VLDL activation of plasminogen activator inhibitor-1 (PAI-1) expression: involvement of the VLDL receptor

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Abstract The potential role of the very low density lipoprotein (VLDL) receptor in mediating VLDL-induced plasminogen activator inhibitor-1 (PAI-1) expression was studied in vitro. Cultured endothelial cells incubated with VLDL showed an increased secretion of PAI-1. This response to VLDL could be completely prevented by the receptor-associated protein (RAP) and partially blocked by rabbit polyclonal anti-VLDL receptor IgG. Furthermore, Chinese hamster ovary (CHO) control cells and cells overexpressing the VLDL receptor were transiently transfected with a PAI-1 promoter–reporter construct and incubated with VLDL. The PAI-1 promoter activity in response to VLDL was significantly higher in the VLDL receptor overexpressing cells compared to the control cells. Addition of RAP completely blocked the VLDL-activated PAI-1 transcription. Electromobility shift assay was performed to investigate whether the enhanced PAI-1 promoter activity seen in the VLDL receptor overexpressing cells in response to VLDL involved induction of the previously described VLDL-inducible factor(s) binding to the −675 to −653 region of the PAI-1 promoter. We found that the binding of the VLDL-inducible factor in VLDL receptor overexpressing cells was markedly enhanced by addition of VLDL as compared to control cells where no increased binding could be seen in response to VLDL. In summary, these results indicate that the VLDL receptor is a strong candidate for mediating VLDL effects on PAI-1 synthesis and secretion in cells expressing this receptor.—Nilsson, L., M. Gåfvels, L. Musakka, K. Ensler, D. K. Strickland, B. Angelin, A. Hamsten, and P. Eriksson. **VLDL activation of plasminogen activator inhibitor-1 (PAI-1) expression: involvement of the VLDL receptor. *J. Lipid Res.* 1999.** 40: **913–919.**

Supplementary key words atherosclerosis • transfection • VLDL response element

Hypertriglyceridemia is associated with an increased risk of coronary heart disease (CHD) (1). Impaired endogenous fibrinolytic function is a frequent finding in subjects with hypertriglyceridemia (2), and it has been suggested that elevated plasma plasminogen activator inhibitor-1 (PAI-1) activity with ensuing attenuation of the fibrinolytic capacity predisposes the hypertriglyceridemic individual to arterial thrombosis and acute CHD (3). Accordingly, in vitro data have shown that triglyceride-rich very low density lipoprotein (VLDL) particles enhance PAI-1 secretion from endothelial cells (4–6) and liver cells (5–7). Furthermore, it has been shown that VLDL stimulation of PAI-1 expression in endothelial cells is mediated through transcriptional activation of the PAI-1 gene, and a VLDL response element has been identified in the promoter region (8). Recently, it has also been demonstrated that unsaturated fatty acids stimulate PAI-1 expression and secretion by endothelial cells (9). It can be envisioned that fatty acids derived from VLDL particles are the actual mediators of VLDL-induced PAI-1 secretion. However, the mechanisms by which endothelial cells bind and process the VLDL particles, eventually leading to increased PAI-1 activity, are largely unknown.

Obvious candidates for mediating the stimulatory effect of VLDL on PAI-1 expression are members of the low density lipoprotein (LDL) receptor superfamily. This family has several members: the LDL receptor (10), the vitellogenin receptor (11), the VLDL receptor (12), the LDL receptor-related protein/α_2-macroglobulin receptor (LRP) (13, 14) and gp 330/megalin (15, 16). The LDL receptor, which displays high-affinity binding of LDL containing apolipoprotein (apo) B-100 as well as of apoE-rich lipoproteins, such as VLDL, intermediate density lipoprotein (IDL), and β-migrating VLDL (β-VLDL), has previously been implicated in the VLDL induction of PAI-1 in human umbilical vein endothelial cells (HUVEC) (4). An-

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; PAI-1, plasminogen activator inhibitor-1; CHD, coronary heart disease; RAP, receptor-associated protein; CHO cells, Chinese hamster ovarial cells; HUVEC, human umbilical vein endothelial cells; CAT, chloramphenicol acetyltransferase; EMSA, electromobility shift assay.

