SOLUBLE GONADOTROPIN RECEPTORS OF THE RAT OVARY

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1. Introduction

Previous studies on the LH/hCG gonadotropin receptors extracted from adult rat testes with the nonionic detergent Triton X-100 have shown that such solubilized receptors behave as a 6.5 S macromolecule of Stokes radius 64 Å [1]. The hormone–receptor complex formed by equilibration of solubilized testis receptors with ^{125}I-labeled human chorionic gonadotropin (hCG) possessed a sedimentation constant of 7.5 S, and the complex extracted with Triton X-100 from prelabeled testis binding particles behaved as an 8.8 S species [3]. In all cases, the Stokes radius of the solubilized receptor molecules determined by gel filtration on columns of Sepharose 6B was 64–67 Å.

Gonadotropin receptors of the rat ovary have been previously shown to bind ^{125}I-labeled hCG with specificity and affinity equivalent to that of the particulate testis receptors [4]. In addition, the ovarian gonadotropin receptors of PMS/hCG-treated immature female rats have been extracted from particulate binding preparations with Triton X-100, and retention of hormonal specificity and high affinity for LH and hCG has been demonstrated [5]. In the present studies, the common receptors for LH and hCG were extracted with Triton X-100 from particulate ovarian binding fractions prepared after homogenization of ovaries from PMS/hCG-treated immature female animals [4, 5], and characterized by gel filtration and density gradient centrifugation.

2. Materials and methods

Pseudopregnant rat ovaries were homogenized in an all-glass homogenizer with two volumes of phosphate-buffered saline (PBS); after centrifugation at 120 *g* for 30 min at 4°C, the supernatant solution was recentrifuged at 20 000 *g* for 30 min at 4°C. The supernatant solution was then discarded, and the pellet was either (a) solubilized by resuspension in 1% Triton X-100 in PBS, for 30 min at 4°C, then diluted to 0.1% Triton with PBS; or (b) resuspended in PBS and equilibrated with ^{125}I-labeled hCG (40 μCi/μg; 20 ng per 3 ovaries) for 16 hr at 4°C, followed by washing three times with 30 ml of cold PBS to remove free hCG. The labeled pellet was then resuspended in 1% Triton X-100 for 30 min at 4°C to solubilize the preformed hormone–receptor complex.

The soluble receptors extracted from unlabeled ovarian particles were diluted to a final volume equivalent to 25 ml per ovary with 0.1% Triton X-100 in PBS, and employed for subsequent binding studies. Binding-inhibition curves were performed with 50 000 cpm (1 ng) of ^{125}I-labeled hCG, and increasing concentrations of unlabeled hCG, from 1–100 ng per assay tube. After incubation at 4°C for 16 hr, separation of receptor-bound and free tracer hCG was performed by precipitation with polyethylene glycol (PEG) as previously described [1, 2]. Gel filtration on columns of Sepharose 6B, and sucrose density gradient centrifugation were performed as previously described [2].

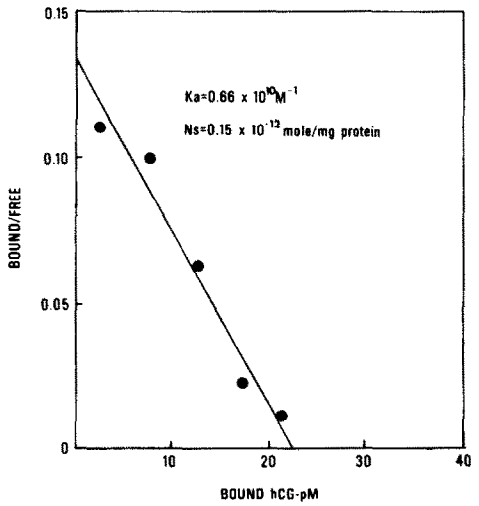

Fig. 1. Scatchard plot of the binding-inhibition data derived by incubation of Triton-solubilized ovarian receptors with ^{125}I-labeled hCG and increasing concentrations (1–100 ng) of unlabeled hCG, at 4°C for 16 hr.

