The very low density lipoprotein (VLDL) receptor binds apolipoprotein E-rich lipoproteins as well as the 39-kDa receptor-associated protein (RAP). Ligand blotting experiments using RAP and immunoblotting experiments using an anti-VLDL receptor IgG detected the VLDL receptor in detergent extracts of human aortic endothelial cells, human umbilical vein endothelial cells, and human aortic smooth muscle cells. To gain insight into the role of the VLDL receptor in the vascular endothelium, its ligand binding properties were further characterized. In vitro blotting experiments documented that lipoprotein lipase (LpL), a key enzyme in lipoprotein catabolism, binds with high affinity to purified VLDL receptor. In addition, urokinase complexed with plasminogen activator-inhibitor type I (uPA-PAI-1) also bound to the purified VLDL receptor with high affinity. To assess the capacity of the VLDL receptor to mediate the cellular internalization of ligands, an adenoviral vector was used to introduce the VLDL receptor gene into a murine embryonic fibroblast cell line deficient in the VLDL receptor and the LDL receptor-related protein, another endocytic receptor known to bind LpL and uPA-PAI-1 complexes. Infected fibroblasts that express the VLDL receptor mediate the cellular internalization of 125I-labeled LpL and uPA-PAI-1 complexes, leading to their degradation. Non-infected fibroblasts or fibroblasts infected with the lacZ gene did not internalize these ligands. These studies confirm that the VLDL receptor binds to and mediates the catabolism of LpL and uPA-PAI-1 complexes. Thus, the VLDL receptor may play a unique role on the vascular endothelium in lipoprotein catabolism by regulating levels of LpL and in the regulation of fibrinolysis by facilitating the removal of urokinase complexed with its inhibitor.

The low density lipoprotein (LDL) receptor family includes the LDL receptor (1), the very low density lipoprotein (VLDL) receptor (2), the LDL receptor-related protein (LRP) (3), and glycoprotein 330 (4). Together, these molecules have important roles in the catabolism of lipoproteins, proteinases, proteinase-inhibitor complexes, and matrix proteins (for reviews, see Refs. 5–8). The members of this receptor family share structural motifs including cysteine-rich epidermal growth factor-like repeats, cysteine-rich ligand binding repeats, repeats containing the tetrapeptide sequence tyrosine-threonine-aspartic acid, and an asparagine-proline-X-tyrosine sequence within the cytoplasmic tail, which is responsible for endocytic signaling in coated pits.

The most recently identified member of this receptor family is the VLDL receptor (2), so named because it appeared to specifically bind VLDL, probably via interaction with apolipoprotein E (apo E). At present, however, the physiological role of the VLDL receptor is uncertain. This receptor is most abundant in skeletal muscle, heart, adipose tissue, and brain (9–11), tissues which metabolize fatty acids as an energy source. This fact, and the observation that the VLDL receptor recognizes apo E-containing lipoproteins, has led to the hypothesis that the VLDL receptor may play an important role in the delivery of triglyceride-rich lipoproteins to peripheral tissues (8). Interestingly, a number of tissues that express high levels of the VLDL receptor also express lipoprotein lipase (LpL) (12), a key enzyme in the metabolism of triglyceride-rich lipoproteins. It has been suggested that LpL may play an important role in conjunction with the VLDL receptor in the catabolism of lipoproteins (8).

A chicken receptor has been identified that is responsible for the endocytosis of VLDL and vitellogenin (13). The primary sequence of this receptor has a high degree of similarity with that of the mammalian VLDL receptors, indicating that it represents a chicken homologue. Insight into a function for the chicken VLDL receptor has been gained by identifying a mu-
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tant hen that is missing this receptor. Hens with this defect are characterized by hereditary hyperlipidemia and the absence of egg laying (14). These observations indicate that the chicken VLDL receptor plays a critical role in mediating the transport of triglycerides into growing oocytes.

