Identification and Characterization of the Endothelial Cell Surface Lipoprotein Lipase Receptor*

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The hydrolysis of triglycerides in plasma lipoproteins is mediated by lipoprotein lipase (LPL) that is bound to vascular endothelial cells. The specific endothelial cell surface protein(s) with which LPL associates has not been characterized. To identify this LPL binding protein(s), radioiodinated cell surface proteins from cultured bovine aortic endothelial cells were chromatographed using bovine LPL-Sepharose. A single radioiodinated protein of apparent molecular mass 220 kDa was specifically retained by the gel and eluted with 0.4 M NaCl. A LPL-binding protein of similar size was obtained after metabolic labeling of the cellular proteoglycans with 35SO4, indicating that the 220-kDa protein is a proteoglycan. After heparitinase or nitrous acid treatments the molecular mass of the LPL-binding protein decreased to approximately 50 kDa, suggesting that it contains heparin sulfate chains. A 220-kDa protein from the basal cell surface was also identified using LPL-Sepharose chromatography. 125I-LPL was cross-linked to the endothelial cell surface using ethylene glycol (succinimidylsuccinate). A single ligand-receptor complex, approximately 350 kDa, was obtained. Heparin and unlabeled LPL decreased the cross-linking of radioiodinated LPL to the cell surface receptor. To examine whether the receptor mediates the internalization of cross-linked 125I-LPL, cells containing 125I-LPL complexed to the surface were incubated at either 37 or at 4°C. The amount of 125I-LPL internalized by the cells was 74% greater at 37°C than at 4°C. This suggested that LPL cross-linked to the receptor was internalized in a temperature-dependent manner. Thus, a 220-kDa heparan sulfate proteoglycan functions as an endothelial cell surface receptor for LPL.

Lipoprotein lipase (LPL), an important enzyme in the metabolism of lipoprotein triglycerides, functions while bound to a poorly characterized endothelial cell binding site. After synthesis in parenchymal cells, LPL is secreted and transported across the endothelium before binding to the luminal surface of endothelial cells (1). Hydrolysis of triglycerides in plasma lipoproteins is thought to be mediated only by the pool of LPL bound to the luminal endothelial cell surface (2). Previous studies suggest that LPL binds to the endothelial cell surface via electrostatic interactions with cellular proteoglycans (3–6). In these studies, pretreatment of endothelial cells with enzymes that degraded heparan sulfate chains or addition of heparin to the culture media decreased LPL binding to cell surfaces. These results provided indirect evidence that LPL binds to heparan sulfate proteoglycan(s) (HSPG). Another study attempted to identify the LPL binding protein in porcine aorta (7). Radioiodinated LPL was mixed with purified chondroitin sulfate and dermatan sulfate proteoglycans and a LPL-proteoglycan complex was demonstrated by gel filtration. It was concluded that LPL can bind to a chondroitin sulfate-dermatan sulfate proteoglycan. However, LPL was released from this complex more effectively by heparin or heparan sulfate than dermatan sulfate or chondroitin sulfate. This suggests that HSPG is the proteoglycan with the highest affinity for LPL.

Vascular endothelial cells synthesize a variety of proteoglycans containing heparan sulfate, chondroitin sulfate, and dermatan sulfate (8, 9). At least three different species of HSPG are synthesized by cultured endothelial cells (10, 11). The three HSPGs differ in their molecular weights and cellular locations; they are either secreted into the medium, retained intracellularly, or displayed at the cell surface. One high molecular mass HSPG, >400 kDa, is incorporated into the subendothelial basement membrane (12). Only a cell surface HSPG is likely to function as a receptor for LPL.

Previous studies from our laboratory have examined the cellular metabolism of LPL using cultured endothelial cells (13). When LPL was bound to the surface of cells, the enzyme was internalized and recycled back to the cell surface without degradation. To study endothelial cell transcytosis of LPL, the cells were grown on permeable polycarbonate filters (14). Active LPL was transported across the monolayer from the basal to the apical cell surface. In both of these studies, there was an absolute requirement for LPL to first bind to the endothelial cell surfaces. Conditions that blocked the binding, resulted in marked decreases in the recycling or transcytotic pathways for LPL. For example, pretreatment of the cells with heparinase to degrade cell surface HSPGs reduced the participation of LPL in both pathways. Therefore, an endothelial cell HSPG receptor is critical for LPL internalization and targeting into recycling or transport pathways.

