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Properties of Follicle-Stimulating-Hormone Receptor in Cell Membranes of Bovine Testis

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A simple method for preparing plasma membranes from bovine testes is described. Bovine testicular receptor has a high affinity and specificity for ^{125}I-labelled human FSH (follicle-stimulating hormone). The specific binding of ^{125}I-labelled human FSH to the plasma membranes is a saturable process with respect to the amounts of receptor protein and FSH added. The association and dissociation of ^{125}I-labelled human FSH are time- and temperature-dependent, and the binding of labelled human FSH to bovine testicular receptor is strong and not readily reversible. Scatchard [*Ann. N.Y. Acad. Sci.* (1949) **51**, 660–672] analysis indicates a dissociation constant, K_d, of 9.8×10^{-11} M, and 5.9×10^{-14} mol of binding sites/mg of membrane protein. The testicular membrane receptor is heat-labile. Preheating at 40°C for 15 min destroyed 30% of the binding activity. Specific binding is pH-dependent, with an optimum between pH 7.0 and 7.5. Brief exposure to extremes of pH caused irreversible damage to the receptors. The ionic strength of the incubation medium markedly affects the association of ^{125}I-labelled human FSH with its testicular receptor. Various cations at concentrations of 0.1 M inhibit almost completely the binding of ^{125}I-labelled human FSH. Nucleotides and steroid hormones at concentrations of 1 mM and 5 μg/ml respectively have no effect on the binding of FSH to its receptor. Incubation of membranes with trypsin and chymotrypsin resulted in an almost complete loss of binding activity, suggesting that protein moieties are essential for the binding of ^{125}I-labelled human FSH. Binding of ^{125}I-labelled human FSH to bovine testicular receptor does not result in destruction or degradation of the hormone.

Numerous studies have been reported on the specific uptake of radioiodinated human chorionic gonadotrophin and human luteinizing hormone by bovine corpora lutea (Gospodarowicz, 1973*a*,*b*; Haour & Saxena, 1974; Rao, 1974), rat testes (DeKretser *et al.*, 1969, 1971; Catt *et al.*, 1971; Leidenberger & Reichert, 1972*a*,*b*), ovaries (Danzo *et al.*, 1972; Kammerman & Canfield, 1972; Lee & Ryan, 1970, 1973; Lee *et al.*, 1973; Rajaniemi & Vanha-Perttula, 1972; Rao & Saxena, 1973; Tsuruhara *et al.*, 1972), isolated granulosa cells (Channing & Kammerman, 1973) and adrenocortical carcinoma (Schorr *et al.*, 1971). The binding of gonadotrophins to cell membranes of the intact ovary has been correlated with the activation of membrane-bound adenylate cyclase and steroidogenesis (Marsh & Savard, 1966; Marsh, 1970; Rao & Saxena, 1973). In a few studies, specific receptors for FSH* have been demonstrated in rat testes (Bhalla & Reichert, 1974; Means & Vaitukaitis, 1972; Reichert & Bhalla, 1974) and ovaries (Vaitukaitis *et al.*, 1971). Further, FSH has been reported to stimulate biosynthesis of proteins and nucleic acid in rat testes (Steinberger, 1971). However, at present, relatively little information is available on the biochemical properties of the membrane-bound receptor for FSH. I describe some of the properties of the FSH-specific receptor or more specifically a binding component with high affinity for FSH in the plasma membranes of bovine testes.

* Abbreviation: FSH, follicle-stimulating hormone.

Materials and Methods

Materials

Purified human follicle-stimulating hormone (LER-1575C), human luteinizing hormone (LER-960), human chorionic gonadotrophin (CR-115), reference standard of sheep follicle-stimulating hormone (FSH-SI) and rabbit anti-(human FSH) serum (batch no. 3) were obtained from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md., U.S.A. Bovine serum albumin (fraction V) was

obtained from Miles Laboratories, Kankakee, Ill., U.S.A. Lactoperoxidase was from Calbiochem, LaJolla, Calif., U.S.A. Trypsin (bovine pancreas treated with L-tosylamido-2-phenylethyl chloromethyl ketone), α-chymotrypsin (bovine pancreas, 45units/mg) and collagenase (*Clostridium histolyticum*, type III, 125units/mg) were purchased from Worthington Biochemical Corp., Freehold, N.J., U.S.A. Neuraminidase (*Vibrio cholerae*) was obtained from Schwarz/Mann, New York, N.Y., U.S.A. Pepsin (hog stomach mucosa, 2500–3200units/mg), ribonuclease A (bovine pancreas, type III-A), deoxyribonuclease I (bovine pancreas), progesterone, testosterone, 17β-oestradiol and dibutyryl cyclic AMP, cyclic AMP, ATP, CTP and GTP were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A. $Na^{125}I$ (carrier-free) was purchased from New England Nuclear Corp., Boston, Mass., U.S.A. H_2O_2 (30%, v/v) was obtained from Fisher Scientific, Fair Lawn, N.J., U.S.A.

