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Lipoprotein lipase enhances the binding of chylomicrons to low density lipoprotein receptor-related protein

(chylomicron catabolism/apolipoprotein E receptor)

U. BEISIEGEL*†, W. WEBER*, AND G. BENGTSSON-OLIVECRONA‡

*Medizinische Kernklinik und Poliklinik, Universitätskrankenhaus Eppendorf, Martinistrasse 52, 2000 Hamburg 20, Federal Republic of Germany; and ‡Department of Medical Biochemistry and Biophysics, University of Umea, S-901 87 Umea, Sweden

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ABSTRACT **Chylomicron catabolism is known to be initiated by the enzyme lipoprotein lipase (triacylglycero-protein acylhydrolase, EC 3.1.1.34). Chylomicron remnants, produced by lipolysis, are rapidly taken up by the liver via an apolipoprotein E (apoE)-mediated, receptor-dependent process. The low density lipoprotein (LDL) receptor-related protein (LRP) has been suggested as the potential apoE receptor. We have analyzed the binding of human chylomicrons to HepG2 cells in the absence and presence of lipoprotein lipase. Bovine and human lipoprotein lipases were able to increase the specific binding of the chylomicrons by up to 30-fold. This effect was not dependent on lipolysis but appeared to be due to the lipase protein itself. It was not found when a structurally unrelated, bacterial lipase was used. Using β-migrating very low density lipoproteins (β-VLDLs), known as a good ligand for LRP, binding studies were performed on LDL receptor-negative human fibroblasts. The binding was increased 40-fold by addition of lipoprotein lipase. Crosslinking experiments on cells with ^{125}I-labeled apoE liposomes or lipoprotein lipase showed that both proteins were able to bind to LRP on the cell surface. The binding of apoE to LRP was highly increased by the addition of lipase. We conclude that lipoprotein lipase strongly enhances the binding of apoE-containing lipoproteins to LRP and therefore might play an important role in chylomicron catabolism not only because of its lipolytic activity but also because of its structural properties.**

Chylomicron catabolism has been studied in cell culture systems, on liver membrane preparations, and in animal models (1–6). The two main steps in the catabolism are lipolysis, which forms chylomicron remnants (CRs), and clearance of the CRs by receptor-mediated uptake in the liver. Lipolysis is achieved by the endothelial-bound enzyme lipoprotein lipase (LPL; triacylglycero-protein acylhydrolase, EC 3.1.1.34), which is present in many extrahepatic tissues (7–9).

Previous experiments (2–6, 10) showed that the uptake of CRs is mediated by apolipoprotein E (apoE) but is independent of apoB (11). Since the low density lipoprotein (LDL) receptor is able to recognize apoE with high affinity, one line of thinking assumed that the LDL receptor was responsible for CR catabolism (12), and this appears to be true in part. However, tissue culture studies as well as *in vivo* experiments have shown that most of the apoE-mediated uptake of CR is independent of the LDL receptor (13, 14). Moreover, patients homozygous for LDL receptor defects do not express notable defects in CR catabolism.

Several earlier attempts to characterize the potential apoE receptor protein (15, 16) were not successful. Recently, Herz *et al.* (17) described the LDL receptor-related protein (LRP) with the structural potential to be a lipoprotein receptor. We could demonstrate with chemical crosslinking that this 600-kDa protein was able to bind apoE on the surface of HepG2 cells (18). Further characterization of the LRP (19–22) increased the evidence that this protein might be the postulated apoE/CR receptor. LRP is present in several different cell types, including HepG2 cells (18) and human LDL receptor-negative fibroblasts (19). It has, however, not yet been possible to show that LRP is responsible for the CR catabolism *in vivo*.

A recent intriguing development of this field is the discovery by Strickland *et al.* (23) and Kristensen *et al.* (24) that the α_2-macroglobulin receptor is structurally identical to the LRP. These findings would propose that LRP is a multifunctional receptor.

