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Lipoprotein lipase (*EC* 3.1.1.34) targeting of lipoproteins to receptors

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The role of lipoprotein lipase (*EC* 3.1.1.34; LPL) and hepatic lipase (*EC* 3.1.1.3; HL) in the hydrolysis of lipoproteins has been extensively studied. Recently, however, it has been reported that these enzymes have a second important function; both lipases can mediate the binding and subsequent uptake of lipoproteins into cells (Beisiegel *et al.* 1991; Chappell *et al.* 1992; Nykjaer *et al.* 1993; Kounnas *et al.* 1995; Krapp *et al.* 1996). Although this function has been clearly demonstrated *in vitro* for various cell types, the physiological relevance remains hypothetical until final elucidation *in vivo*. Our current knowledge on the role of postprandial hyperlipidaemia in CHD (Groot *et al.* 1991; Clifton, 1994; Karpe *et al.* 1994; Karpe & Hamsten, 1995), however, suggests that defects in this lipase-mediated uptake of remnants might be a risk factor for atherosclerosis.

The function of lipases in lipoprotein uptake is dependent on the direct interaction of the enzymes with the lipoproteins and with cellular recognition molecules. An interaction of LPL with lipoproteins in rats was proposed by Felts *et al.* (1975) and recently confirmed in human subjects (Zambon *et al.* 1996). LPL and HL are known to bind to heparan sulfate and several laboratories have demonstrated an interaction between LPL and cell surface proteoglycans (Eisenberg *et al.* 1992; Ji *et al.* 1993, 1995; Mulder *et al.* 1993; Schuster *et al.* 1993; Beisiegel *et al.* 1994; Obunike *et al.* 1994; Beisiegel, 1995; Kounnas *et al.* 1995; Ma & Kovanen, 1995). These lipases, therefore, can target lipoproteins to the cell surface. The lipase-mediated endocytotic uptake of lipoproteins has been shown to be dependent on receptors belonging to the LDL-receptor (LDLR) family (Beisiegel *et al.* 1991; Chappell *et al.* 1992).

Our studies demonstrate that after *in vitro* hydrolysis of human triacylglycerol-rich lipoproteins (TRL) the enzymes remain associated with the remnant lipoproteins. We used these remnants to study the LPL-mediated uptake into cells which express different members of the LDLR family (Hilpert *et al.* 1995; Niemeier *et al.* 1996). We were unable to demonstrate an effect of lipases on lipoprotein uptake via the LDLR. The LDLR-related protein (LRP), however, directly interacts with LPL (Beisiegel *et al.* 1991) and HL (Krapp *et al.* 1996), and gp330 (Willnow *et al.* 1992) and the VLDL receptor (VLDLR) recognizes LPL (Argraves *et al.* 1995; Niemeier *et al.* 1996). The *in vivo* relevance of these lipase–receptor interactions has not yet been finally elucidated.

LIPASES ASSOCIATED WITH LIPOPROTEINS AFTER HYDROLYSIS

As proposed by Felts *et al.* (1975), LPL can be associated with lipoproteins *in vivo* and, therefore, serve as a recognition marker for cellular receptors. We were able to show that in post-heparin plasma LPL was bound to TRL (Zambon *et al.* 1996). The lipoproteins were isolated using fast protein liquid chromatography. This is in contrast to data published by Vilella *et al.* (1993) who found LPL associated with LDL. The difference, however, can be explained by the fact that in the absence of LPL inhibitor, *ex vivo* hydrolysis converts the

TRL into more dense lipoproteins. In our experiments we used Orlistat® (La Roche, Basle) to inhibit the LPL activity in the plasma samples.

To verify the association of lipases with lipoproteins after hydrolysis, we performed *in vitro* experiments with TRL from LPL-deficient patients. This lipoprotein fraction contains VLDL and chylomicrons which could not be hydrolysed *in vivo*. Bovine LPL and HL, derived from human hepatoma cells, were used to perform *in vitro* hydrolysis. Analysis of the remnant lipoproteins was carried out after re-isolation of the particles in a sucrose density gradient. It is important to avoid salt gradients since LPL dissociates from the particles at high salt concentration. SDS-PAGE with subsequent immunoblotting revealed that both enzymes remain associated with the particles (J. Heeren & U. Beisiegel, unpublished results). LPL was used alone, while HL was only added following an initial incubation with LPL, since remnants rather than TRL are considered to be the physiological substrate for HL. HL does not seem to displace the LPL from the particles.