Preparation of plasma membranes from bovine testis

Fresh bovine testes were collected in ice from a local slaughterhouse from calves. Each testis weighed approx. 220–280g. Details of the procedure for preparation of plasma membranes from bovine testes have been published elsewhere (Cheng, 1975). The testis was rinsed with cold 0.025M-Tris–HCl buffer, pH7.2, containing 0.3M-sucrose, and minced and homogenized with a Polytron homogenizer (Brinkmann) type PT-10 at maximum speed for 30s at a concentration of 5ml of buffer/g of tissue. The homogenate was filtered through four layers of cheesecloth, and the filtrate was again filtered through eight layers of cheesecloth. The filtrate was then centrifuged at 12000*g* for 30min at 4°C, and the supernatant was centrifuged further at 100000*g* for 1h at 4°C. The pellet was resuspended in cold 0.025M-Tris–HCl buffer, pH7.2, containing 10mM-$MgCl_2$, at a concentration of 1ml of buffer/g of tissue (original weight of the testis). The purified plasma membranes were stored at −70°C in batches of 10ml/vial until used. Before use, the thawed membranes were homogenized by hand with a glass homogenizer and diluted with the Tris–$MgCl_2$ buffer to 5mg of protein/ml for use. The protein content was determined by the method of Lowry *et al.* (1951) after solubilization of a 0.1ml portion of the membrane fraction with 0.3ml of 1.0M-NaOH by heating in boiling water for 30min. Bovine serum albumin without boiling was used as protein standard.

Radioiodination of hormone

Human FSH (LER-1575C) was a gift from the National Institute of Arthritis, Metabolic, and Digestive Diseases, National Institutes of Health, Bethesda, Md., U.S.A., and was iodinated by the lactoperoxidase method (Miyachi *et al.*, 1972) with slight modifications (Cheng, 1975). The specific radioactivity of ^{125}I-labelled human FSH ranged from 16 to 20μCi per μg. Details of the procedure of iodination and assessments of the specific binding of radioiodinated human FSH to bovine testicular plasma membranes have been reported (Cheng, 1975).

Binding of labelled hormone to bovine testicular plasma membranes

Studies on specific binding of ^{125}I-labelled human FSH to the testicular plasma membranes were carried out in culture tubes (12mm×75mm; Kimble Glass Co., Owens, Ill., U.S.A.). To each tube, 0.2ml of 0.025M-Tris–HCl buffer (pH7.2), containing 10mM-$MgCl_2$ and 0.1% bovine serum albumin, 0.1ml of buffer or unlabelled hormone in the same buffer, 0.1ml of ^{125}I-labelled human FSH tracer (50000 c.p.m., approx. 2ng) and finally 0.1ml of testicular plasma membranes at protein concentrations of 5mg/ml were added to make a final volume of 500μl/tube. All the above solutions were kept at 4°C for use. The tubes were then shaken vigorously and incubated at 25°C for 20h. After incubation the reaction was stopped by adding 3.0ml of cold buffer. After centrifugation at 4000rev./min (IEC PR 6000 centrifuge) for 30min, the supernatant was drained and the pellet remaining in the tube was counted for radioactivity in an automatic gamma spectrometer. Specific binding (%) is defined as $(C_B-C_N)\times 100/C_T$, where C_B is the radioactivity (c.p.m.) bound to the plasma membranes (pellet), C_N is the non-specifically bound radioactivity which cannot be displaced by 1000-fold molar excess of unlabelled hormone, and C_T is the total radioactivity put into the tube. In every assay, calculations were based on specific uptake of ^{125}I-labelled human FSH by the testicular receptor by subtracting non-specifically bound radioactivity.

Results

Distribution of ^{125}I-labelled human FSH-binding activity in subcellular fractions of bovine testes

With the procedure described in the Materials and Methods section, 1g of bovine testis tissue yielded approx. 5–8mg of protein/ml. The specific binding of ^{125}I-labelled human FSH to subcellular fractions at different steps of isolation by centrifugation of bovine testicular homogenate is shown in Table 1. The highest specific binding for ^{125}I-labelled human FSH was observed in the plasma-membranes fraction (100000*g* pellet), which accounted for approx. 60% of the total binding activity of the testicular homogenate. The 12000*g* pellet (nuclear and mitochondrial fractions) also contained slight binding activity, which is probably due to contamination by appreciable amounts of larger fragments of plasma membranes. For comparison, the specific binding of ^{125}I-labelled human luteinizing hormone was also studied, and in

Table 1. *Distribution of ^{125}I-labelled human FSH- and ^{125}I-labelled human luteinizing hormone-binding activities in subcellular fractions in the purification of receptor from homogenates of bovine testis*

Specific binding was determined with 500 μg of protein from each fraction. For full details see the text.

