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Hydrodynamic Properties of the Gonadotropin Receptor from a Murine Leydig Tumor Cell Line Are Altered by Desensitization

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ABSTRACT: The murine Leydig tumor cell line 1 (MLTC-1) contains gonadotropin receptors (GR) that are coupled to adenylate cyclase through the stimulatory guanine nucleotide binding protein (G_s). The binding of human choriogonadotropin (hCG) causes MLTC-1 cells to accumulate cAMP. With time, the ability of MLTC-1 cells to respond to hCG is attenuated by a process called desensitization. The hydrodynamic properties of GR from control and desensitized MLTC-1 cells were studied. Sucrose density gradient sedimentation in H_2O and D_2O and gel filtration chromatography were used to estimate the Stokes radius (a), partial specific volume (v_c), sedimentation coefficient ($s_{20,w}$), and molecular weight (M_r) of the detergent-solubilized hormone–receptor complex (hCG–GR). [^{125}I]hCG was bound to MLTC-1 cells under conditions that allow (37 °C) or prevent (0 °C) desensitization, and hCG–GR was solubilized in Triton X-100. In the absence of desensitization, control hCG–GR had a M_r of 213 000 (a = 6.2; v_c = 0.76; $s_{20,w}$ = 7.3), whereas desensitized hCG–GR had a M_r of 158 000 (a = 6.1; v_c = 0.71; $s_{20,w}$ = 6.6). Deglycosylated hCG (DG-hCG) is an antagonist that binds to GR with high affinity but fails to stimulate adenylate cyclase or cause desensitization. [^{125}I]DG-hCG was bound to MLTC-1 cells and DG-hCG–GR solubilized in Triton X-100. The hydrodynamic properties of DG-hCG–GR (M_r 213 000; a = 5.8; v_c = 0.77; $s_{20,w}$ = 7.6) were the same as that for control hCG–GR. There was no evidence for the association of adenylate cyclase or G_s with GR in Triton X-100 solubilized preparations. When hCG was cross-linked to GR and solubilized with sodium dodecyl sulfate (SDS), the M_r was found to be 116 000 (a = 4.9; v_c = 0.75; $s_{20,w}$ = 5.2), which was similar to that determined by SDS–polyacrylamide gel electrophoresis and less than that of the Triton X-100 solubilized control hCG–GR.

Chemical or photoaffinity cross-linking of radiolabeled gonadotropin to its receptor in conjunction with SDS–PAGE[1] has been used to determine the molecular weight and possible subunit structure of the gonadotropin receptor. Studies employing these techniques have not led to agreement on either point. Conflicting results may reflect differences in the tissue source and in experimental technique. A number of studies contend that the hormone-binding subunit of the GR is in the range of M_r 70 000–90 000 (Dattatreytamurty et al., 1983; Rapoport et al., 1984; Hwang & Menon, 1984; Ascoli & Segaloff, 1986; Bruch et al., 1986; Wimalasena et al., 1986). Some studies indicate that this is the only polypeptide that makes up the GR (Metsikko & Rajaniemi, 1982; Metsikko, 1984; Kusuda & Dufau, 1986). Other studies suggest that the GR is heterooligomeric (Hwang & Menon, 1984; Ji et al., 1985; Bruch et al., 1986). Further complicating the issue are results suggesting that the heterooligomeric nature of the receptor seen by some investigators is the result of proteolysis (Kellokumpu & Rajaniemi, 1985; Ascoli & Segaloff, 1986).

Hydrodynamic studies on the gonadotropin receptor, though fewer in number, have been less conflicting in their results. Although these studies have not approached the subject by rigorous application of hydrodynamic techniques, they do indicate that the receptor is larger than the 70–90 kDa suggested for the hormone-binding subunit of GR by most of the SDS–

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[1] Abbreviations: hCG, human chorionic gonadotropin; DG-hCG, deglycosylated hCG; EGS, ethylene glycol bis(succinimidyl succinate); GR, gonadotropin receptor; G_s, stimulatory guanine nucleotide binding protein; M_r, molecular weight; PAGE, polyacrylamide gel electrophoresis; PBS, Dulbecco's calcium–magnesium-free phosphate-buffered saline; SDS, sodium dodecyl sulfate; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid; cAMP, adenosine cyclic 3′,5′-phosphate; ATP, adenosine 5′-triphosphate; NAD, nicotinamide adenine dinucleotide.