Our present studies are based on the fact that chylomicrons are taken up in the liver only after lipolysis (7, 25). Moreover, they refer to the early data of Felts *et al.* (26), who proposed in 1975 that LPL attached to CRs might be the signal that allows the liver to specifically recognize these particles. Following these lines, we performed a series of binding studies with human chylomicrons on HepG2 cells and human LDL receptor-negative fibroblasts in the absence or presence of LPL. Parallel experiments were done for binding of β-migrating very low density lipoprotein (β-VLDL) and apoE-containing liposomes. With crosslinking experiments, we demonstrated that LRP is the protein responsible for the lipase-enhanced binding of chylomicrons.

MATERIAL AND METHODS

Patients. To obtain human chylomicrons, we used plasma of an LPL-deficient patient. The 17-year-old girl is a compound heterozygote for defects in the LPL gene. In one allele, a missense mutation in codon 207, described as a common defect in affected French Canadians, was detected (27, 28). The defect in the other allele has not yet been characterized.

Cells. Human hepatoma cells (HepG2) were grown in Dulbecco's modified Eagle's medium (DMEM; GIBCO) with 10% fetal calf serum, penicillin, and streptomycin at 37°C in 5% CO_2/95% air. Under these conditions, HepG2 cells express LRP and low levels of LDL receptor. LDL receptor-deficient human skin fibroblasts were a kind gift from B. Bihain (New Orleans). The cells were derived from a French Canadian patient (designated here FC), who is homozygous for a >10-kilobase deletion that eliminates the promoter and the first exon of the LDL receptor gene (29). The fibroblasts were maintained under the same conditions as the HepG2 cells.

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Abbreviations: LPL, lipoprotein lipase; LDL, low density lipoprotein; LRP, LDL receptor-related protein; CR, chylomicron remnant; apoE, apolipoprotein E; β-VLDL, β-migrating very low density lipoprotein; BSA, bovine serum albumin; NP-40, Nonidet P-40.

†To whom the reprint requests should be addressed.

Lipid and Protein Quantifications. Cholesterol, triglycerides, phospholipids, and free fatty acids were determined with enzymatic colorimetric assays from Boehringer Mannheim. The protein content of the lipoproteins and the cell proteins was determined by the Lowry technique.

Chylomicrons. Chylomicrons were isolated from the patient's plasma by a 45-min spin in a swing-out rotor (SW 41; Beckman) at 280,000 × *g* and 4°C. Lipolysis was performed in 7–10% bovine serum albumin (BSA) (fraction V; Sigma) in DMEM, containing 0.02 M Hepes (pH 7.5). The triglyceride concentrations were 2.2–2.5 mg/ml. Chylomicrons were treated with 1 μg of LPL per ml, which produced 80% lipolysis in 30 min at 37°C. After lipolysis, the CRs were reisolated by centrifugation at 4°C in a TLA 100.2 rotor (TL100 ultracentrifuge; Beckman) for 5 min at 95,000 rpm. For the reisolation, sucrose was added to the CRs to a final concentration of 15% and the CRs were then layered under 0.9% NaCl (typically 400 μl under 600 μl of NaCl).

For iodination of the chylomicrons, the ICl method was used (30). The sodium iodide $Na^{125}I$ was purchased from Amersham Buchler. The specific radioactivity in the protein was between 5 and 15 × 10^3 cpm/μg. Around 35% of the total label was in lipid constituents. The chemical composition of the three different labeled preparations was 2.8 ± 0.9 mg of cholesterol per ml (15%), 13.2 ± 2.8 mg of triglyceride per ml (71%), 2.1 ± 0.5 mg of phospholipid per ml (11%), and 0.6 ± 0.08 mg of protein per ml (3%). The chylomicrons contained the apoE isoform E-3.

Rabbit β-VLDL. β-VLDL ($d < 1.006$ g/ml) was isolated from the blood of cholesterol-fed rabbits (31). β-VLDL was labeled with ^{125}I by the ICl method of McFarlane (30). After incubation with ^{125}I-labeled LPL (^{125}I-LPL), the β-VLDL was reisolated in the TL100 ultracentrifuge at 4°C, for 2 h at 95,000 rpm in a TLA 100.2 rotor. The chemical composition of the labeled preparation was 6.1 mg of cholesterol per ml (58%), 1.1 mg of triglyceride per ml (10%), 0.87 mg of phospholipid per ml (8%), and 2.5 mg of protein per ml (24%). The specific activity was 68 × 10^3 cpm/μg.