Recently, Battey et al. (15) discovered that a 39-kDa protein, termed the receptor-associated protein (RAP), binds with high affinity to the VLDL receptor and regulates its ligand binding properties. RAP was discovered when it copurified with LRP during ligand affinity chromatography (16, 17). While the biological function of RAP remains unknown, it binds tightly to LRP, gp330, and the VLDL receptor and modulates their ligand binding activities (15, 18–20). The localization of RAP within the endoplasmic reticulum (21) and studies in which the RAP gene was disrupted in mice (22) suggest that RAP may play an important role in the early processing of these receptors, perhaps in preventing association of the newly synthesized receptors with ligands or in regulating receptor transport or trafficking to the cell surface.

The high affinity interaction between RAP and the VLDL receptor suggested that the VLDL receptor, like LRP and gp330, may interact with additional ligands, and the present studies were undertaken to more fully define the ligand binding characteristics of this newly discovered receptor. These studies demonstrate that the VLDL receptor is a multiligand receptor and may play an important role in lipoprotein catabolism, by binding and internalizing both VLDL and lipoprotein lipase, and in proteinase catabolism, by mediating the cellular uptake of urokinase (uPA) complexed to its inhibitor, plasminogen activator inhibitor type 1 (PAI-1).

MATERIALS AND METHODS

Proteins—Human VLDL receptor was purified from detergent extracts of human embryonic kidney 293 cells infected with the adenovirus containing the human VLDL receptor cDNA (Ad-VLDL receptor) (see below). 25 150-mm plates of infected cells were extracted in 10 ml of ice-cold 50 mM HEPEs, pH 7.4, 0.5 M NaCl, 0.05% Tween 20, 1% Triton X-100, containing the following proteinase inhibitors: 1 mM phenylmethylsulfonyl fluoride, 25 μg/ml leupeptin, 5 μg/ml PPACK, and 2 μg/ml pepstatin (extraction buffer). The cell extract was sheared with a 21-gauge needle and then centrifuged at 14,000 rpm for 10 min. The VLDL receptor was purified by RAP-Sepharose as described by Battey et al. (15). An aliquot of each fraction was subjected to SDS-PAGE, transferred to nitrocellulose, and analyzed by immunoblotting to identify the VLDL receptor-containing fractions. Receptor concentrations were determined by the assay of Bradford (23) using bovine serum albumin as a standard. Human RAP was expressed in bacteria as a fusion protein with glutathione S-transferase (GST) and purified free of GST as described by Williams et al. (19). Bovine milk LPL was isolated as described (24). The carboxyl-terminal domain of human LPL (LPLC) was produced as a fusion protein with GST in bacteria as described (25). Human pro-uromucine (pro-uPA), and two chain uPA were provided by Dr. J. Henkin (Abbott Laboratories, Abbott Park, IL). Active recombinant human RAP was isolated from the medium of recombinant cells grown in either 100- or 150-mm dishes, washed twice in 5 ml of isotonic phosphate-buffered saline, and then solubilized in 300 μl of extraction buffer and used directly for immunoblotting and RAP-ligand blotting experiments. In immunoblot experiments of human umbilical vein endothelial cells, 150-mm dishes of cells were solubilized in 1.8 ml of extraction buffer and centrifuged at 14,000 rpm to remove any cell debris. The supernatant was applied to a RAP-Sepharose column. The column was washed and eluted as described (15), and an aliquot from the eluted fraction was subjected to immunoblot analysis. Protein concentrations were determined by the method of Bradford (23) using BSA as a standard.

Immunoblotting and Ligand Blotting—Cell extracts were subjected to SDS-PAGE on gradient gels (4–12% Tris-glycine gels from Novex, San Diego, CA) under non-reducing conditions and electrophoretically transferred to nitrocellulose membranes. Filters were blocked with 3% non-fat milk in 50 mM Tris, pH 8.0, at room temperature for 30 min. Hens with this defect are characterized by hereditary hyperlipidemia and the absence of egg laying (14). These observations indicate that the chicken VLDL receptor plays a critical role in mediating the transport of triglycerides into growing oocytes.