PAGE studies. It is generally agreed that the hCG–GR complex, when solubilized in a nonionic detergent, has an estimated molecular weight between 210000 and 240000, suggesting that the holoreceptor is approximately twice the size of the hormone-binding subunit. This has led to speculation (Dufau et al., 1975; Rebois et al., 1981; Wimalasena et al., 1986) that the receptor is a homooligomer. We have undertaken careful hydrodynamic studies in an attempt to determine if the detergent-solubilized GR from MLTC-1 cells demonstrates hydrodynamic properties consistent with the proposed molecular weight based on SDS–PAGE. We used both ionic (SDS) and nonionic (Triton X-100) detergents to determine their effect on the hydrodynamic properties of GR. We have also desensitized the gonadotropin-responsive adenylate cyclase system to investigate what effects it had on the hydrodynamic properties of the receptor. Here we report the results of those studies.

Materials and Methods

Materials. hCG (13450 IU/mg, CR 121) was provided by Dr. R. Canfield, Columbia University, through the Center for Population Research for the National Institute of Child Health and Human Development. The hCG was iodinated as previously described (Rebois, 1982). DG-hCG was prepared and iodinated as described previously (Rebois & Fishman, 1983). [^{3}H]H_2O (1 mCi/g), [^{3}H]cAMP (33.5 Ci/mmol), [α-^{32}P]ATP (39.9 Ci/mmol), [α-^{32}P]NAD (987 Ci/mmol), and ^{14}C-labeled protein standards were obtained from New England Nuclear (Boston, MA). Forskolin and choleragen were from Calbiochem-Behring (San Diego, CA). Apoferritin, catalase, and fumerase were obtained from Sigma Chemical Co. (St. Louis, MO), and alcohol dehydrogenase and malate dehydrogenase were from Boehringer Mannheim (Indianapolis, IN). Lactate dehydrogenase was purchased from Worthington Enzymes (Freehold, NJ) and ethylene glycol bis(succinimidyl succinate) from Pierce Chemical Co. (Rockford, IL).

Preparation of Solubilized Hormone–Receptor Complex. MLTC-1 cells were grown as described previously (Rebois & Fishman, 1983). Hormone at a concentration of 0.2–1.0 nM was bound to the cells in medium at 37 °C for 30 min except in some experiments with hCG where a 2-h incubation at 0 °C was used. After binding of [^{125}I]hCG, the cells were washed free of unbound hormone with PBS. When SDS was used for solubilization, the complex was cross-linked with EGS. A 100 mM stock solution of EGS in dimethyl sulfoxide was diluted 100-fold into PBS and added to the cells. Cells were incubated for 30 min at 0 °C, washed with PBS, and incubated for 10 min at 0 °C with 50 mM Tris-HCl, pH 7.4, 125 mM NaCl, and 3 mM KCl to block remaining unreacted cross-linker. [^{125}I]hCG that was not cross-linked to the cell membrane was removed by treatment with glycine-buffered saline (Rebois & Fishman, 1983) prior to preparation of membranes and solubilization with SDS. Membranes were prepared as described previously (Rebois, 1982) and the hormone–receptor complex was solubilized in 2 mM Tris-HCl buffer, pH 7.4, containing 1% Triton X-100 or SDS for 30 min at 0 or 15 °C, respectively. The detergent-solubilized preparations were centrifuged at 100000g for 60 min, and the supernatant was used for gel filtration and sucrose density gradient centrifugation experiments.

Gel Filtration. Samples (200 μL) of detergent-solubilized hormone–receptor complex or standard proteins were applied to a 0.9 × 34 cm column of Ultrogel AcA 34 (LKB, Uppsala, Sweden) and eluted with PBS containing 0.1% of the appropriate detergent at a flow rate of 8 mL/h. The column temperature was maintained with a water jacket at 4 or 15 °C for elution with buffer containing Triton X-100 and SDS, respectively. Column fractions (0.5 mL) were assayed for radiolabeled material by scintillation spectrometry or for enzyme activity.

Sucrose Density Gradient Centrifugation. Linear 5–20% sucrose density gradients were prepared from solutions made up in H_2O or D_2O with a Jule gradient maker (Jule Inc., Trumbull, CT). The solutions were buffered with 50 mM Tris-HCl, pH 7.4, and contained 0.1% Triton X-100 or SDS. Detergent-solubilized hormone–receptor complex or standard proteins were applied in a volume of 100 μL. Centrifugation was for 24 h (H_2O gradients) or 48 h (D_2O gradients) at 4 °C. The gradients were fractionated with an Isco Model 185 density gradient fractionator. The fractions were assayed for radiolabeled material by γ or scintillation spectrometery or for enzyme activity.

Sedimentation of G_s. Membranes were prepared from MLTC-1 cells and ADP-ribosylated with [α-^{32}P]NAD and choleragen as described by Rebois (1982), except that [^{125}I]DG-hCG was also included in the incubation. The membranes were washed and treated as described for sucrose density gradient centrifugation on H_2O gradients. After fractionation of the gradient, samples were made up to 20% glycerol, 5% β-mercaptoethanol, and 2% SDS and heated to 100 °C for 5 min in a boiling water bath. Samples were separated on a 5–20° linear SDS–polyacrylamide gel with a 4.5% stacking gel (Rebois et al., 1981). The gel was dried, and radioactive protein bands were assayed by autoradiography. The location of GR on the gel was revealed by the presence of the ^{125}I-labeled α-subunit of DG-hCG (apparent M_r 26000) and that of the α-subunit of G_s by a ^{32}P-labeled band of apparent M_r 49000. The amount of radioactivity was quantitated with a scanning densitometer.

