Binding of Apolipoprotein A-I and A-II after Recombination with Phospholipid Vesicles to the High Density Lipoprotein Receptor of Luteinized Rat Ovary*

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To determine the apolipoprotein specificity of high density lipoprotein (HDL) receptor, apolipoprotein A-I (apo-AI) and apolipoprotein A-II (apo-AII) purified from high density lipoprotein3 (HDL3) were reconstituted into dimyristoyl phosphatidylcholine vesicles (DMPC) and their ability to bind to luteinized rat ovarian membranes was examined. Both 125I-apo-A-I-DMPC and 125I-apo-A-II-DMPC were shown to bind to ovarian membranes with Kd = 2.87 and 5.70 µg of protein/ml, respectively. The binding of both 125I-apo-A-I-DMPC and 125I-apo-A-II-DMPC was inhibited by unlabeled HDL3, apo-A-I-DMPC, apo-A-II-DMPC, apo-C-I-DMPC, apo-C-II-DMPC, apo-C-III-DMPC, and apo-C-III2-DMPC, but not by DMPC vesicles, bovine serum albumin (BSA) or low density lipoprotein. Since the binding labeled apo-A-I-DMPC and apo-A-II-DMPC was inhibited by the DMPC complexes of apo-C groups, the direct binding of 125I-apo-C-II-DMPC was also demonstrated with Kd = 9.6 µg of protein/ml. In addition, unlabeled apo-A-I-DMPC, and apo-A-II-DMPC, as well as apo-C-DMPC, inhibited 125I-HDL3 binding. 125I-apo-A-I, 125I-apo-A-II, and 125I-apo-C-III2 in the absence of DMPC also bind to the membranes. These results suggest that HDL receptor recognizes apolipoprotein AI, AII, and the C group and that the binding specificity of the reconstituted lipoproteins is conferred by their apolipoprotein moiety rather than the lipid environment. In vivo pretreatment of rats with human chorionic gonadotropin resulted in an increase of 125I-apo-A-I-DMPC, 125I-apo-A-II-DMPC, and 125I-apo-C-III-DMPC binding activities. However, no induction of binding activity was observed when the apolipoprotein was not included in DMPC vesicles. An examination of the equilibrium dissociation constant and binding capacity for 125I-apo-A-I-DMPC and 125I-apo-A-II-DMPC after human chorionic gonadotropin treatment revealed that the increase in binding activity was due to an increase in the number of binding sites rather than a change in the binding affinity. These results further support our contention that apo-A-I, apo-A-II, and the apo-C group bind to HDL receptor. In conclusion, the HDL receptor of luteinized rat ovary recognizes apolipoproteins A-I, A-II, and the C group but not low density lipoprotein, and the binding is induced by human chorionic gonadotropin in vivo.

Plasma lipoproteins are known to bind to receptors with high affinity on the plasma membranes in several different cell types (for review see Refs. 1 and 2). The two classes of lipoproteins that have been demonstrated to bind to rat luteal cell surface receptors are the low density lipoprotein (LDL) and high density lipoprotein (HDL). These lipoproteins have been shown to enhance steroidogenesis in the enzyme-dispersed luteal cells (5-8). The stimulation of steroidogenesis by lipoproteins involves the binding of lipoproteins to specific cell surface receptors followed by endocytosis and lysosomal hydrolysis to release free cholesterol which is then utilized as a substrate for progesterone synthesis (4, 9).

Several studies have indicated that the binding specificity of lipoprotein is conferred by its protein component (10, 11). Recent investigations have shown that LDL receptor can recognize both apo-B and apo-E (12-14). However, which apolipoprotein is recognized by HDL receptor is not well defined although it has been suggested that apo-A-I may be the HDL apolipoprotein recognized by the HDL receptor on rat testicular membranes (15). This study was, therefore, undertaken in an effort to examine the apolipoprotein binding specificity of HDL receptor. Since HDL3 contains two major apolipoproteins, apo-A-I and apo-A-II, experiments were carried out to purify apo-A-I and apo-A-II from human HDL3, followed by an examination of their ability to bind to rat ovarian membranes. It has also been reported that both apo-A-I and apo-A-II can insert into dimyristoyl phosphatidylcholine vesicles (16-19). We have followed these procedures in reconstituting apo-A-I and apo-A-II into phospholipid vesicles to examine their ability to bind to ovarian membranes and to investigate the binding specificity of the resulting complexes. Our results suggest that HDL receptor recognizes apolipoprotein A-I, A-II, and the C group but not LDL, and that the lipid environment may not be required for binding.

