptor to form disulfide bonds (49). Other studies, however, have suggested that the native LH/hCG receptor is a homodimer and its functional form may, in fact, be a homodimer of the 90-kDa species (44). As regards to the evidence on follitropin receptor structure, it is important to emphasize that further investigations are needed to determine the functional form of the follitropin receptor in the Sertoli cell plasma membrane. Finally, the availability of follitropin receptor in a highly purified and stable state should facilitate studies on its molecular biology and structure-function relationships.

**REFERENCES**

FSH Receptor of Bovine Calf Testis

**FSH Receptor of Bovine Calf Testis**

**Analytical Methods**

**Protein purification** - The protein content of the testicular homogenate, sperm membrane preparations or column elution fractions was determined by the method of Lowry et al. (1951). Bovine serum albumin was used as a standard in both assays.

**Homogenization**: Highly purified bovine FSH (LH) 174/1, 200 IU/mg and HCG (CR 119) were radiolabeled using the lactoperoxidase method (16) with some modifications (17). We used either gel filtration through a column of Sephadex G-100 or anion-exchange chromatography on a DEAE-Toyopearl column. Fracton X-100 was eluted with 0.5 M NaCl, pH 7.0, containing 0.005% NaN3 to yield 5.0 to 5.5 mg of FSH receptors from 100 mg of FSH or HCG in 24-32 ml of buffer per column.

**Radioimmunoassay**: Bovine dispersed receptor preparations and column effluent fractions were assayed for FSH in LH- and FSH-binding activities, as described earlier (13).

**Binding of [125I]FSH to the Bovine Calf Testis FSH receptor**

**Characterization of the FSH Receptor**

**Cross-linking of receptor-32P-FSH complex**: A mixture of [32P]-FSH (10-10 M) and receptor preparation was incubated in 20 ml HEPES buffer, pH 7.4, containing 1 mM MgCl2 and 0.68% FSH in the absence or presence of excess nonlabeled FSH (10-5 M) overnight at 4°C. After the reaction, half of the incubation solution was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and the gel was sliced into small pieces, dissolved in 9 M urea and subjected to autoradiography. The remaining amount was subjected to radioactive analysis by scintillation counting.

**SDS-PAGE** - Polyacrylamide gel electrophoresis (Sanger et al. 1961) was performed as described by Davis (24). The gel was cut into small pieces, dissolved in 9 M urea and subjected to autoradiography. The remaining amount was subjected to radioactive analysis by scintillation counting.
FSH Receptor of Bovine Calf Testis

Molecular assembly and oligomerization of the follistatin receptor: purification and characterization. A4-5 was coimmunoprecipitated with a full-length recombinant protein containing the extracellular domain of the FSH receptor from bovine testis. The purified protein was used in immunoprecipitation and immunoblotting experiments. The protein was purified using a monoclonal antibody against the extracellular domain of the FSH receptor.

METHODS

Preparative centrifugation in isosmotic sucrose density gradient. Concentrated fractions were collected and dialyzed against PBS. The final protein concentration was determined using a bicinchoninic acid assay. The protein was stored at -80°C.

Results

The data showed that the purified FSH receptor was able to bind to immobilized FSH and was able to stimulate cAMP production in transfected cells.

Discussion

The purified FSH receptor was able to bind to immobilized FSH and was able to stimulate cAMP production in transfected cells. These results suggest that the purified FSH receptor is a functional receptor that can bind to FSH and stimulate cAMP production.

Fig. 1: Preparative centrifugation in isosmotic sucrose density gradient of concentrated 7,000 g supernatant. Approximately 12.5 g of protein was loaded on a linear sucrose density gradient.

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Concentration of Sepharose 4B-Fr-2 by Amicon 200-ml column. The column was run using an Amicon 200-ml column. The flow rate was 10 ml/min.

Fig. 2: A flowchart of a typical procedure developed for the purification of follistatin receptor from the detergent-soluble fraction of calf testis.

Concentration of Sepharose 4B-Fr-2 by Amicon 200-ml column. The column was run using an Amicon 200-ml column. The flow rate was 10 ml/min.

Concentration of Sepharose 4B-Fr-2 by Amicon 200-ml column. The column was run using an Amicon 200-ml column. The flow rate was 10 ml/min.