Calculations. The equation used to estimate the M_r of a detergent-solubilized hormone–receptor complex was

$$M_c = \frac{6\pi N \eta_{20,w}}{1 - v_c \rho_{20,w}} a s_{20,w} \quad (1)$$

where M_c is the molecular weight of the detergent–protein complex, N is Avogadro's number, $\eta_{20,w}$ is the viscosity of water at 20 °C, v_c is the partial specific volume of the detergent–protein complex, $\rho_{20,w}$ is the density of water at 20 °C, a is the Stokes radius, and $s_{20,w}$ is the sedimentation coefficient of the detergent–protein complex in water at 20 °C. In order to do the calculation, values for a, $s_{20,w}$, and v_c must be experimentally determined. This was done as described by Clarke and Farber (1973) and Martin and Ames (1961). The Stokes radius was determined from gel filtration experiments, and $s_{20,w}$ and v_c were determined form sucrose density gradient experiments.

The Stokes radius of the detergent-solubilized hormone–receptor complex was determined from a standard curve produced by plotting the Stokes radii of the standards versus their distribution coefficients (K_d):

$$K_d = \frac{V_e - V_0}{V_t - V_0} \quad (2)$$

The included volume (V_t) of the gel filtration column was determined with [^{3}H]H_2O. The void volume (V_0) was determined with blue dextran. The elution volumes (V_e) of the following standard proteins were used in calibrating the column (Table I): cytochrome c, ovalbumin, bovine serum albumin, catalase, γ-globulin, fumerase, and apoferritin. Triton X-100 did not affect the distribution coefficient of the standards.

Table I: Standard Proteins Used for Gel Filtration and Sucrose Density Gradient Centrifugation[a]

	a (nm)	v_c (cm^3/g)	$10^{13}s_{20,w}$ (s)	ref
alcohol dehydrogenase		0.73	7.4	Meunier et al., 1972
apoferritin	5.9			Edsall, 1953
bovine serum albumin	3.6	0.734	4.31	Edsall, 1953
catalase	5.0			Edsall, 1953
cytochrome c	1.9			Siegel & Monty, 1966
fumarase	5.3	0.738	9.09	Haga et al., 1977
γ-globulin	5.2	0.729	7.0	Edsall, 1953; Pollet et al., 1979
hCG		0.689	2.98	Bahl, 1973
lactate dehydrogenase		0.74	7.3	Haga et al., 1977
malate dehydrogenase		0.74	4.3	Haga et al., 1977
ovalbumin	2.8	0.748	3.66	Edsall, 1953; Smith, 1970

[a] Values for the Stokes radius (a), partial specific volume (v_c), and sedimentation coefficient ($s_{20,w}$) of standard proteins were taken from the indicated references.

Neither SDS nor increasing the temperature to 15 °C altered the V_t or V_0 of the column, so we assumed that the same calibration curve could be used for determining the Stokes radius of the hormone–receptor complexes solubilized with SDS.

To determine $s_{20,w}$ and v_c from sucrose density gradient experiments, it is necessary to know the density and viscosity of the gradient. Density is a linear function of the sucrose concentration and consequently of the refractive index. The refractive index of each fraction was measured at 24 °C with a Reichert Abbe Mark II refractometer. Density was determined from standard curves correlating refractive index measured at 24 °C with density at 4 °C. Viscosity is not a linear function of the sucrose concentration. Viscosity may be calculated from the sedimentation behavior of standard proteins whose $s_{20,w}$ and v_c are known. Viscosity, determined experimentally for standard proteins in gradients containing Triton X-100, was correlated with refractive index to produced standard curves. Proteins used for these calculations were (Table I) ovalbumin, malate dehydrogenase, bovine serum albumin, alcohol dehydrogenase, lactate dehydrogenase, and fumerase. Viscosity (Winsor, 1954), density, and refractive index were insignificantly affected by the substitution of SDS for Triton X-100. Thus, standard curves correlating refractive index with either the density or viscosity of solutions containing Triton X-100 should be the same as the corresponding solutions containing SDS. This was confirmed for the relationship between refractive index and density. However, SDS binds to proteins, and consequently alters the way they sediment. Therefore, the correlation between refractive index and viscosity could not be experimentally verified for solutions containing SDS, and we assumed that the relationship established for Triton X-100 containing solutions was applicable.