Subcellular fraction	Yield of fraction (mg of protein/g of tissue)	Specific binding of ^{125}I-labelled human FSH (%)	Specific binding of ^{125}I-labelled human luteinizing hormone (%)
Homogenate (after cheesecloth filtration)	74.0	4.5	1.9
12000*g* pellet	29.1	0.9	0.4
12000*g* supernatant	35.5	1.9	0.8
100000*g* pellet (purified receptor)	8.4	13.6	4.3
100000*g* supernatant	28.9	0.3	0.2

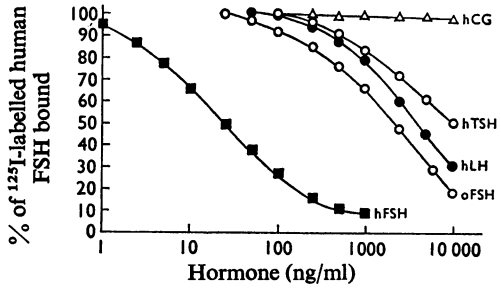

Fig. 1. *Inhibition curves for the specific binding of ^{125}I-labelled human FSH to plasma membranes from bovine testis*

Results are expressed as specific binding (%) of ^{125}I-labelled human FSH. Human FSH (hFSH; LER-1575C), sheep FSH (oFSH; FSH-S1), human luteinizing hormone (hLH; LER-960), human thyroid-stimulating hormone (hTSH; HS-2) and human chorionic gonadotrophin (hCG; CR-115) were obtained from National Institute of Arthritis Metabolism, and Digestive Diseases, National Institutes of Health. In this assay system (Cheng, 1975), 200 μg of protein per tube was used, and the incubation was carried out at 25°C for 20h.

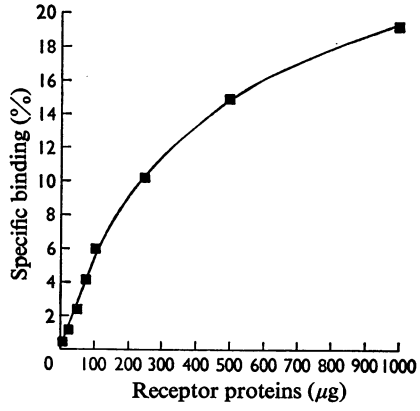

Fig. 2. *Specific binding of ^{125}I-labelled human FSH to bovine testicular receptor as a function of protein concentration*

A constant amount of ^{125}I-labelled human FSH (50000 c.p.m.) was incubated with the indicated amounts of receptor protein at 25°C for 20h. Data represent results of three experiments.

bovine testes the binding activity for luteinizing hormone was only 30% of that for FSH (Table 1).

Specific binding of FSH to bovine testicular plasma membranes

Unlabelled human FSH (LER-1575C) inhibited the binding of ^{125}I-labelled human FSH to the membranes in a dose-dependent manner (Fig. 1). Sheep FSH (FSH-S1 from NIH) also inhibited the binding of ^{125}I-labelled human FSH, but to a much lesser degree, and the slope of both dose–response curves is parallel. The difference in degree of inhibition reflects the degree of the purity of the sheep FSH reference standard preparation. Highly purified human luteinizing hormone and human thyroid-stimulating hormone inhibited the binding of ^{125}I-labelled human FSH to the testicular plasma membranes only at concentrations 200-fold higher than human FSH. However, highly purified human chorionic gonadotrophin, human growth hormone, sheep prolactin, pig insulin, human neurophysin and [arginine]vasopressin, up to 1000-fold greater concentrations than human FSH, showed no inhibition of the binding of ^{125}I-labelled human FSH to testicular plasma membranes (Fig. 1).

Effect of concentrations of hormone and membrane protein on the specific binding of ^{125}I-labelled human FSH

The binding of ^{125}I-labelled human FSH to testicular receptors increased with the amount of receptor protein present, as shown in Fig. 2. Generally 500–600 μg of membrane protein was used per tube for incubation, and 14–20% specific binding of ^{125}I-labelled human FSH was observed. The specific bind-

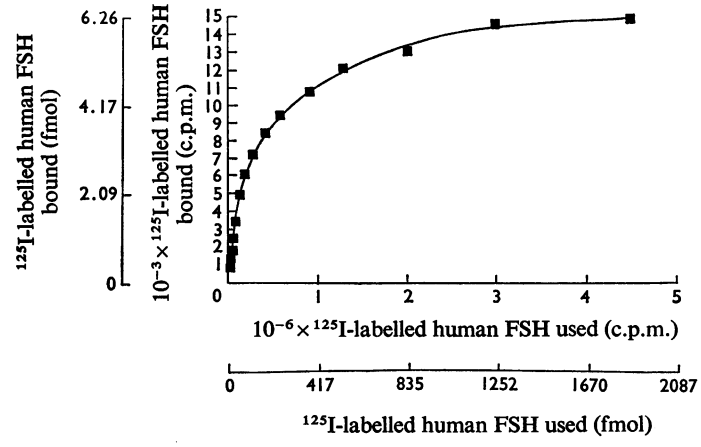

Fig. 3. *Specific binding of ^{125}I-labelled human FSH to bovine testicular receptor as a function of concentration of ^{125}I-labelled human FSH*

Constant amount of receptor protein (100μg/tube) was incubated with the indicated amount of ^{125}I-labelled human FSH at 25°C for 20h. The calculation of the amount of ^{125}I-labelled human FSH at 25°C for 20h. The calculation of the amount of ^{125}I-labelled human FSH (fmol) was based on the known specific radioactivity of the radioiodinated FSH (Cheng, 1975) and an assumed mol.wt. of 30000. Data represent results of three experiments.