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Solid Phase Binding Assay—The binding of uPA-PAI-1 complexes to microtiter wells coated with the purified VLDL receptor was performed essentially as described earlier (15). Wells of microtiter plates (Dynatech Immulon 2, Dynatech Laboratories Inc., Chantilly, VA) were coated with 100 μl of 20 μg/ml affinity-purified anti-VLDL receptor IgG in 0.1 M sodium bicarbonate, pH 9.0, overnight at 4°C. The wells were then blocked with 5% BSA in 50 mM Tris, pH 7.4, 150 mM NaCl, 5 mM CaCl₂ for 1 h at 25°C. The VLDL receptor, purified as described above, was captured on the antibody-coated microtiter wells overnight at 4°C, and following washing, various concentrations of uPA-PAI-1 were added to the wells and also to control wells coated with just BSA. After an overnight incubation at 4°C, the wells were washed and incubated with a mouse monoclonal antibody to uPA (1 μg/ml) for 1 h at 25°C. Following washing, the IgG that was bound was detected with a goat anti-mouse IgG conjugated to horseradish peroxidase using the substrate 3,3',5,5'-tetramethylbenzidine (Kirkegaard & Perry, Gaithersburg, MD). Data were analyzed by nonlinear regression analysis using Equation 1,

\[ A = A_{\text{max}} \left( \frac{[L]}{K_D + [L]} \right) + A_{\text{min}} \]  

where \( A \) is the absorbance value at 650 nm, \( A_{\text{max}} \) is the absorbance value at saturation, \( [L] \) is the molar concentration of free uPA-PAI-1 complexes, and \( K_D \) is the dissociation constant. Since the free uPA-PAI-1 concentration is unknown in these experiments, the use of this equation assumes that the amount of added ligand is greater than the amount of receptor bound to the microtiter wells. Under these conditions, the amount of free uPA-PAI-1 is approximately equal to the total uPA-PAI-1 concentration.

For the analysis of LPL and GST-LPLC binding to VLDL receptor, microtiter wells were coated with 100 μl of LPL or GST-LPLC (10 μg/ml) in 0.1 M sodium bicarbonate, pH 9.0, overnight at 4°C. The wells were blocked as described above, and various concentrations of purified VLDL receptor were added in 0.05% Tween 20, 50 mM CaCl₂, 0.05% Tween 20, 5% BSA was added to 50 mM Tris buffer for 1 h at 25°C. After an overnight incubation at 4°C, the wells were washed and incubated with affinity-purified anti-VLDL receptor IgG (1 μg/ml) in the same buffer for 1 h at 25°C. The bound antibody was detected with a goat anti-rabbit IgG conjugated to horseradish peroxidase using the substrate 3,3',5,5'-tetramethylbenzidine and analyzed as mentioned above.

RESULTS

Infection of PEA13 Fibroblasts with Ad-VLDLR—Results In the expression of VLDL receptor—To investigate the role of the VLDL receptor in mediating the cellular uptake of lipoproteins, a suitable cell line that expresses relatively large amounts of the VLDL receptor and relatively small amounts of other LDL receptor family members was required. For this purpose, PEA13 fibroblasts were employed. PEA13 fibroblasts are genetically deficient in LRP(26). In addition, they do not express RAP binding proteins were not detected in parental PEA13 fibroblasts or from PEA13 fibroblasts infected with Ad-lacZ. The RAP blotting appears to be specific since no RAP (with the exception of trace amounts of endogenously produced protein) was detected without RAP. In controls, RAP was omitted from the procedure (Fig. 1B, right panel).