EXPERIMENTAL PROCEDURES

Materials

Human serum, collected from healthy donors, was obtained from the Blood Bank, University of Michigan, Ann Arbor, MI. [14C]dimyristoyl phosphatidylcholine and sodium [125I]iodide (carrier-free in aqueous solution, pH 8-10) were purchased from New England Nuclear. QAE-Sephadex A-25 and Sephadex G-200 were bought from Pharmacia Fine Chemicals, Inc. Gonadotropin from pregnant mare's serum (PMSG), human chorionic gonadotropin (hCG), and dimyristoyl phosphatidylcholine were the products of Sigma. Bovine albumin

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1 The abbreviations used are: LDL, low density lipoprotein; HDL, high density lipoprotein; DMPC, dimyristoyl phosphatidylcholine vesicles; hCG, human chorionic gonadotropin; BSA, bovine serum albumin; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PMSG, pregnant mare's serum gonadotropin.

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monomer standard protein powder was obtained from Miles Laboratories, Inc. Apolipoprotein C-I, C-II, C-III, and C-IV were a generous gift from Dr. Richard L. Jackson, College of Medicine, University of Cincinnati, Cincinnati, OH. Reagents for electrophoresis were purchased from Bio-Rad. Kodak XAR-5 film, lightning Plus intensifying screen, and X-Omatic cassette (8 x 10 inches) were obtained from Eastman Kodak Co. All other reagents used were of analytical reagent grade.

Methods

Preparation of Serum Lipoprotein and Purification of Apo-A-I and Apo-A-II from Human HDL

HDL was purified by ultracentrifugation, fitted with a 75 mm (10 inches) tube having KBr and NaCl for density adjustment as described by Havel et al. (20). Human HDL was delipidated by solvent extraction (21) and the apolipoproteins were purified by Sephadex G-200 column chromatography (22, 23). Prior to use, apo-A-I and apo-A-II were denatured in 8 M urea and refolded by slow removal of the denaturing agent (18).

Iodination of Apolipoproteins—Apo-A-I, apo-A-II, and apo-C-III, were iodinated with 125I using a modification of the iodine monochloride procedure (24). Free 125I ion was removed from the 125I-apo-A-I, 125I-apo-A-II, or 125I-apo-C-III, by ion exchange chromatography on a 25 cm column (18 x 1.5 cm) which had been equilibrated with 0.1 M Tris-HCl buffer containing 0.15 M NaCl, pH 7.0 (25). The specific activity of 125I-apo-A-I, 125I-apo-A-II, and 125I-apo-C-III, averaged 300-500 cpm/ng.

Preparation of DMPC Vesicles—apo-A-I:DMPC, apo-A-II:DMPC, apo-C-III:DMPC—DMPC vesicles were prepared by the procedure of Roth et al. (26). Briefly, 1 ml of buffer, containing 0.15 M NaCl, 10 mM Tris-HCl, 1 mM EDTA, with 1 mM NaN3, pH 7.6, was added to 10 mg of DMPC that had been dissolved in 1 ml of benzene and dried by lyophilization. The solution was sonicated for 20 min at 50 °C using a Sonifier Cell Disruptor, Model VC-25 (Heat Systems-Ultrasonics, Inc.) equipped with a microtip at a setting of 8 (30 watts). The sonicated preparation of DMPC was then added to the 125I-apo-A-I, 125I-apo-A-II, or 125I-apo-C-III, (specific activity for 125I-apo-A-I, 125I-apo-A-II, and 125I-apo-C-III, was adjusted to 50, 40, and 20 cpm/ng, respectively, by adding an appropriate amount of unlabeled apo-A-I, apo-A-II, or apo-C-III, in the original buffer at a lipid-to-protein ratio of 4:1 (w/w). The mixture was vortexed for 10 s and left overnight at room temperature. The protein-lipid complexes were isolated by density gradient ultracentrifugation performed in a SW 50.1 swinging bucket rotor at 45,000 rpm and 25 °C for 60 h as described by Roth et al. (26). Potassium bromide was removed by dialysis prior to the use of the protein-lipid complexes for binding studies.

