Multiple Forms of Solubilized Gonadotropin Receptors from the Rat Testis

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SUMMARY

Soluble preparations of the testicular gonadotropin receptor for luteinizing hormone (LH) and human chorionic gonadotropin (hCG) exhibit a spectrum of physical characteristics according to the conditions employed for extraction with detergents. Gonadotropin receptors in particulate testis binding fractions were solubilized before or after equilibration with 125I-hCG by detergents, including Triton X-100, Lubrol PX, Lubrol WX, and sodium deoxycholate, and were shown to remain in solution after centrifugation at 350,000 × g. Characterization of such soluble receptors and hormone-receptor complexes by gel filtration and sucrose density gradient centrifugation revealed the presence of several physical forms. In addition to the 6.5 S form of the free receptor extracted by Triton X-100, and the 7.5 S hormone-receptor complex resulting from hCG binding to free receptors extracted with Triton X-100, dialysis of the 7.5 S complex against detergent-free solutions caused reversible conversion to an 8.8 S form of the complex. Extraction of prelabeled testis particles with Triton X-100 gave only the 8.8 S complex, which aggregated reversibly upon dialysis. Lubrol PX extracts of prelabeled particles contained a 7 S complex which also aggregated during dialysis and converted to the 8.8 S form in Triton X-100. The 7 S form of the complex was also observed after extraction of labeled particles with Lubrol WX.

Exposure of the soluble receptors to trypsin and phospholipase A abolished subsequent hormone binding; neuraminidase, RNase, and DNase did not reduce the uptake of 125I-hCG. Phospholipase A had no effect upon preformed 7.5 S and 8.8 S hormone-receptor complexes, while phospholipase C had no effect on the 7.5 S complex and caused aggregation of the 8.8 S form. The less pronounced effects of phospholipase A on preformed hormone-receptor complexes than on unoccupied particulate or soluble receptors suggest that binding activity is influenced by an essential phospholipid component.

Interpretation of the several forms of the gonadotropin receptors extracted from testis particles is complicated by the differential effects of detergent binding and molecular conformation upon sedimentation velocity during density gradient centrifugation. Because the changes in density of the complexes extracted under various conditions are not sufficient to account for the observed sedimentation behavior of the hormone-receptor complexes, it is likely that changes in the symmetry or degree of association of the complexes are responsible for the multiplicity of forms detected under different conditions of detergent extraction.

Receptors for luteinizing hormone (LH) and human chorionic gonadotropin (hCG) in the rat testis and ovary have been previously rendered soluble by extraction with the nonionic detergent Triton X 100 (1-4). The soluble gonadotropin receptors retain hormonal specificity and high affinity for LH and hCG and exhibit the physical characteristics of an asymmetric molecule in the presence of the detergent (3). Uncharged receptors, and the receptor-hCG complex formed after extraction with Triton X-100, have sedimentation coefficients of 6.5 S and 7.5 S, respectively; the Stokes radius of both forms determined by gel filtration is 64 Å. Additional forms of the hormone-receptor complex, with sedimentation coefficients of 7.0 S and 8.8 S, have been identified in extracts of prelabeled interstitial cell fragments treated with various detergents including Lubrol PX, Lubrol WX, and sodium deoxycholate. The characteristics of these multiple forms of the gonadotropin receptors, and their modification by enzyme digestion, are described in the present report.

MATERIALS AND METHODS

Human chorionic gonadotropin (10,000 i.u. per mg) was a gift from Dr. R. Canfield, Department of Medicine, Columbia University, New York. Triton X-100 (Rohm and Haas) was obtained from Packard; carrier-free 125I from Amersham-Searle, Chicago; polyethylene glycol (Carbowax 6000) from Union Carboride; Sephadex G-200 and Sepharose 6B from Pharmacia Fine Chemicals, New Jersey; sucrose, cesium chloride, bovine serum albumin, bovine γ-globulin, fibrinogen, apoferritin, and myoglobin from Schwarz-Mann; thyroglobulin was a gift from Dr. J. M. Bilstad, National Institutes of Health, Bethesda. Concanavalin A was obtained from Calbiochem; cyanogen bromide from Eastman; trypsin, trypsin inhibitor, neuraminidase (Clostridium perfringens), phospholipase A (Vipera russelli), phospholipase C (Clostridium perfringens).

