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Solubilization and Characterization of a Lactogenic Receptor From Human Placental Chorion Membranes

Christopher J. Ormandy, Christine L. Clarke, and Robert L. Sutherland

Garvan Institute of Medical Research, St. Vincent's Hospital, Sydney, New South Wales 2010, Australia

Prolactin has a wide range of actions, including osmoregulation and the control of mammary gland development and lactation. These effects are mediated through a high-affinity cell surface receptor, which has been well characterized in a number of animal tissues. The molecular characteristics of the human receptor are unknown, however. The present studies were initiated, therefore, to determine the binding and molecular characteristics of the lactogenic receptor of human placental chorion membranes. Subcellular fractionation studies showed that the bulk of the receptor sedimented in the microsomal fraction at 45,000g_{av}. Endogenous ligand was dissociated from the receptor with 3.5 M $MgCl_2$ or 0.05 M acetate buffer (pH 4.8) with preservation of binding activity. The microsomal receptor bound human growth hormone (hGH), human prolactin (hPRL), ovine prolactin (oPRL), and human placental lactogen (hPL) but not non-primate growth hormones, indicating a narrow specificity for lactogenic hormones. The binding was only partially reversible in agreement with the known binding kinetics of animal lactogenic receptors. The receptor was solubilized with 45% yield from the microsomes using 16 mM 3-[(3-cholamidopropyl)dimethylammonio]-1-propane sulphonate (CHAPS) detergent-250 mM NaCl, and the binding activity was fully restored by a two-fold dilution in the binding reaction to reveal a K_D of 0.8 nM for hGH and a binding capacity of 200 fmol of specifically bound hGH per mg of microsomal protein. Gel filtration chromatography indicated the minimum molecular weight of the ligand-receptor complex was approximately 60,000 daltons, and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of covalently cross-linked ^{125}I-hGH-receptor complexes revealed a molecular size of 58,000 daltons. When account was taken of the contribution of the ligand, a molecular weight of 36,000 for the receptor's binding domain was obtained. These data indicate that the chorion lactogenic receptor has very similar binding and molecular characteristics to the lactogenic receptors from other mammalian species. Chorion membranes are thus a convenient source of material for the further purification and characterization of the human lactogenic receptor.

Key words: placentae, prolactin, growth hormone

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Prolactin is an anterior pituitary hormone with a wide range of biological actions, which are mediated through binding to a specific high-affinity receptor located on the target cell plasma membrane [1,2]. In mammals, prolactin affects the physiology of a wide spectrum of tissues including the prostate and testis in males and the ovaries and mammary glands in females.

The lactogenic receptor of animal origin has been well characterized: In neoplastic tissue, extensive studies of carcinogen-induced rat mammary carcinoma have established that prolactin regulates tumour growth rates and development [3]. The animal receptor has been purified [4–6] and several molecular weight forms have been identified by affinity labeling, allowing analysis of possible receptor subunit interactions in the holoreceptor [7–10]. More recently, the receptor protein has been purified and partial sequence data obtained for the rat liver and rabbit mammary gland [11–13]. These data have facilitated cDNA cloning of the rat receptor that corresponds to a protein of 291 amino acids. This clone has been used to identify cDNAs from rabbit mammary gland and human hepatoma cDNA libraries coding for proteins of 592 and 598 amino acids respectively [14]. Recently, three different lactogenic receptor mRNA sequences have been identified in mouse liver [15], indicating that lactogenic receptors may be a family of related proteins.

The lactogenic receptor has wide tissue distribution in humans [16–22] and has been studied in most detail in human breast cancer, where up to 70% of tumour biopsies are lactogenic receptor positive [23,24]. Lactogenic receptor levels are correlated with oestrogen receptor levels in breast tumours and breast cancer cell lines [25], and the lactogenic receptor has been examined as a prognostic indicator in breast carcinoma [26,27]. Prolactin has been proposed as a mitogen for cultured breast and prostate cancer cells [28–33].

Although these data indicate a potentially important role for lactogenic hormones in human neoplasia as well as in normal human physiology, the human receptor has received scant attention at the molecular level. Little has been published concerning solubilization of the receptor, and the receptor's subunit structure and molecular weight are unknown.

In this paper, we report the initial partial characterization of the human lactogenic receptor, describing the conditions required for solubilization of the human placental chorion receptor with retention of binding activity and the molecular weight of this receptor as assessed by affinity cross-linking and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).

EXPERIMENTAL PROCEDURES

Chemicals

3-[(3-cholamidopropyl)dimethylammonio]-1-propane sulphonate (CHAPS), bovine gamma globulin, bovine serum albumin-RIA grade (BSA), Tris (hydroxymethyl)aminomethane hydrochloride (Tris-HCl), chloroamine T, and sodium metabisulphate were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.); disuccinimidyl suberate was from Pierce Chemical Co (Rockford, IL, U.S.A.); $Na^{125}I$ from Amersham (Australia); Sephacryl S-200, superose 6 and superose 12 FPLC columns were from Pharmacia Fine Chemicals (Uppsala, Sweden); dimethylsulphoxide from Merck (Darmstadt, Germany); and SDS-PAGE reagents from Bio-Rad (Richmond, CA, U.S.A.).