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other obvious candidate receptor to mediate this effect is the VLDL receptor, discovered in 1992 by Takahashi and co-workers (12). The VLDL receptor binds VLDL, IDL, and β-VLDL with high affinity (12, 17–20), whereas LDL particles containing apoB and no or limited amounts of apoE are weakly bound to the VLDL receptor. Recently, Lp[a] has also been shown to bind to the VLDL receptor via its apo[a] moiety (21). The binding and endocytosis processes of all described ligands of the VLDL and LDL receptors are inhibited by the 39 kDa receptor-associated protein (RAP) (17, 21, 22).

The VLDL receptor is highly expressed in adipose tissue, muscle, and heart (12, 23–26), tissues that display high metabolism of fatty acids. Certain cell types in the vascular wall, such as endothelial cells, smooth muscle cells, and macrophage-derived foam cells in atherosclerotic plaques, also express VLDL receptors (21, 27–30). Considering the ligand specificity and tissue distribution, it has been suggested that the VLDL receptor might be of importance for the binding and uptake of triglyceride-rich apoE-containing lipoproteins in extrahepatic tissues. Earlier studies by Desai, Gotlieb, and Steiner (31) have shown that binding of VLDL to vascular endothelial cells could be mediated by an unidentified receptor that is different from the LDL receptor. Whether vascular endothelial VLDL receptors are involved in the binding and processing of VLDL particles that may lead to increased PAI-1 expression in endothelial cells is unknown.

In the present study we investigated the possible role of the VLDL receptor in mediating VLDL-induced PAI-1 secretion from endothelial cells and VLDL-stimulated enhancement of PAI-1 promoter activity.

MATERIALS AND METHODS

Cell culture

HUVEC were isolated from umbilical cords obtained at normal deliveries. The umbilical vein was cannulated and perfused with 50 ml phosphate-buffered saline (PBS) to remove any blood, whereafter the vein was filled with 20 ml of 0.1% collagenase dissolved in PBS and incubated for 15 min at 37°C. The collagenase solution was drained from the cord and collected, and the cord was gently flushed with 20 ml PBS, which was added to the collagenase solution. The cells in these pooled solutions were recovered by centrifugation at 200 *g* for 5 min and seeded on 90-mm culture dishes in M199 medium with 20% fetal calf serum (FCS), antibiotic/antimycotic (Sigma A-9909) and 25 μg/ml endothelial cell growth supplement (Sigma E-2759). When confluent, the cells were subcultured onto 0.2% gelatin (in PBS) coated dishes. Cells from pooled multiple cords were used for experiments up until the fourth passage.

The Chinese hamster ovary (CHO) mutant cell line ldl a-7 (a kind gift from Dr. Monty Krieger, MIT, Cambridge, MA) was used to create a stable cell line overexpressing the human VLDL receptor in the absence of functional LDL receptors. The VLDL receptor overexpressing cell line V7 and the control cells C2 were created as described (32).

The V7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 5% fetal calf serum, streptomycin (100 μg/ml), and penicillin (100 U/ml). To maintain a continuous high expression of receptor protein, 400 μg/ml G-418 (Geneticin, Boehringer-Mannheim) was included in the medium. As control, ldl a-7 cells cultured in identical medium without G-418 were used, as control experiments showed no difference between ldl a-7 cells and C2 control cells stably transfected with empty vector alone. ldl a-7 cells are derived from CHO cells and have a low basal expression of VLDL receptor, LRP and lipoprotein lipase (32, 33). Rabbit polyclonal antibodies directed against the purified VLDL receptor were prepared as described (21).

Preparation of rabbit β-VLDL and human VLDL

β-VLDL used for ligand blot determination of LDL receptors were prepared from plasma of rabbits fed a diet rich in cholesterol (34). The lipoprotein particles were labeled with iodine-125 using the method described by McFarlane (35) including purification over PD-10 columns (Pharmacia-Biotech). After purification and dialysis the protein concentration of each batch was determined using the method descibed by Bradford (36). The specific activity obtained was typically around 200 cpm/ng protein.