In view of the important role of the putative endothelial cell surface receptor in the cellular metabolism of LPL, we conducted studies to identify and characterize this receptor. Affinity chromatography using LPL immobilized on Sepha-
rose and covalent cross-linking of LPL to endothelial cell surfaces were utilized to identify the cell surface receptor for LPL. In addition, the proteoglycan nature of the receptor and its mode of association with the cell surface were examined.

MATERIALS AND METHODS

Endothelial Cell Culture—Bovine aortic endothelial cells were isolated and cultured as described for porcine aortic endothelial cells (15). The cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% calf bovine serum. Cells between the 3rd and 10th passage were used.

Bovine Milk LPL—Bovine milk LPL was purified from bovine milk and radiiodinated as described previously (15). Protein was determined by the method of Lowry et al. (16) using bovine serum albumin (BSA) as standard. The typical specific activity of the purified enzyme was 25-35 mmol of free fatty acids/h/mg of protein. Radiolabeled LPL had a specific radioactivity of approximately 500 cpm/ng of protein.

Radioiodination of Endothelial Cell Surface Proteins—Endothelial cells were grown to confluence in 75-cm² culture flasks (Falcon 3020, Becton Dickinson, Lincoln Park, NJ). The cells were extensively washed with 150 mM NaCl, 10 mM sodium phosphate, pH 7.4 (PBS). A reaction mixture containing 1 μCi of Na125I, 180 μg of lactoperoxidase, 90 μg of glucose oxidase, and 20 mM glucose in 10 ml of PBS was added to the cells. The cells were incubated at 4 °C for 45 min. After the incubation, the reaction mixture was removed and the cells washed three times with 10 ml PBS. The cells were then extracted with 1% CHAPS, and 1% n-octyl β-D-glucopyranoside (octylglucoside) in 10 mM Tris-HCl, pH 7.4, containing 1 mM EDTA, 1 μM aprotinin, and 2 mM phenylmethylsulfonyl fluoride for 3 h at 4 °C (17). Greater than 90% of the radioactivity associated with the cells was extracted using this procedure.

Bovine aortic endothelial cells were specifically labeled as described by Lisanti et al. (18). Endothelial cells were grown to confluence on permeable polycarbonate filters (Nucleopore, Pleasanton, CA) (14). The media from the bottom and top chambers were removed and the cells were washed three times with PBS. To the bottom chamber was added 2.6 ml of PBS containing 2.6 mg glucose, 42 μg of lactoperoxidase, 5.6 μg of glucose oxidase, and 0.16 μg of Na125I. The cells were then incubated at 4 °C for 45 min. After the incubation, the reaction mixture from the bottom chamber was removed, and the basal cell surface was washed with PBS. The cells were then released from the filters using 2.5 mM EDTA in PBS and the cellular proteins were extracted using CHAPS and octylglucoside as described above.

Affinity Chromatography of Radioiodinated Endothelial Cell Surface Proteins on LPL-Sepharose—LPL-Sepharose and Sepharose containing no coupled LPL (blank-Sepharose) were prepared as described previously (19). Approximately 1 mg of LPL/ml was coupled to the Sepharose. Detergent extracts containing the radioiodinated cell surface proteins were dialyzed against 10 mM Tris-HCl buffer, pH 7.4, containing 1% CHAPS and 1 μM aprotinin and then incubated with LPL-Sepharose or blank-Sepharose for 3-4 h at 4 °C. The gel slice was washed with PBS to remove unincorporated 125I and then incubated with heparinase (0.1 unit, Seikagaku Kogyo, Tokyo, Japan) for 12 h at 42 °C with chondroitinase ABC (5 units, Sigma) for 3 h at 37 °C. After the incubation, the supernatants were precipitated with trichloroacetic acid and analyzed by SDS-PAGE.

RESULTS

Binding of Radioiodinated Endothelial Cell Surface Proteins to LPL-Sepharose—To identify specific proteins present on the endothelial cell surface that interact with LPL, endothelial cell surface proteins were radioiodinated, solubilized, and mixed with LPL-Sepharose affinity gel. As shown in Fig. 1A, greater than 80% of the radioactivity applied did not bind to the affinity gel. About 3-5% of the applied radioactivity was eluted with 0.4 M NaCl buffer. No additional radioactivity was eluted with 1.5 M NaCl or 0.1 M glycine-HCl buffer. 2. Fig. 1B shows an autoradiogram of a SDS-PAGE gel of the total cell surface radiolabeled proteins and proteins eluted with 0.4 M NaCl buffer. The 0.4 M NaCl eluate contained two proteins. One protein had an approximate molecular mass of 220 kDa, and the second had a molecular mass of about 180 kDa. The mobility of both proteins did not change when SDS-PAGE was performed under reducing conditions.