Polyethyleneglycol-6,000 (PEG) was from BDH (Kilsyth Vic). All other chemicals were of AR grade from BDH or Sigma. All buffers were adjusted to the stated pH at 22°C.

Hormones and Iodination

The preparation of human growth hormone (hGH) and human prolactin (hPRL) and 20 K hGH has been described previously [34,35] and were kindly donated by Dr. G.E. Chapman. Human placental lactogen (hPL) purified as previously described [36] was kindly donated by Dr. L.C. Teh. Ovine growth hormone (oGH) (NIAMDD-oGH-12) and ovine prolactin (oPRL) (NIAMDD-oPRL-16) were gifts of the National Hormone and Pituitary Program (National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD, U.S.A.). Porcine growth hormone (pGH) (USDA-pGH-B-1), porcine prolactin (pPRL) (USDA-pPRL-B-1), bovine growth hormone (bGH) (USDA-bGH-B-1), and bovine prolactin (bPRL) (USDA-bPRL-B-1) were gifts of the United States Department of Agriculture. Hormones were dissolved at 1 mg/ml in 1 mM NaOH, then immediately diluted in 25 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, 0.1% (w/v) BSA (TMB buffer) to the required concentration. Human GH and oPRL were iodinated [37] to a specific activity of 50–70 Ci/g, and the mixture was immediately chromatographed on Sephacryl S-200 (30 × 0.9 cm) in TMB buffer. Iodinated hGH was aliquoted (250 μl + 50 μl ethanol) and stored at −20°C. The ^{125}I-hGH was rechromatographed using Sephacryl S-200 on the day of use.

Tissue Collection and Homogenization

Placentae were obtained immediately after birth from the Royal Hospital for Women, Paddington, Sydney. Chorion membranes were separated from the amnion membrane and cut approximately 1 cm from the decidua basalis. The chorion was washed in 25 mM Tris-HCl pH 7.4, 0.3 M sucrose (TS), and stored at −20°C. The chorion membranes (150 g) were homogenized from the frozen state in a final volume of 750 ml of ice cold TS for 1 min at top speed using a kitchen blender, followed by a Polytron PTA 36/4 at setting 3 for 5 min (continuous), with cooling in an ice water bath.

Subcellular Fractionation

The homogenate was centrifuged at $200g_{av}$ for 20 min, and the pellet (designated 200p) and surface layer (designated 200s) were retained. The remaining homogenate was then centrifuged sequentially at $800g_{av}$ (20 min), $5{,}000g_{av}$ (20 min), $15{,}000g_{av}$ (20 min), $45{,}000g_{av}$ (60 min), and $100{,}000g_{av}$ (60 min), retaining the pellet after each centrifugation. All centrifugations were at 4°C using a Beckman JA-14 rotor (200–$15{,}000g_{av}$) or a Beckman Ti-45 rotor ($45{,}000$–$100{,}000g_{av}$). The pellets were resuspended in 25 mM Tris-HCl, pH 7.4 (T buffer), halved and centrifuged at $100{,}000g_{av}$ 60 min at 4°C. One of the pellets was resuspended in 5 M $MgCl_2$ for 20 min at 4°C, then diluted to 1 M $MgCl_2$ with T buffer and centrifuged at $100{,}000g_{av}$ 60 min at 4°C. Both pellets were then resuspended in T buffer, assayed for protein [38], adjusted to 10 mg/ml protein with T buffer, and assayed for ligand binding. Receptors in the supernatant of the $100{,}000g_{av}$ fraction were assessed by tenfold concentration in a centricon-10 concentrator (Amicon) and assay of ^{125}I-hGH binding by the method for solubilized receptors (see below). Unbound hormone was separated from bound using PEG, or the mixture was loaded onto superose 12 and chromatographed as described below.

Microsomes were routinely prepared by retaining the pellet of the $100{,}000g_{av}$ centrifugation after previous centrifugation at $800g_{av}$.

Stripping of Endogenous Ligands

Chorion microsomes were resuspended in TM buffer, and 5 mg of protein was aliquoted into tubes and pelleted by centrifugation at $3500g_{av}$ for 20 min at 4°C. The pellets were resuspended at 4°C in 1 ml of 0.05 M ammonium acetate/acetic acid buffer at various pHs for 10 min or 1 ml of various concentrations of $MgCl_2$ for 20 min followed by centrifugation and two resuspensions-centrifugations in TM buffer to wash the treated microsomes. The pellets were resuspended in 500 μl of TM buffer, and the binding of ^{125}I-hGH and protein was then measured in each pellet by the method described below. Changes in specific binding due to treatment are expressed as a percentage of the untreated control specific binding.

