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Low Density Lipoprotein Receptor Internalizes Low Density and Very Low Density Lipoproteins That Are Bound to Heparan Sulfate Proteoglycans via Lipoprotein Lipase*

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It has previously been shown that lipoprotein lipase (LPL) enhances the binding of low density lipoproteins (LDL) and very low density lipoproteins (VLDL) to HepG2 cells and fibroblasts, up to 80-fold. This increase in binding is LDL receptor-independent and is due to a bridging of LPL between extracellular heparan sulfate proteoglycans (HSPG) and the lipoproteins. In the present paper, we show that preincubation of the cells with LPL, followed by washing prior to the binding experiment, increased binding to the same extent as occurs when the binding is performed in the presence of LPL. This indicates that the formation of a complex of LPL with the lipoproteins is not a prerequisite of binding. Binding curves and Scatchard analyses reveal that both the number of binding sites and the affinity of the binding are increased 20–30-fold by the addition of 3.4 μg/ml LPL. The addition of LPL also resulted in an enhanced uptake and subsequent lysosomal degradation of both LDL and VLDL when compared with binding, although to a lesser extent (up to 25-fold when measured after 5 h at 37 °C). Strikingly, enhanced uptake did not occur in LDL receptor-negative fibroblasts. In addition, down-regulation of the LDL receptor activity by preincubation of the cells for 48 h with either LDL or β-VLDL resulted in a parallel decrease in the uptake of LPL-mediated HSPG-bound LDL, whereas the LPL-mediated binding itself was not diminished. These observations indicate that the uptake of LPL-mediated HSPG-bound LDL and VLDL mainly proceeds via the LDL receptor. Binding of labeled LDL to the cells at 4 °C for 2 h followed by a chase period at 37 °C revealed that in absolute terms, the initial rate of internalization of HSPG-bound LDL is comparable with that of LDL receptor-bound LDL (0.58 and 0.44 ng/min/mg of cell protein, respectively). We conclude that in LDL receptor-positive cells, the LPL-mediated binding of LDL and VLDL to HSPG is followed by internalization of the lipoproteins mainly through the rapid process of the classical LDL receptor recycling system, whereas only a minor portion is internalized via the much slower process of HSPG uptake.

In the circulation, chylomicrons and very low density lipoproteins (VLDL)[1] are partly lipolyzed through the action of endothelium-bound lipoprotein lipase (LPL). The resulting chylomicron remnants and VLDL remnants are rapidly taken up after binding to hepatic receptors, mainly through one of their major protein constituents, apolipoprotein E (apoE). Liver cells possess two different types of lipoprotein receptors. One receptor recognizes both apoB and apoE and is designated as the B, E, or LDL receptor. The other receptor recognizes only apoE and is designated as the apoE or remnant receptor (1). The LDL receptor-related protein (LRP) described by Herz *et al.* (2) appeared to be a potential candidate for the remnant receptor (3, 4) and was observed to be structurally identical to the α_2-macroglobulin receptor (5). The LRP proved to be a multifunctional receptor. It is not yet certain whether the LRP actually is the remnant receptor.

Chylomicrons have been reported to be taken up exclusively through the remnant receptor (6), although the involvement of the LDL receptor in chylomicron remnant clearance has also been suggested (7). Uptake of VLDL and VLDL remnants by the liver is reported to be mediated exclusively through the LDL receptor (8, 9), although others have found that the remnant receptor is also involved in the processing of these lipoproteins (10). Harkes *et al.* (11) and De Water *et al.* (12) have shown that in the rat liver, almost all β-VLDL is taken up via a putative remnant receptor on parenchymal liver cells, which is different from the liver α_2-macroglobulin recognition site (13).

Recently, it has been found that the binding of chylomicrons and β-VLDL to either HepG2 cells or LDL receptor-negative fibroblasts was strongly increased when bovine or human LPL was added to the medium (14). It has been suggested that the LPL protein stimulates the interaction of apoE with LRP. Recently, we found that the stimulating effect of LPL on lipoprotein binding also holds for apoE-free LDL (15).

In addition, we provided evidence that neither the LDL receptor nor the LRP is responsible for the LPL-mediated stimulation of the binding of LDL and VLDL. We found that the enhancing effect of LPL on the binding of these lipoproteins could be prevented by preincubating the cells with heparinase, which is known to prevent high affinity binding

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[1] The abbreviations used are: VLDL, very low density lipoprotein(s); LDL, low density lipoprotein(s); HDL, high density lipoproteins; LPL, lipoprotein lipase; apo, apolipoprotein; LRP, LDL receptor-related protein; HSPG, heparan sulfate proteoglycans; DMEM, Dulbecco's modified Eagle's medium; HSA, human serum albumin; BSA, bovine serum albumin; PBS, phosphate-buffered saline; Lp(a), lipoprotein(a).

of LPL to heparan sulfate proteoglycans (HSPG) (16, 17). This led us to conclude that the stimulation of the binding is caused by the bridging of LPL between proteoglycans present on the plasma membrane and the lipoproteins.