Preparation of Unlabeled Apo-A-I:DMPC, Apo-A-II:DMPC, Apo-C-III:DMPC, Apo-C-III:DMPC—DMPC vesicles containing apo-A-I, apo-A-II, or apo-C-III, apoproteins were prepared by the procedure of Roth et al. (26) followed by the isolation of individual lipid-protein complex by sucrose density gradient centrifugation as described in the previous section dealing with the preparation of 125I-apoprotein complexes.

Electrophoresis and Autoradiography—The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) described by Laemmli (27) was used to examine the purity of apo-A-I and apo-A-II. Electrophoresis was typically carried out at 3 mA/sample for approximately 5 h on 13.5 mm-gels containing 15% acrylamide with 4% stacking gel. The gels were stained over night in a staining solution containing 0.05% Coomassie Blue R-250, 25% isopropanol, and 10% acetic acid and were destained in 10% acetic acid. The gel was dried prior to autoradiography. Autoradiographic exposure of the gels was performed at −70 °C for 20 h with Kodak XAR-5 film using the Dupont Cronex Lightning Plus enhancing screen which has been shown to improve the sensitivity and speed of autoradiography (28).

Animals and Hormonal Treatment—Twenty-one-day-old female Sprague-Dawley rats (Spartan Research Inc., Hazlett, MI) were employed in the present studies. Highly luteinized ovaries were obtained following a regimen described by Parlow (29). Rats were injected subcutaneously with 50 IU of PMSG followed 56 h later with 25 IU of HCG. Day 0 was taken as the day of hCG injection and animals were usually killed on day 7.

Isolation of Partially Purified Plasma Membranes—Fresh, highly luteinized rat ovaries were weighed and homogenized at 4 °C in an Eppendorf homogenizer fitted with a Teflon pestle, and plasma membranes were prepared according to the procedure described by Goslinowicz (30). The relative purification of membrane preparations was ascertained by assaying 5'-nucleotidase activity. The extent of mitochondrial, lysosomal, and microsomal contamination was checked by assaying specific marker enzymes (31). Relative to 5'-nucleotidase activity present in the original rat ovarian homogenate, enrichment of this marker enzyme in isolated plasma membranes averaged 10-fold with negligible mitochondrial, lysosomal, and microsomal contamination. Membranes were prepared on the same day as the binding assay.

Binding Assays—Aliquots of membrane preparations were incubated in disposable glass tubes (12 x 75 mm) precoated overnight with 10% BSA. The incubations usually were carried out in a total volume of 0.25 ml of 25 mM Tris-HCl buffer, pH 7.4, containing 125I-apo-A-I:DMPC, 125I-apo-A-II:DMPC, or 125I-apo-C-III, DMPC (10 μg of protein/ml; specific activity for 125I-apo-A-I, 125I-apo-A-II, and 125I-apo-C-III, was 50, 40, and 20 cpm/ng, respectively, either alone (total binding) or in the presence of 3 mg of unlabeled HDL/ml (nonspecific binding). All the incubations were performed at 37 °C, for 60 min unless otherwise specified. Following incubation, the receptor-bound 125I-apo-A-I:DMPC, 125I-apo-A-II:DMPC, or 125I-apo-C-III, DMPC was separated from free 125I-apo-A-I:DMPC, 125I-apo-A-II:DMPC, or 125I-apo-C-III, DMPC by the addition of 1 ml of precooled buffer (25 mM Tris, pH 7.4) to each tube followed by centrifugation at 3000 x g for 20 min at 4 °C in an IEC (DPF-6000) centrifuge, with a 949 rotor. The supernatants were removed by suction. The pellets were washed once and then counted in an automatic γ counter (Searle Analytical, Model 1195). The specific binding was determined by subtracting the nonspecific binding from the total binding. All assays were carried out in triplicate. The slope of the line obtained from Scatchard plot is equal to −Kd/Ka (32).