Assay of Microsomal and Solubilized Receptors

Microsomal membranes (1 mg protein in 100 μl of 25 mM Tris-HCl, 10 mM $MgCl_2$ [TM buffer]) were incubated for 20 h at 20°C with 60,000 cpm (approximately 0.03 nM final concentration) of iodinated ligand (in 100 μl of TMB buffer) in the absence or presence of various concentrations of uniodinated ligand (in 100 μl of TMB buffer). The final volume was adjusted to 500 μl with TMB buffer. The reaction was terminated by the addition of 1 ml of ice cold TMB buffer, and the microsomes were immediately collected by centrifugation ($3500g_{av}$, 20 min). Tubes were inverted, drained, and counted in an LKB 1282 Compugamma for 1 min. Specific binding is expressed as total binding less the nonspecific binding measured in the presence of 1 μg/ml of unlabeled hGH.

Solubilized microsomes (1 mg protein in 250 μl of 16 mM CHAPS, 250 mM NaCl, TM buffer) were assayed as above. To precipitate the receptor-ligand complex, 500 μl of ice cold 0.1% (w/v) bovine gamma globulin in TM was added, followed by 1 ml of 32% (w/v) polyethylene glycol in 0.1 M phosphate buffer pH 7.4. Tubes were vortexed, then centrifuged, and counted as described above.

Solubilization of Microsomal Receptors

Microsomes that were resuspended at 10 mg/ml in TM were made 250 mM with NaCl, and 16 mM with powdered CHAPS. The mixture was stirred gently for 30 min at 26°C, then centrifuged at $100{,}000g_{av}$ in a Beckman 45 Ti rotor for 60 min. The clear supernatant was separated from the pellet and used immediately. Initial experiments tested a range of CHAPS and NaCl concentrations, both added as powder under the conditions described above. Specific binding was measured in the pellet and solubilized supernatant after centrifugation ($100{,}000g_{av}$, 4°C, 60 min). Results were expressed as the percentage of total binding extracted into the supernatant.

Gel Chromatography of the Receptor-Ligand Complex

Receptors solubilized with 16 mM CHAPS but no NaCl were diluted to 8 mM CHAPS with TMB and were combined with 60,000 cpm of ^{125}I-hGH per mg protein in the presence and absence of 1 μg/ml of unlabeled hGH for 20 h at 20°C followed by fivefold concentration in a centricon-10 concentrating unit. Five hundred microliters of each incubate was loaded onto a superose 12 or superose 6 column and eluted at room temperature at 0.5 ml/min with 8 mM CHAPS in 10 mM sodium phosphate buffer pH 7.4. Fractions (400 μl) were collected, and the radioactivity counted.

Covalent Cross-Linking of the Receptor-Ligand Complex

Peak fractions from superose 12 chromotography were pooled, concentrated to 2 ml in a centricon-10 concentrator, and made 0.5 mM with disuccinimidyl suberate from a freshly prepared 25 mM solution in dimethylsulphoxide, and incubated on ice for 15 min, followed by the addition of 200 μl of 2 M Tris-HCl pH 7.4. The mixture was analysed by SDS-PAGE.

SDS-PAGE

Samples were made 3% (w/v) with SDS and 5% (v/v) with β-mercaptoethanol and denatured at 90°C for 5 min in a one-fifth volume of 0.06 M Tris-HCl pH 6.8 containing glycerol 10% (v/v). Bromophenol blue was added to 0.01% (w/v), the sample was microfuged and loaded onto 7.5% (w/v) 1.5 mm thick polyacrylamide gels according to the method of Laemmli [39]. Pharmacia low molecular weight standard proteins and Bio-Rad pre-stained standard proteins were used as markers. Gels were dried between dialysis membranes and autoradiographed with a Cronex Lightening-Plus intensifying screen and Kodak X-Omat Film at −70°C. Autoradiographs were analysed by laser absorption densitometry.

RESULTS

Subcellular Fractionation

The lactogenic receptor from human chorion sedimented over the entire range of centrifugal forces studied but was most enriched in the 45,000g_{av} pellet after $MgCl_2$ treatment (Fig. 1). Treatment with 5 M $MgCl_2$ resulted in an increase in specific binding per mg of protein in all fractions and especially in the 800 and 45,000g_{av} pellets. No binding activity was detected in the cytosol by PEG precipitation or superose chromatography. Almost 40% of the total binding recovered remained in the 200g_{av} fractions and was composed mainly of unhomogenized material. Maximal recovery of the receptor

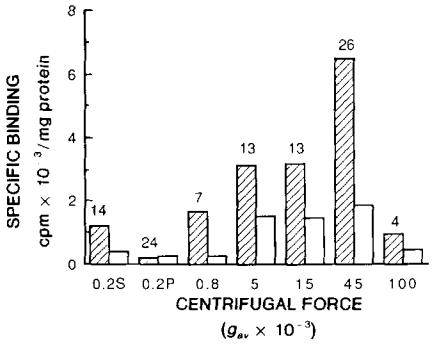

Fig. 1. Subcellular fractionation of human chorion membrane homogenates. Chorion membranes were homogenized in 25 mM Tris-HCl pH 7.4, 0.3 M sucrose at 4°C, and centrifuged sequentially at the centrifugal forces indicated. Pelleted material was assayed for specific ^{125}I-hGH binding with (crosshatched bars) and without (open bars) prior treatment with 5 M $MgCl_2$, as described in the experimental section. S and P refer to the pellet (P) and surface (S) layer of the 200g_{av} fraction. No binding was found in the 100,000g_{av} supernatant fraction. Numbers above the crosshatched bars represent the percentage of the total specific binding recovered in that fraction.

