Heparan Sulfate Proteoglycans Participate in Hepatic Lipase- and Apolipoprotein E-mediated Binding and Uptake of Plasma Lipoproteins, Including High Density Lipoproteins*

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High density lipoprotein (HDL) particles and HDL cholesteryl esters are taken up by both receptor-mediated and non-receptor-mediated pathways. Here we show that cell surface heparan sulfate proteoglycans (HSPG) participate in hepatic lipase (HL)- and apolipoprotein (apo) E-mediated binding and uptake of mouse and human HDL by cultured hepatocytes. The HL secreted by HL-transfected Mca-RH7777 cells enhanced both HDL binding at 4 °C (2–4-fold) and HDL uptake at 37 °C (2–5-fold). The enhanced binding and uptake of HDL were partially inhibited by the 39-kDa protein, an inhibitor of low density lipoprotein receptor-related protein (LRP), but were almost totally blocked by heparinase, which removes the sulfated glycosaminoglycan chains from HSPG. Therefore, HL may mediate the uptake of HDL by two pathways: an HSPG-dependent LRP pathway and an HSPG-dependent but LRP-independent pathway. The HL-mediated binding and uptake of HDL were only minimally reduced when catalytically inactive HL or LRP binding-defective HL was substituted for wild-type HL, indicating that much of the HDL uptake required neither HL binding to the LRP nor lipolytic processing. To study the role of HL in facilitating the selective uptake of cholesteryl esters, we used HDL into which radiolabeled cholesteryl ether had been incorporated. HL increased the selective uptake of HDL cholesteryl ester; this enhanced uptake was reduced by more than 80% by heparinase but was unaffected by the 39-kDa protein. Like HL, apoE enhanced the binding and uptake of HDL (2–fold) but had little effect on the selective uptake of HDL cholesteryl ether. In the absence of HL, apoE did not further increase the uptake of HDL, and at a high concentration apoE impaired or decreased the HL-mediated uptake of HDL. Therefore, HL and apoE may utilize similar (but not identical) binding sites to mediate HDL uptake. Although the relative importance of cell surface HSPG in the overall metabolism of HDL in vivo remains to be determined, cultured hepatocytes clearly displayed an HSPG-dependent pathway that mediates the binding and uptake of HDL. This study also demonstrates the importance of HL in enhancing the binding and uptake of remnant and low density lipoproteins via an HSPG-dependent pathway.

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Lipoprotein Catabolism via HSPG Pathway

LDL receptor gene family members, including gp330 (46), the VLDL receptor (47), and the apoE receptor 2 (48). Apolipoprotein E can associate with HDL and mediate HDL particle uptake by either the LDL receptor (12, 49) or the LRP (20). However, its role in the selective uptake of HDL cholesteryl esters is uncertain because conflicting results have been reported (13, 50). In contrast, HL-mediated selective uptake of cholesteryl esters has been demonstrated in cultured hepatoma cells (36, 37) and in Chinese hamster ovary cells transfected with glycoporphadilinostiol-anchored HL (51).

Our previous studies demonstrated that cell surface HSPG are involved in both HL- and apoE-mediated uptake of remnant lipoproteins (52–55). Kounnas et al. (56) showed that HL uptake is mediated at least in part by the LRP in cultured cells and also by an LRP-independent pathway that we have shown to be HSPG-mediated (55, 57). In this process, both HL and apoE promote the binding of remnant lipoproteins to HSPG or to the HSPG-LRP complex, enhancing uptake by cultured cells and by intact liver (55, 57). A similar process also has been found in lipoprotein lipase-mediated binding and uptake of lipoproteins (58–60). The goal of the present study was to determine whether the HSPG-LRP pathway or HSPG alone participate in HDL metabolism. These pathways may be important not only in the liver, where a large fraction of HDL is catabolized, but also in the adrenal and ovary, where HDL (possibly in association with HL and/or apoE) may play a major role in cholesterol homeostasis (for reviews see Refs. 61 and 62).