Values for the frictional ratio (f/f_0) were calculated by random pairing of values for the estimated Stokes radius from gel filtration experiments with the partial specific volume and molecular weight of the complex calculated from centrifugation experiments according to the equation (Siegel & Monty, 1966):

$$f/f_0 = a/[3v_cM_c/(4\pi N)]^{1/3} \quad (3)$$

Standard Proteins. Standard proteins used in these experiment are listed in Table I along with values for a, $s_{20,w}$, and v_c. Bovine serum albumin, cytochrome c, γ-globulin, hCG, and ovalbumin were radiolabeled and could be assayed by scintillation or γ spectrometry. Fumarase was assayed according to the method of Kanarek and Hill (1964). Apoferritin was assayed by the method of Lowry et al. (1951). Alcohol dehydrogenase, catalase, lactate dehydrogenase, and malate dehydrogenase were assayed as described in the Worthington Enzymes Manual (1982).

Other Methods. Adenylate cyclase was assayed as described previously (Rebois, 1982; Rebois & Patel, 1985). Protein was assayed by the method of Lowry et al. (1951).

Statistics. Centrifugation experiments were done at least 7 times in each medium. Gel filtration experiments were done at least 6 times. Values are reported as the mean ± the standard deviation. Confidence levels for differences between hydrodynamic parameters were determined by applying Student's t-test.

Results

From sucrose density gradient experiments, we determined that there was little, if any, free hormone in preparations solubilized with Triton X-100. These preparations generated two peaks of radioactivity on sucrose density gradients in both H_2O (Figure 1) and D_2O (Figure 2). The peak that had the majority of associated ^{125}I-hormone was taken to be the hormone–receptor complex. The other peak sedimented ahead of the hormone–receptor complex and presumably represents some aggregated hormone–GR complex. There was large variation in the portion of total ^{125}I-hormone associated with this peak but no apparent correlation between experimental conditions and the size of the peak. There was no significant difference ($p > 0.10$) between the partial specific volumes or the sedimentation coefficients of the control hCG–GR complex and the DG-hCG–GR complex (Table II).

Desensitization of the receptor caused an observable change in the sedimentation behavior of the hormone–receptor complex. Under the same experimental conditions, the desensitized hCG–GR complex sedimented more slowly in H_2O gradients than did control hCG– or DG-hCG–GR complexes (Figure

Table II: Summary of Hydrodynamic Properties for Detergent-Solubilized Hormone–Gonadotropin Receptor Complexes[a]

complex	detergent	a (nm)	v_c (cm^3/g)	$10^{13}s_{20,w}$ (s)	M_r[b]	f/f_0
control hCG–GR	Triton	6.2 ± 0.3	0.76 ± 0.02	7.3 ± 0.6	213 ± 30	1.53 ± 0.13
DG-hCG–GR	Triton	5.8 ± 0.3	0.77 ± 0.01	7.6 ± 0.3	213 ± 18	1.44 ± 0.09
desensitized hCG–GR	Triton	6.1 ± 0.2	0.71 ± 0.03[c]	6.6 ± 0.4[d]	158 ± 23	1.74 ± 0.15[d]
hCGXGR	SDS	4.9 ± 0.3[c]	0.75 ± 0.03	5.2 ± 0.5[c]	116 ± 23	1.52 ± 0.15

[a] hCG–GR complexes were formed under conditions that prevented (control hCG–GR) or favored (desensitized hCG–GR) desensitization or by binding DG-hCG to the receptor (DG-hCG–GR) before solubilization in Triton X-100. When SDS was used to solubilize the hCG–GR complex, it was first cross-linked (hCGXGR). The values for the Stokes radius (a), partial specific volume (v_c), sedimentation coefficient ($s_{20,w}$), molecular weight (M_r), and frictional ratio (f/f_0) were calculated as described under Materials and Methods. [b] M_r represents the molecular weight of the complex, which includes receptor, hormone, and detergent. [c] $p < 0.005$ when compared to control hCG–GR by applying Student's t-test. [d] $p < 0.01$ when compared to control hCG–GR by applying Student's t-test.