1 The abbreviations used are: LH, luteinizing hormone; hCG, human chorionic gonadotropin; PBS, phosphate-buffered saline (0.9% NaCl solution). The trivial names for steroids are: estradiol, extra-1,3,5-10-tiene-3,17β-diol; testosterone, 17β-hydroxyandrost-4-en-3-one; dihydrotestosterone, 17β-hydroxy-5a-androstan-3-one; progesterone, preg-4-ene-3,20-dione.
tritium porifringenes), Lubrol PX, Lubrol WX, sodium dodecyl sulfate, and sodium deoxycholate from Sigma.

Solubilization of Unlabeled Testis Particles—Testes from adult male Sprague-Dawley rats were decapsulated and teased apart in Dulbecco phosphate-buffered saline (PBS), pH 7.4, to release interstitial cell fragments with high binding affinity for LH and hCG (5, 6). The yield of interstitial cell particles was increased by swaying the mass of dispersed tubules for 5 to 10 min as previously described (5). After filtration through cotton wool, the particulate suspension was centrifuged at 12,000 × g for 20 min. The supernatant solution then was centrifuged at 27,000 × g for 30 min to sediment particles with gonadotropin binding activity, and the pellets were resuspended in 1% Triton X-100 at 4° for 30 min. After dilution to 0.1% Triton with PBS, the solution was centrifuged at 27,000 × g for 20 min to remove undissolved particles. The solubilized binding sites were not sedimented by further centrifugation at 360,000 × g for 3 hours.

Solubilization of Particulate Fractions—Interstitial cell particles preincubated with 125I-hCG were utilized to examine the actions of various detergents upon preformed hormone-receptor complexes. Particles were incubated at 4° for 16 hours, with 600,000 cpm (150 pg) of labeled hCG, then washed three times with 40 ml of PBS to remove free hCG, and recovered by centrifugation at 20,000 × g. Up to 80% of the labeled hCG was taken up by the particulate fraction and remained bound during storage of the particles at 4° for several days. Aliquots of such prelabeled particles were extracted with detergents including Triton X-100, Lubrol WX, and sodium deoxycholate and centrifuged at 20,000 × g for 20 min before analysis of the supernatant solutions by gel filtration and density gradient centrifugation. The supernatant of the supernatant solutions for 3 hours at 360,000 × g was performed to confirm that the binding activity represented completely solubilized hormone-receptor complexes.

Preparation of 125I-hCG—Purified hCG (10,000 i.u. per mg) was labeled with 125I (Amersham-Searle) to an average specific activity of 50 μCi per μg and purified by affinity chromatography on agarose-concanavalin A as previously described (7). The specific activity of each labeled hormone preparation was determined by solid phase radioimmunoassay (8) and retention of full biological activity by the radioiodinated hormone was confirmed by a highly sensitive bioassay based on the testosterone production of isolated testes during incubation with LH or hCG in vitro (9, 10).

Assay of Receptor Binding—The receptor assay based upon double precipitation of the hCG-receptor complex with polyethylene glycol has been previously described in detail (3). Briefly, 0.5 ml aliquots of receptor solution (500 μg of protein) were mixed with 0.1 ml of PBS containing known quantities of unlabeled hCG, followed by 0.1 ml of PBS containing 125I-hCG. Nonspecific binding was determined from tubes containing labeled hormone and receptor in the presence of an excess (20 μg) of unlabeled hCG. After incubation for 16 hours at 4°, 200 μl of a solution of bovine γ-globulin (5 mg per ml in PBS) were added, followed by 0.5 ml of 30% polyethylene glycol (w/v) in PBS; after mixing, the tubes were centrifuged at 2000 × g for 20 min. The precipitates were redissolved in 0.9 ml of 0.1% Triton X-100 in PBS and reprecipitated with 0.5 ml of 30% polyethylene glycol. The bound hormone present in each sample was determined by counting the radioactivity of the precipitates in a gamma spectrometer after aspiration of the supernatant solutions.