(approximately 50% of detected binding after $MgCl_2$ treatment) was achieved by first centrifuging at $800g_{av}$ and then at $100{,}000g_{av}$ prior to treatment of the $100{,}000g_{av}$ pellet with 3.5 M $MgCl_2$.

Stripping of Endogenous Ligands

The ability of $MgCl_2$ and pH to increase the specific binding of ^{125}I-hGH to microsomes was investigated by treating the microsomal receptor with $MgCl_2$ (0.5–5 M) or pH (4–7) and assaying for ligand binding. The method employed measured the increase in specific binding sites independently of protein loss. Figure 2 shows that a threefold increase in the binding of the microsomes could be achieved using 2–3 M $MgCl_2$ (Fig. 2A) or pH 4.8 (Fig. 2B). Further increases in $MgCl_2$ concentration or lower pH caused a permanent loss of binding activity. Treatment with $MgCl_2$ caused a concentration-dependent loss of protein to a maximum of 50% of control, whilst pH had no such effect. As a consequence, treatment of microsomes with 3 M $MgCl_2$ resulted in a sixfold increase in specific activity over the starting material and a twofold increase over microsomes treated at pH 4.8.

Time Course of Receptor-Ligand Association

Binding of ^{125}I-hGH to the microsome receptor at room temperature reached equilibrium at 16–20 h (Fig. 3). At 5, 10, and 20 h, unlabeled hGH was added to a

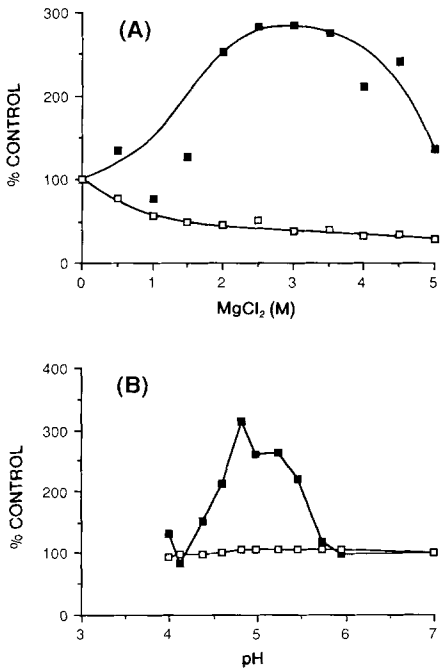

Fig. 2. Effect of $MgCl_2$ and pH treatment on ^{125}I-hGH binding. Microsomes obtained from the 800 to $100{,}000g_{av}$ fraction of homogenized chorion membranes were treated at 5 mg protein/ml with various concentrations of $MgCl_2$ (**panel A**) for 20 min at 4°C or at various pH (**panel B**) for 10 min at 4°C, pelleted and washed. The effects on specific ^{125}I-hGH binding (■) and protein content (□) were measured as described in the experimental section. Results are expressed as the percentage of untreated control preparations.

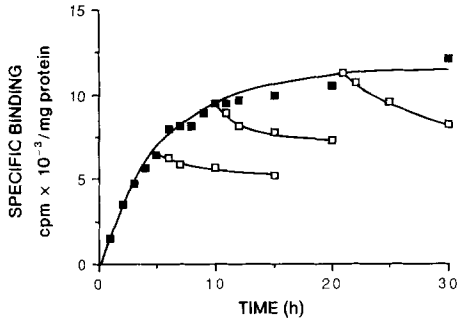

Fig. 3. Time course of association of ^{125}I-hGH to chorion microsomes. Microsomal preparations (1 mg of protein) were combined with 60,000 cpm of ^{125}I-hGH in the presence and absence of 0.5 μg of hGH (final volume 500 μl), and at the indicated times, 1 ml of ice cold 25 mM Tris-HCl, 10 mM $MgCl_2$, 0.1% (w/v) BSA was added, and the tubes were centrifuged, drained, and the radioactivity in the pellet was counted to assess ^{125}I-hGH binding (■). At 5, 10, and 20 h, 5 μg of hGH was added to a parallel series of tubes and incubated for the indicated times prior to addition of 25 mM Tris-HCl, 10 mM $MgCl_2$, 0.1% (w/v) BSA, centrifugation, and counting to assess the ability of a large excess of hGH to displace ^{125}I-hGH already bound (□).

parallel series of tubes at a final concentration of 10 μg/ml, and the binding was followed with time. Unlabeled hormone at saturating concentration (approximately 570 × K_D) and in approximately 15,000-fold molar excess over the labeled hormone was unable to displace more than 25–30% of the labeled hormone already bound. This indicated that most of the ^{125}I-hGH was irreversibly bound to the receptor and could not be exchanged.