Protein Assay—Protein was determined by the colorimetric procedure of Lowry et al. (33).

RESULTS

The identity and purity of apo-A-I and apo-A-II, isolated from human HDLs by chromatography apo-HDLs on a Sephadex G-200 column (100 x 2.5 cm) equilibrated with buffer, containing 10 mM Tris-HCl, 1 mM EDTA, and 8 M urea, pH 8.6, was examined by SDS-PAGE (27). Thirty micrograms of apo-A-I and apo-A-II gave single bands with molecular weights of 28,000 and 17,000, respectively, when analyzed by electrophoresis on a 0.1% SDS, 15% polyacrylamide gel under nonreducing conditions (Fig. 1A). The molecular weights of apo-A-I and apo-A-II obtained in this study are consistent with the values reported by other investigators (34-36). Bovine serum albumin (BSA), purified apo-A-I, and apo-A-II were then iodinated with radioactive 125I. The iodinated products were then examined by electrophoresis on a 0.1% SDS, 15% polyacrylamide gel under nonreducing conditions followed by autoradiography. The 125I-apo-A-I and 125I-apo-A-II appeared as single bands in the autoradiogram, and the 125I-BSA preparation had slight impurity as shown in Fig. 1B. These results further show that apo-A-I and apo-A-II are homogeneous preparations.

Since HDL contains two major apolipoproteins, apo-A-I and apo-A-II and apo-C group (apo-C-I, -C-II, -C-III), and -C-IV as minor constituents, it was of interest to examine which apolipoprotein in HDL recognizes the HDL receptor. The result of an examination of the ability of BSA, apo-A-I, apo-A-II, and apo-C-III, to bind to rat ovarian membranes is shown in Table I. The 125I-BSA and 125I-Apo-A-I:DMPC were used as controls, since BSA is not expected to specifically bind to the HDL receptor. As expected, 125I-BSA and 125I-BSA:DMPC failed to bind to plasma membranes. However, 125I-apo-A-I, 125I-apo-A-II, and 125I-apo-C-III, irrespective of whether the radiolabeled ligand was present in a reconstituted form with DMPC vesicle or solely as a protein in solution, bound significantly to membrane preparations. In addition, the binding of 125I-apolipoprotein and 125I-apoA-I:DMPC complexes was inhibited by an excess of unlabeled
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of 0.0625 to a run on a 0.1% SDS, 15% polyacrylamide slab gel under nonreducing conditions. The molecular weight markers are shown in

formed at -70 °C for 20 h using Kodak XAR-5 film. The migration of the molecular weight markers is shown to the left of the autoradiogram. B, K represents M, in thousands.

TABLE I

Binding ability of apolipoproteins purified from HDL₃ to rat ovarian membranes

Aliquots of labeled ligands (¹²⁵I-BSA, 10.2 µg/ml, 50 cpmp/ng; ¹²⁵I-apo-A-I, 10.4 µg/ml, 50 cpmp/ng; ¹²⁵I-apo-A-II, 11.6 µg/ml, 40 cpmp/ng; ¹²⁵I-apo-C-III, 11.0 µg/ml, 20 cpmp/ng; ¹²⁵I-BSA-DMPC, 10.8 µg of protein/ml, 50 cpmp/ng; ¹²⁵I-apo-A-I-DMPC, 12.2 µg of protein/ml, 40 cpmp/ng; ¹²⁵I-apo-A-II-DMPC, 10.2 µg/ml, 20 cpmp/ng) were incubated with plasma membranes (135 µg of membrane protein) at a concentration of 12.5-15 µg of protein/ml and the nonspecific binding was also increased linearly as a function of the labeled ligand concentration. The Scatchard analysis indicated one class of binding sites with Kₛ for ¹²⁵I-apo-A-I-DMPC to the plasma membranes of 9.6 µg of membrane protein/ml (Fig. 2C, inset).