**EXPERIMENTAL PROCEDURES**

Materials—Lactose, tyramine, and heparinase I were purchased from Sigma Chemical Co. The H-labeled cholesteryl linoleyl ether (CLE) and sodium 125I were purchased from Amersham Life Sciences. Plasmid DNA coding for human apoE (a gift from Dr. James E. Blangero, University of Texas Southwestern Medical School, Dallas, TX) was transfected into Escherichia coli, and the 39-kDa protein was purified as described (63). Human apoE2, apoE3, and apoE4 were provided by Dr. Karl Weissgraber (Gladdstone Institute of Cardiovascular Disease).

Preparation of Lipoproteins—Human and mouse HDL and HDL subfractions were prepared by ultracentrifugation as described (64). Unless otherwise indicated, the densities of human and mouse HDL used in this study were 1.063–1.21 g/ml and 1.07–1.21 g/ml, respectively. The HDL were iodinated as described by Billehei et al. (65). Free iodine was removed by PD10 column chromatography. The HDL were labeled with 1,1-dioctadecyl-3,3,3,3-tetramethylindocarbocyanine (DiI) as described (66). Mouse HDL were double-labeled with 125I-dilactitol tyramine (DLT) and 125I-CLE by two separate procedures (67, 68). Briefly, HDL (2 mg of protein) were mixed with 350 μl of 125I-DLT (in 100 μl of toluene) and dried under N2. Mouse HDL (10 mg of protein) were added to the culture medium, and the cells were washed twice with serum-free medium, and incubated with fresh serum-free medium at 37 °C for 2 h. The cells were placed on ice and equilibrated for 20 min in a chamber with 7% CO2. Labeled lipoproteins or lipoproteins plus apoE were added to the culture medium, and the cells were incubated in the chamber on ice in the cold room (4 °C) for 2 h, washed five times with cold phosphate-buffered saline (PBS) containing 0.2% bovine serum albumin (BSA) and once with PBS alone, and dissolved in 0.1 N NaOH. Bound radiolabeled lipoproteins were measured by γ counting, and the protein concentration of the cells was determined by Lowry’s method (80).

**Cell Association of HDL—**Nontransfected and HL-transfected McA-RH7777 cells were plated in 22-mm dishes for 4–5 days (90% confluence), washed twice with serum-free medium, and incubated with 125I-DLT-labeled HDL (60 μl) in the presence of 1 M NaCl (79). Wild-type HL and the HL variants were characterized by their heparin-binding activities of HL-LRP complexes, and positive clones were identified by lipase activity and Western blot assays of cell culture medium with a monoclonal HL antibody (a gift of Dr. André Bensadoun, Cornell University, Ithaca, NY). Clones that produced similar amounts of immunoreactive HL by Western blot assay were used in these studies.

Wild-type HL and the HL variants were characterized by their heparin-binding properties (75–77) as follows. Conditioned medium from transfected cells was applied to a heparin-Sepharose column and eluted with an NaCl gradient (0.4–1.2 M). Fractions (1.1 ml) were collected and assayed for catalytically active and immunoreactive HL as described below. The salt concentration of every other fraction was determined by conductance measurements.

**LRP Binding of Wild-type HL and HL-LRP—**To compare the LRP binding activities of HL-LRP and wild-type HL, rat liver LRP was prepared (78) and examined in a ligand binding assay with unlabelled anti-human HL antibody. Rat liver membrane proteins were separated by SDS-polyacrylamide gel electrophoresis with 5–8% gels and transferred to nitrocellulose membranes. The position of the LRP was determined by incubating the membranes with biotinylated β-VLDL enriched with human apoE (69) and visualized with 125I-labeled streptavidin.