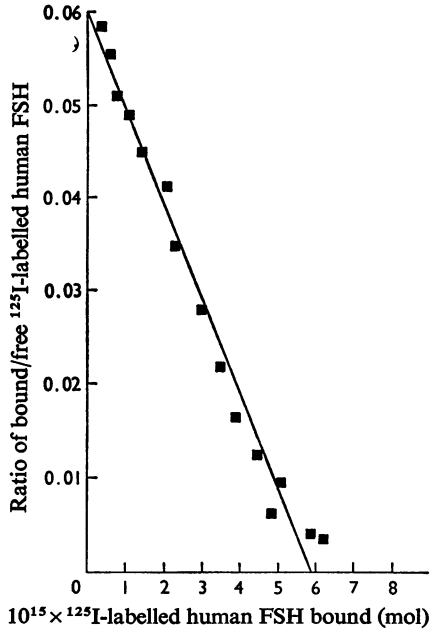

Fig. 4. *Scatchard plot of data in Fig. 3 for the specific binding of ^{125}I-labelled human FSH to receptor at different concentrations of ^{125}I-labelled human FSH*

Slope of the plot yields $-1/K_d$, and the intercept on the abscissa yields the total ^{125}I-labelled human FSH bound by the amount of receptor protein used (100μg). $K_d = 9.8 \times 10^{-11}$M, and binding sites = 5.9×10^{-14}mol/mg of protein.

ing of ^{125}I-labelled human FSH increased proportionally to the amount of plasma membranes present.

Fig. 3 shows that the specific binding of ^{125}I-labelled human FSH to receptors in testes is a saturable process; the specific binding of ^{125}I-labelled human FSH increased with the amounts of labelled hormone added. Fig. 4 depicts the Scatchard (1949) plot analysis of the data in Fig. 3, and the intercept on the abscissa gives 5.9fmol/100μg of protein (5.9×10^{-14}mol/mg) as the maximum amount of FSH bound, and the reciprocal of the slope yields the dissociation constant, K_d, which is 9.8×10^{-11}M.

Effects of time and temperature on the association of ^{125}I-labelled human FSH to bovine testicular receptor

The specific binding of ^{125}I-labelled human FSH to bovine testicular receptor is dependent on both the duration and temperature of incubation, as shown in Fig. 5. At 37°C, equilibrium was attained after about 4h, and a decrease in specific binding was observed when incubation was continued for longer than 6h. However, at 25°C (room temperature), specific binding increased continuously, and maximum binding was attained after 24h of incubation. The binding at 4°C increased very slowly, and equilibrium was not obtained after even 48h of incubation.

The dissociation of bound ^{125}I-labelled human FSH from bovine testicular receptor is also time- and temperature-dependent (Fig. 6). At 37°C and in the presence of excess of unlabelled hormone (1000-fold

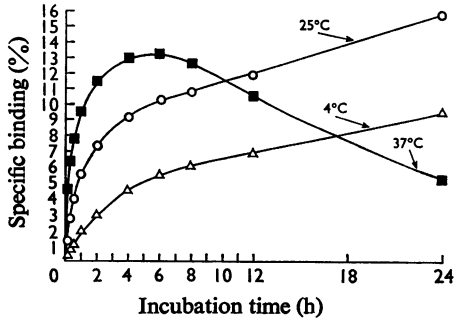

Fig. 5. *Effect of temperature and time of incubation on the specific binding of ^{125}I-labelled human FSH to bovine testicular receptor*

Constant amounts of ^{125}I-labelled human FSH (50000 c.p.m.) and receptor protein (500μg) were incubated for the indicated time-intervals at 4°C, 25°C or 37°C. Values are means of three experiments.

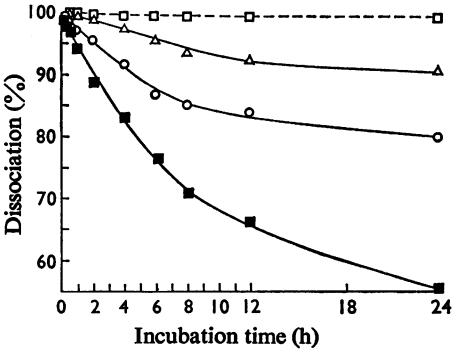

Fig. 6. *Effect of temperature and time of incubation on dissociation of ^{125}I-labelled human FSH from bovine testicular receptor*

Receptor protein (500μg) was incubated with ^{125}I-labelled human FSH (50000c.p.m.) for 12h at 25°C. After incubation, bound and unbound ^{125}I-labelled human FSH were separated by centrifugation, and the pellet was washed three times with several volumes of ice-cold 0.025M-Tris–HCl containing 0.1% bovine serum albumin. The washed pellet was resuspended in 0.5ml of incubation medium. To one set of the tubes, 1000-fold molar excess of unlabelled human FSH was added, and to another identical set only incubation buffer was added. The tubes of ^{125}I-labelled human FSH–receptor complex were then incubated at the indicated temperatures for various time-intervals. Broken line (□): incubated in absence of unlabelled hormone. Solid lines: incubated in the presence of unlabelled human FSH at 4°C (△), 25°C (○) and 37°C (■).

molar excess), the dissociation of ^{125}I-labelled human FSH from its receptor was a slow process. About 50% of the total bound ^{125}I-labelled human FSH was dissociated after 24h of incubation at this temperature. At 25°C, after 24h only 20% of the bound labelled hormone was dissociated, and at 4°C the dissociation was less than 10%. No significant dissociation of bound ^{125}I-labelled human FSH was observed when the receptor complex was incubated in a hormone-free medium after 24h at the above temperatures studied. It seems that the ^{125}I-labelled human FSH–receptor complex, once formed, is very stable.