To examine the specificity of binding of the two proteins to LPL, the detergent extract was applied to a column containing blank-Sepharose. When that column was eluted using 125I-LPL bound to the cell surface by incubating the cells with PBS containing 0.15 mM ethylene glycolbis(succinimidy1succinate) for 45 min at 4 °C. In some experiments, this incubation mixture also contained 500 units of heparin (20 μg). At the end of the incubation, the cells were washed with PBS and then solubilized with the detergent containing buffer as described above.

Experiments were performed where the cellular internalization of cross-linked 125I-LPL was studied. The enzyme was first cross-linked to the surface as described above. The cells were then incubated with 100 units of heparin for 15 min to release the LPL that was bound to the surface but was not cross-linked. The cells containing cross-linked 125I-LPL were then incubated at either 4 or 37 °C or at 37 °C in the presence of 400 μM cytochalasin B (13). At the end of the incubation, the media were removed, the cells washed with DMEM-BSA and cell surface non-cross-linked LPL was removed using 0.4 M NaCl. The gel slice was washed three times with PBS. '*'I-LPL bound to the cells was cross-linked to the cell surface by incubating the cells with PBS containing 100 units of heparin (100 μCi) at 4 °C for 10 min, and 2.0 × 105 cpm of trypan at 37 °C for 3 min (13). The radioactivity associated with the cells (representing internalized 125I-LPL) was then measured.

SDS-PAGE—To concentrate the extracted cell proteins for SDS-PAGE, detergent extracts were dialyzed overnight, and the proteins were precipitated with trichloroacetic acid (10% v/v) after the addition of 20-30 μg of BSA. The precipitates were then analyzed using either 1-2% gradient gels (Pharmacia LKB Biotechnology Inc.) or 7.5% gels (13). The gels were subsequently subjected to autoradiography using X-Omat AR film (Eastman Kodak Company, Rochester, NY).

Enzymatic and Nitrous Acid Treatment of LPL-binding Proteins—Radioiodinated cell surface proteins which bound to LPL-Sepharose were separated by SDS-PAGE, visualized by autoradiography, and the 220-kDa band was excised. To obtain sufficient amounts of radioactivity in the specific 220-kDa band, in some experiments, additional proteins obtained from LPL-Sepharose were separated by SDS-PAGE. After this separation the gel region corresponding to the 220-kDa band was excised. The gel slide was extensively washed with a solution containing 25% isopropanol alcohol and 10% methanol, rinsed with PBS, and then the 220-kDa proteins was radioiodinated while still on the gel slice (12). The gel slice was washed with PBS to remove unincorporated 125I and then incubated with heparinase (0.1 unit, Seikagaku Kogyo, Tokyo, Japan) for 12 h at 42 °C or with chondroitinase ABC (5 units, Sigma) for 3 h at 37 °C in 10 mM Tris-HCl buffer, pH 7.4, containing 50 mM NaCl, 3 mM CaCl2, 0.1 mg/ml BSA, and 1 mM phenylmethylsulfonyl fluoride. After the incubation, the supernatants were precipitated with trichloroacetic acid and analyzed by SDS-PAGE.

Proteins that eluted with 0.4 M NaCl from LPL-Sepharose were also treated with nitrous acid in the presence of 10 mM HCl for 3 h at room temperature (12). After the incubation, the mixture was neutralized with 10 N NaOH and then the proteins were analyzed by SDS-PAGE.