The binding specificity of ¹²⁵I-apo-A-I-DMPC binding showed a linear plot indicating that the membranes contain one class of binding sites for ¹²⁵I-apo-A-I-DMPC with a Kₛ = 5.70 µg of protein/ml (Fig. 2B, inset). The binding of ¹²⁵I-apo-A-I-DMPC to the plasma membranes (150 µg of membrane protein) with increasing concentrations of the labeled ligand concentration is shown in Fig. 2C. The binding was increased with increasing concentrations of the labeled ligand up to 25 µg of protein/ml and reached saturation at 40-90 µg/ml. Nonspecific binding increased as a function of ligand concentration. The Scatchard analysis indicated one class of binding sites with Kₛ = 9.6 µg/ml (Fig. 2C, inset).

To characterize the binding properties of the major apolipoprotein of HDL₃, the binding of ¹²⁵I-apo-A-I-DMPC and ¹²⁵I-apo-A-II-DMPC, as a function of membrane protein concentration and incubation time, was examined. Fig. 3, A and B, show the binding of ¹²⁵I-apo-A-I-DMPC and ¹²⁵I-apo-A-II-DMPC to ovarian membranes as a function of membrane protein concentration. The binding of ¹²⁵I-apo-A-I-DMPC and ¹²⁵I-apo-A-II-DMPC increased linearly with increasing amounts of membrane protein in the assay solution up to 0.75 and 0.45 mg of membrane protein/ml, respectively.

HDL₃. These results suggest that plasma membranes possess specific binding sites for apo-A-I, apo-A-I-DMPC, apo-A-II, apo-A-II-DMPC, apo-A-II-DMPC, apo-C-III, and apo-C-III-DMPC and these binding sites may be involved in the previously reported HDL receptor (3).

To examine whether the binding sites of apo-A-I-DMPC, apo-A-II-DMPC, and apo-C-III-DMPC are the putative HDL receptors, the binding of these labeled apolipoprotein-DMPC complexes to plasma membranes as a function of ligand concentrations, membrane protein concentration, incubation time, and binding specificity was examined. If apo-A-I, apo-A-II-DMPC, apo-A-II-DMPC, apo-C-III, and apo-C-III-DMPC bind to the putative receptor, the binding of these ligands to a fixed amount of plasma membranes should reach saturation with increasing concentrations

of ligand. For this study, the lipid-protein complexes, ¹²⁵I-apo-A-I-DMPC, ¹²⁵I-apo-A-II-DMPC, and ¹²⁵I-apo-C-III-DMPC, were used instead of ¹²⁵I-apo-A-I, ¹²⁵I-apo-A-II, and ¹²⁵I-apo-C-III, to compare the binding kinetics obtained with HDL₃ since native HDL₃ is also a protein-lipid complex. The specific activity of ¹²⁵I-apo-A-I-DMPC, ¹²⁵I-apo-A-II-DMPC, and ¹²⁵I-apo-C-III-DMPC was adjusted to 50, 40, and 20 cpmp/ng of protein, respectively, by the addition of unlabeled apo-A-I-DMPC, apo-A-II-DMPC, or apo-C-III-DMPC complex. When aliquots of plasma membranes (200 µg of membrane protein) were incubated with various concentrations of ¹²⁵I-apo-A-I-DMPC, the specific binding of ¹²⁵I-apo-A-I-DMPC increased with increasing concentrations of the ligand up to 5.0 µg of protein/ml and the binding process was saturated at a concentration of 7.5-12.5 µg of protein/ml (Fig. 2A). The nonspecific binding was also increased linearly as a function of labeled ligand concentration. Scatchard analysis of ¹²⁵I-apo-A-I-DMPC binding showed a linear plot indicating that the membranes contain one class of binding sites for ¹²⁵I-apo-A-I-DMPC (Fig. 2A, inset).
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**Fig. 2.** The binding of 125I-apo-A-I·DMPC (A), 125I-apo-A-II·DMPC (B), and 125I-apo-C-III·DMPC (C) to rat ovarian membrane preparations with increasing ligand concentrations. Two hundred micrograms of membrane protein were incubated with various concentrations of 125I-apo-A-I·DMPC from 0.5 to 15 μg of protein/ml (50 cpm/ng) (A); one hundred thirty-five microgram of membrane protein were incubated with various concentrations of 125I-apo-A-II·DMPC from 1.5 to 15 μg of protein/ml (40 cpm/ng) (B) in a total volume of 0.4 ml of binding assay solution (total binding). One hundred fifty microgram of membrane protein were incubated with various concentrations of 125I-apo-C-III·DMPC from 5 to 90 μg of protein/ml (20 cpm/ng) in a total volume of 0.3 ml of binding assay solution (total binding). Incubation was carried out at 37°C for 60 min. For estimation of nonspecific binding, unlabeled HDL3 was added at a final concentration of 3 mg/ml. The specific binding was calculated by subtracting the nonspecific binding from the total binding. The inset represents the transformation of binding data to Scatchard plot. ○, total binding; O, nonspecific binding; $\triangle$, specific binding.