Effect of pH on binding of ^{125}I-labelled human FSH to receptor

The specific binding of ^{125}I-labelled human FSH to bovine testicular receptor is dependent on the pH of the incubation medium, and occurs over a fairly

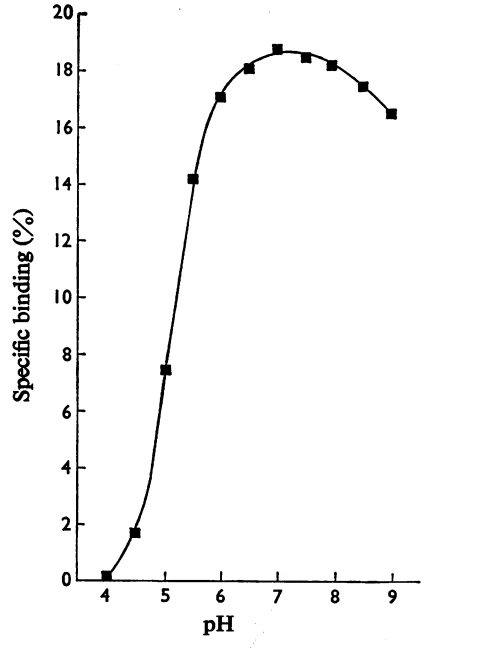

Fig. 7. *Effect of pH on the specific binding of ^{125}I-labelled human FSH to bovine testicular receptor*

Membrane fraction (600μg) was incubated in buffers of the indicated pH with ^{125}I-labelled human FSH (50000c.p.m.) at 25°C for 20h. In another set of tubes, receptor protein (600μg) was exposed to buffers of the indicated pH for 1h, and specific binding was tested after centrifugation and resuspension in 0.025M-Tris–HCl. Buffers were 0.1M-sodium acetate (pH4–5.5), 0.1M-sodium phosphate (pH6–6.5), 0.1M-Tris–HCl (pH7–8) and 0.1M-$NaHCO_3$ (pH8.5–9.0). The pH of the buffers was adjusted with the appropriate solution of 1M-acetic acid, 1M-phosphoric acid, 1M-HCl or 1M-NaOH. Values are means of two experiments each.

wide range of pH, as shown in Fig. 7. Maximal binding occurred at pH7–7.5. It is noteworthy that receptor retained over 85% of its binding activity even at pH9. However, at pH5 or below, little or no specific binding was observed. When the membrane-bound receptor was pretreated for 1 h at the pH range indicated (Fig. 7) no recovery of the lost specific binding was observed after resuspending the treated cellular particles in a medium of pH7.2. This indicates that the binding activity of bovine testicular receptor was destroyed irreversibly by a brief exposure to extremes of pH.

Effect of pretreatment of testicular receptor at various temperatures

The membrane-bound receptor is heat-labile. The specific binding of ^{125}I-labelled human FSH to testicular receptor was maximal at 25°C. After a brief exposure of 15min at 50°C, the receptor lost completely its specific binding of ^{125}I-labelled human FSH, as shown in Fig. 8. A gradual decrease in binding activity was observed at 40° and 45°C. Approx. 30% of the binding activity of the receptor was destroyed by pretreating the testicular receptor at 40°C for 15min. Non-specific binding increased slightly after a brief treatment at higher temperatures.

Effect of salts and EDTA on the binding of ^{125}I-labelled human FSH to testicular receptor

Fig. 9 shows the inhibitory effect of NaCl, $CaCl_2$ and $MgCl_2$ present in the incubation medium on the specific binding of ^{125}I-labelled human FSH to its testicular receptor. A slight inhibitory effect was observed even at concentrations below 0.005M, whereas concentrations above 0.01M almost completely inhibited the specific binding between ^{125}I-labelled human FSH and its receptor. Similarly, EDTA at concentrations higher than 0.01M had a significant inhibitory effect on the binding activity. It seems that these inhibitory effects are related to changes of the ionic environment of the receptor molecule in the plasma membrane.

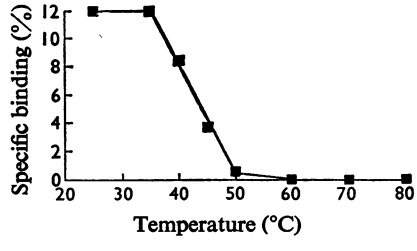

Fig. 8. *Effect of heating bovine testicular receptor on the specific binding of ^{125}I-labelled human FSH*

Receptor protein (500μg) was heated for 15min at the indicated temperatures and returned to ice temperature. Specific binding was tested by incubating with ^{125}I-labelled human FSH at 25°C for 20h. Values are means of two experiments.