ApoE-Containing Liposomes. ApoE (isoform 3 or a mixture of isoforms 3 and 4) was isolated by preparative SDS/PAGE (13%; Desaga apparatus), and 220 μg of this protein in 1 ml of 50 mM borate buffer (pH 8) containing 23.4 mg of sodium cholate was added to a lipid film consisting of 2 mg of egg lecithin and 40 μg of cholesterol. The solution was mixed in a Vortex for 1 min, gently shaken for 2 h at 24°C, and dialyzed against borate buffer (pH 8) for 48 h at 4°C. The apoE was radiolabeled with ^{125}I by the iodogen method before introduction into liposomes (32). The specific activity of apoE was usually 312 × 10^3 cpm per μg of protein.

Lipases. Bovine LPL was purified from milk as described (33). The specific activity of the preparation using a phospholipid stabilized emulsion of soy oil (Intralipid; Kabi-Nutrition, Stockholm) at pH 8.5 and 25°C, with human serum (10%; vol/vol) as source of apoCII, was 570 units/mg. One unit corresponds to 1 μmol of fatty acid released per min. Protein concentrations were calculated by using the absorbance at 280 nm and the absorption coefficient ($A_{1\%}$) 16.8 cm^{-1}. Human LPL was purified from postheparin plasma by adsorption to heparin/agarose. The preparation was treated with antibodies against human hepatic lipase (34) before gradient elution from a second column of heparin/agarose. The activity of the final preparation (in 0.1% BSA) was 40 units/ml, which would correspond to ≈0.1 mg of protein per ml assuming a similar specific activity as that for the bovine LPL.

Bovine LPL was iodinated using lactoperoxidase and glucose oxidase and was repurified on heparin-Sepharose as described (35). The lipase was stored in 10 mM Tris·HCl, pH 7.4/20% (vol/vol) glycerol/0.1% Triton X-100/≈1 M NaCl/2 mg of BSA per ml. The labeled lipase preparations contained ≈1 ng of protein per μl and had a specific activity of 10–30 × 10^3 cpm/ng.

The bacterial lipase was a kind gift from Amano Pharmaceutical (Nagoya, Japan). It was derived from *Pseudomonas fluorescens* and was dissolved at a concentration of 0.5 mg/ml in 20 mM Tris·HCl/5 mM deoxycholate/0.1 mM SDS, pH 8.5.

Antibodies. We obtained the polyclonal anti-LRP from J. Herz. The preparation and characterization of the antibody have been described (17). The polyclonal anti-LPL antibody used in Fig. 5 was raised in rabbits against purified bovine LPL.

Binding Experiments. For the binding experiments, the cells were grown in six-well plates from Costar. Typically, 1.5 × 10^6 HepG2 cells or 1 × 10^6 fibroblasts were plated and used as confluent monolayers on day 2. The experiments were performed in 3 ml of DMEM containing 5% BSA (fraction V; Sigma) and 0.02 M Hepes (pH 7.4). Concentrations of ^{125}I-chylomicrons, ^{125}I-β-VLDL, or ^{125}I-apoE liposomes, as well as the time and temperature of the incubation, are indicated in the figure legends. To determine the binding fraction, the cells were first washed several times with phosphate-buffered saline (PBS) (pH 7.4) containing BSA (2 mg/ml). The last wash was done without BSA. Then, the bound particles were released by PBS containing 5 mg of heparin per ml (153.3 units/mg; Hoffman–La Roche). All binding data with chylomicrons and β-VLDL were corrected for the radioactivity in the lipids. The cell monolayers were then dissolved in 1 M NaOH for measurement of cell-associated radioactivity and cell protein. The amount of cell protein per well was comparable in all experiments: 0.4 ± 0.05 mg for the HepG2 cells and 0.05 ± 0.005 mg for the fibroblasts.