Gel Filtration—Sephadex 6B and 6B columns (0.9 × 100 cm) were equilibrated with 50 mM Tris-HCl buffer, pH 7.4, and subjected to gel filtration. Two discrete peaks of ultraviolet-absorbing aggregated material and radioactivity which correspond to the position of the blue dextran elution of the free receptor, 0.7 ml of each fraction was incubated with 32,000 cpm of 125I-hCG in the presence or absence of 10−7 M hCG for 16 hours at 4°, and the bound radioactivity was isolated by polyethylene glycol precipitation. Nonspecific values determined in tubes containing excess hCG were subtracted from the total precipitable radioactivity. Bottom, fractions corresponding to the hCG-receptor peak (above) were pooled and concentrated with Sephadex G-25. An aliquot containing 5000 cpm then was subjected to density gradient centrifugation (11). Two discrete peaks of radioactivity were resolved, corresponding to free hCG and the hormone-receptor complex, which was 90% precipitable by polyethylene glycol. BSA, bovine serum albumin.

RESULTS

Effect of dialysis on 7.6 S hormone-receptor complex formed by equilibration of soluble testis receptors with 125I-hCG

Triton-solubilized receptors (0.5 S) were incubated with 125I-labeled hCG for 16 hours at 4° to give an equilibrium mixture of the 7.5 S hormone-receptor complex and free hCG. When this preparation was dialyzed against 50 mM Tris-HCl buffer, pH 7.4, and subjected to gel filtration on a column (0.9 × 100 cm) of Sepharose 6B, three radioactive peaks were observed (Fig. 1, top, closed circles). That corresponding to the void volume was due to aggregated complexes, of which a similar but less prominent peak was previously noted during gel filtration of the 7.5 S receptor (3). A further peak with Kav = 0.27 represented the corresponding peak. That corresponding to the void volume was due to aggregated complexes, of which a similar but less prominent peak was previously noted during gel filtration of the 7.5 S receptor (3). A further peak with Kav = 0.27 represented the
hormone-receptor complex and was abolished by preincubation of the soluble receptor with excess unlabeled hCG; and the third peak was free hCG with \( K_{av} = 0.56 \). In addition, the 6.5 S unlabeled soluble receptor preparation was dialyzed against 50 mM Tris-Cl buffer, pH 7.4, and subjected to gel filtration on Sepharose 6B. The binding activity of the eluant fractions was determined by equilibration of an aliquot from each fraction with \(^{3}H\)-labeled hCG, followed by precipitation with polyethylene glycol. The major peak of binding activity was eluted with \( K_{av} = 0.31 \) (Fig. 1, top, open circles).

Concentration and refiltration of the aliquots from the peak corresponding to the dialyzed \(^{3}H\)-hCG hormone-receptor complex on two occasions gave \( K_{av} \) values of 0.27 and 0.28. A further aliquot of the same peak was also subjected to density gradient centrifugation in 5 to 20% sucrose for 16 hours at 190,000 \( \times g \), giving the sedimentation pattern shown in the lower panel of Fig. 1. The mean sedimentation coefficient of the soluble dialyzed hormone-receptor complex calculated by comparison with reference proteins (12) was determined to be \( 8.8 \pm 0.30 \) (S.D.) in three separate experiments.

To examine the reversibility of the conversion between the 7.5 S and 8.8 S forms of the hormone-receptor complex, a further preparation of the 8.8 S dialyzed hormone-receptor complex was exposed again to 0.1% Triton X-100. The sedimentation pattern of this preparation showed that reversion to the 7.5 S form had occurred in the presence of the detergent (Fig. 2).

**Extraction of Prelabeled Testis Particles with Various Detergents**

A variety of ionic and nonionic detergents were employed for solubilization of the preformed hormone-receptor complex prepared by equilibration of testis interstitial cell particles with \(^{3}H\)-hCG. The efficacy of extraction was 80 to 90% with the nonionic detergents at concentrations greater than 0.1%, employing 0.1 ml per mg of protein; a similar yield was obtained at concentrations of the ionic detergent sodium deoxycholate above 0.5% (Fig. 3).

**Properties of Hormone-Receptor Complex Solubilized by Triton X-100 from Prelabeled Testis Particles**

Extraction of prelabeled particles with Triton-X-100 yielded a soluble hormone-receptor complex which sedimented as an 8.8 S species in sucrose gradients (Fig. 4, top). When this preparation was subjected to gel filtration on Sepharose 6B, the elution pattern showed a single peak of bound hormone-receptor complex with \( K_{av} \) of 0.31, and the calculated Stokes radius was 64 A. When free detergent was removed by dialysis, the hormone-receptor complex converted to a broad and rapidly sedimenting peak (Fig. 4, middle); such a pattern could be consistent with the formation of aggregates, and such dialyzed preparations were eluted in the void volume on Sepharose 6B columns. This phenomenon was reversible on addition of Triton X-100 to a concentration of 0.1%, which converted the aggregated complexes to the original 8.8 S form (Fig. 4, bottom).