Concentration of Sepharose 4B-Fr-2 by Amicon 200-ml column. The column was run using an Amicon 200-ml column. The flow rate was 10 ml/min.
DEAE-cellulose chromatography: Approximately 12.7 mg protein aliquots of Sepharose 4B-Fr-2 was further purified each time by ion exchange chromatography. As shown in figure 4, most CBO-binding, CBO(NH)P-binding activities, and residual 5'-nucleotidase enzyme activity present in 4B-Fr-2 were adsorbed by DEAE-cellulose and could be eluted by 0.5 M ammonium acetate, pH 7.5 (DE-Fr-3) and 0.2 M ammonium acetate, pH 6.4 (DE-Fr-4). The FSH-binding activity of 4B-Fr-2, however, was unadsorbed by DEAE-cellulose and could be recovered in breakthrough (DE-Fr-1) and buffer wash (DE-Fr-2) fractions. The average recovery of protein in these fractions was 29% of total protein. Fractions 180-Fr-1 and DE-Fr-2 contained high affinity follitropin receptors (Table 1) with specific activity 36-fold higher than in 4B-Fr-1. The CBO(NH)P-binding activity in DE-Fr-2 and DE-Fr-4 was extremely low, and no significant differences between the two activities was achieved by DEAE-cellulose chromatography (Fig. 4).

Lectin affinity chromatography: Fractions DE-Fr-1 and DE-Fr-2 were pooled and further purified by affinity chromatography using agave-linked wheat germ agglutinin (WGA), a lectin that specifically binds to N-acetyl glucosamine and sialic acid residues. As shown in figure 5, greater than 80% of the total FSH receptor activity present in fractions (DE-Fr-1 and DE-Fr-2) was adsorbed by immobilized lectin, and could be eluted with 0.2 M N-acetyl glucosamine (WGA-Fr-1) and 2.4 M N-acetyl glucosamine (WGA-Fr-4). Total amounts of CBO(NH)P-binding activity present in the applied sample were unadsorbed by the lectin. The resulting fractions (WGA-Fr-1) and WGA-Fr-4 contained purified high affinity follitropin receptors (Table 1). With an average FSH-binding capacity of 3-4.7 (1-4.3) nM/mg protein, which corresponds to 0.31 nM FSH bound per mg of receptor (249 kDa).

Yield and activity of the follitropin receptors: Table 1 shows the comparative data on recovery of FSH receptor activity and increase in specific FSH-binding activity of the fractions isolated from several batches (11.5 kg) of FSH receptor. A 49% recovery of protein (peak 3,000 x g supernatant) represents an yield of only the high affinity and low capacity binding sites present in this fraction. Therefore, in Table 1, the recovery of total receptor activity and yield of different stages of purification are expressed as percent of total receptor activity initially present in 1,000 g x g supernatant. Approximately 5% of high affinity FSH receptor protein was recovered after lectin affinity chromatography, from 11.5 kg of bovine calf testes. This results in a 19.75% yield of 1,000 x g supernatant. The purified receptors, when stored in 20 mM Hepes buffer, pH 7.5, containing 10 mM MgCl₂, 0.1% NaN₃, 0.2% Triton X-100 and 30% glycerol, was stable for several months at -80°C. However, for a week at 4°C.
Characterization of the Folliculin Receptor

Non-cytoplasmic gel electrophoresis. The purified fraction obtained after ferritin affinity chromatography, when fractionated by electrophoresis on a linear gradient of sucrose, showed a single band with $K_a = 7.400$ and a comparison of $25-30$ bands stained by the lighter membrane fraction or fraction, indicating a high degree of purity and electrophoretic homogeneity of the purified receptor (Fig. 6). After treatment with 70% DMSO (doxorubicin) and 21.05% for 30 min at 100°C, the purified receptor showed a single minor stained band with $K_a = 3.600$ (Fig. 6).

Non-denaturing electrophoretic analysis of the $^{125}$I-labeled-binding data revealed the presence of only a single class of high affinity $^{125}$I-labeled-binding sites and all active fractions obtained during the various stages of purification (Table 1). The dissociation constant ($K_d$) of the receptor-growth in deproteinized testicular fraction, supernatant 60 (fraction 1), and HCG fraction 30 were $0.64 \times 10^{-12}$, $1.8 \times 10^{-12}$, and $3.9 \times 10^{-10}$, respectively, suggesting that there was only a small change in the affinity of the receptor during purification. The high affinity, low capacity binding sites in the highly purified folliculin receptor ranged from $3.7 \times 10^{-13}$ to $5.6 \text{ mol} / \text{mg}$ of protein.

Specificity of $^{125}$I-labeled-binding to the purified receptor preparations. A major exception by which receptors are characterized is their specificity for binding known ligands. As shown in figure 4, unlabeled hCG, hHMG, and hCG effectively inhibited binding of $^{125}$I-labeled hCG purified receptor in a concentration-related manner. UHCG, hHMG and hCG did not inhibit $^{125}$I-labeled-binding at concentrations up to 50 ng (ML, BPH, or 25 ng (HCG)). These results substantiate a high degree of hormone specificity as well as absence of species specificity in FSH interaction with the purified receptor.

FIG. 7. Scatchard plot for the binding of $^{125}$I-hCG to purified FSH receptor. Purified receptor was incubated with various amounts of $^{125}$I-hCG and increasing amounts of unlabeled FSH as described under MATERIALS. The data were expressed in the form of a Scatchard plot.