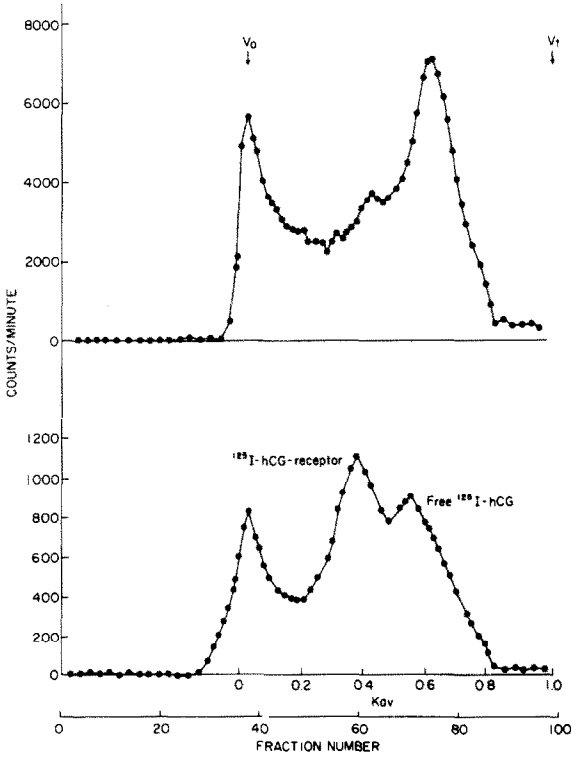

Fig. 2. Gel filtration on Sepharose 6B (1× 60 cm) of solubilized ovarian receptors following equilibration with ^{125}I-labeled hCG for 16 hr at 4°C. The large peak of aggregated material at the void volume (V_o) is followed by a shoulder of hCG–receptor complex and a larger peak of free ^{125}I-labeled hCG (above). Concentration and re-filtration of the hormone–receptor region gave an elution profile characterized by a major peak of the complex between an aggregated front peak and a peak of free hCG (below).

3. Results and discussion

Scatchard plots of the data obtained from such binding-inhibition studies revealed a single order of binding sites with $K_a = 0.5–1 \times 10^{10}$ M^{-1} (fig. 1). The binding capacity of the soluble ovarian receptor fraction was approximately 0.15×10^{-12} moles per mg protein. Gel filtration of the equilibrium mixture of the hormone–receptor complex and free hormone, on columns of Sepharose 6B equilibrated with 0.1% Triton X-100 in 50 mM Tris–HCl buffer, pH 7.4, showed a small peak of hormone–receptor complex ($K_{av} = 0.36$) followed by a more retarded peak of free ^{125}I-labeled hCG ($K_{av} = 0.56$) (fig. 2, above). The hormone–receptor peak was abolished by previous incubation with excess unlabeled hCG (20 μg), and was precipitable with 11% PEG, previously shown to be suitable for precipitation of the testicular hormone–receptor complex formed after extraction of Leydig cell binding fractions with Triton X-100 [2]. In addition, the ovarian hormone–receptor complex was adsorbed by Blue Dextran during gel filtration, as previously observed during characterization of the Triton-solubilized testicular hormone–receptor complex [2]. Concentration and re-filtration of the fractions containing the ovarian hormone–receptor complex on Sepharose 6B (fig. 2, below) showed a more prominent peak of the complex, followed by a smaller peak of free hCG. Unlike the pattern obtained with the testicular hormone–receptor complex, a prominent peak of aggregated material was observed during refiltration of the ovarian hormone–receptor complex. The Stokes radius of the ovarian complex ($K_{av} = 0.36$) was determined to be 60 Å by reference to standard proteins, including bovine serum, albumin, myoglobin, bovine gamma globulin, thyroglobulin, and apoferritin.

Sucrose density gradient centrifugation of the hormone–receptor complex formed by equilibration of the Triton-extracted ovarian receptors with ^{125}I-labeled hCG revealed a 7.5 S peak of hormone–receptor complex, followed by a 2.9 S peak of free hCG (fig. 3).