Recently, Williams *et al.* (18) have also reported that LPL enhances the binding of apoB100-rich lipoproteins, such as LDL and Lp(a), via binding to HSPG. They found that the LPL-mediated cell association of Lp(a) is completely LDL receptor-independent, whereas the subsequent degradation of this lipoprotein is partly LDL receptor-dependent. In the case of LDL and nascent apoB-containing lipoproteins, the LPL-mediated cell association and degradation both appeared to be independent of LDL receptor activity. Rumsey *et al.* (19) also reported that the LDL receptor is not involved in the LPL-mediated binding and uptake of LDL by both fibroblasts and THP-1 macrophages. With the results presented in this paper, we obtained strong evidence that the LDL receptor is responsible for the major part of the uptake of LPL-mediated HSPG-bound LDL and VLDL, whereas only a minor part of HSPG-bound LDL and VLDL is directly internalized, thus without the LDL receptor. We also show that the rate of internalization of HSPG-bound LDL via the LDL receptor is comparable with that of LDL, which is directly bound to the LDL receptor.

MATERIALS AND METHODS

Lipoproteins—Blood was obtained from healthy volunteers after an overnight fast. Serum was separated from the cells by centrifugation at 500 × *g* for 15 min at room temperature. LDL (density 1.035–1.06 g/ml), VLDL (density <1.019 g/ml), and heavy HDL (density 1.16–1.20 g/ml) were isolated by ultracentrifugation using the procedure as previously described (8). β-VLDL was obtained from fasted serum of male Wistar rats that were maintained on a cholesterol-rich diet (Hope Farms, Woerden, The Netherlands) containing 2% cholesterol, 5% olive oil, and 0.5% cholic acid. β-VLDL were isolated according to Redgrave *et al.* (20) followed by a second identical centrifugation step.

Protein contents of the lipoprotein fractions were determined according to Lowry *et al.* (21). Total cholesterol, free cholesterol, triacylglycerols, and phospholipids were determined with enzymatic colorimetric assays (Boehringer Mannheim and Wako Chemicals, GmbH, Neuss).

Labeling of Lipoproteins—After isolation, the lipoproteins were immediately iodinated using the [^{125}I]iodine monochloride method described by Bilheimer *et al.* (22). After iodination, the lipoproteins were dialyzed and stored as described previously (8). The specific radioactivity ranged from 150 to 500 cpm/ng of protein.

Lipoprotein Lipase—Bovine LPL was isolated from skimmed milk as described by Tajima *et al.* (23). Inactive LPL was obtained by incubation of the lipase for 4 h at 50 °C. Complete loss of activity of the enzyme was then checked using as substrate serum-activated [9,10-^{3}H]oleic acid-labeled trioleoyl glycerol emulsified with phosphatidylcholine (24).

Binding Studies—HepG2 cells, normal fibroblasts, and LDL receptor-negative fibroblasts were cultured in 2-cm^2 multiwell dishes (Costar) using Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) fetal calf serum as previously described (8). LDL receptor-negative fibroblasts were obtained from a patient with homozygous familial hypercholesterolemia (25). Twenty-four h before the start of the experiment, DMEM supplemented with 1% (w/v) human serum albumin (HSA) instead of fetal calf serum was added to the cells. The binding of ^{125}I-LDL and ^{125}I-VLDL to the cells in the presence or in the absence of LPL was determined after a 2.5-h incubation with 10 μg/ml ^{125}I-labeled lipoprotein at 0–4 °C. After removing the medium, the cells were washed five times with ice-cold phosphate-buffered saline (PBS) containing 1% (w/v) bovine serum albumin (BSA), followed by one wash with PBS without BSA. Cells were then dissolved in 0.5 ml of 0.2 N NaOH. Protein content was measured according to Lowry *et al.* (21). The radioactivity in an aliquot of the sample represents the binding.

To measure binding, intracellular presence, and degradation of lipoproteins separately, cells were incubated for 4 h at 37 °C with 10 μg of ^{125}I-LDL or ^{125}I-VLDL/ml either in the presence or in the absence of LPL. At the end of the incubation, the medium was removed for determination of lipoprotein degradation as described previously (8). The cells were then washed five times with ice-cold PBS/BSA (0.1%, w/v), followed by one wash with PBS without BSA. The cells were then released from the culture dishes by incubation with trypsin (0.05%, w/v) in a 137 mM NaCl, 5 mM KCl, 4 mM $NaHCO_3$, 5 mM D-glucose, 0.02% EDTA buffer (pH 7.4) for 10 min at 37 °C. The viability of the cells was checked, using trypan blue. Trypsin removes both cell-bound lipoproteins and cell-bound lipoprotein lipase (26). The cells were placed on ice to prevent further proteolysis and then immediately centrifuged for 1 min at 13,000 × *g* at 4 °C. Radioactivity was determined in an aliquot of the supernatant, reflecting the binding of the labeled lipoproteins to the exterior of the cells. The cell pellet was resuspended in PBS and centrifuged for 5 min at 10,000 × *g*. The pellet was dissolved in 0.5 ml of 0.2 N NaOH. The radioactivity found in the pellet represents the amount of lipoprotein that is intracellularly present (trypsin resistant). Protein was measured in an aliquot of the sample.

Treatment with heparinase (Sigma) was performed by incubating the cells at 37 °C in the presence of 2.4 units/ml heparinase.