EFFECT OF LIPASES ON LIPOPROTEIN UPTAKE INTO CELLS

It has been shown in many studies that apolipoprotein (apo) E is important for the catabolism of TRL, particularly for remnant lipoproteins. After hydrolysis, apo E is in a more-accessible configuration on the surface of the particle. Recent data indicate that lipases in combination with apo E are important recognition signals for remnant uptake into cells.

To demonstrate the proposed effect of the lipases on lipoprotein uptake, we used LPL- and/or HL-containing particles produced by *in vitro* hydrolysis as described previously. Receptor-mediated uptake of remnants into cells was studied using several different cell lines. In all experiments it was shown that hydrolysis by LPL alone increases the uptake of TRL into the cells compared with 'native' TRL from LPL-deficient patients. The increase on human hepatoma cells was approximately 230%. Additional hydrolysis by HL increased the uptake even more to about 350%. We postulate, therefore, that for an optimal catabolism *in vivo* both lipases are important. Perfusion studies with chylomicrons in rat liver provide evidence for such an *in vivo* effect (Skottova *et al.* 1995). This work shows that chylomicron clearance is not only dependent on the lipolytic activity, but also that LPL increases the clearance independently of its catalytic activity.

Mann *et al.* (1995) described a coordinate effect of apo E and LPL, such that both proteins are involved in the uptake mechanism, and defects in one of them might by partly compensated by the other. Several laboratories have studied the structural features of LPL which may be responsible for the interaction with cell-surface receptors. Several receptors of the LDLR family have been investigated as potential LPL binding receptors (see p. 734). The most detailed studies, however, were performed with the LRP. Krapp *et al.* (1995) studied the structural features of LPL necessary for mediation of lipoprotein binding to receptors and found that the LPL has to be in the dimeric form to target lipoproteins to their receptors. However, it does not need to be catalytically active. The binding site for LRP in the enzyme was localized in the C-terminus within residues 313–448 by Williams *et al.* (1994) and within 380–425 by Nykjaer *et al.* (1994). Krapp *et al.* (1995) proposed residues 390–421 as being responsible for the interaction with LRP.

The amount of LPL associated with remnants *in vivo* is difficult to determine. All data at present available, however, indicate that it is not more than one molecule per every second particle. More studies with fresh human plasma in the presence of LPL inhibitors need to be performed to finally answer this question. Particles reaching the liver cell

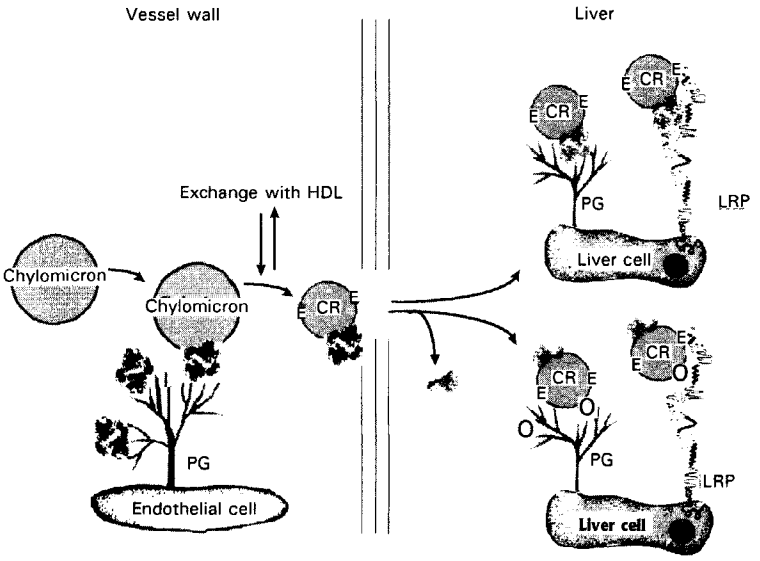

Fig. 1. Model for lipoprotein lipase (*EC* 3.1.1.34, LPL) targeting of lipoproteins to receptors. On the endothelial cells in the blood vessels LPL is bound to the proteoglycans (PG). After hydrolysis of the triacylglycerol-rich lipoproteins (TRL; chylomicrons) the LPL might be carried on the remnant particles (CR) as an intact dimer (●) or as a monomer ( ). In the latter case, the monomeric form is not able to mediate the binding of the CR to the cell surface and the proteoglycan-bound hepatic lipase (*EC* 3.1.1.3; HL; ○) might compensate for the lack of LPL dimer. With the dimeric LPL the CR can be targeted to the cell-surface PG and subsequently to the endocytotic receptor. The LDL-receptor-related protein (LRP) is the main LPL-binding receptor on liver cells. E, apolipoprotein E.