**Cell Association of HDL—**Nontransfected and HL-transfected cells were grown in 22-mm dishes for 4–5 days (90% confluence), washed twice with serum-free medium, and incubated with 125I-DLT-labeled HDL (60 μl) in the presence of 1 M NaCl (79).

Cell Association of HDL—Nontransfected and HL-transfected McA-RH7777 cells were plated in 22-mm tissue culture dishes and grown to ~90% confluence, washed twice with fresh serum-free medium, and incubated for 37 °C for 2 h. After the addition of 125I-DLT-labeled HDL (60 μl) to cells, the cells were incubated at 37 °C for 2 h, washed on ice in the cold room (4 °C), washed five times with PBS containing 0.2% BSA and once with PBS alone, and dissolved in 0.1 N NaOH. The radioactivity of the cells was measured by γ counting, and the protein concentration of the cells was determined by Lowry’s method (80).

Degradation of HDL—Nontransfected and HL-transfected cells were grown to ~90% confluence, washed twice with serum-free medium, and incubated for 6 h with 125I-HDL at 37 °C. After incubation, the cells were placed on ice; the culture medium was collected for the degradation assay (70), and the cells were washed five times in 0.1 M PBS containing 0.2% BSA and once with PBS alone, and dissolved in 0.1 N NaOH. The radioactivity of the cells was measured by γ counting, and the protein concentration of the cells was determined by Lowry’s method (80).

Selective Uptake of 125I-DLT—H-CLE-labeled HDL—Nontransfected and HL-transfected cells were grown in 22-mm dishes to ~90% confluence, washed twice with serum-free medium, and incubated with fresh

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serum-free medium at 37 °C for 2 h. Then 125I-DLT-3H-CLE-labeled HDL with or without exogenous apoE were added to the culture medium, and the cells were incubated for 3 h. After incubation, the cells were placed on ice, washed five times with PBS containing 0.2% BSA and once with PBS alone, and dissolved in 1 ml of 0.1 N NaOH. Aliquots (0.5 ml) were precipitated with an equal volume of trichloroacetic acid to determine trichloroacetic acid-soluble and -insoluble radioactivity (70, 81). Another aliquot was extracted with n-hexane and isopropanol (3:2) to measure 3H radioactivity. The relative activities of 125I-DLT-3H-CLE-labeled HDL particles were used to estimate the ratio of HDL protein to CLE. Trichloroacetic acid-soluble 125I represented degraded peptides that accumulated in the cells; trichloroacetic acid-insoluble 125I represented intact HDL particles. Therefore, the amount of internalized CLE could be calculated based on the amount of internalized protein. The additional 3H-CLE represented the amount taken up selectively by cultured cells.

Cell Treatment with HL and the 39-kDa Protein—The cells were treated with heparinase as described (52). Briefly, heparinase I was added to nontransfected and HL-transfected McA-RH7777 cells at 37 °C for 2 h before they were incubated with lipoproteins or lipoproteins plus apoE. Unless otherwise indicated, heparinase was used at a concentration of 10 units/ml.

Nontransfected and HL-transfected cells were incubated with fresh medium at 37 °C for 1.45 h. Fifteen minutes later, the 39-kDa protein was added to the culture medium. The labeled lipoproteins were added to the cultured cells and incubated for an additional 2 h.

RESULTS

Binding of HDL—To determine if secreted HL mediates the direct binding of HDL to the cell surface, nontransfected and HL-transfected McA-RH7777 cells were incubated at 37 °C for 2 h to allow HL to accumulate in the medium as described previously (52) and placed on ice for 20 min in the cold room (4 °C) to minimize the catalytic activity of the secreted HL. Under these conditions, the catalytic activity of HL was typically reduced by more than 95% compared with that at 37 °C (data not shown). Then 125I-HDL were added to the culture medium at 4 °C for 2 h, and the amount of 125I-HDL bound to the cell surface was determined. The mouse HDL displayed 3–4-fold greater binding, and human HDL displayed about 2-fold greater binding, to transfected than to nontransfected cells (Fig. 1). Therefore, even with minimal catalytic activity, HL mediated the enhanced cell surface binding of HDL at 4 °C.