RESULTS

We have previously found that the LPL-mediated enhancement of the binding of LDL and VLDL occurs via bridging of LPL between HSPG and lipoproteins, as it could be inhibited by pretreatment of the HepG2 cells with heparinase (15). In Fig. 1, it is shown that preincubation of HepG2 cells with LPL for 1 h at 4 °C followed by washing also results in an increase of the binding of LDL. This enhancement of the LDL binding is similar to that found if the binding experiment is performed in the presence of LPL. Therefore, these results indicate that the complex formation between the lipoproteins and the lipase prior to the binding is not a prerequisite and, thus, sustain the hypothesis that LPL forms a bridge between HSPG and lipoproteins. In Fig. 1*A*, LPL concentrations in the μg/ml range are used. In Fig. 1*B*, it is shown that the LPL-mediated binding of LDL is already evident at more physiological concentrations of LPL (ng/ml range).

Fig. 2 shows the binding of increasing concentrations of LDL to HepG2 cells that had been preincubated with either medium alone or with medium supplemented with 1.7 μg/ml LPL for a period of 1 h at 0 °C. The results show an approximately 20-fold higher maximum binding of LDL to the cells that had been preincubated with LPL. The Scatchard plots, shown in the *insets* of the *graphs*, suggest a comparable increase of the binding affinity.

To investigate whether LPL also enhances the uptake of LDL and VLDL, we incubated HepG2 cells with either ^{125}I-LDL or ^{125}I-VLDL at 37 °C either in the presence or in the absence of heat-inactivated LPL for a period of 4 h. In Fig. 3, it is shown that in the presence of heat-inactivated LPL, not only the binding of LDL and VLDL is enhanced (about 14- and 31-fold for LDL and VLDL, respectively) but also the internalization (expressed as the amount of intracellular plus degraded lipoprotein) is increased, although to a lesser extent (6- and 23-fold for LDL and VLDL, respectively). In our previous paper (15), we have shown that the major part of LPL-mediated binding is prevented by pretreating the cells with heparinase, indicating that the binding is mediated via HSPG. Fig. 3 shows that besides the inhibition of the LPL-mediated binding of LDL and VLDL, treatment of the cells with heparinase also resulted in inhibition of the LPL-mediated internalization of both lipoproteins. These results indicate, therefore, that at least part of the lipoproteins that are bound via LPL to HSPG are subsequently internalized and degraded as well.

As shown in Table I, degradation of both LDL and VLDL is inhibited in the presence of 50 μM chloroquine to 24 and 36% of the control value, respectively, when the experiment

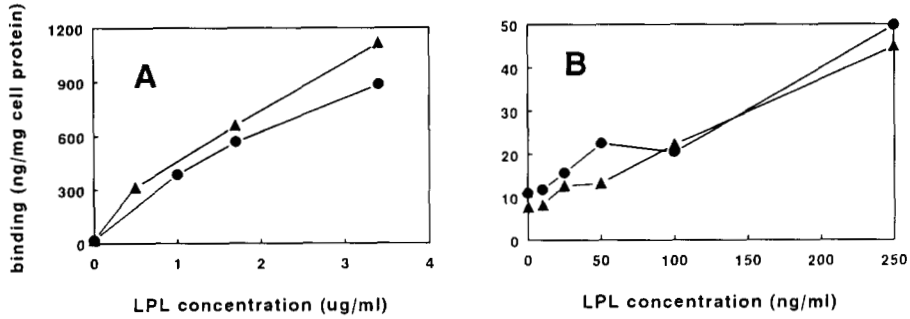

FIG. 1. **Effect of LPL on the binding of LDL.** The binding experiment was performed either with HepG2 cells in medium containing increasing concentrations of LPL (▲) or with HepG2 cells that had been preincubated for a period of 1 h with increasing concentrations of LPL at 0 °C followed by washing in medium without LPL (●). Binding of ^{125}I-LDL was measured after 2.5 h of incubation with 10 μg/ml ^{125}I-LDL at 4 °C, as described under "Materials and Methods." Values are presented as the mean of three measurements. *A*, LPL concentrations in μg/ml range. *B*, LPL concentrations in ng/ml range.