Human VLDL (Svedberg flotation rate (S_f) 20–400) were prepared by density gradient ultracentrifugation (37). The endotoxin content in the VLDL preparations was tested using a Limulus Amebocyte lysate assay (COATEST Endotoxin, Endosafe Inc.). Endotoxin levels were shown to be lower than 0.1 ng/mg protein.

Solubilization of cells and ligand blotting procedures

To obtain cell membranes for ligand blotting, detergent solubilized cell lysates were prepared by homogenizing cell pellets by a Polytron (Kinematica Co., Luzern, Switzerland) in a buffer containing 50 mm Tris, pH 6.5, 5 mmol/l $CaCl_2$, 1% Triton X-100, 0.02 mg/ml leupeptin, and 1 mmol/l phenylmethylsulfonylfluoride (PMSF). The homogenates were centrifuged at 20,000 *g* for 15 min, and the clear supernatant was thereafter recovered. Protein concentration was determined according to Bradford (36).

Membrane proteins were separated under non-reducing conditions by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 4–15% gradient gel. A molecular marker (SDS-PAGE Mw standard high range, Bio-Rad, CA) was loaded parallel to the samples. After electrophoresis, the proteins were electrotransferred to a nitrocellulose membrane (BA 85 0.45 μm, Schleicher & Schuell, Germany). VLDL receptor expression in HUVEC was analyzed by ligand blotting using 39 kDa RAP (17, 32). RAP bound to the filter was detected using purified polyclonal anti-RAP IgG (17). As a control for these experiments, membrane extracts from V7 VLDL receptor overexpressing cells were run in parallel. Earlier control experiments had shown that the specific anti-RAP antibody did not react directly with the VLDL receptor. Immunoreactive bands were demonstrated by enhanced chemoluminescence (Amersham). In this type of ligand blot, no binding of RAP to LDL receptors has been demonstrated. Expression of LDL receptors in HUVEC was determined on identical samples ran on the same gel, but the filters were ligand blotted using ^{125}I-labeled rabbit β-VLDL (32, 34). Bands binding β-VLDL were demonstrated by autoradiography.

Determination of PAI-1 protein secretion

To analyze the effect of RAP and VLDL receptor antibodies on VLDL-induced PAI-1 secretion, semiconfluent cultures of HUVEC were incubated for 8–10 h in M199 containing 0.1% BSA. The cells were then preincubated with RAP (50–500 nm) or anti-VLDL receptor IgG (100 μg/ml) for 2 h or 1 h, respectively, before VLDL (50 μg/ml) were added and cells were incubated for another 14 h. After collecting the conditioned medium and centrifugation at 9000 *g* for 5 min, the PAI-1 protein concentration in the medium was quantified using an ELISA (TintELIZE PAI-1, Biopool) which detects active and inactive (latent) forms of PAI-1, as well as t-PA/PAI-1 complexes. The cells were either

trypsinized and counted or lysed with 0.01 M NaOH, followed by measurement of total protein according to Bradford (36). PAI-1 secretion was expressed as the percent of control (vehicle containing the same amount of PBS added).

Transfection assay

CHO cells were transfected using a calcium phosphate-precipitation method (38). pRSV-β-galactosidase control vector (Promega) was cotransfected as an internal control. The construction of the PAI-1 CAT plasmids has been described elsewhere (8). The 4G-PAI-pCAT construct comprises the human PAI-1 sequences −804 to +17 of the 4G allele.

The cells were transfected at 80–90% confluence. At 1–3 h before transfection, the dishes received fresh complete medium. Cells were incubated for 5–6 h with calcium phosphate-precipitated DNAs (15 μg plasmid per 90 mm dish). After a 2-min 15% (vol/vol) glycerol shock, fresh medium containing 0.1% bovine serum albumin was added. After incubation for 14 h, RAP was added to the cells, and 2 h later freshly prepared VLDL were added. Cells were incubated with VLDL for 6 h. After 6 h, the medium was replaced by fresh medium containing 0.1% BSA, and cells were incubated for another 16 h before being harvested for analysis of transient expression. CAT activity was analyzed according to Sambrook, Fritsch, and Maniatis (38).