Crosslinking Experiments. For the crosslinking experiments, binding was performed on the cells as described above but on 10-cm-diameter Petri dishes in 5 ml of DMEM for 1 h on ice. After the final wash with PBS (pH 7.4), the cells were washed once with PBS (pH 7.0). Subsequently, the bound ligand was linked to its receptor by the chemical crosslinker 1-ethyl-3(3-dimethylaminopropyl)carbodiimide in combination with *N*-hydroxysulfosuccinimide. The reagents (purchased from Pierce) were both used in a final concentration of 1 mg/ml in PBS (pH 7). The cells were incubated with the crosslinking reagents for 30 min at room temperature, followed by a wash with PBS. The cells were then scraped from the plastic dish, spun at 400 × *g* for 10 min, and solubilized in buffer A [20 mM Tris·HCl, pH 7.5/150 mM NaCl/2 mM $MgCl_2$/1% Nonidet P-40 (NP-40)]. The insoluble proteins were removed by a 55,000 rpm spin for 10 min at 4°C in a TLA 100.2 rotor. The NP-40-solubilized proteins were applied to an SDS/5% polyacrylamide gel with subsequent electroblotting to nitrocellulose (0.45 μm; Schleicher & Schuell), which was exposed to x-ray film.

RESULTS

Binding of ^{125}I-chylomicrons to HepG2 cells was studied for 1 h on ice in the absence of LPL and with increasing amounts of human LPL in the medium (Fig. 1*A*). Binding of the ligand was defined as the heparin-releasable fraction. In parallel experiments, trypsin release was determined and the results were identical. For the chylomicrons alone, we found only 8 ng of protein specifically bound per mg of cell protein. However, with the addition of 0.17 μg of human LPL per ml (Fig. 1*A*) or 0.22 μg of bovine LPL per ml (data not shown), the specific binding was already increased to 78 and 129 ng per mg of cell protein, respectively. Maximal binding was reached with 2 μg of lipase per ml for both enzymes.

To evaluate the effect of LPL, binding studies were performed with increasing amounts of chylomicrons in the

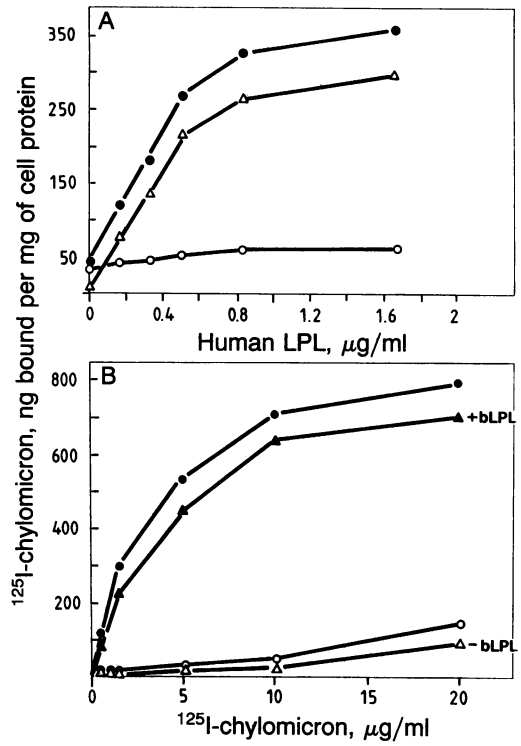

FIG. 1. Effect of LPL on the binding of human chylomicrons to HepG2 cells. Binding experiments were performed on HepG2 cells with chylomicrons from a lipase-deficient patient. Incubation was for 1 h on ice. (*A*) The indicated amount of partially purified human LPL was given to each well and subsequently a constant amount of ^{125}I-chylomicrons (4.4 μg of protein per ml of medium; specific activity, 5075 cpm per μg of protein) was added. ●, Total heparin-releasable binding in ng per mg of cell protein; ○, unspecific binding in the presence of a 13-fold surplus of unlabeled chylomicrons (56 μg/ml); △, specific binding calculated by subtracting unspecific from total binding. (*B*) Two binding experiments were performed with the indicated amount of chylomicron protein (specific activity, 15,691 cpm/μg). One experiment in the absence of LPL (○ and △) and the other in the presence of 0.43 μg of purified bovine LPL per ml (● and ▲). Total (○ and ●) and specific (△ and ▲) binding is shown. Binding was suppressed by an 18-fold surplus of unlabeled chylomicrons.

presence or absence of bovine lipase (Fig. 1*B*). In a parallel experiment, binding in the presence of 0.33 μg of human LPL per ml was performed and saturation was reached at a similar concentration of chylomicrons, as with bovine LPL (data not shown).