The VLDL Receptor Mediates the Cellular Catabolism of Lipoprotein Lipase—The tissue distribution of the VLDL receptor is quite similar to that of LpL (12), and thus LpL has been proposed to play an important role in conjunction with the VLDL receptor in the catabolism of lipoproteins (8). Consequently, studies to examine whether the VLDL receptor mediates the catabolism of LpL were initiated. The ability of the VLDL receptor to mediate the cellular internalization of 125I-LpL was examined using PEA13 cells infected with Ad-VLDLR. The results of these experiments are shown in Fig. 2. PEA13 fibroblasts that express the VLDL receptor efficiently internalize (Fig. 2A) and degrade (Fig. 2B) 125I-LpL. Both of these processes are blocked when RAP is included in the culture media. PEA13 fibroblasts infected with Ad-lacZ internalized and degraded a small amount of 125I-LpL; these processes were not blocked by RAP. Parental PEA13 fibroblasts were identical to fibroblasts infected with Ad-lacZ (data not shown). These experiments confirm that the VLDL receptor, like LRP (31), mediates the cellular internalization of LpL leading to its degradation.

The VLDL Receptor Binds to the Carboxyl-terminal Domain

**Fig. 1.** Immunoblot analysis (A) and RAP ligand blot analysis (B) of cell extracts from murine PEA13 fibroblasts and adenovirus-infected fibroblasts. A, left panel, cell extracts prepared from PEA13 fibroblasts or from PEA13 fibroblasts infected with Ad-lacZ or Ad-VLDLR were subjected to SDS-PAGE on 4–12% gradient gels under non-reducing conditions, transferred to nitrocellulose, and incubated for 1 h with 25 nM RAP. After incubation, the blots were washed and incubated with a goat anti-rabbit IgG conjugate. The bands were visualized by use of the Renaissance chemiluminescence kit. 14 μg of total protein were applied to each lane. Right panel, same as above, except the anti-VLDL receptor IgG was omitted from the blotting protocol. B, left panel, cell extracts prepared from PEA13 fibroblasts or from PEA13 fibroblasts infected with Ad-lacZ or Ad-VLDLR were subjected to SDS-PAGE on 4–12% gradient gels under non-reducing conditions, transferred to nitrocellulose, and incubated for 1 h with 25 nM RAP. After incubation, the blots were washed and incubated with a goat anti-rabbit IgG conjugate. The bands were visualized by use of the Renaissance chemiluminescence kit. 14 μg of total protein were applied to each lane. Right panel, same as above, except that RAP was omitted from the protocol.
of Lipoprotein Lipase—Previous studies have determined that the carboxyl-terminal domain of LpL, termed LpLC, when expressed as a fusion protein with GST, binds to LRPs (25), and thus it was of interest to determine whether this region of LpL also interacts with the VLDL receptor. For these studies, an enzyme-linked immunosorbent assay was employed. This assay revealed that the purified VLDL receptor binds to microtiter wells coated with LpL (25). The interaction between uPA and uPA-PAI-1 complexes and mediates their cellular catabolism.

The VLDL Receptor Binds Pro-uPA and uPA-PAI-1 Complexes and Mediates Their Cellular Catabolism—It was of interest to determine if other LRP ligands, such as uPA, also interact with the VLDL receptor. For these studies, an enzyme-linked immunosorbent assay was employed. This assay revealed that the purified VLDL receptor binds to microtiter wells coated with LpL, but not to BSA-coated microtiter wells (Fig. 3A). The apparent KD of 1 nM is comparable to that measured for the binding of LpL to microtiter wells coated with LpL (25). Fig. 3B demonstrates that the purified VLDL receptor also binds to GST-LpLC coated microtiter wells, and the apparent KD of 1.2 nM is close to that measured for the binding of LRP to GST-LpLC using a similar assay (25).

The interaction between uPA-PAI-1 complexes and the VLDL receptor was also confirmed by ligand blotting experiments. For these experiments, purified VLDL receptor was subjected to SDS-PAGE, transferred to nitrocellulose, and incubated with uPA-PAI-1 complexes. The binding of uPA-PAI-1 complexes to the immobilized VLDL receptor was visualized using an anti-uPA IgG. The results demonstrate that uPA-PAI-1 complexes bind to the VLDL receptor (Fig. 4B, left panel). In the presence of excess RAP, the binding was completely inhibited (Fig. 4B, right panel). Together, these in vitro binding experiments document that uPA-PAI-1 complexes interact with the VLDL receptor with high affinity, and that RAP blocks ligand binding.