Cell Association and Degradation of HDL—To determine the effect of secreted HL on the binding and uptake of HDL, nontransfected and HL-transfected McA-RH7777 cells were incubated at 37 °C for 2 h with 125I-HDL (as a tracer of the HDL protein) or with Dil-labeled HDL (as a tracer of HDL lipids). The binding and uptake of 125I-mouse HDL were 3–5-fold greater by HL-transfected cells than by nontransfected cells (Fig. 2A). The binding and uptake of 125I-human HDL were also enhanced about 2-fold by HL (Fig. 2B). After incubation with Dil-labeled HDL at 37 °C for 2 h, the HL-transfected cells showed more intense fluorescence than the nontransfected cells (data not shown), indicating that the lipid core of the HDL had been internalized. In addition, the degradation of 125I-HDL by HL-transfected cells was 2–3-fold greater than that by nontransfected cells (data not shown). Therefore, the secreted HL facilitated the binding, uptake, and degradation of 125I-HDL by cultured hepatocytes.

Cell Association of HDL Subfractions—To ascertain if the secreted HL could differentially mediate the binding and uptake of HDL subfractions, nontransfected and HL-transfected McA-RH7777 cells were incubated with mouse HDL (d = 1.07–1.21) and 1.09–1.21 g/ml) or human HDL (d = 1.063–1.21, 1.063–1.125, and 1.125–1.21 g/ml) at 37 °C for 2 h. Binding and uptake of all three fractions of mouse and human HDL were increased equivalently in HL-transfected cells compared with nontransfected cells (data not shown).

Effects of Heparinase and the 39-kDa Protein on HL-mediated Enhanced Binding and Uptake of HDL—Previous studies have shown that HL binds to heparin and heparin-like molecules (77, 82, 83) and that HL binding to cell surface HSPG can serve as a “bridge” to enhance the binding and uptake of remnant lipoproteins (52). To determine if HL has a similar role in the binding and uptake of HDL, nontransfected and HL-transfected cells were pretreated with heparinase for 2 h or with the 39-kDa protein for 15 min at 37 °C and then incubated with mouse 125I-HDL for 2 h at 37 °C. Heparinase had little effect on the binding and uptake of 125I-HDL by nontransfected cells but inhibited ~90% of the enhanced binding and uptake mediated by HL in the transfected cells (3A). The 39-kDa protein did not affect 125I-HDL binding and uptake by nontransfected cells; however, it inhibited only about 35% of the enhanced binding and uptake mediated by HL in transfected cells (3A). At the concentration used in these studies, the 39-kDa protein completely blocked the binding and degradation of 125I-α₂-mac-
In parallel studies, the binding and uptake of mouse 125I-β-VLDL and 125I-LDL by nontransfected McA-RH7777 cells in conditioned media were also examined. The enhanced cell association of β-VLDL was 3.4-fold greater in medium containing wild-type HL than in control (nontransfected cell) medium and was slightly lower in medium containing HL-LRP (2.6-fold enhancement); however, in the presence of HL-LRP, the enhanced binding and uptake were reduced by ~50% (Fig. 5B). In contrast, the enhanced cell association of 125I-LDL was essentially identical with wild-type HL, HL-CAT, and HL-LRP (Fig. 5C). Therefore, although the binding and uptake of β-VLDL may be enhanced slightly more by wild-type HL than by HL-CAT, the HL-LRP is clearly less effective than wild-type HL. With LDL, HL appears to act more as a ligand. With LDL, HL-LRP acts almost entirely HSPG-mediated pathway.