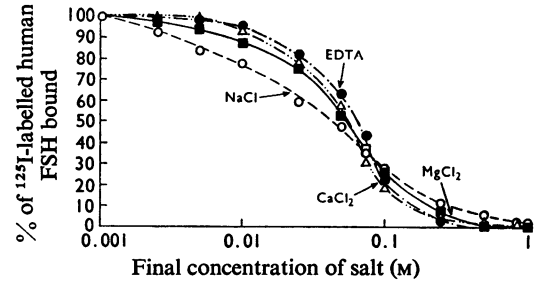

Fig. 9. *Effect of various salts and EDTA on specific binding of ^{125}I-labelled human FSH to bovine testicular receptor*

Membrane protein (450μg) was incubated with ^{125}I-labelled human FSH (50000c.p.m.) at 25°C for 20h at the indicated concentrations of various salts (NaCl, ○; $CaCl_2$, △; $MgCl_2$, ■) or EDTA (●).

Effect of enzyme treatments on membrane receptor-binding activity

The chemical nature of the receptor was investigated by treating the plasma membranes with different enzymes, followed by testing for specific binding to ^{125}I-labelled human FSH as shown in Table 2. Proteolytic enzymes, such as trypsin and α-chymotrypsin, at an enzyme/substrate ratio of 1:100(w/w), abolished over 80–90% of the binding activity of the membrane receptor for its specific hormone. Removal of sialic acid from plasma membranes with neuraminidase slightly increased (20%) the specific binding of ^{125}I-labelled human FSH. Incubation of plasma membranes with deoxyribonuclease and ribonuclease had no significant effect on the testicular receptor for binding ^{125}I-labelled human FSH. These results suggest that the bovine testicular FSH-specific receptor molecule is protein in nature.

Effect of nucleotides and steroid hormones on specific binding of ^{125}I-labelled human FSH

Various low-molecular-weight compounds that are frequently found in physiological fluids were examined for interference with specific binding of ^{125}I-labelled human FSH to bovine testicular receptor. Steroid hormones, including testosterone, progesterone and 17β-oestradiol were tested by incubating at final concentrations of 0.1, 1.0 and 5.0μg/ml with the membrane receptor and ^{125}I-labelled human FSH. No effect on the binding of FSH to the testicular receptor was observed. Similarly, nucleotides, including dibutyryl cyclic

AMP, cyclic AMP, ATP, CTP and GTP, at concentrations of 0.1, 0.5 and 1.0mM, showed no inhibitory effect on the binding between FSH and its testicular receptor.

Integrity of receptor-bound ^{125}I-labelled FSH

After incubation for 12h at 25°C, the unbound ^{125}I-labelled human FSH was separated from the bound hormone and receptor by centrifugation at 1000*g* for 30min at 4°C. The supernatant (unbound ^{125}I-labelled human FSH) was kept for later studies. The receptor–hormone complex (pellet) was washed three times with 50vol. of ice-cold 0.025M-Tris–HCl containing 0.1% bovine serum albumin; each step of washing was followed by centrifugation. The bound ^{125}I-labelled human FSH was then dissociated from its receptor by resuspending the pellet in 0.001M-HCl (one-quarter of the original volume) for 5min, followed by centrifugation. Under these conditions, over 90% of the bound ^{125}I-labelled human FSH was dissociated. The supernatant (bound ^{125}I-labelled human FSH) was immediately adjusted to pH7.2 with 0.01M-NaOH. For comparative studies, solutions of unbound, bound and non-treated ^{125}I-labelled human FSH were diluted appropriately to give approx. 100000c.p.m./ml, and subjected to the tests shown in Table 3. It is noteworthy that 'bound' ^{125}I-labelled human FSH yielded the best percentage of specific binding with the receptor as well as the antiserum. Trichloroacetic acid at a final concentration of 10% precipitated only 40% of FSH in solution.

The integrity of bound ^{125}I-labelled human FSH was further examined by polyacrylamide-gel electrophoresis (Davis, 1964), as depicted in Fig. 10. From the peak of distribution of radioactivity (gel segment 9, Fig. 10), it is obvious that bound ^{125}I-labelled human FSH had identical electrophoretic mobility with standard human FSH. These findings indicate that the specific testicular receptor binds only the biologically intact molecules, and that the interaction of FSH with its specific receptor does not result in a significant alteration or degradation of the hormone itself.

Table 2. *Effect of various enzyme treatments of bovine testicular receptor on specific binding of ^{125}I-labelled human FSH*

Membrane receptor protein (500μg) was incubated with different enzymes at 37°C for 1h in 0.5ml of 0.025M-Tris–HCl buffer, pH7.2. The treated receptor was resuspended in the same buffer, after five washings with 4ml of cold buffer and centrifugations. Specific binding was tested by incubating with ^{125}I-labelled human FSH (50000c.p.m.) at 25°C for 20h. Values are means±S.D. of three experiments.