Ligand Specificity of the Chorion Lactogenic Receptor

The ligand specificity of the chorion lactogenic receptor was investigated by determining the ability of various concentrations of known lactogenic and somatogenic hormones to compete for the binding of ^{125}I-hGH to the receptor (Fig. 4). In panel A, the ability of hGH and its 20 K variant to compete with ^{125}I-hGH, is shown. Human growth hormones competed with ^{125}I-hGH, and the native hGH was more potent than the 20 K variant. In contrast, growth hormones of non-primate origin represented by pGH in panel B did not compete with ^{125}I-hGH for the receptor in this concentration range. Ovine GH and bGH gave the same result (data not shown). Panel C indicates that both oPRL and hPRL also competed with hGH for binding to this receptor. Bovine PRL and pPRL were equipotent with hPRL (data not shown), and oPRL was the most potent prolactin. Human PL (panel D) was also able to bind to the receptor but with considerably less potency than either hGH or hPRL.

Solubilization of the Chorion Lactogenic Receptor with CHAPS and NaCl

The effect of increasing concentrations of CHAPS on the extraction of the receptor from microsomes was investigated (Fig. 5A). CHAPS at 15 mM allowed maximal extraction of 30% of total protein and receptor. Concentrations of CHAPS above 8 mM in the binding reaction caused a decrease in specific binding and concomitant underestimation of receptor extraction. If the concentration of CHAPS was allowed to fall below 8 mM after solubilization, a loss of receptor binding activity resulted (data not shown). No increase in total binding sites was produced by solubilization. The effect of NaCl on

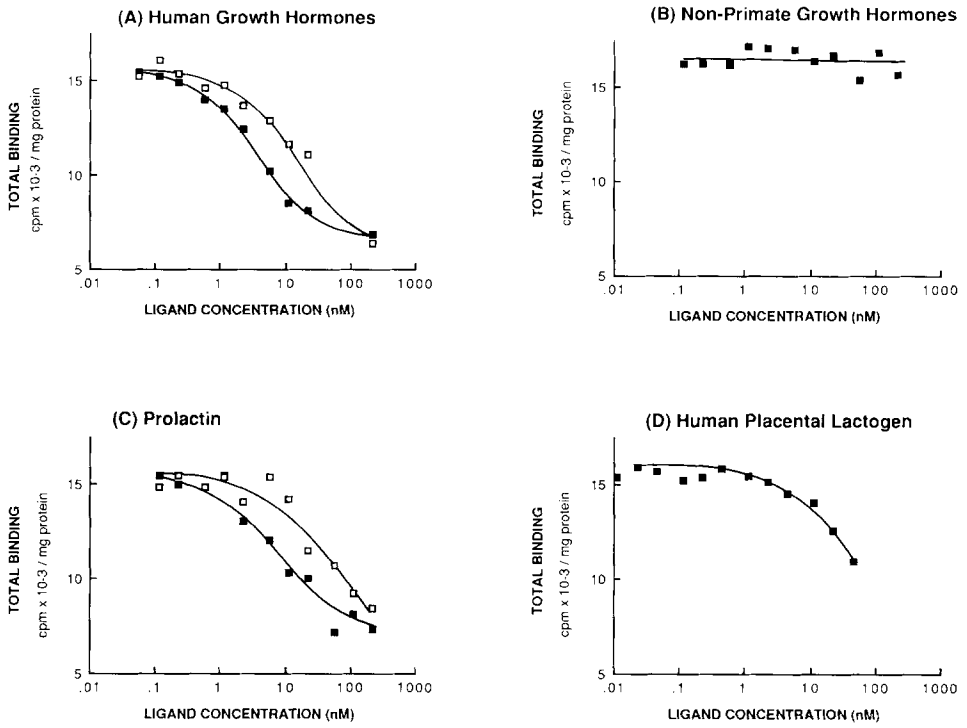

Fig. 4. Specificity of ligand binding to chorion microsomal receptor. Chorion microsomes were combined in a final volume of 500 μl with 60,000 cpm of ^{125}I-hGH in the presence of various concentrations of hGH (■), 20 K hGH (□), pGH (■), oPRL (■), hPRL (□) and hPL (■) for 20 h at 20°C, followed by addition of 1 ml of ice cold 25 mM Tris-HCl, 10 mM $MgCl_2$, 0.1% (w/v) BSA, centrifugation, and counting of radioactivity in the pellet. Results are expressed as total ^{125}I-hGH bound per mg of microsomal protein.

extraction was also examined (Fig. 5B). Increasing concentrations of NaCl in combination with 16 mM CHAPS resulted in increased extraction of receptor, reaching a maximum of 45% at 250 mM. NaCl reduced receptor binding significantly only above 125 mM (data not shown). The procedure adopted, therefore, was to solubilize with 16 mM CHAPS and 250 mM NaCl and to dilute twofold in the binding assay to negate the effects of high detergent and high salt concentrations on binding. In other experiments, the following parameters were tested and found to give optimal extraction or binding: solubilization temperature = 26°C; solubilization time = 20–30 min; microsomal protein concentration = 10 mg/ml; PEG concentration = 16% (w/v); bovine gamma globulin concentration = 0.1% (w/v).