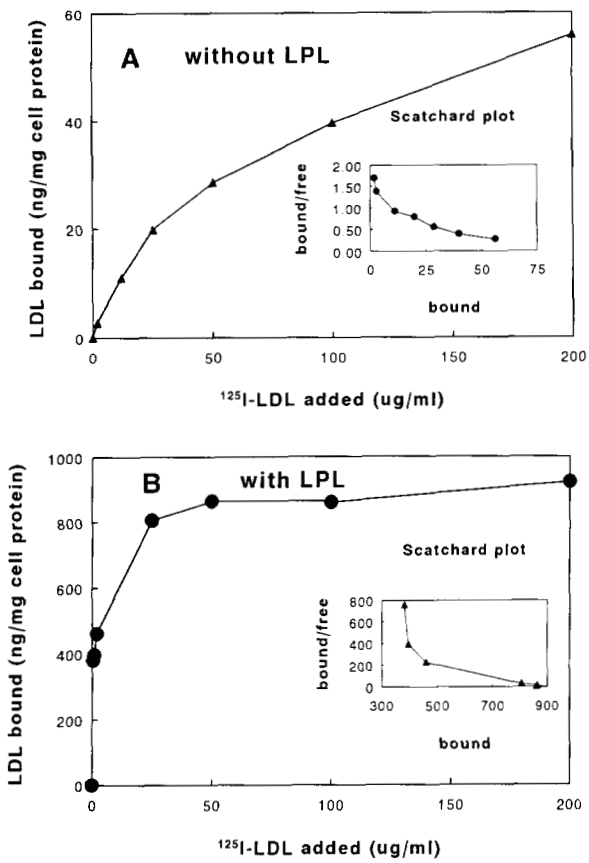

FIG. 2. **Binding curves of ^{125}I-LDL to HepG2 cells preincubated with DMEM/HSA alone (*A*) or with DMEM/HSA supplemented with 1.7 μg/ml LPL (*B*).** The cells were preincubated for a period of 1 h at 4 °C in the presence of DMEM/HSA alone or DMEM/HSA supplemented with LPL. After three washes with DMEM/HSA, the cells were incubated with increasing concentrations of ^{125}I-LDL at 4 °C for a period of 2.5 h. Binding was then measured as the amount of ^{125}I-LDL that became cell-associated as described under "Materials and Methods." Values are the mean of two measurements. *Insets* represent the respective Scatchard analyses.

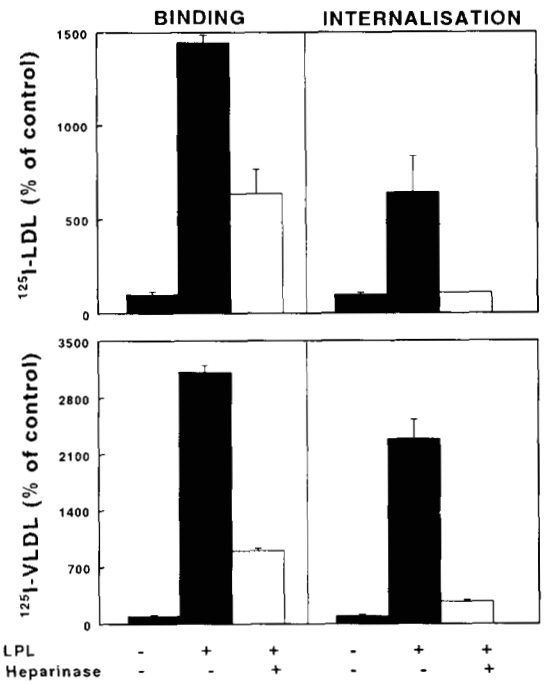

FIG. 3. **The effect of LPL and heparinase on the binding and internalization of ^{125}I-LDL and ^{125}I-VLDL by HepG2 cells.** Lipoprotein binding and internalization (expressed as intracellular plus degraded lipoprotein) were measured upon incubation of the cells with 10 μg/ml labeled lipoproteins at 37 °C for a period of 4 h, in the absence (*solid bars*) (control values 100%) or in the presence (*tightly dotted bars*) of 3.4 μg/ml heat-inactivated LPL. For the heparinase treatment, 2.4 units/ml heparinase were present during the 4 h of incubation of the cells with labeled lipoprotein in order to prevent regeneration of HSPG on the cell membrane during this incubation period (*dotted bars*). Binding and internalization are expressed as a percentage of the control values (incubations in the absence of LPL) and were determined as described under "Materials and Methods." Incubation with heparinase did not affect the control binding and internalization. The values represent the mean ± S.D. of four measurements.

is performed in the absence of LPL and to 32 and 38%, respectively, when performed in the presence of LPL. In the presence of 100 μM chloroquine, the degradation of LDL and VLDL is further reduced to 10 and 15% in the absence of LPL and to 14 and 18% in the presence of LPL. 10 mM NH_4Cl reduces the degradation of LDL and VLDL to less than 10%, irrespective of the presence or absence of LPL. From these results, we conclude that the LPL-mediated HSPG-bound LDL and VLDL are also taken up and directed to the lysosomes for degradation.

We wondered whether or not lipoprotein receptors such as the LDL receptor and/or the putative remnant receptor are involved in the internalization of LPL-mediated HSPG-bound LDL and VLDL. To answer this question, we first measured the binding and the internalization of ^{125}I-LDL and

TABLE I

The effect of chloroquine and ammonium chloride on the degradation of ^{125}I-LDL and ^{125}I-VLDL in the presence and in the absence of 3.4 μg/ml heat-inactivated LPL

Twenty-four h before the start of the experiment, cells were incubated with DMEM/HSA (1%, w/v). Degradation was determined after 4 h of incubation of the cells with 10 μg/ml ^{125}I-labeled lipoproteins in the presence or in the absence of LPL at 37 °C with DMEM/HSA alone or DMEM/HSA supplemented with chloroquine or ammonium chloride as indicated. The degradation of the lipoproteins by the cells without any addition and the degradation of the lipoproteins in the presence of LPL but without any further addition were taken as respective control values (100%). Values given represent the mean ± S.D. of four measurements. The absolute control values were in nanograms of lipoprotein degraded per milligrams of cell protein: LDL, 140; LDL + LPL, 302; VLDL, 65; VLDL + LPL, 300.