surface without an LPL molecule, however, might interact with HL and use this for binding to endocytotic receptors (Fig. 1).

HL has also been shown to act as a ligand in the uptake of lipoproteins (Diard *et al.* 1994; Kounnas *et al.* 1995; Krapp *et al.* 1996) and due to its structural similarity to LPL this was not unexpected. Nykjaer *et al.* (1994) showed that LRP directly binds to HL and LPL, and that β-VLDL, as a model lipoprotein, interacts directly with both lipases. The role of HL in remnant catabolism has been studied in rat liver perfusion experiments (Shafi *et al.* 1994) and *in vivo* in rabbits (Fan *et al.* 1994). In both animal models it could be shown that HL facilitates remnant uptake into the liver. Shafi *et al.* (1994) demonstrated that heparin treatment and anti-HL antibodies decreased the clearance of chylomicrons in rat liver perfusion experiments. In transgenic rabbits overexpressing human HL, Fan *et al.* (1994) showed that both HDL and IDL were decreased.

LIPASE INTERACTION WITH PROTEOGLYCANS

LPL and HL are located on the endothelial cell surface in blood vessels due to their high-affinity binding to proteoglycans, in particular to heparan sulfate. The observed effect of lipases on lipoprotein binding to cells was thought, therefore, to be mainly due to this kind of interaction (Eisenberg *et al.* 1992). Proteoglycan-deficient cells are a suitable model for studying the role of this molecule in lipoprotein uptake. We found that the binding and uptake of remnants was reduced by 31–80 % on the proteoglycan-deficient chinese hamster ovary (CHO) cells (Esko *et al.* 1988; Beisiegel *et al.* 1994; Fig. 2) compared with control

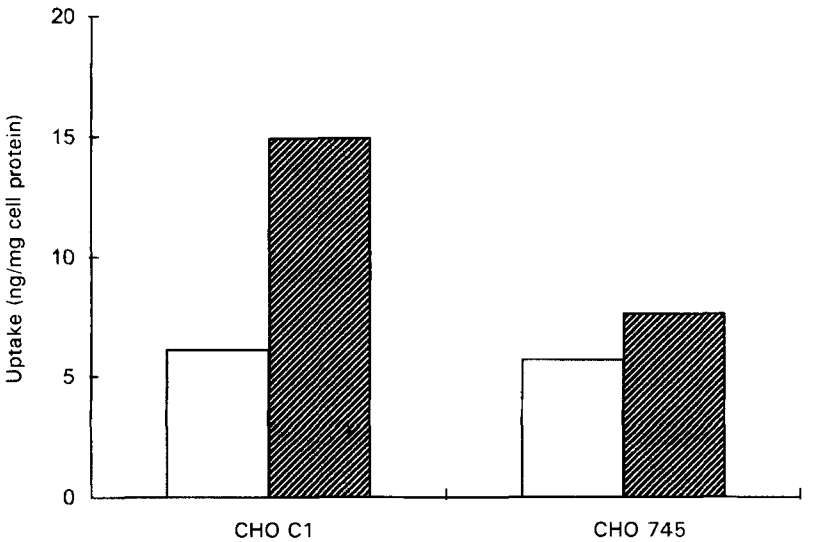

Fig. 2. Uptake of ^{125}I-labelled chylomicrons (□) and lipoprotein lipase (*EC* 3.1.1.34) and hepatic lipase (*EC* 3.1.1.3)-treated remnants (final remnants; ▨) into chinese hamster ovary (CHO) cells (C1 are normal CHO cells and CHO745 are proteoglycan-deficient). The uptake experiments (mean of two) were performed at 37° for 90 min. The uptake of the chylomicrons is very low and not very much influenced by the presence of proteoglycans, while the lipase-mediated uptake is reduced about 50 % in the absence of proteoglycans.