The ability of PEA13 fibroblasts infected with Ad-VDLDR to mediate the cellular uptake and degradation of 125I-uPA-PAI-1 complexes was next investigated. The results of a representative experiment are shown in Fig. 5 and demonstrate that while PEA-13 fibroblasts infected with Ad-lacZ are unable to internalize or degrade 125I-uPA-PAI-1 complexes, PEA13 fibroblasts that express the VLDL receptor following infection with Ad-VDLDR are effective in internalizing and degrading 125I-uPA-PAI-1 complexes.
The internalization of pro-uPA. The amount of 125I-pro-uPA process that is antagonized by RAP. These data are consistent with VLDLR but not with Ad-

Adequate its internalization (28), it was of interest to examine the adequacy of these complexes are completely blocked by the addition of RAP ligand blotting experiments. Further, the investigators demonstrated that COS cells transfected with the chicken VLDL receptor also used, and these cells also mediate the cellular internalization (Fig. 6A) and degradation (Fig. 6D) of 125I-pro-uPA. This process is blocked when RAP is included during the incubation. As a control, mouse embryo fibroblasts that express LRP, are also unable to internalize or degrade 125I-pro-uPA-1 complexes (data not shown). Together, these data provide compelling evidence that the VLDL receptor mediates the cellular uptake of uPA-PAI-1 complexes leading to their degradation.

Since LRP is known to also directly bind pro-uPA and mediate its internalization (28), it was of interest to examine the potential role of the VLDL receptor in the catabolism of this molecule. Fig. 6 demonstrates that cells infected with Ad-VLDLR but not with Ad-lacZ mediate the cellular internalization (Fig. 6A) and degradation (Fig. 6D) of 125I-pro-uPA. This process is blocked when RAP is included during the incubation. As a control, mouse embryo fibroblasts that express LRP were also utilized, and these cells also mediate the cellular internalization (Fig. 6A) and degradation (Fig. 6D) of pro-uPA in a process that is antagonized by RAP. These data are consistent with previous studies (28), demonstrating that LRP mediates the internalization of pro-uPA. The amount of 125I-pro-uPA internalized by cells expressing the VLDL receptor or LRP represents about 7% of the amount of 125I-uPA-PAI-1 complexes that are internalized by these cells (compare Fig. 6A with 6B). These results indicate that the preferred ligand for both of these receptors is the uPA-PAI-1 complex.

The VLDL Receptor Does Not Mediate the Cellular Uptake of Activated αM—A recent study (32) reported that both native and activated αM bind to the chicken VLDL receptor in ligand blotting experiments. Further, the investigators demonstrated that COS cells transfected with the chicken VLDL receptor cDNA were able to internalize activated αM. Since COS cells are known to express large amounts of LRP (33), it is not easy to demonstrate that the VLDL receptor is functional in internalizing activated αM using these cells. Consequently, it was of interest to investigate if the human VLDL receptor could mediate the cellular uptake of activated αM. The results demonstrate that PEA13 fibroblasts expressing the VLDL receptor following infection with Ad-VLDLR were unable to internalize (Fig. 6C) or degrade (Fig. 6F) 125I-labeled αM activated by treatment with methylamine. In the same experiment, these cells were able to mediate the internalization of 125I-uPA-PAI-1 complexes (Fig. 6B), indicating that the VLDL receptor is indeed functional. In contrast, mouse embryo fibroblasts, which express LRP, were efficient in internalizing and degrading 125I-labeled activated αM in a process that was inhibited by RAP (Fig. 6, C and F). These experiments indicate that the human VLDL receptor does not mediate the cellular uptake of activated αM.