^{125}I-Labeled lipoproteins	Incubation with			
	No addition	Chloroquine		NH_4Cl, 10 mM
		50 μM	100 μM	
	% of control degradation			
LDL	100	24 ± 5	10 ± 3	2 ± 0.3
LDL + LPL	100	32 ± 1	14 ± 1	2 ± 0.1
VLDL	100	36 ± 9	15 ± 1	10 ± 0
VLDL + LPL	100	38 ± 5	18 ± 2	6 ± 0.2

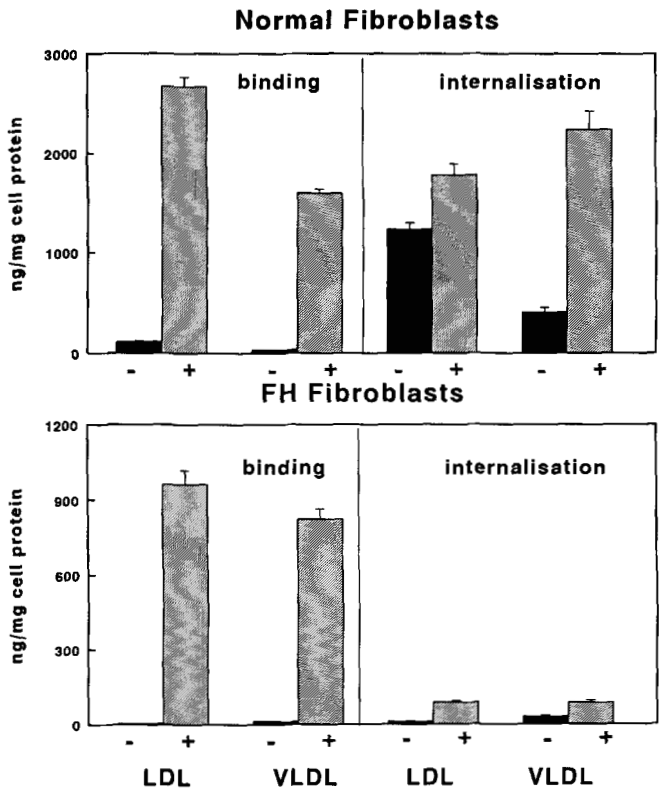

FIG. 4. **Effect of LPL on the binding and the internalization of LDL and VLDL by normal fibroblasts and LDL receptor-negative fibroblasts.** The cells were incubated for 4 h at 37 °C in the presence of 10 μg/ml ^{125}I-LDL and ^{125}I-VLDL without (*solid bars*) or with (*hatched bars*) the addition of 3.4 μg/ml LPL. (The presence or absence of LPL is also indicated by + and −, respectively.) Binding and internalization are measured as described under "Materials and Methods." The values represent the mean ± S.D. of four measurements.

^{125}I-VLDL in the presence and in the absence of LPL in normal fibroblasts and in LDL receptor-negative fibroblasts. Fig. 4 shows that, in normal receptor-positive (*upper*) and receptor-negative cells (*lower*), the total amount of LDL and VLDL that is bound in the presence of LPL (*hatched bars*) is of the same order of magnitude. However, in contrast to the binding, the internalization of LDL and VLDL in receptor-negative fibroblasts did not reach the same order of magnitude as that measured for receptor-positive fibroblasts. Thus, although the LPL-mediated binding of LDL and VLDL occurs via HSPG, the major part of the subsequent internalization of these lipoproteins is mediated via the LDL receptor.

Further evidence for this statement is provided by the results presented in Fig. 5. Preincubation of HepG2 cells with either 200 μg/ml rat β-VLDL or 300 μg/ml human LDL resulted in down-regulation of the binding of ^{125}I-LDL to about 65% (*A*), whereas these conditions exerted an increase in the LPL-mediated binding to HSPG of about 1.5-fold (*B*). Preincubation of the cells with β-VLDL or LDL also resulted in a decreased internalization of LDL receptor-bound ^{125}I-LDL (40 and 25% of the control value, respectively) (*C*). Strikingly, parallel results were obtained for the uptake of LPL-mediated HSPG-bound LDL (50 and 45% of the control value, respectively) (*D*). From these results, we conclude that the LDL receptor is involved in the uptake of lipoproteins following the binding of these lipoproteins via an LPL-mediated bridging between lipoproteins and HSPG.