Effect of ApoE on Binding and Uptake of 125I-HDL by Nontransfected Cells—To determine the effect of added apoE on the cell association of 125I-HDL, nontransfected McA-RH7777 cells were incubated at 4°C for 2 h with 125I-HDL that had been preincubated with exogenous human apoE2, apoE3, or apoE4 at 37°C for 1 h. The binding of the apoE-enriched mouse or human 125I-HDL at 4°C was ~1.5–2-fold greater than that of control 125I-HDL without added apoE (data not shown). Similarly, in cell association studies at 37°C, apoE2, apoE3, and apoE4 enhanced the binding and uptake of mouse and human 125I-HDL by nontransfected cells ~1.8–2-fold compared with that in medium containing wild-type HL. Although apoE also enhances the binding and uptake of HDL by cultured cells. Interestingly, apoE2, which is defective in binding to the LDL receptor (42), was at least as active as apoE3 in enhancing the binding and uptake of HDL by the nontransfected cells, whereas apoE4 appeared to be slightly less active.

Effect of Heparinase and the 39-kDa Protein on ApoE-enhanced Binding and Uptake of 125I-HDL by Nontransfected Cells—To determine the effect of heparinase on the cell association of 125I-HDL, nontransfected McA-RH7777 cells were incubated in serum-free medium for 2 h at 37°C with or without heparinase (10 units/ml for 2 h) or the 39-kDa protein (10 μg/ml) for 15 min. The labeled lipoproteins were added, the cells were incubated at 37°C for 2 h, and the cell association was determined as described under “Experimental Procedures.” Values are the mean ± S.D. of two experiments performed in duplicate.
The selective uptake of $^3$H-CLE was measured by incubation of nontransfected and HL-transfected McA-RH7777 cells with $^{125}$I-DLT-$^3$H-CLE-labeled mouse HDL at 37 °C for 3 h. Both $^{125}$I-DLT and $^3$H-CLE accumulated inside cells when the double-labeled HDL were degraded (67, 68, 81).

In the nontransfected cells, the selective uptake of $^3$H-CLE was 348 ± 72 ng/mg of cell protein, which was about 2.5-fold greater than that taken up in parallel with HDL particles (127 ± 14 ng/mg of cell protein as determined by $^{125}$I-DLT labeling of HDL protein). This selective uptake was inhibited ~25% by heparinase and only minimally by the 39-kDa protein (Fig. 7). Apolipoprotein E had little or no effect on the selective uptake of $^3$H-CLE (mouse HDL, HDL + apoE2, HDL + apoE3, and HDL + apoE4 had selective uptakes of approximately 350, 370, 340, and 340 ng of $^3$H-CLE/mg of cell protein, respectively).

In the HL-transfected cells, the selective uptake of $^3$H-CLE was ~2.7-fold greater than in the nontransfected cells (Fig. 7). Heparinase inhibited this enhancement by ~80%, whereas the 39-kDa protein had little or no effect (Fig. 7). The addition of apoE to the double-labeled mouse HDL slightly inhibited the selective uptake of the $^3$H-CLE by the HL-transfected cells (data not shown).

**Effects of Catalytic and LRP Binding Activities of HL on Selective Uptake of $^3$H-CLE of HDL**—The effects of the catalytic and LRP binding activities of HL on the selective uptake of HDL cholesteryl esters are shown in Fig. 8. Selective uptake of $^3$H-CLE by nontransfected cells was 2-fold greater in medium containing wild-type HL than in control medium from nontransfected cells. This HL-mediated enhancement of selective uptake was only slightly reduced in medium containing HL-LRP$^-$ but was ~30% lower in medium containing HL-CAT$^-$ ($p < 0.01$). Thus, the enhanced selective uptake of HDL cholesteryl esters mediated by HL appears to involve catalytic activity but not binding to the LRP and, as shown in Fig. 7, is largely mediated via the heparinase-sensitive, HSPG pathway.