Enzyme	Ratio (w/w) enzyme/receptor protein	Specific binding of ^{125}I-labelled human FSH (% of control)
Control	—	100
Neuraminidase	1:10	121±17
Trypsin	1:10	4±1
	1:100	11±3
Chymotrypsin	1:10	6±2
	1:100	26±5
Pepsin	1:10	84±14
	1:100	92±22
Collagenase	1:10	36±11
	1:100	60±15
Ribonuclease A	1:10	85±13
	1:100	95±15
Deoxyribonuclease I	1:10	81±9
	1:100	104±14

Discussion

At the present time relatively little is known about the mechanism of action and precise effects of gonadotrophins on mammalian testes. The initial event in hormonal action is generally considered to be the binding of the hormone to specific cellular receptors at the target organ. An early event in the action of gonadotrophins, luteinizing hormone and FSH is the activation of testicular adenylate cyclase (Kuehl

Table 3. *Tests for the integrity of the receptor-bound or unbound ^{125}I-labelled human FSH after incubation with bovine testicular plasma membranes*

The unbound ^{125}I-labelled human FSH after 12h of incubation with the receptor preparation was separated by centrifugation. After three washings, the bound ^{125}I-labelled human FSH was dissociated from its receptor with 0.001M-HCl, and the pH was immediately adjusted to 7.2. Solutions of non-treated, unbound and bound ^{125}I-labelled human FSH (50000c.p.m. each) were subjected to the various tests.

Test	^{125}I-labelled human FSH		
	Non-treated	Unbound	Bound
Specific binding by fresh receptor (%)	12.7	6.7	24.9
Specific binding by excess of antiserum (%)	56.1	42.2	93.4
Precipitation by 10% (w/v) trichloroacetic acid (%)	44.2	42.3	30.8
Precipitation by 90% (v/v) acetone (%)	99.1	98.5	99.5

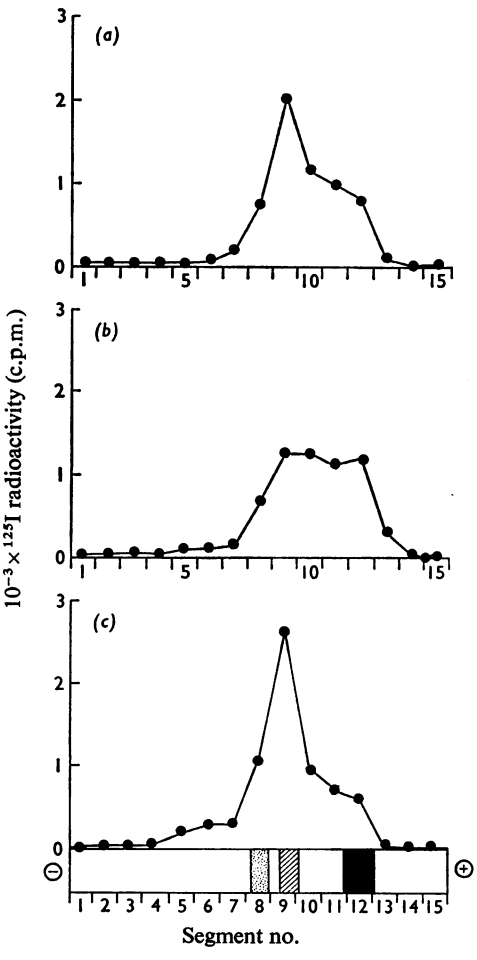

Fig. 10. *Comparison of electrophoretic mobility of non-treated ^{125}I-labelled human FSH (a) and receptor-bound (c) and unbound ^{125}I-labelled human FSH (b) after incubation with bovine testicular plasma membranes*

Each sample of ^{125}I-labelled human FSH (10000c.p.m.) in 0.025M-Tris–HCl buffer, containing 0.1% bovine serum albumin, was mixed with 50μg of unlabelled standard human FSH. Electrophoresis was carried out with 2mA per gel tube for 1h in 7.5% (w/v) polyacrylamide gels at pH8.9. Gels were stained with 1% Amido Black 10B in 7% (v/v) acetic acid for 1h, and destained in 7% acetic acid. Each gel was then segmented according to the stained protein bands as indicated. Radioactive ^{125}I-labelled human FSH in each segment was counted in an automatic gamma spectrometer. Stained protein bands at segments 8 and 12 are different components of bovine serum albumin, and the protein band at segment 9 is unlabelled human FSH.

et al., 1970; Murod *et al.*, 1969). It has been established that luteinizing hormone stimulates androgen production in the interstitial cells of the testes (Hall & Eik-Nes, 1962; Wakabayashi & Tamaoki, 1966). FSH increases adenylate cyclase activity in isolated seminiferous tubules (Dorrington *et al.*, 1972; Dorrington & Fritz, 1974; Kuehl *et al.*, 1970) and the Sertoli cell may be the principal cell type within the seminiferous tubule responding to FSH (Dorrington & Fritz, 1974).