We wondered whether the rate of internalization of HSPG-bound LDL is comparable with that of LDL receptor-bound LDL. To study this, the cells were first incubated with ^{125}I-

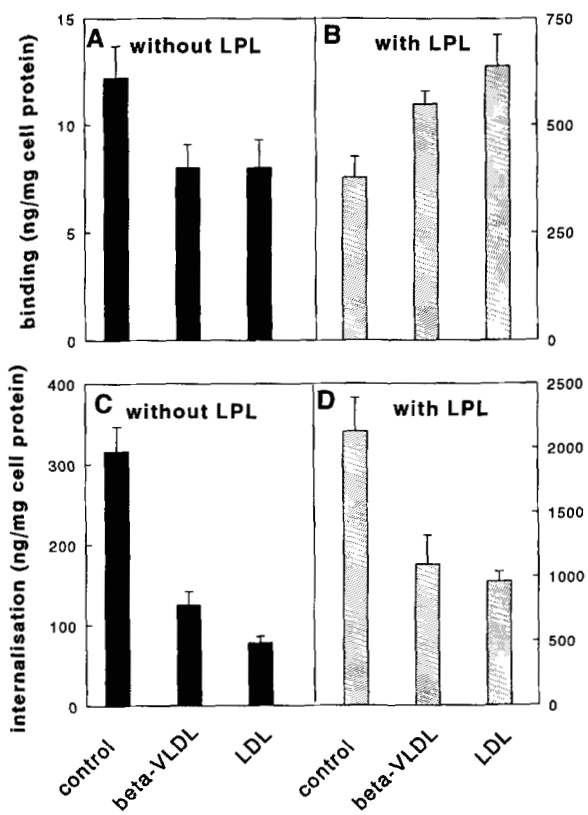

FIG. 5. **Effect of down-regulation of the LDL receptor on the binding and internalization of ^{125}I-LDL in the absence and in the presence of LPL.** HepG2 cells were incubated in DMEM/HSA alone, DMEM/HSA supplemented with 200 μg/ml rat β-VLDL, or DMEM/HSA supplemented with 300 μg/ml human LDL, as indicated, at 37 °C for a period of 24 h. At the end of these incubations, the cells were washed three times with DMEM/HSA and incubated for 4 h at 37 °C with 10 μg/ml ^{125}I-LDL in the absence or in the presence of 3.4 μg/ml inactive LPL. Results are expressed as nanograms of lipoprotein bound or internalized per mg of cell protein. Values of binding and internalization in the absence of LPL (*A* and *C*) are indicated on the *left y axis*, and values obtained after incubation in the presence of LPL (*B* and *D*) are indicated on the *right y axis*.

LDL, either in the presence or in the absence of LPL, at 4 °C for a period of 2 h, followed by three washes at 4 °C. To allow the lipoproteins to be internalized, the cells were then incubated at 37 °C in medium without any addition for increasing periods of time. If expressed in absolute amounts of LDL internalized, it is obvious that the initial rate of internalization of LPL-mediated HSPG-bound LDL is comparable with the internalization rate of LDL receptor-bound LDL (0.58 *versus* 0.44 ng of LDL/min/mg of cell protein) (Fig. 6).

DISCUSSION

Previously, it has been reported that LPL, independently of its lipolytic activity, enhances the cellular binding of a number of lipoproteins, including chylomicrons, VLDL, chylomicron and VLDL remnants, β-VLDL, apoE-free LDL, and HDL (14, 15, 27). We found that neither the LDL receptor nor the LRP is involved in the LPL-mediated binding of LDL and VLDL but that binding occurs mainly through bridging of LPL between HSPG on the plasma membrane and lipoproteins (15). This result was confirmed recently by Williams *et al.* (18). Further evidence for this is provided by the observation that preincubation of the cells with LPL followed by three washes resulted in the same increase in the binding of LDL as when the experiment was performed in the presence of the same amount of LPL (Fig. 1). The saturation *curves* shown in Fig. 2 indicate that the LPL-stimulated binding is due to an increase in the maximum binding and an increase in the binding affinity of about 20-fold.

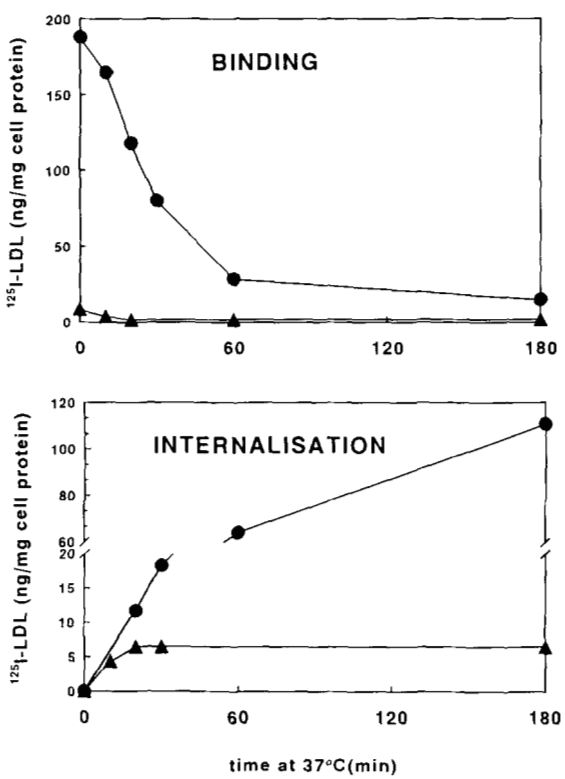

FIG. 6. **Rate of internalization of ^{125}I-LDL bound either in the absence or in the presence of 3.4 μg/ml LPL.** The HepG2 cells were incubated with 10 μg/ml ^{125}I-LDL for 2 h at 4 °C either in the presence (●) or in the absence (▲) of 3.4 μg/ml heat-inactivated LPL. At the end of this incubation period, the cells were washed three times with DMEM containing 1% (w/v) HSA. Cells were then further incubated at 37 °C for increasing periods of time, as indicated, and the binding and internalization were measured separately as described under "Materials and Methods." Values are expressed as nanograms of ^{125}I-LDL per mg of cell protein.