Effects of Pretreatment of Endothelial Cell Surfaces on 125I-LPL Binding—Confluent endothelial cells in 35-mm tissue culture dishes (Falcon 3032), were rinsed three times with PBS and then incubated with either PBS containing heparin (300 units) or 10 mM Tris-HCl, pH 7.4, containing 1 μM NaCl at 4 °C for 1 h. In other experiments the cells were pretreated with purified phosphatidylinositol-specific phospholipase D (3.9 units/ml) from Bacillus thuringiensis (a gift from Dr. Martin Low, Columbia University, NY) at 37 °C for 1 h or with trypsin (0.25%) at 25 °C for 10 min. After the various incubations, the cells were washed three times with DMEM-BSA. The cells were then incubated with 125I-LPL (10 μg) at 4 °C for 2 h to allow the enzyme to bind to the cell surface. 125I-LPL bound was then released by heparin as described previously (13).
Endothelial Cell Lipoprotein Lipase Receptor

FIG. 1. Isolation of radioiodinated cell-surface proteins by LPL-Sepharose affinity chromatography. A, elution of radioiodinated cell-surface proteins from LPL-Sepharose. Radioiodinated cell-surface proteins (3000 × 10⁶ cpm) were incubated with LPL-Sepharose (1 ml) for 4 h. The affinity gel in a column was washed with 10 mM Tris- HCl buffer, pH 7.4, containing 0.1% CHAPS, 0.15 M NaCl. The gel was eluted with 0.4 M NaCl and 1.5 M NaCl in the same buffer. Any proteins remaining bound to the gel were then released with 0.1 M glycine-HCl, pH 2. 1 ml fractions were collected and the radioactivity determined. Greater than 0.4 M NaCl (lane 2) were applied to a 2–16% gradient SDS-PAGE gel. The gel was run at 30 mA for 3.5 h and exposed to X-Omat AR film for 3.5 days. B, SDS-PAGE of radiolaheled cell-surface proteins. Cell surface proteins of endothelial cells were radiolaheled and solubilized using CHAPS and octylglucoside. The total radioiodinated proteins obtained from the cells (lane 1) and those eluted from LPL-Sepharose affinity column by 0.4 M NaCl (lane 2) were applied to a 2–16% gradient SDS-PAGE gel. The gel was run at 30 mA for 3.5 h and exposed to X-Omat AR film for 12 h. Approximately 600 × 10⁶ cpm were applied to lane 1; 90 × 10⁶ cpm were applied to lane 2. The migration of 30, 46, 69, 96, and 200 kDa molecular mass standards are indicated. The proteins retained by LPL-Sepharose were rechromatographed using Sepharose gel without covalently bound LPL (blank-Sepharose) to determine if either protein was nonspecifically retained by the Sepharose. Initial iodinated proteins (lane 3) and those proteins retained by the blank-Sepharose and eluted with 0.4 M NaCl (lane 4) were assessed by SDS-PAGE and autoradiography. The lower molecular mass protein was retained by the blank-Sepharose and is seen in both lanes 3 and 4.

0.4 M NaCl, the 220-kDa protein was not retained. The 180-kDa protein did bind to blank-Sepharose. Similarly, if the proteins isolated by LPL-Sepharose were then eluted through blank-Sepharose, the lower molecular mass protein was retained (Fig. 1). No other proteins were eluted with higher concentrations of NaCl or the glycine buffer. These results suggest that only the 220-kDa protein is a specific LPL-Sepharose binding protein. Retention of the lower molecular mass protein on LPL-Sepharose may represent nonspecific binding of radioiodinated proteins to Sepharose.

Proteoglycan Nature of the LPL-Sepharose Binding Protein—To examine whether the 220-kDa protein isolated in the above experiments is a proteoglycan, endothelial cell proteoglycans were metabolically labeled using ³⁵SO₄ (21) and the cellular proteins extracted. The extract was then applied to the LPL-Sepharose column. FIG. 2A shows an autoradiogram of an SDS-PAGE gel of ³⁵SO₄-labeled proteins which did not bind to the affinity column and those eluted from the column with 0.4 M NaCl buffer. In the 0.4 M NaCl eluate, two bands were visible, one with mobility identical to that obtained after radioiodination of the endothelial cell surface (molecular mass, 220 kDa) and another which was of higher molecular mass. The metabolic labeling of the 220-kDa protein with ³⁵SO₄ suggests that it contains glycosaminoglycan chains. This 220-kDa band was not present in the unbound fractions obtained from LPL-Sepharose (FIG. 2A). The 220-kDa protein was also radiolabeled and retained by the affinity column when the cellular proteins were metabolically labeled with ³⁵S-methionine. Therefore, the cell surface 220-kDa LPL-binding protein is synthesized by endothelial cells and is heavily sulfated.