The localization of radioiodinated human luteinizing hormone and human chorionic gonadotrophin in rat testis (DeKretser *et al.*, 1969, 1971; Catt *et al.*, 1971; Leidenberger & Reichert, 1972*a*,*b*) indicated that specific binding sites for gonadotrophic hormones were in gonadal tissue. The specific binding of ^{3}H-labelled human FSH to rat testes has been demonstrated by Means & Vaitukaitis (1972). Reichert & Bhalla (1974) have shown the specific uptake of ^{125}I-labelled human FSH by rat seminiferous tubules. They described further some of the properties of the human FSH-specific receptor in homogenates of rat seminiferous tubules (Bhalla & Reichert, 1974). The present studies indicate that the plasma membranes of bovine testes contain specific receptors for FSH. It is noteworthy that the binding activity of the bovine testicular plasma membranes for human luteinizing hormone is only 30% of that for human FSH (Table 1). In rat testicular homogenates, the total specific uptake of ^{125}I-labelled human luteinizing is 4.86-fold greater than that of ^{125}I-labelled human FSH (Bhalla & Reichert, 1974); even in homogenates of separated seminiferous tubules, the total binding of luteinizing hormone is still 2.6-fold greater than that of FSH (Bhalla & Reichert, 1974). Our observation of significantly high specific binding of ^{125}I-labelled human FSH by bovine testicular plasma membranes indicates that the bovine testis is a specially good source for FSH receptors. Unfortunately, at present, purified bovine FSH is not available for comparable studies on the binding of radioiodinated bovine FSH to the testicular membrane receptor. However, using this partially purified bovine testicular receptor and ^{125}I-labelled human FSH, I have developed a highly sensitive and specific radioligand-receptor assay for FSH (Cheng, 1975).

The binding of ^{125}I-labelled human FSH to bovine testicular receptor has been shown to be a saturable process with respect to the concentration of labelled hormone. The interaction between FSH and its testicular receptor has a high affinity, and the dissociation constant (K_d) has been demonstrated to be 9.8×10^{-11}M (Fig. 4). This value is consistent with those reported by Means & Vaitukaitis (1972) and Bhalla & Reichert (1974), 7×10^{-9}M and 6.7×10^{-10}M respectively. Further, the binding sites for ^{125}I-labelled human FSH have been estimated to be 5.9×10^{-14}mol/mg of receptor protein (Fig. 4), and are in reasonable agreement with the data obtained previously by other investigators, being 3.5×10^{-15} (Means & Vaitukaitis, 1972) and 6.2×10^{-14} (Bhalla & Reichert, 1974) mol/mg of protein. However, it

should be pointed out that the data obtained by Means & Vaitukaitis (1972) and Bhalla & Reichert (1974) are derived from their studies on rat seminiferous-tubule homogenates, and I am using 'purified' plasma membranes from bovine testes.

Similar to the binding of many other hormones to their receptors, the binding of ^{125}I-labelled human FSH to bovine testicular receptor is a reversible process and is dependent on temperature and time of incubation (Fig. 5). However, in rat seminiferous tubules, maximum binding has been reported to be 1h (Means & Vaitukaitis, 1972) or 3h (Bhalla & Reichert, 1974) at 37°C. The very slow dissociation rate of ^{125}I-labelled human FSH from its receptor (Fig. 6) suggests that the FSH–receptor complex, once formed, is relatively stable and not immediately followed by degradation of the bound hormone. This suggestion is supported further by the fact that bound ^{125}I-labelled human FSH eluted from its receptor has the highest percentage of specific binding with a freshly added receptor as well as a specific antiserum when compared with the non-treated or unbound ^{125}I-labelled human FSH (Table 3). The chemical nature of the receptor-bound ^{125}I-labelled human FSH, as revealed by its electrophoretic mobility (Fig. 10), remains identical with that of the unlabelled standard human FSH.

Bovine testicular receptor is thermally unstable. Preheating of the plasma membranes at 45°C abolishes over 60% of the binding activity for ^{125}I-labelled human FSH (Fig. 8). However, rat seminiferous-tubule receptor is relatively stable at this temperature (Bhalla & Reichert, 1974). Further, the binding of ^{125}I-labelled human FSH to bovine testicular receptor is dependent on pH and ionic concentration of the incubation medium (Figs. 7 and 9). My observation that pretreatment of testicular plasma membranes with extremes of pH causes irreversible inactivation of specific binding is in agreement with that reported on rat seminiferous-tubule homogenates (Bhalla & Reichert, 1974). This finding, however, is in contrast with the properties of the insulin receptor (Cuatrecasas, 1971*b*). Similar to the binding of human luteinizing hormone and human chorionic gonadotrophin to their receptors (Haour & Saxena, 1974; Rao, 1974), high concentrations of salts drastically decreased the binding of ^{125}I-labelled human FSH to bovine testicular receptor. This inhibitory effect of ions at high concentrations may be due to induction of changes in ionic environment of the receptor, in the state of aggregation of membranes, or even solubilization of the receptor from the membranes.

The use of enzymes as a tool to investigate the chemical nature of the macromolecular receptor complex has been reported by many investigators (Cuatrecasas, 1971*a*; Rao, 1974; Rubalcava & Rodbell, 1973). The loss of ^{125}I-labelled human FSH binding after treatment of plasma membranes with trypsin or chymotrypsin, but not with ribonuclease or deoxyribonuclease (Table 2), indicates that bovine testicular receptor is a protein in nature. This finding agrees with earlier reports on the rat seminiferous-tubule homogenates (Means & Vaitukaitis, 1972). A slight increase in binding activityid-plasma membranes after treatment with neuramin of ase suggests that sialic acid moieties of the membrane or receptor, or both, are not essential for FSH binding. However, the ultimate understanding of the biochemical and physiological nature of interactions between FSH and its receptor will be obtained only after the isolation of the membrane-bound receptors.

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