FIG. 8. Specificity of $^{125}$I-labeled-binding to the purified receptor preparation. Aliquots (10 ng) of protein from purified receptor after ferritin affinity chromatography, were incubated with $^{125}$I-hCG (30 ng) for 18 h at $4^\circ$, together with indicated concentrations of unlabeled hCG (C), hFSH (A), hHCG (B), hML (c), hBPH (d), or 500 ng of hCG under standard assay conditions described under MATERIALS. Results (B/F) are expressed as percent of specific $^{125}$I-hCG binding as obtained in the absence of unlabeled hCG or unlabeled other hormones. Each data point represents the mean of triplicate measurements.
The oligomeric nature of the FSH receptor-lectin affinity purified FSH receptor preparation was radiiodinated to specific activities of 130-62 Kcpn. Following treatment of the receptor with 350 K for 90 s at 100°C, and analysis by SDS-PAGE and autoradiography, a major band with an apparent M of 240,000 was observed (Fig. 9A). Occasionally, an additional faint band corresponding to M of 170,000 was also noticed (Fig. 9B). This component, however, was unobserved by the DTT-affinity column, whereas the receptor was eluted and could be eluted by 50 mM sodium acetate buffer, pH 5.5, containing 1 M NaCl (not shown). Treatment of radiiodinated receptor with 4 or 8 M urea for 4 h at 37°C, prior to SDS-PAGE, did not alter the molecular weight (Mr ~ 240,000) of the receptor (Fig. 10) suggesting that the receptor does not contain non-covalently associated dimers. However, the treatment with 250 K for 90 s at 100°C followed by reducing final SDS concentration 0.1% by dialysis and then treatment with DTT (1 to 70 m) for 45 s at 100°C induced a band of M ~ 240,000 and with appearance of an M ~ 60,000 band (Fig. 9C). Exposure of the receptor preparation to 200 K of DTT did not further dissociate the M ~ 60,000 band. Moreover, the band with M of 60,000 identified in 7.5% acrylamide gel under reduced conditions, gave a single band following electrophoresis in the second dimension in 15% polyacrylamide gels (Fig. 11). 4%V, the purified receptor was incubated with labeled FSH and the hormone-receptor complexes were extensively cross-linked with 50% acrylamide under non-reducing conditions followed by autoradiography revealed a single prominent band in the region of M ~ 300,000 (Fig. 12). To further understand the relationship between 240,000 and 60,000 bands of the receptor, we subjected both radioactive bands to peptide mapping by limited proteolysis by toluene-treated mouse if-protector. SDs (SDS)-PAGE analysis (Fig. 13) revealed that the pattern of the polypeptide released after partial proteolytic cleavage, were identical for both 240 K and 60 K bands.

Fig. 9: Non-polyacrylamide gel electrophoresis analysis of purified [125I]-labeled receptor, under non-reduced and reduced conditions. Radiolabeled receptor preparation was taken in 0.0625 M Tris-HCl buffer, pH 7.4, containing 1 M SDS and heated in boiling water for 90 s. The sample, after dialysis against 0.0625 M Tris-HCl buffer, pH 7.4, containing 2 M SDS was either directly electrophoresed in 6% acrylamide separating gel (Panel A) or incubated for 45 s in the presence of 0, 5, 10, 15, 20 or 250 mM DTT and then electrophoresed in 7.5% acrylamide stacking gel (Panel B). Gels were dried before autoradiography. The positions of M, standards are indicated by arrows in the figure.
FSH Receptor of Bovine Calf Testis

Fig. 12: Covalent cross-linking of 125I-labeled FSH to purified FSH receptor. Unlabeled receptor after SDS-polyacrylamide gel electrophoresis, was incubated with 125I-labeled FSH in the absence (lane 1) or presence of excess unlabeled FSH (lane 2). The hormone-receptor complexes were separated free from unbound 125I-labeled by gel filtration, and cross-linked by the bifunctional reagent diisocyanatoguaiacol as described in MATERIALS. The covalently cross-linked hormone-receptor complex was analyzed by SDS-PAGE under non-reduced conditions in an acrylamide separation gel containing 3.1% SDS, followed by autoradiography. The positions of molecular weight standards are indicated by arrows in the figure.

Fig. 13: Peptide maps of purified receptor and subunit after digestion with Streptococcus aureus V8 protease. Electrophoresis of 125I-receptor was first performed (see Fig. 9, Panel B). The gel slices of interest (240 kDa and 60 kDa bands) were sliced, inserted in the sample wells (lane 1 and lane 2, respectively) of a second SDS gel (15% acrylamide) and overlaid with gel with Streptococcus aureus protease (final concentration of 80 μg/ml). Electrophoresis was performed in the usual manner with the exception that the current was turned off for 30 min when the bromophenol blue dye had reached the bottom of the stacking gel. Digestion proceeded directly in the stacking gel. An autoradiogram of a dried gel is shown.
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