Scatchard Analysis of Microsomal and Solubilized Receptor Binding

The binding of hGH to the microsomal and solubilized receptor preparations was compared by Scatchard analysis [40] (Fig. 6). Both preparations displayed a linear plot of equal slope after subtraction of nonspecific binding, indicating a single population of high affinity receptor sites of K_D = 0.8 nM. The binding capacity of 180 fmol/mg

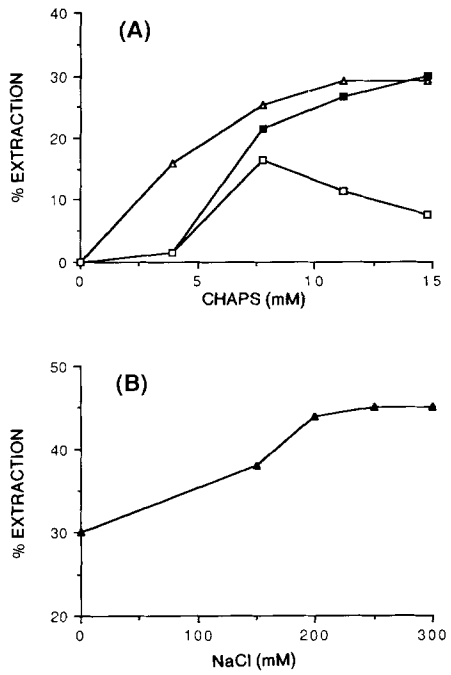

Fig. 5. Solubilization of chorion lactogenic receptor with CHAPS and NaCl. Microsomes were resuspended at 10 mg protein/ml in TM, and various concentrations of CHAPS were added as powder for 20 min at 20°C, followed by centrifugation at 100,000g_{av} for 60 min at 4°C (**panel A**). The clear supernatant was removed from the pellet and assayed for ^{125}I-hGH binding after adjustment to 8 mM CHAPS (■) or at the concentration of CHAPS used to solubilize the microsomes (□). Results are expressed as a percentage of the total binding and protein (△) recovered in both supernatant and pellet fractions. In **panel B**, microsomes were solubilized as before with 16 mM CHAPS in the presence of increasing concentrations of NaCl, and the binding in the supernatant was measured at 8 mM CHAPS and expressed as the percentage of total binding recovered in the pellet and supernatant fractions (▲).

protein was also the same, confirming no enrichment of the receptor with detergent solubilization.

Gel Chromatography of Solubilized Receptor

Solubilized receptors bound to ^{125}I-hGH in the presence and absence of 1 μg/ml hGH were analysed by gel filtration using superose 12 (Fig. 7). In the presence of excess unlabeled hGH, only one peak of radioactivity was seen, corresponding to the elution position of ^{125}I-hGH. In the absence of hGH, two peaks of specific binding appear (peaks A and B in Fig. 7). The size of peak A varied between experiments and was increased by further concentration of the sample. Superose 6 chromatography (data not shown) resolved the two peaks as a single asymmetric peak from 600,000 to 60,000 daltons, indicating that peak A of the superose 12 profile was an artifact caused by the inability of superose 12 to resolve the very large receptor aggregates. The total specific binding recovered in the superose 12 peaks corresponded to the specific binding in the starting material measured by the binding assay, indicating that the concentration of PEG used

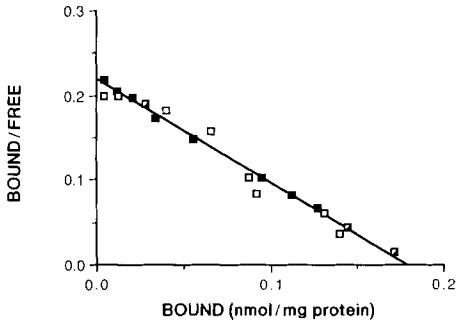

Fig. 6. Scatchard analysis of ^{125}I-hGH binding to microsomal and solubilized chorion lactogenic receptors. Microsomal (■) or solubilized (□) receptor preparations were combined with 60,000 cpm of ^{125}I-hGH (0.03 nM) in the presence of various concentrations of hGH for 20 h at 20°C, and binding was assessed as described in the experimental section. The data were analysed by the method of Scatchard [40]. Results are expressed as the amount of binding of hGH per mg microsomal protein.

to separate receptor bound ligand from free ligand precipitated all the ligand bound receptor.

Covalent Cross-Linking of the Receptor-Ligand Complex

The peak fractions (labeled A and B in Fig. 7) from the superose 12 profile and the corresponding fractions of the nonspecific binding profile (open squares in Fig. 7) were cross-linked with disuccinimidylsuberate and analysed under reducing conditions by SDS-PAGE. Figure 8 shows densitometric scans and photographs of an autoradiograph of the gel. Panel 8B shows material from peak B in Figure 7. At the ion front, a large