Electromobility shift assay (EMSA)

Nuclear extracts were prepared according to Alksnis et al. (39). All buffers were freshly supplemented with 0.7 μg/ml leupeptin, 16.7 μg/ml aprotinin, 0.5 mm PMSF, and 0.33 μl/ml 2-mercaptoethanol. The protein concentration in the extracts was estimated by the method of Kalb and Bernlohr (40). For electromobility shift assay, a double-stranded oligonucleotide comprising the −675 to −653 region of the PAI-1 promoter was designed. The probe was end-labeled with ^{32}P-γ-ATP using T4 polynucleotide kinase. Incubation conditions for EMSA were as described (8). To test for specific interaction of the VLDL-induced factor, 50-fold excess of non-labeled specific and non-specific probes were used as competitors (8).

Statistical methods

Data are shown as means ± SD. Differences in continuous variables between two groups were tested by paired or unpaired *t*-tests.

RESULTS

Serum regulation of LDL and VLDL receptor expression in endothelial cells

Both LDL and VLDL receptors have previously been shown to be expressed in HUVEC (27, 41, 42). In order to assess how lipoprotein receptor expression in HUVEC responds to different amounts of sterol present in the culture medium, cells were cultured for 24 h with or without 20% fetal calf serum. Membrane proteins prepared from these cells were separated on SDS-PAGE, electroblotted to nitrocellulose filters, and the presence of VLDL and LDL receptors was probed with RAP and β-VLDL, respectively (see Materials and Methods).

The VLDL receptor was demonstrated as a 116 kDa band, and the majority of receptor co-migrated with the variant corresponding to the exon 16-containing variant expressed in the V7 cells (**Fig. 1A**). Removal of serum in the incubation medium did not change the abundance of VLDL receptors. HUVEC also contained a 130 kDa band

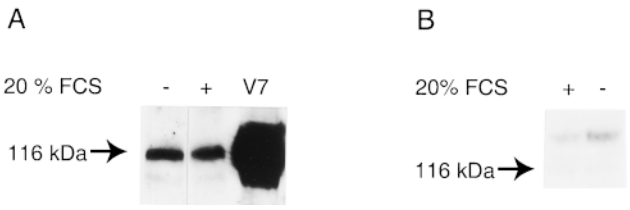

Fig. 1. Ligand blot of VLDL and LDL receptors. HUVEC were cultured for 24 h with or without 20% FCS. Binding of RAP (A) and β-VLDL (B) to cell membranes of HUVEC (50 mg cell protein per lane). In panel A, cell membranes from V7 cells (see Materials and Methods) were included as a control for the VLDL receptor.

that reacted strongly with β-VLDL. This main band, which corresponds to the LDL receptor (43), was clearly increased after a 24-h incubation in the absence of fetal calf serum (Fig. 1B). These results demonstrate the simultaneous expression in HUVEC of VLDL and LDL receptors, both with a potential for endocytosis of VLDL particles. Furthermore, they show that VLDL receptor expression in HUVEC did not change in response to withdrawal of sterols, whereas LDL receptors were up-regulated in agreement with what has been shown for THP-1 cells (44).

Effect of RAP and VLDL receptor antibodies on VLDL-induced PAI-1 secretion

To test whether lipoprotein receptors mediate the VLDL-induced PAI-1 secretion from endothelial cells, HUVEC were preincubated with RAP (50–500 nm) for 2 h, followed by incubation with 50 μg/ml VLDL for 14 h. As seen in **Fig. 2A**, the enhanced PAI-1 secretion caused by VLDL was counteracted by addition of RAP in a dose-dependent manner. Complete inhibition was obtained at a concentration of 100 nm RAP.

Furthermore, to study the specific role of the VLDL receptor, HUVEC were preincubated with anti-VLDL receptor IgG (100 μg/ml) for 1 h, and then incubated with 50 μg/ml VLDL for 4 or 14 h. The stimulatory effect of VLDL on PAI-1 secretion (164 ± 6% and 178 ± 16% of control at 4 or 14 h, respectively) was greatly diminished by the addition of anti-VLDL receptor IgG (118 ± 12% and 130 ± 19% of control at 4 or 14 h, respectively; Fig. 2B).