The higher molecular mass protein labeled by ³⁵SO₄, and retained on LPL-Sepharose (FIG. 2A) is also likely to be a proteoglycan. A high molecular mass HSPG is secreted by endothelial cells and is the principal proteoglycan in basement membrane (12). Because this proteoglycan contains heparan sulfate molecules which can interact with LPL, it is not surprising that it is retained by LPL-Sepharose. Since the higher molecular mass proteoglycan was labeled by ³⁵SO₄ and not labeled by cell surface radioiodination, it is not on the apical cell surface. Therefore, unlike the 220-kDa protein, the high molecular mass sulfated protein is unlikely to function as the cell surface receptor for LPL.

To determine if the 220-kDa proteoglycan contains heparan sulfate glycosaminoglycan chains, the isolated and radioiodinated 220-kDa protein was treated with heparitinase. This treatment resulted in a marked decrease in the molecular mass of the protein on SDS-PAGE. The molecular mass of the core protein was approximately 50 kDa (FIG. 2B). A similar size protein was obtained when nitrous acid was used to remove the glycosaminoglycan chains. Since one of the HSPGs synthesized by endothelial cells in culture contains a small amount of chondroitin sulfate (10, 11), the 220-kDa proteoglycan was also treated with chondroitinase ABC. Treatment with this enzyme resulted in a small change in the mobility of the proteoglycan to approximately 195 kDa. These results demonstrate that the 220-kDa proteoglycan contains predominantly heparan sulfate chains, but it may also contain some chondroitin sulfate chains.

Cross-linking of ¹²⁵I-LPL to the Endothelial Cell Surface—The results presented above indicate that the 220-kDa HSPG is the endothelial cell surface receptor for LPL. To further confirm that this is the LPL receptor, ¹²⁵I-LPL was covalently cross-linked to the endothelial cell surface using ethylene glycolbis(succinimidyl succinate). The cells were then extracted with detergent and the proteins analyzed by SDS-PAGE followed by autoradiography. Two bands were observed, one with mobility corresponding to molecular mass of about 350 kDa and another at 55 kDa (FIG. 3). The band at 55 kDa corresponds to free, monomeric ¹²⁵I-LPL that was not cross-linked to the receptor but was bound to it during solu-
The specific cross-linking of $^{125}$I-LPL to the endothelial cell surface was examined by cross-linking in the presence of heparin, a molecule which inhibits the binding of LPL to the endothelial cell surface (3, 13). As shown in Fig. 3, addition of heparin markedly decreased the LPL cross-linked to the receptor since the intensity of the band corresponding to the $^{125}$I-LPL-receptor complex was reduced (compare lane A with lane B in Fig. 3). The 55-kDa band, corresponding to noncross-linked $^{125}$I-LPL was also reduced. The inclusion of heparin inhibited LPL association with the cell surface, markedly reducing the amount of radiolabeled LPL associated with the solubilized cell. For this reason, the intensity of both bands was decreased. Similarly, cross-linking in the presence of 20 μg of unlabeled LPL also reduced the amount of $^{125}$I-LPL cross-linked to the cells by 31% relative to that found in the absence of unlabeled LPL.

Internalization of $^{125}$I-LPL Cross-linked to the Cell Surface—On the basis of previous findings, we hypothesized that cross-linking of LPL to its functional receptor should allow internalization by the cells (13). To confirm this, $^{125}$I-LPL was first cross-linked to the surface and the cells were then incubated at either 4 or 37 °C or at 37 °C in the presence of 400 μM cytochalasin B. The amounts of LPL internalized during the incubations were then determined. As shown in Fig. 4, cross-linked $^{125}$I-LPL internalized by the cells at 37 °C was 74% greater than the amount of LPL internalized at 4 °C, suggesting that internalization is a temperature-dependent process. The presence of cytochalasin B during the incubation at 37 °C markedly decreased the internalization of cross-linked $^{125}$I-LPL (Fig. 4). Cytochalasin B blocks cellular internalization of proteins (22). These results suggest that $^{125}$I-LPL cross-linked to the surface of endothelial cells is internalized by the cells.