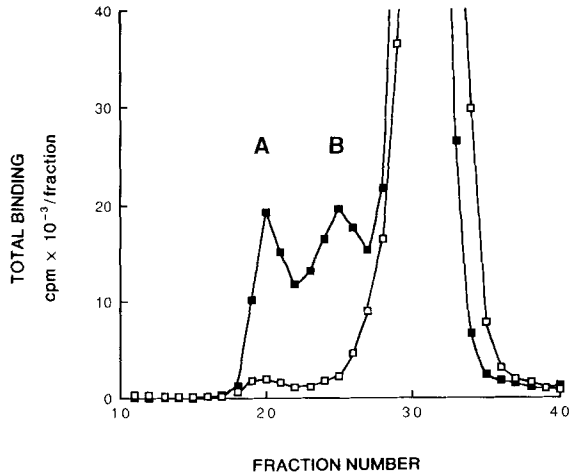

Fig. 7. Superose 12 chromatography of solubilized chorion lactogenic receptor-^{125}I-hGH complexes. Solubilized receptors (25 mg protein) were bound with 600,000 cpm of ^{125}I-hGH in the presence (□) and absence (■) of 1 μg/ml hGH for 20 h at 20°C, concentrated tenfold with a centricon-10 unit, and separated by size exclusion liquid chromatography using a superose 12 column at a flow rate of 30 ml/h of 25 mM Tris-HCl, 10 mM $MgCl_2$, 0.1% (w/v) BSA, 8 mM CHAPS. Fractions measuring 400 μl were collected and counted for radioactivity.

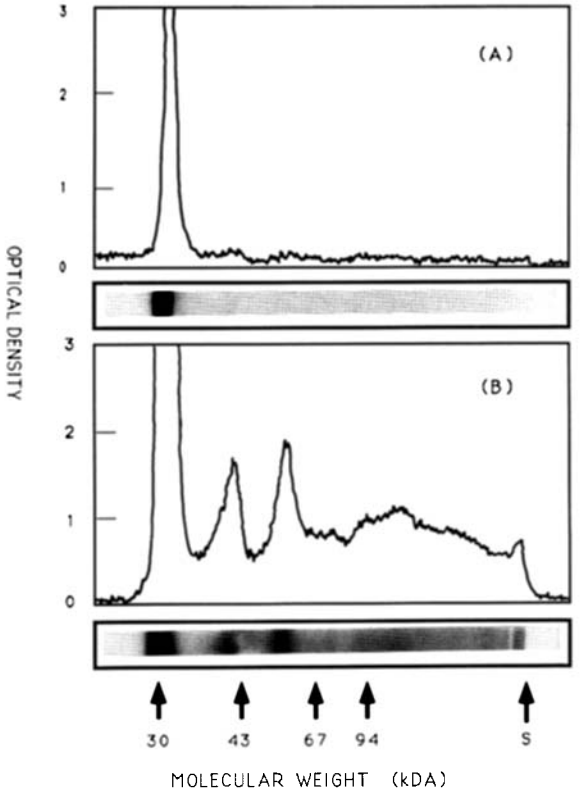

Fig. 8. Affinity cross-linking and SDS-PAGE analysis of solubilized chorion lactogenic receptor-^{125}I-hGH complexes. Fractions of specific ^{125}I-hGH binding (**panel B**) eluting from the superose column in peak B of Figure 7 and corresponding fractions of nonspecific binding (**panel A**) were cross-linked with 0.5 mM disuccinimidal suberate, and 250 μl samples were analysed by SDS-PAGE as described in the experimental section. Autoradiographs of dried gels were scanned by laser densitometry. Molecular weight markers are phosphorylase B (94,000), BSA (67,000), ovalbumin (43,000), and carbonic anhydrase (30,000). S indicates the end of the stacking gel.

peak was seen that corresponds to the position of ^{125}I-hGH in other experiments, two smaller peaks followed at 42,000 and 56,000 daltons, and a broad peak was seen around 100,000 daltons. At the stacking gel interface (S), there was a small peak of material that has only just entered the 7.5% gel. Panel A shows material from the nonspecific binding profile in Figure 7 (open squares, fractions 24, 25, 26), which eluted at the same position as the specific binding peak B. Only one peak coincident with ^{125}I-hGH was present. Material from the superose 12 peak A showed an identical pattern of peaks on the autoradiograph, except that an increased proportion of the material failed to enter the 7.5% gel (data not shown).

DISCUSSION

The normal physiological role of the lactogenic receptor in the human chorion membrane [18,19] is not clear. It has been suggested that prolactin may control amniotic fluid volume [41,42]. Excessive amniotic fluid volume has been correlated with reduced

^{125}I-hGH binding to chorion microsomes [43] and reduced prolactin content of the amniotic fluid [44]. Alternatively, the receptor may be responsible for the transport of decidual prolactin to the amniotic fluid. A similar transport role for the rat choroid plexus receptor has been proposed for the appearance of serum prolactin behind the blood–brain barrier [45].

Irrespective of the physiological role of this molecule, the human placental chorion membrane lactogenic receptor demonstrates a number of properties that are indistinguishable from the lactogenic receptor of other species and tissues—indicating that the chorion receptor is a typical lactogenic receptor.