**Physical Parameters of Extracted Hormone-Receptor Complex**

For calculation of molecular weight and fractional ratio (13), the Stokes radius of 64 A and the sedimentation coefficient of 8.8 S, in solvent of density 0.9876 g cm\(^{-3}\) and viscosity coefficient (\( n \)) of 0.0094 poise (3, 15), were used. The partial specific volume of the 8.8 S hormone-receptor derived from the density of the molecule in cesium chloride gradients was 0.7825 cm\(^3\) per g.

By this method, the apparent molecular weight of the 8.8 S hormone-receptor complex was 270,000. The difference between the estimated molecular weight of the 8.8 S form and the previously described 7.5 S form (224,000) was approximately 46,000. The frictional ratio calculated by neglecting the solvation factor was 1.47, corresponding to an axial ratio (prolate) of 8.8.
Effect of pH upon Dissociation of 8.8 S Hormone-Receptor Complex

No dissociation of the soluble 8.8 S hormone-receptor complex was observed upon incubation with buffer solutions in the pH range between 5.7 and 8.6 at 34°C (5.70 to 6.93 pH, 0.1 M acetate buffer; 7.4 to 8.6, 0.1 M Tris-Cl buffer) when followed by precipitation of the bound complex with polyethylene glycol. However, decreasing the pH to 3.5 for 1 to 5 min by addition of acetic acid immediately dissociated the hormone from the receptor site, as shown by density gradient centrifugation (Fig. 5). Upon neutralization and reincubation at pH 7.4 for 16 hours, the receptor once again bound the hormone. The resulting hormone-receptor complex clearly differed from the original 8.8 S preparations, having acquired the sedimentation characteristics of the 7.5 S hormone-receptor complex (Fig. 5).

Effects of Urea, Guanidine, Steroids, and Nucleotides

Exposure of soluble complexes to 2 M urea and guanidine HCl caused complete dissociation of the hormone from receptor sites, as determined in sucrose density gradients. No effects on the hormone-receptor complex were observed after treatment with steroid hormones of the androsten, androstan, and pregnen series including estradiol, testosterone, dihydrotestosterone, and progesterone at concentrations of 10^-6 M. In addition, cyclic adenosine 3':5'-monophosphate (5 mM), ATP (5 mM), and GTP (1 mM) were without effect on hormone binding and dissociation, and variations in medium calcium concentration from 0 to 3 mM caused no change in binding.

Precipitation of Triton-solubilized Gonadotropic Receptors by Ammonium Sulfate

The equilibrium mixture of the hormone-receptor complex (7.5 S) and free hCG resulting from incubation of soluble receptors with 125I-hCG was treated with increasing concentrations of neutral ammonium sulfate (10 to 80%) and centrifuged at 100,000 x g for 1 hour. In addition, cyclic adenosine 3':5'-monophosphate (5 mM), ATP (5 mM), and GTP (1 mM) were without effect on hormone binding and dissociation, and variations in medium calcium concentration from 0 to 3 mM caused no change in binding.
TABLE I

| Treatment                     | Total mCi-hCG | Protein (mg) | Specific activity (cpm/mg protein) | Recovery (%) |
|-------------------------------|---------------|--------------|-----------------------------------|--------------|
| Prelabeled particles          | 283,000       | 34.0         | 8,320                             | 100          |
| Triton X-100 extract          | 252,000       | 26.8         | 9,400                             | 80           |
| 25 to 50% (NH₄)₂SO₄ fraction | 174,540       | 7.0          | 25,000                            | 69           |
| 5 to 20% sucrose gradient in  |               |              |                                   |              |
| 0.1% Triton                   | 99,080        | 2.6          | 30,000                            | 35           |

Fig. 6. Top, effects of phospholipases A and C upon the sucrose density gradient centrifugation pattern of the 7.5 S ¹²⁵I-hCG-receptor complex prepared by Triton X-100 extraction of testis particles and subsequent equilibration with ¹²⁵I-hCG. Bottom, effects of phospholipases A and C on 8.8 S complex formed by dialysis of the 7.5 S ¹²⁵I-hCG-receptor complex. Control, ●—●; phospholipase A, 0.5 unit, □—□; phospholipase C, 1.0 unit, ○—○.