**Fig. 3.** The binding of 125I-apo-A-I·DMPC (A) and 125I-apo-A-II·DMPC (B) to rat ovarian membrane preparations as a function of membranes protein concentration. Aliquots of 125I-apo-A-I·DMPC (10 μg of protein/ml, 50 cpm/ng) (A) or 125I-apo-A-II·DMPC (10 μg of protein/ml, 40 cpm/ng) (B) were incubated with various protein concentrations of membrane preparation at 37°C for 60 min in a total volume of 0.25 ml of binding assay solution either alone (total binding) or together with 3 mg of unlabeled HDL3/ml (nonspecific binding). The specific binding was calculated by subtracting the nonspecific binding from the total binding. ○, total binding; O, nonspecific binding; $\triangle$, specific binding.
FIG. 4. Effect of incubation time on the binding of $^{125}$I-apo-A-I-DMPC (A) and $^{125}$I-apo-A-II-DMPC (B) to rat ovarian membranes. Aliquots of $^{125}$I-apo-A-I-DMPC (10 µg of protein/ml, 50 cpm/ng) (A) or $^{125}$I-apo-A-II-DMPC (16 µg of protein/ml, 40 cpm/ng) (B) were incubated with 100 µg of membrane protein in a total volume of 0.25 ml of binding assay solution at 37 °C in the absence (total binding) or in the presence (nonspecific binding) of unlabeled HDL₃ at a final concentration of 3 mg/ml. The specific binding was calculated by subtracting the nonspecific binding from the total binding. The binding activity of $^{125}$I-apo-A-I-DMPC and $^{125}$I-apo-A-II-DMPC was measured at different time periods from time 0 to 120 min. ○, total binding; O, nonspecific binding; △, specific binding.

$^{125}$I-HDL₃ binding. Since HDL₃, apo-A-I-DMPC, apo-A-II-DMPC, and apo-C-III₁-DMPC can mutually inhibit each other's binding, it appears that these ligands may bind to the HDL receptor. In addition, the binding of $^{125}$I-apo-A-I-DMPC, $^{125}$I-apo-A-II-DMPC, and $^{125}$I-apo-C-III₁-DMPC was inhibited by unlabeled reconstituted apolipoprotein-DMPC complexes but not by DMPC vesicles, suggesting that the binding ability of the reconstituted lipoproteins is conferred by their apolipoprotein moiety rather than the lipid environment. Taken together, these results suggest that HDL receptor recognizes apolipoprotein A-I, A-II, and the C group, but not LDL.

Since we have previously reported that in vivo pretreatment of rats with hCG results in induction of HDL receptors in the ovary (3), this characteristic was used as an additional criterion to examine whether apo-A-I-DMPC, apo-A-II-DMPC, and apo-C-III₁-DMPC bound to the HDL receptor. If the binding site for apo-A-I-DMPC, apo-A-II-DMPC, and apo-C-III₁-DMPC is the HDL receptor, then the number of binding sites for these ligands should also be increased following hCG treatment. The binding of $^{125}$I-apo-A-I-DMPC and $^{125}$I-apo-A-II-DMPC to the control and the hCG-treated groups as a function of the concentrations of ligand is shown in Fig. 5, A and B. As expected, the binding of $^{125}$I-apo-A-I-DMPC
and $^{125}$I-apo-A-I:DMPC to the ovarian membranes was significantly higher in the hCG-treated group than in the control group.