At 37 °C, most of the LPL-mediated binding and LPL-mediated internalization of LDL and VLDL could be inhibited by heparinase, indicating that the LPL-mediated increases in both binding and internalization are dependent on the presence of HSPG on the plasma membrane. Furthermore, our results show that the degradation of LDL and VLDL is lysosomal both in the absence and in the presence of LPL, which is similar to the results obtained by Williams *et al.* (18) with respect to LDL and Lp(a).

It has been suggested that the receptor involved in the LPL-mediated internalization of lipoproteins may be the LRP (14). We believe, however, that an important role for the LRP in this respect can be excluded, as the binding of apoE-free LDL (15) and Lp(a) (18) is also enhanced by the presence of LPL, while the LRP is assumed to bind only apoE-containing lipoproteins. In addition, as already mentioned by Williams *et al.* (18), most of the LPL-mediated binding is abolished by heparinase or heparitinase, whereas the LRP is assumed to contain no heparan sulfate side chains.

Bihain *et al.* (28) have found that long chain free fatty acids rapidly increase (up to 50-fold) the uptake of LDL. It seems, however, unlikely that a significant part of the LPL-mediated binding of LDL and VLDL is dependent on this "lipolysis-stimulated receptor" as at 4 °C LPL displays little or no activity at all and because heat-inactivated LPL has also been found to increase the binding to a similar extent as native LPL (15).

In their experiments, Rumsey *et al.* (19) found that with receptor-negative fibroblasts, the incubation with LPL also resulted in a dramatic stimulation of the uptake of LDL, thereby stating that LPL increases lipoprotein uptake via a pathway not involving the LDL receptor. Although less pronounced due to a shorter incubation time (4 instead of 8 h) and lower LPL concentration used (3.4 instead of 10 μg/ml), we also show that in LDL receptor-negative fibroblasts, the internalization of LDL and VLDL is increased considerably upon incubation of the cells with LPL (Fig. 4). However, if expressed in absolute amounts of LDL taken up per milligram of cell protein, our results show that the receptor-negative fibroblasts are much less efficient than control fibroblasts in LPL-mediated uptake of lipoproteins. This led us to conclude that the major portion of the LPL-mediated HSPG-bound lipoproteins is taken up via the LDL receptor, whereas, simultaneously, only a minor part of the LPL-mediated binding of LDL is internalized without the action of the LDL receptor. Rumsey *et al.* (19) were not able to draw this conclusion as from their results a comparison of LPL-mediated uptake by receptor-negative fibroblasts with that of control fibroblasts could not be made.

We found that the LPL-mediated binding of lipoproteins is not suppressed by preincubation of the cells with lipoproteins (Fig. 5). This is in full agreement with the results reported by Williams *et al.* (18) and Rumsey *et al.* (19). However, we also found that in contrast to the binding, the uptake of HSPG-bound LDL is suppressed parallel to the down-regulation of the LDL receptor activity (Fig. 5, *C* and *D*). This sustains our conclusion that the LDL receptor is indeed involved in the internalization of LPL-mediated HSPG-bound lipoproteins. Similar results were obtained for the degradation of HSPG-bound Lp(a) by Williams *et al.* (18). However, they found that down-regulation of the LDL receptor in normal fibroblasts did not affect the degradation of LDL in the presence of LPL, suggesting that the subsequent uptake of HSPG-bound LDL is LDL receptor-independent. An explanation for the discrepancy between results obtained

by Williams *et al.* (18) and our results might be that they measured total cell association, thus without discriminating between lipoproteins bound to the outer cell membrane and those that had been internalized.

As shown in Fig. 6, most of the LDL receptor-bound LDL has been internalized within 10–15 min, which is in accordance with the recycling time reported earlier for the classical LDL receptor (28). These results also indicate that the internalization of HSPG-bound LDL, if expressed as nanograms of LDL/min/mg of cell protein, is as fast as the internalization of LDL bound to the classical LDL receptor (in the absence of LPL). Similar experiments have been performed by Rumsey *et al.* (19). However, in contrast to our conclusion, Rumsey *et al.* (19) concluded that the uptake of HSPG-bound LDL is much slower than the uptake of LDL that is bound directly to the LDL receptor. Two facts may explain the discrepancy between their conclusion and our conclusion regarding the rate of uptake of HSPG-bound LDL. (i) They used 1 h as the first sampling time point, whereas we used minutes for initial time intervals, which is, in our opinion, reasonable, as the LDL receptor recycling time is also in the order of minutes (28). (ii) In their time course experiment, Rumsey *et al.* (19) expressed the rate of internalization as "% of total radioactivity." We also found much slower internalization when expressed as % of total radioactivity. However, our results clearly show that the internalization of LPL-mediated HSPG-bound LDL is equally fast as that of LDL receptor-bound LDL, when expressed in absolute terms ("ng of LDL/min/mg of cell protein").