Fig. 7. Effects of phospholipases A and C upon the sucrose density gradient centrifugation pattern of the 8.8 S ¹²⁵I-hCG-receptor complex extracted from prelabeled testis particles with Triton X-100. A, enzyme treatment of particles; B, enzyme treatment after solubilization; C, enzyme treatment of soluble receptor fractionated by precipitation with 25 to 50% (NH₄)₂SO₄. Control, ●—●; phospholipase A, 0.5 unit, □—□; phospholipase C, 1.0 unit, ○—○.

**Extraction of ¹²⁵I-hCG-receptor complex from prelabeled testis particles by Triton X-100, and fractionation by ammonium sulfate and sucrose density gradient centrifugation**

**Treatment**

- Prelabeled particles
- Triton X-100 extract
- 25 to 50% (NH₄)₂SO₄ fraction
- 5 to 20% sucrose gradient in 0.1% Triton

**Table**

| Treatment                     | Total mCi-hCG | Protein (mg) | Specific activity (cpm/mg protein) | Recovery (%) |
|-------------------------------|---------------|--------------|-----------------------------------|--------------|
| Prelabeled particles          | 283,000       | 34.0         | 8,320                             | 100          |
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| 25 to 50% (NH₄)₂SO₄ fraction | 174,540       | 7.0          | 25,000                            | 69           |
| 5 to 20% sucrose gradient in  |               |              |                                   |              |
| 0.1% Triton                   | 99,080        | 2.6          | 30,000                            | 35           |

**Results**

- Similarly unaffected by phospholipase A; however, exposure to phospholipase C caused a moderate degree of aggregation (Fig. 6).

**8.8 S Receptor-Hormone Complex Extracted from Prolabeled Testis Particles**

- When labeled testis particles were exposed to phospholipases A and C before extraction with Triton X-100, the hormone-receptor complexes subsequently extracted from the 27,000 x g residue with detergent showed considerable aggregation on sucrose density gradients (Fig. 7A). The 27,000 x g supernatant solution from prelabeled particles treated with phospholipase A contained much more radioactivity than released from controls and particles treated with phospholipase C (Table II). The radioactivity released from labeled particles by phospholipase A was 50% precipitable by polyethylene glycol; sucrose density gradient centrifugation showed that the phospholipase A supernatant contained roughly equal proportions of aggregated material and free hCG.

- The 8.8 S hormone-receptor complex extracted from prelabeled particulate fraction with Triton X-100 was also aggregated by exposure to phospholipase C, while phospholipase A had virtually no effect upon this species (Fig. 7B). Identical results were ob-
served when the 8.8 S receptor subjected to ammonium sulfate fractionation (25 to 50% saturation) was treated with phospholipase C (Fig. 7C). Under the conditions of this incubation, phospholipase A did not have any effect on the sedimentation behavior of the 8.8 S hormone-receptor complex. However, the presence of a small but definite peak of free hCG (Fig. 7, A, B, and C) suggested that dissociation of the hormone from the receptor occurred during treatment with phospholipase A, comparable to the phospholipase A-induced release of free hCG from prelabeled particles.

Exposure of the various 7.5 and 8.8 S forms of the hormone-receptor complex to RNase, DNase, or neuraminidase had no effect upon the polyethylene glycol precipitability or the sedimentation patterns of the solubilized receptors.

**Hormone-Receptor Complexes Extracted from Prelabeled Testis Particles with Various Nonionic and Ionic Detergents**

While solubilization of the prelabeled particles with Triton X-100 yields only one form of the receptor (8.8 S), extraction with other detergents led to the formation of soluble hormone-receptor complexes other than the 8.8 S form. Extraction with Lubrol PX (1.0%) gave a 7 S form of the hormone-receptor complex. Dialysis of this preparation allowed the hormone-receptor complex to aggregate, and addition of Triton to 0.1% concentration reverted the conglomerate to the 8.8 S form. Treatment of prelabeled particles with 1% Lubrol WX also yielded the 7 S form of the hormone-receptor complex; a more rapidly sedimenting form (8.8-9.2 S) was obtained by extraction with 0.25% sodium deoxycholate.