The subcellular fractionation of the chorion homogenate shows a distribution of the receptor similar to that seen in animal studies [46,47], with the exception of an increased amount of receptor remaining in the unhomogenized fraction, probably due to the structural nature of the chorion membrane. Receptors in the nuclear ($800g_{av}$) fraction may be due to the sedimentation of large sheets of plasma membrane [48] or the presence of lactogenic receptors in the nucleus [49].

Treatment of chorion microsomes with 3 M $MgCl_2$ and pH 4.8 resulted in a threefold increase in binding, indicating that a large number of the sites are unavailable for binding in untreated microsomes. In another experiment, ^{125}I-hGH and ^{125}I-oPRL were bound to microsomal receptors (previously treated with 3.5 M $MgCl_2$) and exposed to the same range of $MgCl_2$ and pH as above. ^{125}I-hGH was removed completely by 4–5 M $MgCl_2$ and pH 4.0, and ^{125}I-oPRL was removed completely by 2–3 M $MgCl_2$ or pH 4.5 (data not shown), reflecting the relative affinity of these hormones for the receptor and suggesting that endogenously bound prolactin may explain the low binding in untreated microsomes. Receptors from other sources exhibit the same response to $MgCl_2$ [50–52]. The human receptor's response to pH has not been examined previously and appears to be slightly more sensitive than the rabbit mammary receptor [53].

The chorion receptor has binding kinetic characteristics typical of other lactogenic receptors. The slow rate of association at room temperature and the irreversible nature of the binding (Fig. 3) are distinguishing features of lactogenic receptors [51,54–60]. Furthermore, the receptor has the general ligand specificity of a lactogenic receptor (Fig. 4), given that there is some controversy surrounding the relative binding affinities of hGH, hPRL, and oPRL to the human receptor [16,17,20,21,25,28,51]. Most of these studies show almost equipotency of hGH and PRL for the receptor (when compared to hPL), and that hPRL is generally more potent than oPRL. Our observations differ in respect to the latter and may be due to differing purities and potencies of the ligand preparations or the expression of a different member of the lactogenic receptor gene family in placenta. Interestingly, recent cloning of a human prolactin receptor gene allowed the identification of an hPRL receptor mRNA in human chorion membranes [61].

Scatchard analysis of binding data indicated equal affinity and capacity for both solubilized and microsomal receptors. The observed K_D (approx 1 nM) is in good agreement with the K_D for other animal and human lactogenic receptors [51,52] and the capacity (200 fmol/mg protein) is at the low end of the range seen in preparations from other sources [4,47]. The linear Scatchard plot after subtraction of nonspecific binding indicates a single class of high-affinity binding sites. In contrast to preparations from some animal tissues where solubilization leads to increased total receptors by revealing

binding sites previously inaccessible to the ligand [5,17,62,63], no increase in specific activity was observed following solubilization of the chorion receptor.

Efficient solubilization of the human receptor is required before any molecular characterization can be attempted. Cholate and the polyoxyethylene detergents Triton X-100 and Lubrol PX can also solubilize the chorion receptor (data not shown) with lower extraction efficiency than CHAPS. The solubilized preparation was analysed by gel permeation chromatography to estimate the molecular size of the receptor-ligand complex. Superose 12 chromatography revealed two peaks of specific binding, one at approximately 60,000 daltons and the other near the void volume. Superose 6 resolved these larger complexes at a range from approximately 600,000 daltons to a peak at around 60,000 daltons. Both columns indicate a minimum molecular weight of approximately 60,000 daltons for the CHAPS solubilized receptor-ligand complex. Assuming a 1:1 ratio for binding of ^{125}I-hGH to the receptor, a minimum molecular weight for the receptor's binding domain of approximately 40,000 daltons can be calculated, including the contribution of CHAPS to the complex's size. To more accurately estimate the binding domain's molecular weight, the complex was covalently cross-linked with disuccinimidyl suberate and analysed by SDS-PAGE.

An autoradiograph of cross-linked material from superose 12 peak B indicated three peaks that could be displaced by unlabeled hGH at around 44,000, 58,000, and a broad peak centred about 109,000 daltons. The 44,000 peak is probably a dimer of ^{125}I-hGH [64]. A fourth peak running at the ion front could not be displaced and represents ^{125}I-hGH not cross-linked to other protein. The high background was also reduced by excess hGH and probably represents coupling of specifically bound ^{125}I-hGH to cellular proteins other than the receptor during the cross-linking reaction. Subtraction of the molecular weight of hGH gives the molecular weight of the peaks as 36,000 and 87,000. A molecular weight of approximately 36,000 and 87,000 for the binding domain is consistent with affinity cross-linking studies using animal tissues [7–9,65] and SDS-PAGE analysis of affinity purified material identified by Western blot analysis [66–68] or probing with ^{125}I-hGH [62,67]. Unidentified bands of these molecular weights have also been seen on coomassie or silver stained gels of affinity purified material including human chorion [5,69].

This study has shown that the lactogenic receptor from human chorion membranes has the characteristics typical of lactogenic receptors from other human and animal sources. The receptor can be extracted from the microsomal membranes with retention of activity by CHAPS detergent and has the same observed molecular weight as lactogenic receptors from other sources. The material is presently being used for further purification and antibody production.

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