Role of the VLDL receptor in activation of PAI-1 gene transcription

To investigate the potential role of VLDL receptors in mediating VLDL activation of the PAI-1 gene, CHO control cells and V7 cells overexpressing the human VLDL receptor cDNA were transiently transfected with a 804 bp fragment of the PAI-1 promoter coupled to a CAT reporter gene. Cells were then cultured with increasing concentrations of human VLDL.

Addition of 25–100 μg/ml VLDL resulted in a dose-dependent increase in PAI-1 promoter activity in cells overexpressing the VLDL receptor as compared to control cells (147% vs. 112% at 50 μg/ml VLDL added, $P < 0.05$; **Fig. 3**). The VLDL-induced PAI-1 promoter activation could be prevented by inclusion of 250–500 nm recombinant 39 kDa

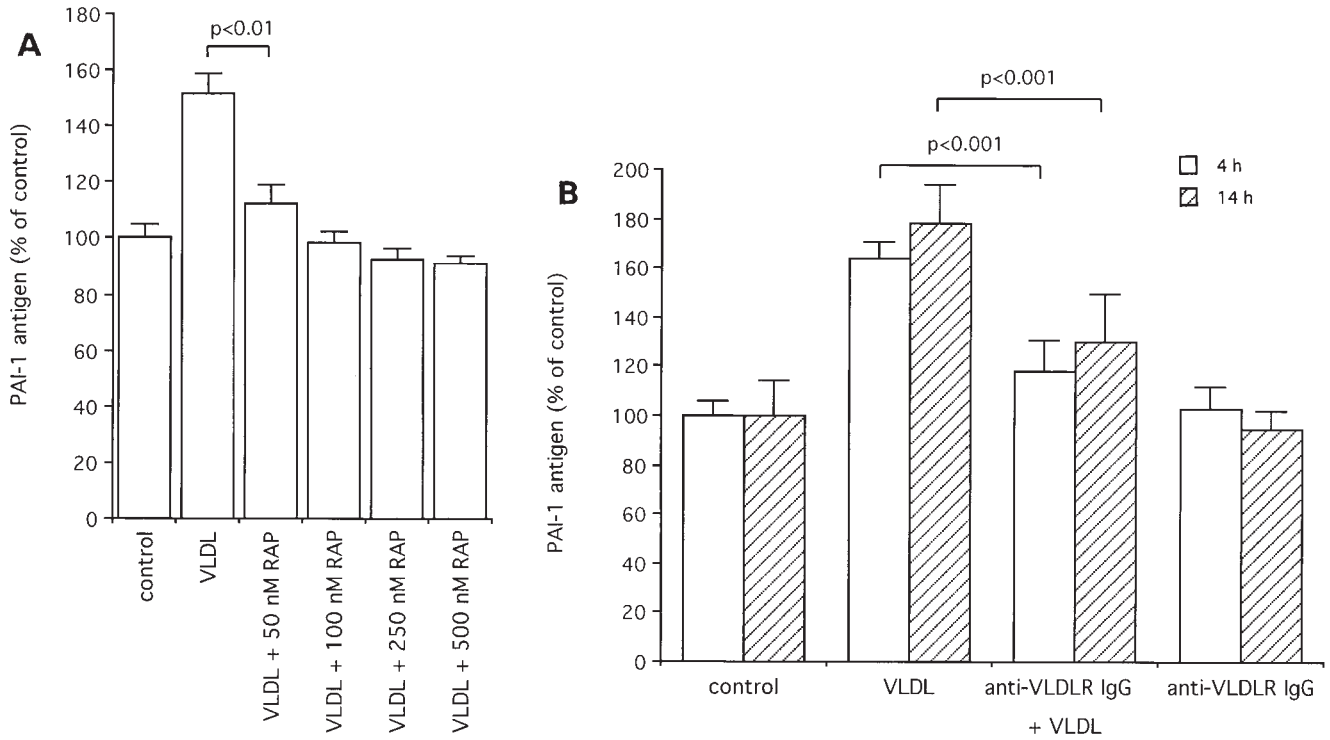

Fig. 2. A: Effect of receptor-associated protein (RAP) on VLDL-induced PAI-1 secretion. HUVEC were preincubated with RAP for 2 h and then incubated with VLDL 50 μg/ml for 14 h, whereafter the PAI-1 content of the culture medium was determined by an ELISA. Results (mean ± SD) are given as % of control. The results were derived from 6 experiments, all performed in triplicate. B: Effect of anti-VLDL receptor IgG on VLDL-induced PAI-1 secretion. HUVECs were preincubated with anti-VLDL receptor IgG for 1 h and then incubated with VLDL 50 μg/ml for 4 or 14 h. Results (mean ± SD) are given as % of control. The results were derived from 3 experiments, all performed in triplicate.

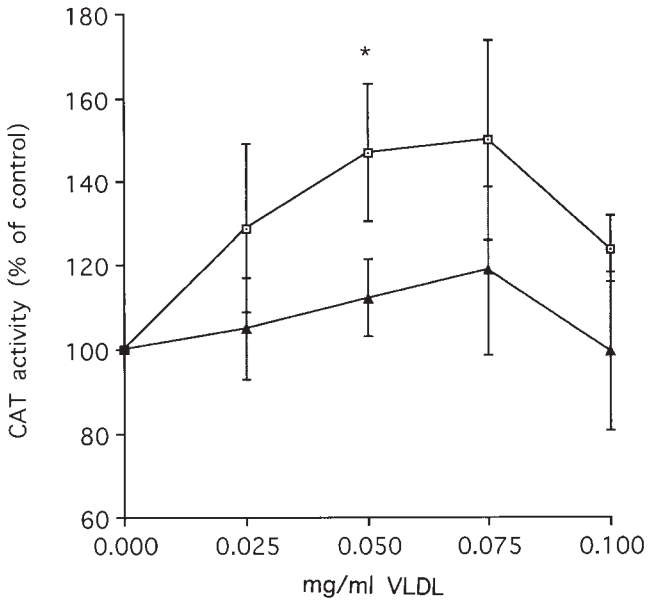