From the results presented in this paper, we propose the mechanism for LPL-mediated uptake of LDL and VLDL as illustrated in Fig. 7; LPL enhances the binding of LDL and VLDL to cells by means of a bridging between the lipoproteins in the medium and HSPG on the plasma membrane. Thereafter, the HSPG-bound LDL and VLDL are internalized mainly via the rapid process of the classical LDL receptor recycling system, if the LDL receptor is present. Simultaneously, the remaining portion of HSPG-bound lipoproteins is internalized together with HSPG, which is a much slower process with a half-life of about 7 h (30). In LDL receptor-negative fibroblast, the total amount of HSPG-bound lipoprotein is internalized via this slow process of HSPG uptake. The fact that in normal cells the uptake of LPL-mediated HSPG-bound LDL continues up to 60 min indicates that the LDL-receptor recycling system is saturated during 4–6 LDL receptor cycles and, consequently, the rate-limiting step in this process.

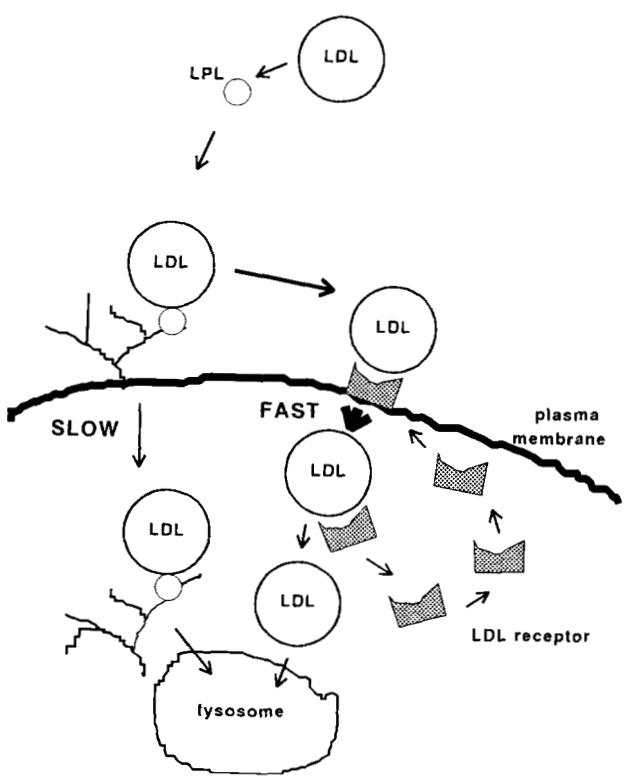

FIG. 7. **Proposed mechanism for LPL-mediated binding and uptake of LDL.** After binding to HSPG, the major part of the LDL is transferred to the LDL receptor, whereafter it is rapidly internalized via the LDL receptor-recycling system. Only a minor portion of the HSPG-bound LDL is taken up directly and at a much slower rate.

The role of LPL in lipoprotein uptake *in vivo* is presently the subject of speculation. Williams *et al.* (18) suggest that LPL may serve as an atherogenic molecule in the arterial wall by stimulating the uptake of apoB-rich lipoproteins by macrophages and smooth muscle cells, leading to foamcell formation. On the other hand, in the liver it would be antiatherogenic by enhancing uptake of apoB-rich atherogenic lipoproteins such as VLDL remnants, LDL, and Lp(a). This possible dual function of LPL *in vivo* may thus relate to its location.

It has been reported that macrophages in atherosclerotic plaques synthesize LPL, which can be found anchored to their cell surface (31, 32). This fact, together with the observation that *in vivo* macrophages synthesize HSPG depending on the amount of intracellular cholesterol ester accumulation (30), strongly sustains the hypothesis that in the arterial wall, LPL may indeed serve as an atherogenic factor.

The concentration of LPL in the circulation is normally kept low because of avid uptake in the liver (33). Although the lipase concentrations used in most of the present experiments are more than 100 times the physiological concentration that ranges between 8 and 25 ng/ml (34), we found that in the presence of 25 ng/ml LPL, the binding of LDL to HepG2 cells also increased (about 1.5-fold, Fig. 1*B*). This sustains the hypothesis that *in vivo* LPL may affect lipoprotein binding. Whether this may also affect the lipoprotein catabolism *in vivo* remains subject to further investigation.

In vivo, most of the circulating LPL is associated with lipoproteins (34), mainly with LDL and HDL and, strikingly, not with VLDL or chylomicrons (35), suggesting a specific role for LPL in directing the LDL and HDL to the liver. Vilaro *et al.* (36) have shown that exogenous LPL bound in the liver caused a dramatic increase in the utilization of a perfused triacylglycerol emulsion. Possibly, LPL fulfills a metabolic role at its binding site in the liver before it is degraded. Hepatic lipase, which is present in the liver, could also act in this way (37).

The relevance of our data, obtained with cells in culture, to the *in vivo* fate of lipoproteins is still unclear. We propose that LPL may accelerate the removal of the atherogenic LDL particles from the blood circulation and, simultaneously, stimulate the reverse cholesterol transport mediated by HDL. Studies are in progress to test these potentially important implications for atherosclerosis.

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