Gonadotropin receptors solubilized from prelabeled testis particles with Lubrol WX and subjected to gel filtration on Sepharose 6B showed identical elution profiles *K* ~ 0.26) in 0.1% Lubrol WX and 0.1% Triton X-100. From the partition coefficient of 0.26 the Stokes radius was determined by reference to standard proteins to be 77 A in either detergent. The density of the 7.0 S hormone-receptor complex extracted with Lubrol WX was determined by isopyknic density gradient centrifugation to be 1.3150, and the apparent molecular weight was calculated to be 230,000.

**DISCUSSION**

The presence of multiple forms of the gonadotropin hormone-receptor complexes in detergent extracts of interstitial cell particles is attributable to at least two mechanisms. First, the nature and state of association of the receptor complex released from cell membranes may differ according to the surfactant employed for extraction; and second, the degree of association between receptors and the individual detergents may be subject to considerable variation. A notable feature of the present studies was the reproducibility with which the several forms of the receptor complex could be extracted under defined conditions by the various detergents. As previously noted, the difference between the 6.5 S receptor extracted from the testis particles with Triton X-100 and the 7.5 S hormone-receptor complex formed by equilibration with 125I-labeled hCG is attributable to binding of 1 molecule of gonadotropin by each molecule of the receptor protein (3). In the present studies, 8.8 S forms of the hormone-receptor complex were obtained both by dialysis of the 7.5 S complex and by extraction of prelabeled testis particles with Triton X-100. The increased sedimentation velocity of the dialyzed 7.5 S hormone-receptor complex in sucrose density gradients could be due to removal of a significant proportion of the bound detergent molecules, with correspondingly increased density of the soluble complex. The markedly hydrophilic properties of the heavily sialylated glycoprotein hormone molecule may contribute to the maintenance of the soluble form of the extracted hormone-receptor complex after dialysis against detergent-free solutions.

The sedimentation properties of the 8.8 S complex extracted from previously labeled particles with Triton X-100 could also be caused by a lower degree of detergent binding, since the hormone-receptor interaction is likely to involve hydrophobic regions of the receptor which are then less accessible for subsequent detergent binding. Reduced binding of detergent would result in higher buoyant density and increased sedimentation velocity of the extracted complex, and could be more readily reduced by dialysis to below a critical value necessary to prevent aggregation of the complex. However, the density of the 8.8 S complex extracted from prelabeled particles (1.277) is not greater than that of the 7.5 S complex (1.289), so that decreased detergent binding cannot be invoked to explain the higher sedimentation velocity of the 8.8 S form. The possibilities remain that the preformed hormone-receptor complex is extracted as a larger or less asymmetric species, or as one containing relatively less phospholipid than that extracted from unlabeled particulate receptors. Since precise measurement of detergent binding by such minute quantities of receptor cannot be performed, such speculations about the nature of the 8.8 S complex are at best of operational value until more direct methods are developed to determine the extent and effects of detergent-receptor interactions under these conditions.

The presence of 7 S and 9 S forms of the solubilized hormone-receptor complex prepared by extraction with Lubrol PX, Lubrol WX, and sodium deoxycholate suggests that these detergents may also exhibit characteristic degrees of binding to the receptor complex or are capable of extracting less associated forms of the receptor. Since the density of the 7.0 S Lubrol-extracted hormone-receptor complex (1.315) is greater than that of the Triton-solubilized forms (1.277) and the apparent frictional ratio is higher (1.87), the lower sedimentation velocity in sucrose gradients is probably caused by extraction of a more highly asymmetric or lower molecular weight form of the receptor. The 7 S form extracted by Lubrol PX is analogous to the 8.8 S form extracted by Triton X-100, with presumably greater detergent binding, and converts to the 8.8 S form after dialysis and equilibration with 0.1% Triton X-100.