In the binding experiments (Fig. 1), performed on ice, the lipase activity is low. To investigate whether the observed effect of LPL was due to its residual lipolytic activity, we preincubated chylomicrons with 0.69 μg of bovine LPL per ml either at 0°C or at 37°C for various times to produce particles that had reached different levels of lipolysis. With increasing lipolysis, the specific binding at 0°C decreased from 2801 ng bound per mg of cell protein with no lipolysis to 1493 ng per mg when 80% lipolysis was reached. The data clearly show that increased lipolysis did not lead to increased binding.

In parallel experiments, we used ^{125}I-LPL to analyze the binding of lipase to the chylomicron particles. Chylomicrons (2.5 μg of protein) were incubated with 10 ng of LPL in 160 μl of 6% BSA at 0°C (to avoid rapid lipolysis) for 30 min. The particles were reisolated as described and 80% (8 ng) were found to be associated. When the incubation was performed at 37°C, we found that with increasing lipolysis the lipase dissociated from the particles together with other proteins (data not shown).

Another approach to evaluate the role of the LPL protein versus its lipolytic activity was to use an unrelated lipase with activity against lipoproteins as the bacterial lipase from *P. fluorescens*. This lipase has lipolytic activity similar to that of LPL, but it is a structurally different protein (35). The addition of 0.86 μg of LPL per ml caused a 29-fold increase of chylomicron binding to HepG2 cells at 0°C, while the addition of 0.83 and 1.7 μg of bacterial lipase per ml had no effect on binding. The lipolytic activity of the bacterial lipase on the chylomicrons was found to be slightly higher than the activity of LPL.

The availability of human skin fibroblasts that lack the LDL receptor (FC) but express LRP provided the possibility to study whether the observed effect was related to LRP. Addition of either human or bovine LPL to binding experiments on FC cells caused a significant increase of ^{125}I-chylomicron binding. Without lipase, the binding was 19 ng per mg of cell protein, while in the presence of 0.87 μg of bovine LPL per ml the binding was 1824 ng/mg and with addition of 0.33 μg of human LPL per ml, 802 ng was bound per mg of cell protein. This and other experiments indicated that the effect of LPL on the binding of chylomicrons to cells might be mediated by LRP rather than by the LDL receptor.

Rabbit β-VLDL had been shown to be taken up by LDL receptor-negative fibroblasts via LRP (19). Therefore, we also used this ligand to perform binding experiments in the absence and presence of LPL. Fig. 2 demonstrates that the addition of increasing amounts of bovine LPL enhanced the binding of β-VLDL to FC cells. Similar effects could be demonstrated with HepG2 cells (data not shown). Binding of ^{125}I-LPL (10 ng) to β-VLDL (125 μg of protein) at 0°C for 30 min was determined after reisolation of the β-VLDL in the ultracentrifuge and 50% of the LPL (5 ng) was found associated.

To investigate whether the observed effect of LPL was on LRP-mediated binding, apoE liposomes were used for which binding to LRP had been shown previously by using different crosslinking reagents (18). Fig. 3 demonstrates the effect of bovine LPL on the binding of apoE liposomes to FC cells. At the highest concentration, a 33-fold increase could be measured.