To examine whether the increase in $^{125}$I-apo-A-I:DMPC and $^{125}$I-apo-A-II:DMPC binding activity to hCG-treated ovarian membranes was caused by an increase in the number of binding sites or by a change in the binding affinity, the data obtained from Fig. 5, A and B, were transformed to a Scatchard plot (Fig. 6). Two linear parallel lines were obtained as shown in Fig. 6A with $K_d$ of $^{125}$I-apo-A-I:DMPC = 2.87 µg of protein/ml for the control group and 2.83 µg of protein/ml for the hCG-treated group. The binding capacity for $^{125}$I-apo-A-I:DMPC was 4.36 and 7.36 µg of protein/mg of membrane protein in control and hCG-treated group, respectively. Similar results were also obtained for $^{125}$I-apo-A-II:DMPC binding. Two linear parallel lines were obtained in Fig. 6B with $K_d$ = 5.70 and 5.36 µg of protein/ml for control and hCG-treated group, respectively. The binding capacity for $^{125}$I-apo-A-II:DMPC was 15.6 µg of protein/mg of membrane protein in control group and 23.6 µg of protein/mg of membrane protein in hCG-treated group. The data derived from Fig. 6, A and B, are summarized in the inset. These results further support the finding that the binding site for apo-A-I:DMPC and apo-A-II:DMPC is the hCG receptor.

Experiments were then carried out to examine the specificity of the hCG effect in inducing apo-A-I, apo-A-II, and apo-C-III binding activities. The binding of $^{125}$I-apo-A-I:DMPC, $^{125}$I-apo-A-II:DMPC, $^{125}$I-apo-C-III:DMPC, $^{35}$S-labeled BSA:DMPC, and $[^{14}C]$DMPC vesicles to hCG-treated control ovarian membranes is shown in Fig. 7. Administration of the maximum stimulatory dose of 25 IU of hCG did not result in an increase in the binding activities of $^{125}$I-labeled BSA:DMPC and $[^{14}C]$DMPC vesicles. However, the binding of $^{125}$I-apo-A-I:DMPC, $^{125}$I-apo-A-II:DMPC, and $^{125}$I-apo-C-III:DMPC in the hCG-treated group did result in an increase, suggesting that the hCG effect to induce lipoprotein binding activity is apolipoprotein-specific.

**DISCUSSION**

In the present study, we have used apo-A-I and apo-A-II isolated from human HDL3, due to the fact that 1) the stimulation of steriodogenic response was previously performed using human HDL (5–7), 2) the characterization of HDL receptor was carried out by using human HDL3 (3, 3) rat and human HDL are similar in apolipoprotein content with apo-A-I being the major apolipoprotein component (37, 38) while rat HDL possesses more of apo-A-IV and apo-E (39, 40) than that seen in human HDL, and 4) unlabeled human HDL inhibits $^{125}$I-rat HDL binding as effectively as unlabeled rat HDL (4).

Previous studies have shown that rat corpus luteum possesses discrete receptors for LDL and HDL3 (3). This conclusion has been further supported by the observation in this study that unlabeled LDL failed to inhibit the binding of both $^{125}$I-apo-A-I:DMPC and $^{125}$I-apo-A-II:DMPC. Furthermore, apo-A-I and apo-A-II bound significantly to ovarian membranes as proteins in solution, unlike apo-E which possesses binding activity only in a form of protein-lipid complex (41). This would suggest that the lipid environment may not be required for apo-A-I and apo-A-II binding. Since the binding assays were performed at 37°C, it could be argued that at this temperature binding assays of proteoliposomes might be artificially affected by phospholipid or protein exchange with membranes or lipoproteins. However, both apo-A-I and apo-A-II, when incubated at 4°C to minimize such exchange reaction, also bound to ovarian membranes (data not shown).

This observation may rule out the possibility that the binding activity of apo-A-I and apo-A-II was due to lipid-protein exchange.