Association of the LPL Receptor with the Cell Surface—HSPGs are associated with the plasma membrane of cells by at least three mechanisms (23). HSPGs bound to protein or glycosaminoglycan receptors can be displaced from the cell surface by heparin or treatment with high ionic strength buffers (24). Some HSPGs are attached to the plasma membrane via a covalent linkage that is hydrolyzed by phosphatidylinositol-specific phospholipase C (PI-PLC) at 37 °C for 1 h or 0.25% trypsin at 25 °C for 10 min. After these treatments, the cells were incubated with $^{125}$I-LPL (10 μg/ml) at 4 °C for 1 h. $^{125}$I-LPL bound to the cell surface was determined after release of $^{125}$I-LPL from the cell with heparin. Results are shown as percent decrease in binding relative to control experiments with DMEM alone. In control experiments, 312 ng of $^{125}$I-LPL was bound to the surface. The data shown are means ± S.E. of experiments performed in triplicate.
Preparation of Endothelial Cells

The endothelial cells were grown on permeable polycarbonate filters (14) and the basal cell surface proteins selectively labeled using lactoperoxidase and glucose oxidase. The radiiodinated proteins were solubilized by detergents and then applied to LPL-Sepharose. The bound proteins were eluted with 0.4 M NaCl (lane 1, 88 × 10⁶ cpm) and radiiodinated proteins eluted from LPL-Sepharose (lane 2, 52 × 10⁶ cpm) were analyzed by SDS-PAGE on a 7.5% gel. The gel was then exposed to X-Omat AR film. Lane 1 was exposed for 12 h, and lane 2 was exposed 48 h. The mobility of the 220-kDa protein is indicated by the arrow.

Identification of the LPL Receptor on the Basal Cell Surface

Identification of the LPL Receptor on the Basal Cell Surface of Endothelial Cells—We have recently reported that the transport of LPL across endothelial cells grown on permeable polycarbonate filters requires the binding of LPL to a heparinase-sensitive proteoglycan on the basal cell surface (14). To identify this binding site, endothelial cells were grown on polycarbonate filters and the basal cell surface specifically radiiodinated at 4 °C. The cells were released from the filters and then extracted with detergents. Several proteins present at the basal cell surface were identified (Fig. 6). After application of the detergent extract to LPL-Sepharose, a 220-kDa protein was eluted with 0.4 M NaCl buffer. These results demonstrate that the basal endothelial cell surface also contains a 220-kDa LPL receptor. A lower intensity, slower migrating band was also observed at the top of the gel (Fig. 6). This high molecular mass band may contain basement membrane proteoglycans radiiodinated by the procedure used.

Discussion

In the present study, we identified a 220-kDa endothelial cell surface LPL-binding protein. Two separate techniques verified that this protein specifically bound LPL. First, the receptor was identified on the basis of its ability to specifically bind to LPL immobilized on Sepharose. Second, soluble ¹²⁵I-LPL was bound to the surface of endothelial cells and then directly cross-linked to the cell surface receptor. A single LPL-receptor complex of approximately 350 kDa was identified. This was the expected size of a complex between dimeric, 110-kDa LPL and the 220-kDa receptor. Thus, both techniques identified a single cell surface LPL-binding protein. It should be noted that Scatchard analyses of LPL binding to endothelial cells had predicted the presence of a single, high affinity binding site for LPL (3, 5, 6, 13).

Several characteristics of the 220-kDa protein are compatible with information obtained from cellular studies of LPL binding to endothelial cells. First, the interaction of LPL and its receptor is thought to be via electrostatic interactions. Elution of the 220-kDa protein from the LPL-Sepharose affinity column by 0.4 M NaCl demonstrated that the LPL receptor interaction was sensitive to ionic strength. Second, this receptor is thought to be a heparan sulfate proteoglycan. The 220-kDa protein was metabolically labeled with ¹³⁵SO₄, suggesting that it contains glycosaminoglycan chains. Furthermore, heparinase treatment markedly increased the migration of this molecule on SDS-PAGE, confirming the presence of heparan sulfate chains. Third, the surface location of this HSPG (also present on the basal cell surface), was confirmed by specific radiiodination of cell surface proteins and by the cross-linking experiments. Finally, LPL is internalized and recycled after interaction with its cell surface receptor. Because ¹²⁵I-LPL cross-linked to the endothelial cell surface was also internalized, the ¹²⁵I-LPL was bound to a functional receptor.

Previous studies have shown that endothelial cells in culture synthesize at least three different types of HSPGs (10, 11). A comparison of the LPL-binding proteoglycan with those previously reported indicates several similarities with one particular HSPG termed HSPG 1 in one study (10) and HSPG 2 in another (11). Both HSPGs were approximately 200 kDa and were located on the plasma membrane. In addition, their core proteins were approximately 50 kDa, similar to the size of the core protein in the LPL-binding proteoglycan. These proteoglycans contained 8–10% heparinase-resistant glycosaminoglycan chains. Thus, the LPL-binding HSPG identified in our studies may be the same as the HSPG 1 or 2 previously reported.