Crosslinking reagents [1-ethyl-3(3-dimethylaminopropyl)-carbodiimide/*N*-hydroxysulfosuccinimide] were used in binding experiments with ^{125}I-apoE liposomes in the absence or presence of LPL on HepG2 and FC cells. In the same experiment, bovine ^{125}I-LPL itself was used as a ligand. Fig. 4 shows the autoradiographs of the experiments and in both

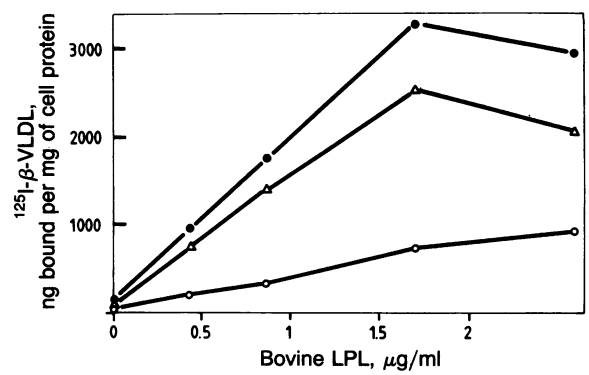

FIG. 2. Effect of LPL on binding of β-VLDL to LDL receptor-negative fibroblasts (FC). Binding experiments were performed with ^{125}I-β-VLDL for 1 h at 4°C. The indicated amount of bovine LPL was given to each well and subsequently 2.2 μg of ^{125}I-β-VLDL/ml was added. In control wells, an 80-fold surplus of unlabeled β-VLDL was added (○) to determine the specific binding. The specific binding of β-VLDL was increased 42-fold by addition of 1.7 μg of bovine LPL per ml. ●, Total binding; △, specific binding.

cell types the increased binding of apoE liposomes in the presence of LPL was reflected by the labeled 600-kDa protein. This protein is in its position identical to LRP as shown by immunostaining of the nitrocellulose after autoradiography. In HepG2 cells, an additional band with a larger molecular mass was detectable. This band was also detected by the anti-LRP. The low amount of ^{125}I-apoE binding in the absence of lipase cannot be seen in this radiograph, but after a longer exposure the band appeared in the same position. The binding of apoE liposomes in the presence of LPL could be suppressed by chylomicrons. In several other binding experiments, it was shown that the three ligands used in this study effectively compete with each other. The ^{125}I-LPL bound to the 600-kDa protein in the crosslinking experiment without being associated with apoE (Fig. 4, lanes 1). The larger molecular mass band on HepG2 cells seen with labeled apoE was also detectable with the LPL. These experiments showed that LPL not only increased binding of ^{125}I-apoE to LRP but also demonstrated that LPL bound directly to this receptor. The addition of heparin, or of an antiserum to LPL, abolished the binding of LPL to LRP in crosslinking experiments, as shown in Fig. 5.

DISCUSSION

Previous studies showed that lipase-treated, small chylomicrons or CRs were rapidly cleared by the liver (7, 10, 25). The uptake mechanism, however, was not understood. For the LRP, the potential CR receptor, the ability to bind apoE-containing liposomes (18) and β-VLDL (19) has recently been demonstrated. The physiological role to act as a CR receptor, however, has not yet been verified. In the current approach, we used lipase-treated chylomicrons as ligands for the LRP, since these particles should most closely resemble the physiologically produced CR.

To obtain human chylomicrons that had not been exposed to LPL, chylomicrons were isolated from a LPL-deficient patient. We showed that ^{125}I-labeled, active lipase was able to bind to these chylomicrons, to β-VLDL, and to apoE liposomes at 4°C. The binding to the reisolated particles was not heparin releasable (data not shown). All binding studies shown in this paper were performed at 0°C or 4°C to avoid rapid lipolysis. The data show that LPL stimulates binding of the chylomicrons to fibroblasts and HepG2 cells independently from its lipolytic activity. This is clearly different from the stimulating effect of free fatty acids described by Yen *et al.* (36) and the effect of lipolysis on VLDL uptake by the LDL receptor (37, 38).