The VLDL Receptor Is Expressed in Vascular Cells—Previous studies have detected VLDL receptor mRNA in human umbilical endothelial cells by Northern blot analysis (29). To determine if protein is expressed in these cells, RAP ligand blotting experiments were performed on cell extracts. Previous studies have shown that this technique is approximately 10-fold more sensitive than immunoblotting using an affinity-purified anti-VLDL receptor peptide IgG (15). The results of RAP blotting studies shown in Fig. 7 (left panel), demonstrate that human aortic endothelial cells, human umbilical vein endothelial cells, and human aortic smooth muscle cells all contain an ~130-kDa polypeptide that comigrates with the VLDL receptor. A 105-kDa polypeptide was also detected in all three types of cells. This polypeptide could represent the VLDL receptor precursor or the variant form of the VLDL receptor lacking the O-linked sugar domain (10). A large polypeptide, with a mobility identical to LRP was detected in the human aortic smooth muscle cells. Only trace amounts of this polypeptide were detected in endothelial cells. This molecule was identified as LRP by immunoblot analysis using an anti-LRP IgG (Fig. 7, middle panel). Human umbilical vein endothelial cells and human aortic endothelial cells do not appear to express much, if any, LRP (Fig. 7, middle panel). Cellular uptake experiments confirmed that 125I-labeled αM-protease complexes are not internalized by human umbilical vein endothelial cells (data not shown), confirming that very little LRP is present in these cells. Taken together, these experiments suggest that endothelial cells express the VLDL receptor but negligible amounts of LRP.

Immunoblotting experiments of the cell extracts using an anti-VLDL receptor IgG failed to detect any protein. This is most likely due to the low sensitivity of this technique when compared to RAP ligand blotting approaches. To confirm that the 130-kDa polypeptide represents the VLDL receptor, an affinity chromatography approach was utilized. Detergent extracts from human umbilical vein endothelial cells were prepared and applied to RAP-Sepharose to concentrate the VLDL receptor. The eluted protein was subjected to SDS-PAGE and transferred to nitrocellulose, and the VLDL receptor was identified by immunoblot analysis using an anti-VLDL receptor IgG. The results of this experiment are shown in Fig. 7 (right panel) and demonstrate that the 130- and 105-kDa polypeptides are detected with the anti-VLDL receptor IgG. As a control, molecular weight standards were incubated with the anti-VLDL receptor IgG to demonstrate the specificity of this antisera. The immunoreactive material detected at approximately 260 kDa is presumed to represent a dimer of the VLDL receptor and has been previously observed in extracts from cells transfected with the VLDL receptor cDNA (15). The immunoblotting results confirm that human umbilical vein endothelial cells express the VLDL receptor. These studies are supported by in situ hybridization studies, which have detected VLDL receptor mRNA in human endothelium (34) and by Northern analysis (29).

To determine if the VLDL receptor is functional in endothelial cells, the ability of these cells to mediate the cellular internalization and degradation of 125I-labeled RAP was investigated. Fig. 8 demonstrates that human umbilical vein endothelial cells rapidly internalize 125I-labeled RAP, leading to its degradation, and this is consistent with the expression of functional VLDL receptors in these cells.
The internalization and degradation of \(^{125}\text{I}\)-labeled uPA-PAI-1 complexes by human endothelial cells were also investigated. The results of this experiment, shown in Fig. 9, demonstrate that human endothelial cells internalize (Fig. 9A) and degrade (Fig. 9B) uPA-PAI-1 complexes. To assess the contribution of the VLDL receptor to this process, RAP was included during the incubation and was found to block approximately 45% of the specific degradation (Fig. 9B). This suggests that a RAP-sensitive receptor, most likely the VLDL receptor, is contributing to the uptake and degradation of this ligand. Thus, these experiments reveal that the VLDL receptor likely plays a major role in regulating uPA-PAI-1 levels on the endothelial cell surface.