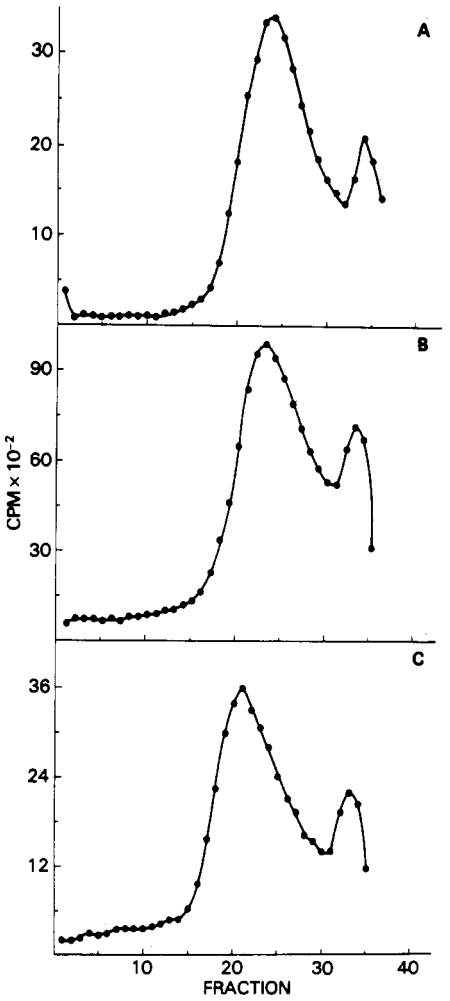

FIGURE 1: Sucrose density gradient centrifugation in H_2O. Triton X-100 solubilized (A) control hCG–GR, (B) DG-hCG–GR, and (C) desensitized hCG–GR complexes were prepared and centrifuged on sucrose density gradients in H_2O as described under Materials and Methods.

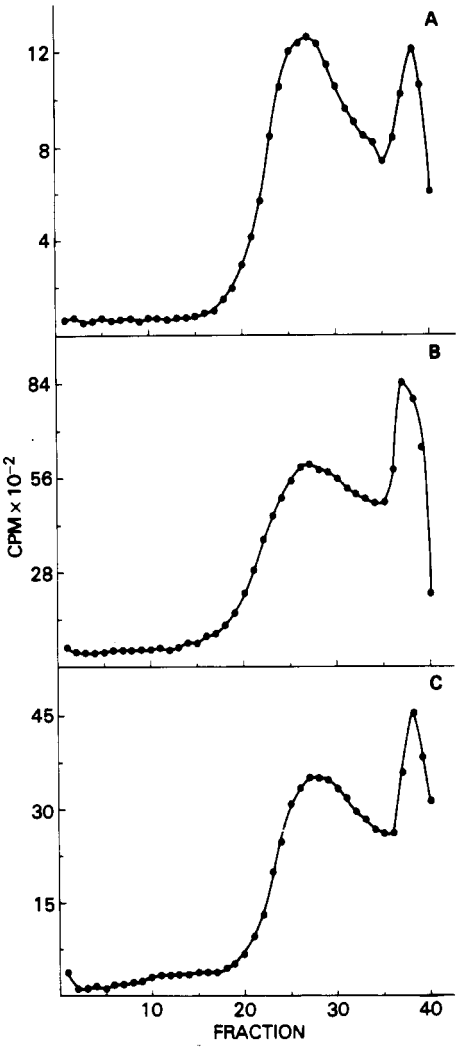

FIGURE 2: Sucrose density gradient centrifugation in D_2O. Triton X-100 solubilized (A) control hCG–GR, (B) DG-hCG–GR, and (C) desensitized hCG–GR complexes were prepared and centrifuged on sucrose density gradients in D_2O as described under Materials and Methods.

1). The rate of sedimentation in D_2O gradients (Figure 2) was not appreciably altered by desensitization. In the less dense H_2O gradients, the rate of sedimentation is faster than in D_2O gradients. Thus, the difference between the rate of sedimentation in H_2O and D_2O was reduced. This suggests an increase in the density of the complex [see eq 8 in Clarke and Farber (1973)] and is reflected in a decreased δ_c. An increase in density would normally result in an increased rate of sedimentation in H_2O gradients, but sedimentation is also dependent upon M_r and the Stokes radius (see eq 1). Gel filtration experiments indicate that the Stokes radius did not change (Figure 3 and Table II). Thus, desensitization must retard the sedimentation rate by reducing M_r. This change is more than enough to offset the increased density, so that the rate of sedimentation on H_2O was significantly slower than for control hCG–GR preparations. The rate of sedimentation in D_2O gradients should also be affected by the noted changes; however, the increased density and decreased molecular mass apparently compensate for each other in such a way that no change was observed in the sedimentation behavior of the desensitized complex in the D_2O gradients. No change in the Stokes radius in the wake of lower molecular mass and increased density can be accounted for by a change in the shape or hydration of the complex. We calculate an increased frictional ratio (Table II) for the desensitized hCG–GR complex when compared to the control hCG–GR complex, suggesting that desensitization has changed the hydration and/or conformation of the receptor molecule.