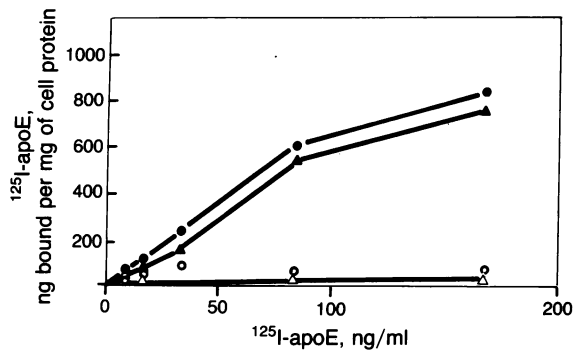

FIG. 3. Effect of LPL on binding of apoE-containing liposomes to LDL receptor-negative fibroblasts (FC). Two binding experiments were performed with the indicated amounts of ^{125}I-apoE-containing liposomes for 1 h at 0°C. In the absence of bovine LPL, binding was nearly undetectable. ○, Total binding; △, specific binding. When bovine LPL (0.7 μg/ml) was added to the wells, specific binding (▲) was increased up to 700 ng per mg of cell protein (●, total binding). For both sets of experiments, control wells were incubated with unlabeled chylomicrons (200-fold excess of protein) to suppress specific binding.

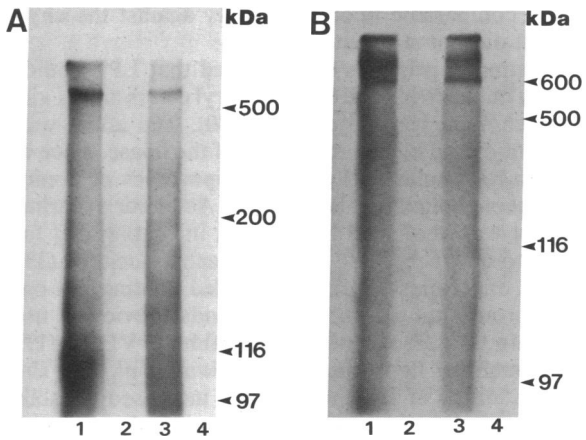

FIG. 4. Crosslinking of apoE and LPL to cells. Binding experiments were performed on HepG2 and FC cells with ^{125}I-apoE liposomes (E-3) in the absence or presence of unlabeled LPL, in the presence of surplus chylomicrons, or with bovine ^{125}I-LPL alone. Incubation was for 1 h at 0°C and subsequently the bound ligand was chemically linked to the receptor by 1-ethyl-3(3-dimethylaminopropyl)carbodiimide/*N*-hydroxysulfosuccinimide. After crosslinking, the cells were solubilized in NP-40, applied to an SDS/polyacrylamide gel, and electroblotted onto nitrocellulose. The radiolabeled bands were detected after 4 and 5 days of exposure to x-ray films. (*A*) FC cells were used and binding experiments were performed with 6 ng of ^{125}I-LPL per ml (lane 1), 0.32 μg of ^{125}I-apoE liposomes per ml (lane 2), 0.32 μg of ^{125}I-apoE liposomes per ml with 0.52 μg of unlabeled LPL per ml (lane 3), and 0.32 μg of ^{125}I-apoE liposomes per ml with 0.52 μg of unlabeled LPL per ml and a surplus of unlabeled human chylomicrons (lane 4). Autoradiography of nitrocellulose after SDS/PAGE is shown. The following amounts of ^{125}I-ligand in the NP-40-solubilized fractions were applied to the gel: lane 1, 0.35 ng of LPL; lane 2, 8 ng of apoE; lane 3, 24 ng of apoE; lane 4, 1.5 ng of apoE. The binding of apoE alone is too low to be detected in the 4-day exposure (lane 2). (*B*) HepG2 cells were used and binding experiments were performed with the same ligands described in *A* (samples were applied in the same order). The radiolabeled bands correspond to the following amounts of ^{125}I-ligand in the NP-40-solubilized fractions: lane 1, 0.42 ng of LPL; lane 2, 5.9 ng of apoE; lane 3, 47 ng of apoE; lane 4, 4.3 ng of apoE.