It is apparent that additional RAP-insensitive mechanisms also exist for the cellular uptake of uPA-PAI-1 complexes. The catabolism of bovine LpL by porcine endothelial cells has been previously investigated (35). Saxena et al. (35) found that LpL bound to the cell surface and was rapidly internalized by cultured endothelial cells. This process was inhibited when heparin was included during the incubation. Interestingly, it was observed (35) that the LpL was not degraded in these experiments but rather was recycled back to the cell surface and could be recovered from the medium. In several of our studies utilizing human umbilical vein endothelial cells, we noted that \(^{125}\text{I}\)-labeled LpL was internalized but not degraded. The internalization was not prevented by RAP. In other experiments, however, we noted extensive degradation of \(^{125}\text{I}\)-labeled LpL that was prevented by RAP. The reason for this discrepancy is not readily apparent but may relate to variable expression of the VLDL receptor in endothelial cells. The results of these studies confirm that the regulation of LpL levels on the endothelium is a complex process that likely involves several cell surface molecules.
DISCUSSION

The VLDL receptor is a newly discovered member of the LDL receptor family whose domain organization is remarkably similar to that of the LDL receptor, with the exception that the VLDL receptor family, such as LRP and gp330, both of which bind to LDL and VLDL, and VLDL (5). On the other hand, the VLDL receptor recognizes apo E-containing lipoproteins such as intermediate density lipoprotein, \( \beta \)-migrating VLDL, and VLDL (5). The VLDL receptor binds and internalizes apo B-100 (LDL) or apo E-containing lipoproteins such as intermediate density lipoprotein, \( \beta \)-migrating VLDL, and VLDL (5). The VLDL receptor recognizes apo E-containing lipoproteins but only binds weakly to LDL (2). The differences in ligand binding properties of these two receptors were further highlighted when recent studies found that RAP binds with high affinity to the VLDL receptor (\( K_D = 0.7 \text{ nM} \)) (15) but binds weakly to the LDL receptor (\( K_D = 300 \text{ nM} \)) (36). Since the biological role of the VLDL receptor is not fully understood, the present investigation was initiated to gain insight into its function by further characterizing the ligand binding properties of this receptor.

The strategy that was employed to measure the capacity of the VLDL receptor to mediate the cellular internalization of ligands involved introducing the VLDL receptor gene into a cell line deficient in this receptor and demonstrating an enhanced uptake of ligands in those cells expressing the VLDL receptor. A murine fibroblast cell line (26) genetically deficient in LRP was utilized for this purpose. An adenoviral vector was chosen to introduce the gene for the VLDL receptor into these cells since adenovirus-mediated gene transfer to mammalian cells in culture has proven to be a highly effective means for introducing genes into a variety of cells (37). Immunoblotting and RAP ligand blotting experiments confirmed that infection of the PEA13 fibroblasts with Ad-VLDLR led to high levels of expression of this receptor.

Using fibroblasts infected with Ad-VLDLR, we documented the ability of the VLDL receptor to mediate the cellular internalization and subsequent degradation of LpL. In this regard, the VLDL receptor is similar to other members of the LDL receptor family, such as LRP and gp330, both of which bind LpL and mediate its cellular catabolism (31, 38). Interestingly, Takahashi et al. (39) recently demonstrated that both LpL and apo E enhance the binding of triglyceride-rich lipoproteins to the VLDL receptor, an effect that has also been noted on LRP-mediated uptake and degradation of triglyceride-rich li-

poproteins (40). LpL is a key enzyme involved in lipoprotein metabolism and is synthesized by parenchymal cells, such as adipocytes (41). A significant portion of newly synthesized LpL appears to be degraded (42), while the remainder is secreted and transferred by an unknown mechanism to nearby vascular endothelium, where it remains bound through interaction with membrane-associated heparan sulfate chains (43, 44). Triglyceride-rich lipoproteins bind transiently to LpL at the vascular endothelium, and the enzyme rapidly hydrolyzes triglycerides enabling tissues to utilize fatty acids from the lipoproteins, thereby transforming large lipoproteins, such as chylomicrons and VLDL into cholesterol-rich remnant lipoproteins, which can be taken up by the liver.