CHO cells. However, the residual binding, as shown by cross-linking experiments, is due to LRP. All published data give evidence that the binding of remnants via apo E, LPL and HL to proteoglycans is the first and very important step for the cellular uptake of these lipoproteins, as demonstrated in Fig. 1.

INTERACTION BETWEEN LIPASES AND MEMBERS OF THE LDLR GENE FAMILY

The LDLR as the first described member of the LDLR family recognizes apo B-100 and apo E. No other ligands have been described. All other members of this gene family are multi-functional receptors with several groups of ligands. Next to lipoprotein ligands, protease–protease inhibitor complexes are the most important.

LPL was first described as interacting with LRP, and the addition of LPL increased the binding of TRL to cells. We found that the addition of LPL to LDL did not stimulate uptake (Fig. 3), while other authors reported (Mulder *et al.* 1993) that the addition of LPL to LDL in the incubation medium leads not only to an accumulation on the surface but also to an increased internalization of the LDL.

The VLDLR was first described as apo E-binding protein (Takahashi *et al.* 1992). Further studies in our laboratory demonstrated that LPL also directly binds to this member of the LDLR family (Niemeier *et al.* 1996). We used LDLR-negative CHO cells with and without overexpression of human VLDLR, and the LPL-mediated uptake of remnants was facilitated by the VLDLR in these cells.

Another multi-functional receptor belonging to the LDLR family is gp330 (Saito *et al.* 1994). Amongst other ligands apo E and LPL have also been shown to bind to this receptor (Willnow *et al.* 1992; Kounnas *et al.* 1993).

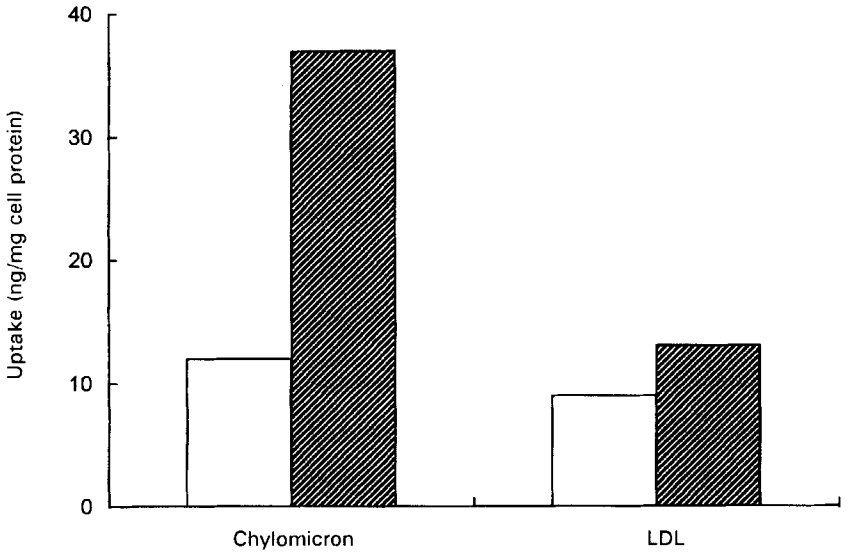

Fig. 3. Uptake experiments with ^{125}I-labelled chylomicrons and ^{125}I-labelled LDL, with (▨) and without (□) the addition of lipoprotein lipase (*EC* 3.1.1.34; LPL). The experiments are performed at 37° for 90 min. There is only a minimal increase of LDL uptake after addition of LPL, while the increase in chylomicron uptake is approximately 300 %.

SUMMARY

Summarizing all available data on the role of lipases in targeting lipoproteins to their receptors, we propose the following model: TRL after hydrolysis by LPL have apo E exposed on their surface and might contain one or more molecules of LPL. Both 'apolipoproteins' direct the particles to the cell surface by high-affinity binding to cellular proteoglycans. HL, bound to the surface of hepatocytes can further hydrolyse the particles and together with apo E and LPL mediate the binding to cellular receptors. The most important receptors recognizing these remnants are LRP and VLDLR. The LRP seems to be mainly responsible for the hepatic uptake of remnant lipoproteins, while the VLDLR, mainly located in adipose tissue and muscle, might target the lipoproteins to these tissues for fatty acid delivery.

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