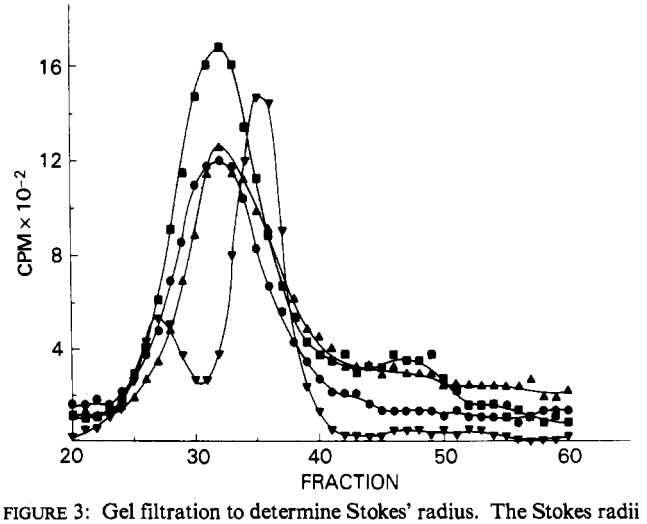

FIGURE 3: Gel filtration to determine Stokes' radius. The Stokes radii of detergent-solubilized hormone–GR complexes were determined by gel filtration as described under Materials and Methods. The curves represent (●) control hCG–GR, (▲) DG-hCG–GR, (■) desensitized hCG–GR solubilized with Triton X-100, and (▼) cross-linked hCG–GR solubilized with SDS.

The detergent, SDS, disrupted the noncovalent interaction of hCG with the GR, making it necessary to covalently

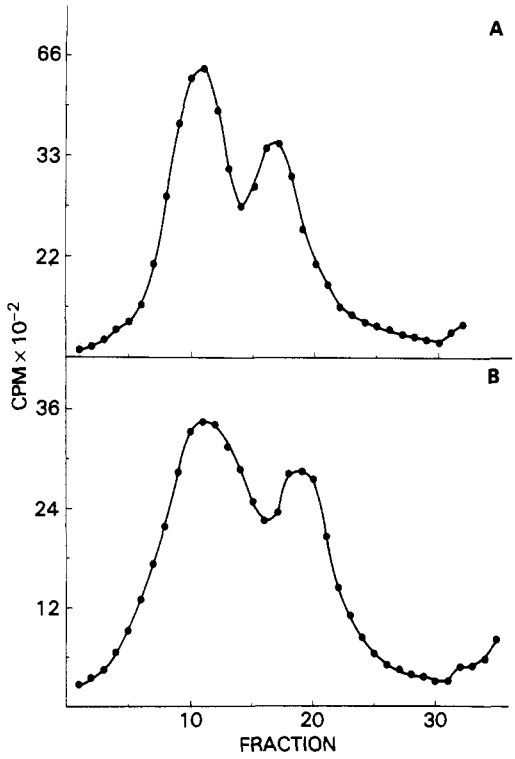

FIGURE 4: SDS-solubilized preparations on sucrose density gradients. SDS-solubilized hormone–receptor complexes were prepared and centrifuged on sucrose density gradient centrifugation in (A) H_2O and (B) D_2O as described under Materials and Methods.

cross-link the [^{125}I]hCG–GR complex before solubilization in SDS. Only a fraction of the hCG was cross-linked by this procedure. Much of the hCG that was not cross-linked could be removed by a low-pH wash. Sucrose density gradient analysis of preparations solubilized with SDS had two peaks of radioactivity (Figure 4). The peak that sediments more slowly on the gradient comigrated with free hCG, indicating that the low-pH wash was not completely effective in removing noncovalently bound hCG from membranes treated with the chemical cross-linker. The second peak was taken to be the covalently cross-linked [^{125}I]hCG–GR complex. The sedimentation coefficient for an SDS-solubilized complex was significantly less than that for a Triton X-100 solubilized complex (Table II). Cross-linking and the low-pH wash had no effect on the sedimentation properties of the Triton X-100 solubilized complex (data not shown). Solubilization with SDS also produced a complex with a significantly smaller Stokes radius when compared to Triton X-100 solubilized control hCG–GR (Table II).

We conducted experiments to determine if G_s and adenylate cyclase were coupled to the GR and, if so, what effect desensitization would have on the coupling. Triton X-100 solubilized preparations responded to forskolin, but not to hCG and only slightly to fluoride (Table III). We studied the sedimentation behavior of G_s. The α-subunit of G_s was identified by ADP-ribosylation with choleragen (Figure 5). This treatment did not alter the sedimentation characteristics of the DG-hCG–GR complex. The data indicate that the α-subunit of G_s in Triton X-100 solubilized preparations sediments more slowly than in the DG-hCG–GR complex.

Discussion

We have conducted hydrodynamic studies on gonadotropin receptors from MLTC-1 cells with three purposes in mind. First, by a careful study, we hoped to determine the molecular weight of the intact hCG–GR complex solubilized in nonionic detergent and the proportions of receptor, hormone, and detergent in the complex. Second, we wanted to determine if the ionic detergent, SDS, which is used in SDS–PAGE, is responsible for disrupting GR subunit interaction. Third, we wanted to determine if any changes occurred in the hydrodynamic properties of GR as a result of desensitization.