Fig. 3. VLDL activates transcription from the PAI-1 promoter in V7 cells. The transcriptional activity in response to VLDL is significantly higher in the VLDL receptor overexpressing cells (⊡) as compared to control cells (▲). Data points represent mean ± SD, and the PAI-1 transcription rate is given as % of control after correction for the β-galactosidase activity. Results are based on 3 experiments, all performed in triplicate; $*P < 0.05$.

RAP in the culture medium (**Fig. 4**), indicating that the enhanced PAI-1 promoter activity seen in V7 cells was to a large extent mediated by VLDL receptors.

Electromobility shift assay (EMSA) analysis of the VLDL response element in the PAI-1 promoter

An EMSA was performed in order to investigate whether the enhanced PAI-1 promoter activity seen in the VLDL-receptor overexpressing cells (V7) in response to VLDL involves induction of the previously described VLDL-inducible factor(s) binding to the −675 to −653 region of the PAI-1 promoter (8). CHO V7 cells and control cells were incubated with 0–75 μg/ml VLDL for 6 h, and nuclear extacts were prepared. As shown in **Fig. 5**, the binding of the VLDL-inducible factor in V7 cells was markedly enhanced by the addition of 25–75 μg/ml of VLDL (Fig. 5, lanes 6–9) as compared to control cells where no increased binding could be seen in response to VLDL (Fig. 5, lanes 2–5). Specificity of the VLDL-inducible factor(s) was demonstrated using non-labeled specific and nonspecific probes as competitors (Fig. 5, lanes 10–12).