In situ hybridization studies (34) have detected VLDL receptor mRNA in human endothelium. These results have been confirmed by Northern blot analysis (29) of mRNA isolated from human umbilical vein endothelial cells. The present studies used RAP ligand blotting and immunoblotting techniques on cell extracts to confirm that the VLDL receptor is expressed in human endothelial cells and smooth muscle cells. To assess the function of the VLDL receptor in mediating the internalization of ligands in human umbilical vein endothelial cells, \( 125^\text{I} \)-labeled RAP was utilized as a ligand. These experiments revealed that the VLDL receptor appears to be functional in these cells since they rapidly internalize and degrade RAP. However, the role of the VLDL receptor in regulating levels of LpL on the endothelium at this time remains ambiguous, since variable results were obtained in our experiments. Possibly, this results from variable expression of the VLDL receptor in endothelial cells and the involvement of other cell surface molecules that bind LpL.

In addition to its role in the catabolism of LpL and apoE-containing lipoproteins, the VLDL receptor may also play an important role in proteinase catabolism by binding and mediating the cellular internalization of uPA-PAI-1 complexes. uPA is synthesized by endothelial cells as a single chain zymogen, pro-uPA, that is converted to the active two chain enzyme (two chain-uPA) by proteolysis. The conversion of pro-uPA to active two chain-uPA is enhanced upon interaction with the urokinase plasminogen activator receptor (uPAR). This molecule is a 55-kDa glycosyl-phosphatidylinositol-anchored cell surface protein (45, 46) that is localized on many cell types, including endothelial cells (47). In addition to facilitating activation of pro-uPA, binding of u-PA to uPAR acts to localize uPA activity on the cell surface (48), where it has been implicated in the process of pericellular proteolysis, cell migration, and tissue remodeling (49). uPA activity is regulated by PAI-1, a rapidly acting inhibitor that is also produced by the endothelium (50).

Once a complex between uPA and PAI-1 forms, it is rapidly internalized and degraded in a process mediated by LRPs (33, 51). The results of the present investigation confirm that the VLDL receptor, like LRPs, can also mediate the cellular catabolism of uPA-PAI-1 complexes. This conclusion is supported by in vitro binding studies, which document a high affinity interaction between uPA-PAI-1 complexes and the purified VLDL receptor. RAP was shown to antagonize the binding. Further, cultured fibroblasts expressing the VLDL receptor following infection with Ad-VLDLR mediate cellular uptake of \( 125^\text{I} \)-labeled uPA-PAI-1 complexes leading to their degradation. Thus, the VLDL receptor, like LRPs (51) and gp330 (52), binds to uPA-PAI-1 complexes and mediates their cellular uptake and degradation. This conclusion is supported by recent findings of Heggard et al. (53). The presence of the VLDL receptor on the vascular endothelium suggests a role for this receptor in regulating fibrinolysis, and our experiments suggest a major role for this receptor on the endothelium in regulating uPA-PAI-1
levels. However, it is apparent that other RAP-insensitive mechanisms exist on the vascular endothelium that contribute to the internalization of uPA-PAI-1 complexes.

Both LRP and the VLDL receptor are able to mediate the cellular uptake of pro-uPA directly, although much less pro-uPA is internalized by either receptor when compared with uPA-PAI-1 complexes. This might relate to a decreased affinity of these receptors for pro-uPA when compared with uPA-PAI-1 complexes (28). Nykjaer et al. (54) found that soluble uPAR blocked the binding of pro-uPA to LRP, suggesting that uPAR may protect pro-uPA from LRP-mediated internalization. This observation may also extend to the VLDL receptor and stresses that a major function of uPAR is to protect uPA from being internalized and subsequently degraded.

In summary, the present studies have found that the VLDL receptor, like other members of the LDL receptor family, is a multiligand receptor and, in addition to apo E-containing lipoproteins, also binds and mediates the cellular catabolism of LpL as well as uPA-PAI-1 complexes. The present studies detected the VLDL receptor in endothelial cells, and cell uptake experiments suggest that the VLDL receptor plays an important role, along with other molecules, in the regulation of uPA-PAI-1 levels on the vascular endothelium.

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