Table III: Stimulation of Adenylate Cyclase Activity in Triton X-100 Solubilized Preparations of MLTC-1 Cells[a]

activator	adenylate cyclase activity (pmol of cAMP/mg of protein)
none	13 ± 1
hCG	15 ± 1
fluoride	30 ± 1
forskolin	420 ± 70

[a] MLTC-1 cells were homogenized in a buffer containing 10 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 0.1 mM EGTA, and 1 mM dithiothreitol. Membranes were collected at 31000g for 20 min and solubilized in the same buffer containing 1% Triton X-100. Insoluble material was removed from the detergent extract by centrifugation at 100000g for 1 h. Samples of the soluble material containing 165 μg of protein were assayed for adenylate cyclase as described under Materials and Methods. The experiment was done 3 times, and the data represent the mean ± SD for triplicate determinations in a representative experiment.

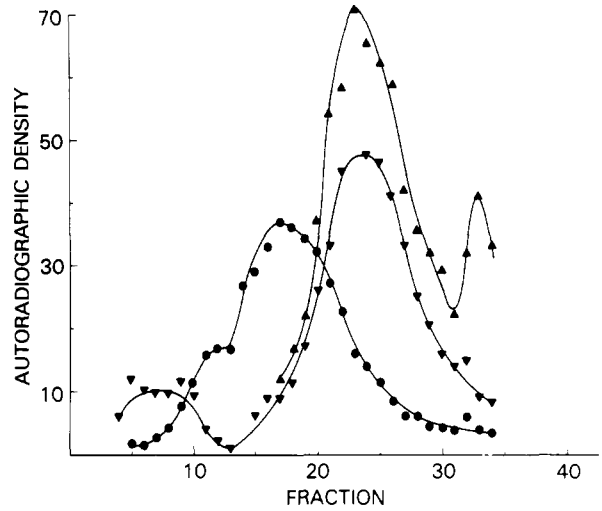

FIGURE 5: G_s α-subunit on sucrose density gradients. Sedimentation of G_s subunits and gonadotropin receptors on sucrose density gradients was determined as described under Materials and Methods. The curves represent (●) the α-subunit of G_s and the gonadotropin receptor in preparations that had (▼) or had not (▲) been treated with choleragen.

We have found from our hydrodynamic studies that the value for the Stokes radius of the Triton X-100 solubilized hCG–GR complex agrees with previously published values (Dufau et al., 1973; Metsikko, 1984). The same was true for the sedimentation coefficient (Dufau et al., 1973). We estimated from the hydrodynamic data a M_r of 213 000 for the complex. This agrees with estimates from our laboratory, determined in less rigorous studies (Rebois et al., 1981), and from other laboratories (Dufau et al., 1973). As in previous studies (Rebois, 1981) we observed a complex at the bottom of H_2O and D_2O gradients that appears to represent receptor aggregates.

From published data, the partial specific volume of the receptor was calculated to be 0.724 (Dattatreyamurty et al., 1983). The partial specific volume for hCG and Triton X-100 are 0.689 (Bahl, 1973) and 0.91 (Pollet et al., 1981), respectively. Each of the components, detergent, hormone, and receptor, contributes to the experimentally determined partial specific volume of 0.76. Assuming that only one hCG molecule (M_r 38 000) is associated with the complex and attributing all

of the experimental error to uncertainty about the molecular weight of the receptor itself, we estimate the receptor alone to be 126 ± 30 kDa. The remaining weight of the complex, approximately 49 kDa, is attributable to Triton X-100 (average M_r 647) and suggests that the complex binds approximately 76 mol of detergent for each mole of receptor. If DG-hCG [M_r 28 000 and v_c = 0.716, calculated from the published molecular composition; see Manjunath and Sairam (1982)] was used as the radiolabeled tracer for binding to GR, similar results (M_r = 131 000 ± 18 000) were obtained.