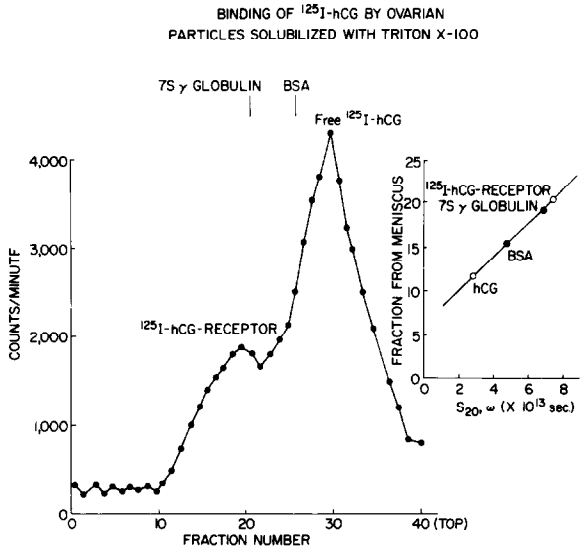

Fig. 3. Density gradient centrifugation of solubilized receptors equilibrated with ^{125}I-labeled hCG, on 5–20% sucrose in 50 mM Tris–HCl buffer, pH 7.4 containing 0.1% Triton X-100. The 7.5 S hormone–receptor peak was abolished by prior incubation with excess unlabeled hCG.

Again, the hormone–receptor peak was abolished by previous equilibration with excess hCG (20 μg). The density of the 7.5 S ^{125}I-labelled hCG–receptor complex during isopycnic density gradient centrifugation in cesium chloride was 1.2859. The apparent molecular weight calculated from these determinations, by the equations derived by Siegel and Monty [6], neglecting the solvation factor, was 228 000; the frictional ratio of the complex was 1.44.

In addition to these studies on the 7.5 S hormone–receptor complex formed by equilibration with hCG after Triton extraction, the free or unlabeled receptors extracted with Triton X-100 were also subjected to gel filtration on Sepharose 6B, and detected in the eluent fractions by subsequent binding of ^{125}I-labeled hCG. This experiment showed a single symmetrical peak of binding activity with K_{av} = 0.36, equivalent to a Stokes radius of 60 Å (fig. 4). Sucrose density gradient centrifugation of the free ovarian receptors solubilized with Triton X-100 was rendered difficult by the presence of large amounts of lipid in the extracts, but on two occasions gave peaks of binding activity with sedimentation constants of 6.0 S and 6.7 S. These values are comparable with that of the free testicular receptor

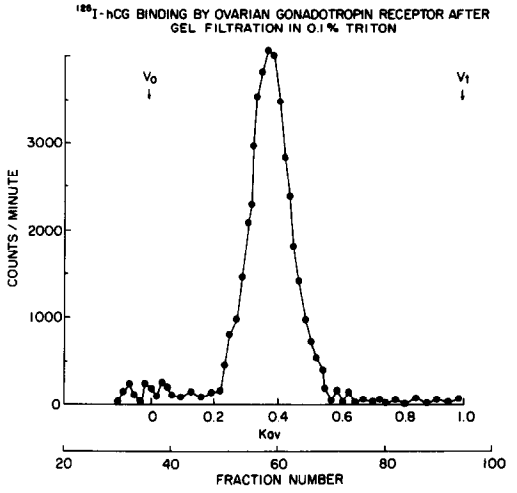

Fig. 4. Gel filtration of free ovarian gonadotropin receptors on Sepharose 6B (1× 60 cm). The peak of binding activity (K_{av} = 0.36) was localized by binding assay of the eluent fractions by equilibration with ^{125}I-labeled hCG for 16 hr at 4°C, followed by PEG precipitation of the bound complex.

extracted by Triton X-100, for which a sedimentation coefficient of 6.5 S was derived [2].

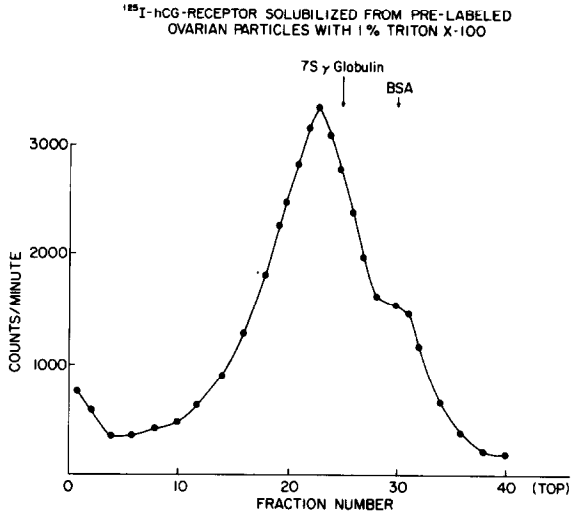

Fig. 5. Density gradient centrifugation on 5–20% sucrose of prelabeled ovarian receptors solubilized with Triton X-100. The major peak of radioactivity corresponding to the 8.8 S hormone–receptor complex was not present when the preceding incubation with ^{125}I-labeled hCG was performed in the presence of excess hCG (20 μg).