DISCUSSION

In the present study, we have demonstrated that endothelial VLDL receptors are important for mediating VLDL

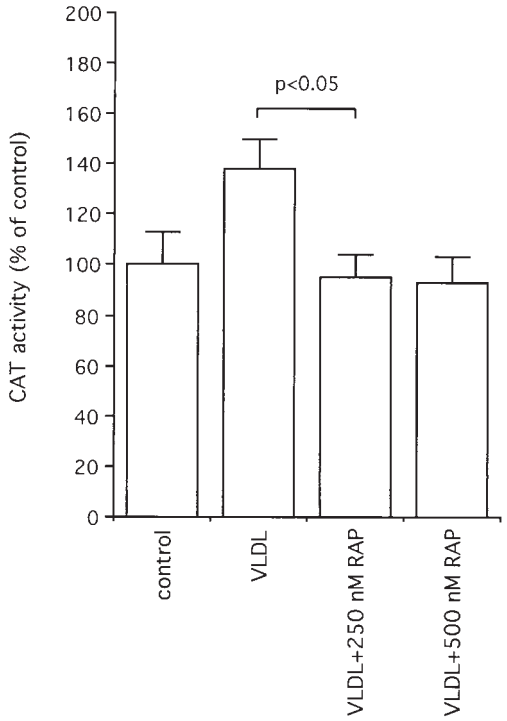

Fig. 4. VLDL receptors mediate the enhanced PAI-1 promoter activity seen in V7 cells after VLDL stimulation.VLDL (50 μg/ml) stimulation of PAI-1 promoter activity in V7 cells could be completely blocked by addition of 250–500 nm of RAP. Results (mean ± SD) are given as % of control. The results were derived from 3 experiments, all performed in triplicate.

effects on PAI-1 secretion in vitro. The finding that PAI-1 promoter activity in response to VLDL was markedly enhanced in the presence of VLDL receptors further supports this notion. The effect on PAI-1 transcription appears to be mediated by a previously described VLDL-inducible transcription factor.

HUVEC express LDL and VLDL receptors, whereas

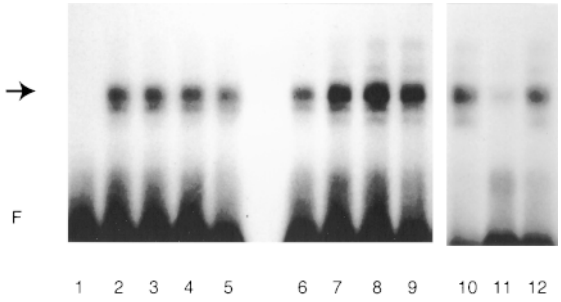

Fig. 5. VLDL induction of the VLDL-inducible transcription factor in CHO cells overexpressing the VLDL receptor. A representative autoradiogram of an EMSA using protein extracts derived from control cells (lanes 2–5) and V7 cells (lanes 6–9) which had been incubated with PBS-vehicle (lanes 2 and 6) or 25, 50, or 75 μg/ml of VLDL (lanes 3–5 and 7–9), and bound to the −675/−653 PAI-1 probe. To test for specific interaction of the VLDL-inducible factor, PBS-incubated V7 cell extract (as in lane 6) was incubated without competitor (lane 10) or with 50-fold excess of non-labeled specific (lane 11) or nonspecific probe (lane 12). F denotes free probe, arrow denotes the VLDL-inducible factor.

only trace amounts of LRP have been demonstrated (27, 41, 42). In the present study, we confirm the expression of both LDL and VLDL receptors in HUVEC and also show, in accordance with studies in THP-1 cells (44), that the level of LDL receptors in HUVEC is suppressed in the presence of serum. This finding is clearly in contrast to the expression of the VLDL receptor which is unchanged by this treatment.

Earlier studies by Stiko-Rahm et al. (4) have indicated that the LDL receptor could mediate the effect of VLDL on PAI-1 expression in HUVEC. However, 30 μg/ml of the C7 monoclonal antibody was used by these authors to block VLDL binding. At this concentration, the C7 antibody may bind unspecifically also to other lipoprotein receptors as fibroblasts from FH patients lacking functional LDL receptors also bind this antibody (45). In the present study, pretreatment of HUVEC with increasing concentrations of RAP significantly decreased VLDL-induced PAI-1 secretion. The concentration of RAP that was effective in inhibiting the effect is more consistent with a role for the VLDL receptor rather than the LDL receptor, as RAP binds only weakly to the LDL receptor, and concentrations of 1 μm or greater are required to antagonize the LDL receptor (22). However, because RAP is not highly specific (46, 47), we sought to identify the receptor involved by using highly specific antibodies directed against the VLDL receptor. The blocking effect on PAI-1 secretion seen after addition of rabbit polyclonal antibodies against the VLDL receptor clearly shows the importance of endothelial VLDL receptors in mediating VLDL-induced PAI-1 secretion. At the concentration used in this study, the anti-VLDL receptor IgG binds solely to the VLDL receptor and to no other lipoprotein receptors (21). However, we cannot exclude that the LDL receptor mediates a minor part of the VLDL effect on PAI-1 in HUVEC, as addition of the anti-VLDL receptor IgG did not completely block the stimulatory effect of VLDL.