When the hCG–GR complex was first cross-linked and then solubilized in SDS, its estimated molecular weight was significantly less than that of the Triton X-100 solubilized complex. This result is not attributable to either the cross-linking or the low-pH wash since these steps did not affect the hydrodynamic properties of the Triton X-100 solubilized cross-linked complex. The relative contribution of the individual components in the SDS-solubilized hCG–GR complex can be calculated just as they were for the Triton X-100 solubilized complex. In doing the calculations, we used the same assumptions, and in addition we assumed that only the α-subunit of hCG [v_c = 0.69 calculated from the structure; see Birken and Canfield (1978)] was cross-linked to the complex (Rebois et al., 1981), contributing approximately 15 kDa. SDS, with a partial specific volume of 0.864 (Reynolds & McCaslin, 1985), contributes approximately 25 kDa to the complex. This corresponds to approximately 87 mol of detergent (SDS = 288 Da) per mole of receptor. The balance of the complex we attribute to the hormone-binding subunit of the GR with an estimated M_r of 76 000 ± 23 000. This value agrees with a number of published reports regarding the M_r of the GR hormone-binding subunit determined by using SDS–PAGE. This subunit appears to be all that is required for the binding of hCG (Keinanen et al., 1987; Wimalasena et al., 1986), and intrachain disulfides are critical for the retention of its binding activity (Keinanen et al., 1987). There remains a difference of approximately 50 kDa between the Triton X-100 solubilized receptor and the receptor solubilized in SDS. Thus, the GR receptor appears to contain more than one polypeptide chain, and SDS effectively disrupts this interaction. The interaction between the proposed subunits is noncovalent since sulfhydryl reagents are not required to give this result. SDS also disrupts the interaction of hormone with the receptor, and the interaction can only be preserved by covalently cross-linking the complex. Cross-linking was unable to preserve the interaction of the hormone-binding subunit with the other subunit(s) of the receptor, indicating that they may be inaccessible under the experimental conditions used in these studies. Furthermore, our results suggest that the additional subunit(s) is (are) not equal in size to the hormone-binding subunit, though we cannot rule this possibility out statistically.

The presence of two dissimilar subunits, a hormone-binding subunit of approximately 76 kDa and a second component of approximately 50 kDa, is consistent with the observations of other investigators. Dattatreyamurty et al. (1983) have purified GR from bovine corpora lutea and shown two components with molecular weights of 85 000 and 35 000. Wimalasena et al. (1985) also purified GR from porcine corpora lutea using different techniques. They found two components and, on the basis of intensity of staining and radioiodination, designated them major and minor components. The differential staining and radiolabeling may be due to the chemical composition of the minor component rather than its quantity. In a subsequent study (Wimalasena et al., 1986), they identified the major component as having M_r 77 000 and the minor component as being 66 000 on reducing SDS–PAGE. On nondenaturing gels, the purified GR retained its ability to bind hormone and enabled the investigators to identify receptors of higher molecular weight. These they attribute to homooligomers of the major component, though the larger receptor species could also be accounted for by receptors composed of the 77- and 66-kDa subunits.

The response of MLTC-1 cells to gonadotropin undergoes a time-dependent attenuation referred to as desensitization (Rebois & Fishman, 1986). We found that desensitization had significant effects on the hydrodynamic properties of the GR. There was a decrease in the partial specific volume as well as a significant decrease in its molecular weight with no change in the Stokes radius. Since G_s does not seem to be associated with the receptor before desensitization, we cannot attribute the change in molecular weight to dissociation of G_s from GR during desensitization. Applying the same assumptions used for the control hCG–GR complex, we calculate the molecular weight of the receptor alone to be 125 000 ± 23 000, suggesting that desensitization did not alter the molecular mass of the receptor itself. Curiously, the receptor and bound hormone taken together account for almost the entire mass of the complex, suggesting that little or no detergent is associated with the complex. This seems to be a tenuous conclusion since the receptor is considered to be a transmembrane protein. However, detergent is not necessary to solubilize a large portion of the gonadotropin receptor from rat ovary (Wimalasena & Dufau, 1982), suggesting that some forms of the receptor do not require bound detergent to remain in solution. The changes in the values for the frictional ratio of GR may reflect changes in the hydration of receptor or changes in the conformation with the receptor becoming more elongated or flattened, as a result of desensitization. These observations suggest that desensitization leads to changes that make the receptor more hydrophilic.

At low temperatures hCG is able to activate adenylate cyclase (Rebois & Fishman, 1986), albeit at a reduced rate, but is unable to change the hydrodynamic properties of the receptor. But altered hydrodynamic properties were observed under conditions that resulted in desensitization of the hormone response. These data make it likely that the proposed changes in hydrophilicity and molecular shape are associated with events other than the activation of adenylate cyclase. Desensitization may reflect a reduced ability of the altered receptor to couple to components of the adenylate cyclase system (Rebois & Fishman, 1986). In addition, the changes may facilitate removal of the occupied receptor from the membrane during subsequent down-regulation (Rebois & Fishman, 1984), and/or they make the GR more susceptible to proteolysis (Kellokumpu & Rajaniemi, 1985). If the latter circumstance exists, it may explain the heterooligomeric structure proposed by some investigators. The first step in some studies is to bind the hormone to the receptor on intact cells or to membranes under conditions where the changes that we have observed would occur. If proteolysis of the receptor ensues, it could generate the observed patterns on SDS–PAGE. Whether or not the changes that occur during desensitization are brought about by covalent modification of the receptor, as has been suggested (Rebois & Patel, 1985; Rebois & Fishman, 1985), is still under investigation.

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