LPL-Sepharose affinity chromatography was previously used to isolate proteoglycans from rat brain homogenates (27). Interestingly, in these studies the major species of proteoglycan which bound to the affinity column was a HSPG of 220 kDa molecular mass. A small percentage (3–8%) of the glycosaminoglycan chains were chondroitinase ABC-sensitive. This proteoglycan appears to be similar to the LPL receptor identified in the present study. Thus, LPL immobilized on Sepharose recognizes a distinct HSPG from cultured endothelial cells and brain tissue. It should be noted that brain tissue includes blood vessels and is a site of LPL synthesis (28, 29).

Endothelial cell surface proteoglycans serve as receptors for several plasma proteins (23). Thrombomodulin is a well-characterized receptor for thrombin on endothelial cells (30). This proteoglycan contains both heparan sulfate and chondroitinase ABC-sensitive glycosaminoglycans (31). The molecular mass estimated for purified bovine thrombomodulin is 84 kDa making it unlikely to be the same proteoglycan as that which binds to LPL-Sepharose in our studies. Another molecule which binds to HSPG on the vascular endothelium is antithrombin III (22). Bovine macrovascular cells in culture synthesize a species of HSPG which binds antithrombin III (33). This species constitutes only 1–10% of the total HSPG produced by the cells. The location of the antithrombin III binding HSPG was predominantly in the basement membrane with only small quantities present on the cell surface (34). In addition, this HSPG did not appear to contain hydrophobic domains and may not be an integral protein of the cell membrane. Based on these data it appears that the antithrombin III binding HSPG is different from the LPL receptor isolated in the present studies. The metabolic fate of LPL bound to endothelial cells differs from both thrombin and antithrombin III (34, 35). Thrombin is internalized and de-
graded (34); antithrombin III is bound but not internalized (34); whereas LPL is bound, internalized, and recycled back to the cell surface and the medium without degradation (13). These differences in the metabolism of thrombin, antithrombin, and LPL by endothelial cells may result from interaction with different proteoglycans on the endothelial cell surface.

LPL receptors from other cell types have not been identified and characterized. The nondegradative recycling pathway observed in endothelial cells (13) is different from the metabolism of LPL in adipocytes, cardiac myocytes, and liver cells. In these latter cells, the enzyme is internalized and degraded in lysosomes (36–39). Since the binding of LPL to the endothelial cell surface was increased in acidic pH (13), we postulated that an LPL-receptor complex was not dissociated in these observations raise the possibility that distinctive metabolism of LPL in endothelial cells, compared to the other cell types, is due to a unique endothelial cell LPL receptor protein. In muscle cells, LPL binds to a pool of HSPG that is released by treatment with phosphatidylinositol-specific phospholipase C (40). We could not find a similar pool of phosphatidylinositol-linked HSPG on the endothelial cells. LPL binds to the surface of adipocytes with at least 5–10-fold higher affinity than LPL binding to endothelial cells (3, 13, 15). Thus, different LPL binding proteins may be present on these cells. Future studies may permit the comparison of cell surface receptors for LPL on different cell types. This may help elucidate the mechanisms causing differences in metabolism of LPL by various cell types.

A similar 220-kDa LPL-binding protein was found on the apical and basolateral surfaces of endothelial cells. A special feature of the interaction of LPL with monolayers of cultured endothelial cells is its transcytosis. We have hypothesized that this transcytosis requires interaction of LPL with a specific basolateral cell surface proteoglycan. If a LPL-proteoglycan complex is then transported across the cell, the same proteoglycans should be found on both sides of the cell. The 220-kDa protein satisfies this requirement. It should be noted that an additional LPL-binding protein of higher molecular mass was found after iodination of the basal cell surface. Whether this protein is a component of the basement membrane or is an integral cell membrane protein remains to be determined.

In summary, a single proteoglycan that has several properties expected of a LPL receptor on endothelial cells was identified. This identification should allow for further characterization of the 220-kDa HSPG and its role in the specialized interactions of LPL with endothelial cells. Furthermore, the regulatory role of the LPL receptor in the physiological functions of LPL can be explored.

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