For the effect of LPL on chylomicron binding, it did not matter whether the lipase was associated with the particle before the binding assay, was added in the medium during the assay, or was allowed to bind to the cells before chylomicrons were added. The latter effect could be abolished with a heparin wash. These results indicated that enhanced binding is due to the LPL protein itself. This was further supported by experiments using a structurally different bacterial lipase

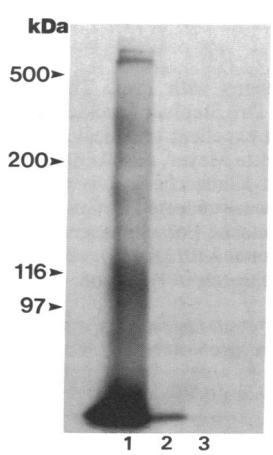

FIG. 5. Crosslinking of LPL to HepG2 cells. Binding experiments and crosslinking were performed with bovine ^{125}I-LPL as described in Fig. 4. HepG2 cells were used and binding experiments were performed with 10 ng of ^{125}I-LPL alone (lane 1) or with the same amount of lipase but either in presence of rabbit anti-bovine LPL antiserum (100 μl) (lane 2) or in the presence of heparin (5 mg/ml) (lane 3). Autoradiography of nitrocellulose after SDS/PAGE is shown. NP-40-solubilized fractions that were applied to the gel contained the following amounts of ^{125}I-LPL: lane 1, 1.7 ng; lane 2, 0.1 ng; lane 3, 0.01 ng.

that had comparable lipolytic activity against the chylomicrons but did not stimulate binding.

Interestingly, previous data showed that LPL could dramatically enhance uptake of cholesteryl esters and of vitamin E from chylomicrons to cells (39, 40). The effect was not dependent on the hydrolytic ability of the lipase, since it was also seen for cholesteryl ethers in liposomes of nonhydrolyzable phospholipid analogues (41). As in our experiments, the effect was abolished by heparin, indicating that for the transfer to occur the LPL had to be cell associated (39, 40).

In our experiments, LPL stimulated binding not only of chylomicrons, but also of other ligands previously used to investigate the CR receptor—i.e., rabbit β-VLDL (19) and apoE-containing liposomes (18). Kowal *et al.* (19) showed that the addition of apoE to β-VLDL increased the ability of LDL receptor-negative cells to internalize these particles.

A low amount of LPL normally circulates in plasma. According to Goldberg *et al.* (42) the major part of this LPL is associated with lipoproteins. In humans, the level of LPL activity is 0.3–1 milliunit/ml. After a meal high in fat this level is often increased to 2–3 milliunits/ml (unpublished data). These levels are still low compared to those obtained in postheparin plasma (150–300 milliunits/ml). The levels of LPL protein mass in preheparin plasma have been determined by Kern *et al.* (43) in six normal subjects. They vary between 8 and 25 ng/ml. Babirak *et al.* (44) measured postheparin LPL protein mass in plasma and, in normal probands, found ≈0.2 μg/ml. In our studies, we used LPL concentrations of 0.1–2.0 μg/ml, which corresponds to the postheparin range.

By crosslinking experiments, we could show that LPL markedly stimulated the binding of apoE to LRP and LPL on its own appeared to bind to the same receptor. Addition of heparin abolished the binding of LPL to the 600-kDa protein. We suggest that the increased particle binding to LRP is due to the attachment of both apoE and LPL to the same protein.

It is known that circulating LPL is avidly taken up in the liver. Binding appears to occur both to heparin-sensitive and to heparin-insensitive sites (45, 46). An interesting question is whether all uptake of the enzyme molecule occurs together with the apoE-containing lipoproteins by the LRP or whether there are still other binding mechanisms.

In summary, this study shows that LPL enhances binding of chylomicrons, β-VLDL, and apoE-containing liposomes to cells. Crosslinking experiments showed that LPL increased binding of ^{125}I-apoE-containing liposomes to LRP severalfold, and that LPL itself can be a ligand for the LRP. It was recently shown that LRP is the receptor for activated α_2-macroglobulin (23, 24) and the relation between binding of α_2-macroglobulin and apoE-containing lipoproteins was recently reviewed by Brown *et al.* (47). The data presented here support the view that LRP might be the long-sought CR receptor, recognizing chylomicrons after their association with LPL.

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