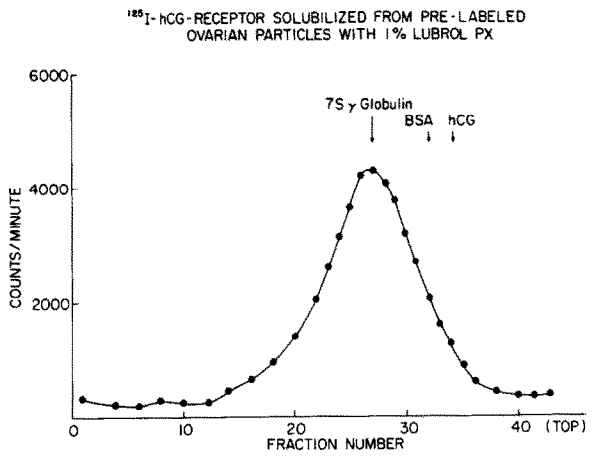

Fig. 6. Density gradient centrifugation on 5–20% sucrose of prelabeled ovarian receptors extracted with Lubrol PX. The solubilized hormone–receptor complex behaved as a single species with sedimentation coefficient of 7.5 S.

The hormone–receptor complex extracted from prelabeled ovarian particles was significantly different from the post-labeled receptor complex during gel filtration and sucrose density gradient centrifugation. On Sepharose 6B columns, the K_{av} of the prelabeled complex was 0.30, equivalent to a Stokes radius of 71 Å. During density gradient centrifugation, the complex manifested a sedimentation coefficient of 8.8 S (fig. 5), and the density on isopycnic gradient centrifugation was 1.2734. Extraction of labeled ovarian particles with another non-ionic detergent, Lubrol PX, gave a hormone–receptor complex of sedimentation coefficient 7.5 S, analogous to that observed on similar extractions of labeled testis particles (fig. 6).

These studies on the gonadotropin receptors of the rat ovary have revealed marked similarities between the detergent-extracted species and that derived from the interstitial cells of the rat testis (table 1). Such findings indicate that the structural properties of the two gonadal receptors are closely similar, as may be

Table 1
Comparison of physico-chemical characteristics of solubilized testicular and ovarian receptors.

Parameter	Ovarian receptors	Testicular receptors
Association constant (K_a)	0.66×10^{10} M^{-1}	0.60×10^{10} M^{-1}
Binding capacity of initial extract	0.15×10^{-12} mole/mg protein	0.12×10^{-12} mole/mg protein
Stokes radius:		
Free receptor	60 Å	64 Å
hCG–receptor complex formed after extraction	60 Å	64 Å
hCG–receptor complex extracted from prelabeled particles	71 Å	64 Å
Sedimentation constant:		
Free receptor	6.0–6.8 S	6.5 S
hCG–receptor complex formed after extraction	7.5 S	7.5 S
hCG–receptor complex extracted from prelabeled particles	8.8 S	8.8 S
Density:		
7.5 S hCG–receptor complex	1.283	1.289
8.8 S hCG–receptor complex	1.273	1.278

expected from the high sensitivity and specificity of both testicular and ovarian cells to stimulation by LH and hCG [7, 8]. Receptors extracted from both tissues with Triton X-100 show detectable loss of binding affinity in the soluble form (approximately 50% of that of the particulate receptor), but retain hormonal specificity and exhibit several similarities during physicochemical characterization. The only significant difference observed in the present studies was the slightly higher apparent hydrodynamic radius of the Triton-extracted ovarian hormone–receptor complex (71 Å vs 64 Å), though the sedimentation constants of the two species were identical (7.5 S). Although further fractionation of the two gonadal receptors may reveal individual differences between the specific gonadotropin binding sites of each tissue, the present results suggest that a common macromolecular configuration is shared by the gonadotropin receptors extracted from testis and ovary with Triton X-100.

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