The 7 S form of the receptor extracted by Lubrol WX and Lubrol PX exhibits an even more marked disproportion between Stokes radius (77 A) and sedimentation constant (7.0 S) than the Triton-extracted forms of the complex (*a* = 64 A; *S* = 7.5 and 8.8). This cannot be attributed to detergent binding with consequently low buoyant density since the density of the 7 S form determined by isopyknic density gradient centrifugation is higher than that of 8.8 S species (1.315 versus 1.277). It should be noted that the labeled ligand (hCG) bound to the receptors is

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**Table II**

*Extraction of prelabeled particles with phospholipase A*

| Treatment          | 125I hCG particles | Supernatant | Polyethylene glycol precipitation |
|--------------------|--------------------|-------------|----------------------------------|
| Control            | 28,630             | 1,320 (4.6) | 93 (7.0)                         |
| Phospholipase A    | 20,510             | 9,350 (45.5)| 4,720 (50.5)                     |
| Phospholipase C    | 28,460             | 1,280 (4.5) | 112 (8.8)                        |

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a heavily sialylated glycoprotein which also exhibits a larger Stokes' radius (34 Å) than expected from its sedimentation constant (2.9 S) and molecular weight (37,000). This is caused by the relatively large carbohydrate content of the hCG molecule (30 to 33% by weight, of which about 3% is sialic acid) and not by detergent binding since the physical properties of hCG are not altered in the presence of 0.1% Triton. The density of hCG in cesium chloride gradients containing 0.1% Triton X-100 (1.304) is quite close to that of the hormone-receptor complex (7.5 S, 1.280; 8.8 S, 1.277), and in neither case can substantial binding of detergent be invoked to explain the marked disparity between sedimentation velocity and hydrodynamic radius. For this reason, the 7.0 S form of the hCG-receptor complex could represent a more highly asymmetric form of the 8.8 S complex, while the difference between the 7.5 and 8.8 S forms is attributable to changes in detergent binding and symmetry of the hormone-receptor complexes.

The effects of detergents on the hydrodynamic properties of the cholineric receptor protein of the electric organ has been recently described by Meunier et al. (16). Their studies indicated that the unusual hydrodynamic behavior of the receptor protein in Triton X-100 was attributable to decreased density caused by detergent binding, rather than to intrinsic molecular asymmetry. In the case of the cholineric receptor, the disproportion between hydrodynamic radius (73 Å) and sedimentation velocity (12.5 S) was somewhat less marked than with the soluble gonadotropin receptor, and detergent binding provides only a partial explanation for the anomalous hydrodynamic properties of the latter protein. The presence of lipid and carbohydrate residues in the receptor, the glycoprotein nature of the ligand, and a degree of asymmetry in the receptor molecule probably all contribute to the observed disparity between Stokes radius and sedimentation velocity and combine to exaggerate the apparent asymmetry of the receptor protein as calculated from the hydrodynamic properties of the complex.

The effects of enzyme treatment on the sedimentation and binding properties of the hormone-receptor complexes also provide some indication of the structural complexity of the receptor molecule. It was previously shown that the binding activity is destroyed by trypsin and that reduced binding activity is observed after treatment of particulate and soluble (0.5 S) receptors with phospholipase A (3). In the present studies, exposure of the 7.5 S receptor and its dialyzed 8.8 S derivative to phospholipase A and C had relatively little effect other than the moderate aggregation observed in sedimentation patterns after exposure to phospholipase C. Comparable effects of phospholipases A and C were observed after enzyme treatment of prelabeled testis particles and of the 8.8 S receptor extracted from labeled particles with Triton X-100. In each case, phospholipase C caused aggregation of the extracted hormone-receptor complex.

Phospholipase A treatment of labeled testis particles released considerable quantities of the bound hormone, in the form of aggregated complexes and free hCG. By contrast, phospholipase A had little effect on the already extracted 8.8 S hormone-receptor complex. Although these various results are not simple to interpret, phospholipase A has a more pronounced effect upon the unoccupied binding sites of the gonadotropin receptor, and on the particulate receptors prior to solubilization, than on the soluble and preformed receptor complexes. By contrast, phospholipase C was never observed to destroy binding activity, although it frequently caused aggregation of the extracted complexes. For these reasons, it is probable that phospholipids form an essential component of the receptors and that binding activity in particular is strongly influenced by a phospholipid moiety which is susceptible to hydrolysis by phospholipase A. Since the receptor molecules do not exhibit the physical properties of conventional lipoproteins, it is likely that the phospholipid components make up only a small proportion of the receptor complex, but are of considerable functional importance in the interaction between hormone and receptor site.

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