**DISCUSSION**

The mechanism of the HL-mediated enhanced binding and uptake of HDL by cultured cells appears to involve several processes. Hepatic lipase acts as a ligand that mediates an increased cell surface binding of HDL particles. Binding studies at 4 °C with conditioned medium from cells secreting HL-CAT$^-$ indicated that a significant portion of the HL-enhanced binding and uptake of mouse and human HDL is directly related to the ligand function of HL, possibly reflecting its ability to associate with HDL (29) and to mediate binding to HSPG or the LRP (52, 82, 83, 85). This role for HL is similar to that suggested by previous studies with remnant lipoproteins, in which HL enhanced their binding and uptake via HSPG or the HSPG-LRP pathway on the cell surface (52, 86).

The results of the present study strongly suggest that at least two pathways are involved in the HL-mediated enhanced uptake of HDL particles by cultured cells. The first pathway, by which about 30–40% of HDL particles are internalized, is sensitive to the 39-kDa protein. The 39-kDa protein binds to the LRP with very high affinity and blocks all known ligands of the LRP (87, 88). Therefore, the 39-kDa protein-sensitive portion of HDL uptake may be mediated by the LRP. However, the

**TABLE I**

| Heparinase | 39-kDa protein |
|------------|---------------|
| Control    | 27.9 ± 8.8     | 30.1 ± 6.7   |
| 125I-HDL   | 58.5 ± 7.1     | 29.0 ± 8.9   |
| 125I-HDL + apoE2 | 48.5 ± 8.2     | 29.7 ± 7.2   |
| 125I-HDL + apoE3 | 42.8 ± 4.5     | 26.3 ± 6.3   |

* Mean ± S.D. of two studies performed in duplicate.
Our current findings indicate that HL is involved in the HSPG-mediated uptake of HDL particles, although the type of HSPG required and exactly how it mediates the uptake of HDL or other lipoproteins remain unknown. These data implicate an HL-HSPG pathway in HDL catabolism and are similar to our previous data demonstrating the involvement of HL in the HL-HSPG pathway in HDL catabolism and are similar to our other lipoproteins. These data implicate an HSPG required and exactly how it mediates the uptake of HDL particles, although the type of uptake (52, 55, 57, 60, 91).

FIG. 5. Binding and uptake of 125I-labeled lipoproteins by nontransfected McA-RH7777 cells in medium conditioned by cells transfected with wild-type HL, HL-CAT, or HL-LRP. The cells were washed twice with serum-free medium and then incubated in the conditioned media at 37 °C for 2 h with mouse 125I-HDL (5 μg of protein/ml) (A), rabbit 125I-β-VLDL (2 μg of protein/ml) (B), or human 125I-LDL (2 μg of protein/ml) (C). The control medium was obtained from the nontransfected cells. Values are the mean ± S.D. of two experiments performed in duplicate for each lipoprotein.

FIG. 6. Effect of human apoE isoforms on binding and uptake of 125I-HDL by nontransfected and HL-transfected McA-RH7777 cells. Nontransfected (A and B) and HL-transfected (C and D) cells were incubated with fresh serum-free medium at 37 °C for 2 h. Mouse 125I-HDL (A), human 125I-HDL (B), mouse 125I-HDL plus apoE (C), and human 125I-HDL plus apoE (D) were added, and the cells were incubated at 37 °C for 2 h. Before addition to the cells, the 125I-HDL (5 μg) and apoE (7.5 μg of protein/ml) were incubated together at 37 °C for 1 h. After incubation, the cells were washed five times with 0.1 M PBS containing 0.2% BSA and once with 0.1 M PBS and dissolved in 0.1 N NaOH. Protein concentration and radioactivity were measured as described under “Experimental Procedures.” Values are the mean ± S.D. of two separate experiments performed in duplicate.

39-kDa protein can also interact with HSPG and block ligand binding (57, 89). Therefore, HSPG may also be involved in the 39-kDa protein-sensitive pathway. Moreover, the binding and uptake of 125I-HDL by nontransfected cells in medium containing HL-LRP were reduced ~20%, which supports the idea that the LRP is involved in only a portion of the HL-enhanced uptake of HDL particles.