From the binding specificity study it is apparent that the HDL receptor has broad specificity since apolipoprotein A-I, A-II, and the C group inhibited $^{125}$I-HDL binding. It has been shown that apolipoprotein A-I, A-II, and the C group share no primary sequences in common (42). However, the secondary structure of these apolipoproteins shows typical amphipathic helical structure (42). Thus, this amphipathic helical structure may represent the common binding domain for the HDL receptor which in turn confers broad binding specificity.
The total binding. The data points of the control are the same as binding was calculated by subtracting the nonspecific binding from DMPC from 0.5 to pg binding assay solution (total binding). Incubation was carried out at 37°C for 60 min.

For estimation of nonspecific binding, unlabeled HDL3 was added at a final concentration of 3 mg/ml. The specific binding was calculated by subtracting the nonspecific binding from the total binding. The data points of the control are the same as those in Fig. 2. O, control group; •, hCG-treated group.

Since apolipoprotein alone bound to ovarian membranes as effectively as apolipoprotein-DMPC complexes, this may suggest that the binding domain of these apolipoproteins resides in the hydrophilic portion rather than in the lipid-binding determinants. Since apolipoproteins have been shown to increase amphipathic helical formation upon recombination with DMPC (42), a question may be then raised as to why apo-A-I, apo-A-II, and apo-C-III, effectively bind to the HDL receptor when supplied solely as a protein in solution. This may be due to the fact that apo-A-I, apo-A-II, and apo-C-III, were already in an amphipathic helical structure since they were denatured in 8 M urea and refolded by slow removal of the urea before being used for the binding assay.

125I-apo-A-I-DMPC, 125I-apo-A-II-DMPC, and 125I-apo-C-III-DMPC are shown to bind to the same receptor, since the binding of each labeled apolipoprotein-DMPC complex is inhibited by the other unlabeled apolipoprotein-DMPC complexes. However, it may be noticed that the binding capacity for 125I-apo-A-I-DMPC, 125I-apo-A-II-DMPC, and 125I-apo-C-III-DMPC observed in this study may be attributed to the following reasons: 1) the average number of apolipoprotein molecules in each DMPC vesicle may be different or 2) the stoichiometry of the ligand-receptor interaction may be different for apolipoprotein-DMPC vesicles. The former possibility is supported by the finding that the average number of apo-A-I molecules in the 125I-apo-A-I-DMPC complex is less than that of apo-A-II in 125I-apo-A-II-DMPC complexes (data not shown). In view of the interaction of apo-A-I and apo-A-II with lipid vesicles, it has been shown that lipid vesicles have higher affinity for apo-A-II than apo-A-I (44–46). This may result in an increase in the average number of apo-A-II molecules in one apo-A-II-DMPC complex. However, in spite of the evidence in support of the first possibility, we cannot completely rule out the latter possibility at the present time.

Although the Kd values for 125I-apo-A-I-DMPC, 125I-apo-A-II-DMPC, and 125I-apo-C-III-DMPC obtained in this study were slightly lower than the apparent Kd of native HDLs, these values were comparable (Kd for apo-A-I-DMPC, apo-A-II-DMPC, apo-C-III-DMPC, and native HDLs are 2.87, 5.70, 9.6, and 17.8 μg of protein/ml, respectively). A likely explanation for this difference, however, is that apo-A-I in the reconstituted apo-A-I-DMPC complex may be more exposed to the medium than in the native HDLs. Thus, apo-A-I in the reconstituted apo-A-I-DMPC complex may be more exposed than in the native HDLs. Thus, apo-A-I in the reconstituted apo-A-I-DMPC complex may be more exposed than in the native HDLs. Thus, apo-A-I in the reconstituted apo-A-I-DMPC complex may be more exposed than in the native HDLs. Thus, apo-A-I in the reconstituted apo-A-I-DMPC complex may be more exposed than in the native HDLs.
of their binding sites in the ovary. We conclude from this study that HDL receptor recognizes apolipoprotein A-I, A-II, and the C group, that the apolipoprotein binding activity can be induced by hCG treatment, and that lipid environment probably is not required for apo-A-I and apo-A-II binding to the ovarian membranes.

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