To study whether the VLDL receptor could contribute to the VLDL activation of PAI-1 transcription, we used CHO cells overexpressing VLDL receptors in the absence of functional LDL receptors (48). We and others have earlier demonstrated that VLDL receptors overexpressed in this context bind and endocytose rabbit β-VLDL, human VLDL and chylomicron remnants (12, 17, 32, 49). Patel et al. (49) have shown that β-VLDL has the same apparent affinity to VLDL receptors as LDL receptors and that both receptors stimulate β-VLDL degradation as well as oleate incorporation. Thus, the VLDL receptor could mediate an increased lipid flow into cells in a mode identical to the LDL receptor. Although the presence of other lipoprotein receptors, i.e., LRP and proteoglycans, as well as the presence of a low activity of lipoprotein lipase in the ldla-7 CHO cells (33) could be responsible for the baseline activation of PAI-1 promoter activity, our results demonstrate that VLDL receptor overexpression potentiates a concentration-dependent activation of PAI-1 promoter activity by VLDL. The stimulatory effect of VLDL could be fully inhibited by RAP, a well-known inhibitor of VLDL binding to VLDL receptors (17). It is not likely that LRP contributes

to this stimulatory effect as LRP is only expressed at low levels in CHO cells, and no difference in expression level has been detected in control versus VLDL receptor overexpressing cells. In the VLDL receptor overexpressing cells, VLDL induced a dose-dependent increase of a previously found VLDL-inducible transcription factor. EMSA analysis has previously demonstrated induction of what appears to be the identical transcription factor in HUVEC cells (8). In an earlier study, we have found that fatty acids can induce PAI-1 promoter activity and expression of the VLDL-induced factor. A similar mechanism may be assumed where VLDL receptor binding of VLDL could drive a lipid flux into the cell, subsequently affecting PAI-1 promoter transcription and PAI-1 synthesis.

VLDL receptors have been shown to be expressed in vivo in vascular endothelium as well as in foam cells in atherosclerotic plaques (21, 28, 29). VLDL receptors are markedly up-regulated during atherogenesis in rabbits (50). By its affinity for lipoproteins, lipoprotein lipase and uPA:PAI-1 complexes, the VLDL receptor may therefore serve an important function in regulating metabolic processes relating to lipid homeostasis, atherogenesis, and hemostasis. The relative role of endothelial VLDL and LDL receptors for VLDL effects on PAI-1 in vivo is unknown, however, and should be further studied.

The physiological significance of VLDL-mediated regulation of PAI-1 expression in vivo also needs to be clarified. Currently, the concept that VLDL impair the endogenous fibrinolytic function is based on the striking positive relationship between the plasma VLDL triglyceride concentration and plasma PAI-1 activity (51), and on the consistent, concentration-dependent increase in PAI-1 secretion from HUVEC (4–6) and Hep G2 cells (5–7) in culture upon incubation with VLDL. However, fibrate compounds that markedly lower the plasma concentrations of VLDL have shown widely different effects on plasma PAI-1 activity (52–56). Furthermore, inferences from cell culture studies to the situation in vivo should for many reasons be made with caution. PAI-1 synthesis occurs in a number of different cell types in culture and is regulated by a large number of substances (reviewed in ref. 57). Needless to say, the behavior of cultured cells may not be identical to that of the same cell type in vivo. Accordingly, studies of PAI-1 expression in the arterial wall and other relevant tissues of hypertriglyceridemic animal models are warranted.

In conclusion, the present work indicates that the VLDL receptor is a strong candidate for mediating VLDL effects on PAI-1 synthesis and secretion in cells expressing this receptor.

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