The second pathway is solely dependent on HSPG. Hepatic lipase on the cell surface or in the medium may associate with HDL particles, and the resultant complex may be taken up directly by cell surface HSPG. Heparinase abolished almost all (~80–90%) of the enhanced binding and uptake. Previously we established that heparinase affects neither LDL binding to the LDL receptor nor α2-macroglobulin binding to the LRP (59). Oswald et al. (90) reported earlier that cell surface proteoglycans can mediate the cellular uptake of triglyceride-enriched emulsions, and other studies provided evidence for a receptor-independent, proteoglycan-dependent pathway of lipoprotein uptake (52, 55, 57, 60, 91).

Our current findings indicate that HL is involved in the HSPG-mediated uptake of HDL particles, although the type of HSPG required and exactly how it mediates the uptake of HDL or other lipoproteins remain unknown. These data implicate an HL-HSPG pathway in HDL catabolism and are similar to our previous data demonstrating the involvement of HL in β-VLDL and LDL metabolism (52). The present study also indicates that the HL-enhanced binding and uptake of β-VLDL are mediated to a major extent via the HSPG pathway and to a lesser extent via the LRP pathway; β-VLDL cell association was blocked almost completely by heparinase but only partially by the 39-kDa protein and was partially decreased by HL-LRP compared with wild-type HL. In contrast, the HL-enhanced binding and uptake of LDL occurred almost exclusively by the HSPG pathway, were blocked by heparinase, and were not dependent on the catalytic or LRP binding activity of HL.

Hepatic lipase also plays an important role in the selective uptake of HDL-CLE, a nonmetabolized surrogate for HDL cholesterol esters. Although the mechanism of the selective uptake of HDL-CLE by cultured cells and by perfused liver is unknown, the present data are consistent with previous observations that HL stimulates the selective uptake of HDL cholesterol esters (36, 37, 51). Recently the SR-BI receptor has been proposed as an HDL receptor for the selective uptake of HDL cholesterol esters (18, 19, 21), and HL, but not apoE, was found to regulate this receptor and facilitate selective uptake of HDL cholesterol esters (21). Interestingly, in the present study the selective uptake of HDL-CLE was sensitive to heparinase (Fig. 7). Thus, the HSPG pathway on the cell surface appears to be an important factor in the selective uptake of HDL cholesterol esters by cultured cells. The HSPG interaction with the HDL-HDL complexes may facilitate the transport of HDL cholesterol esters across the plasma membrane of cells, based on the lipid gradient between the intracellular and extracellular membranes. Furthermore, HSPG binding may create a microenvironment on the cell surface for the lipolysis of HDL by HL or other phospholipases, releasing cholesterol ester from the
lipoprotein core and thereby facilitating selective uptake. Alternatively, HSPG binding of the HL-HDL complex may facilitate the uptake of HDL cholesteryl ester by anchoring the HDL in the proximity of the SR-BI receptor (18, 19, 21).

Although it may not be required for the ligand function of the molecule, the catalytic activity of HL clearly plays an important role in HDL metabolism. This activity may be involved in the uptake of HDL particles to a limited extent but clearly appears to be important in the selective uptake of cholesteryl esters of HDL. The enhanced cell association of 125I-HDL and the enhanced selective uptake of 3H-CLE of HDL in medium containing HL-CAT were reduced ~15 and 30%, respectively, compared with those in medium containing wild-type HL (Figs. 5 and 8). Enzymatic hydrolysis of the HDL particles could expose their apolipoproteins (apoA-I or apoE) to a receptor (13) or could prepare the particle for binding to other sites on the cell surface. Although it is unclear how the catalytic activity of HL contributes to the selective uptake of HDL cholesteryl esters, our results are consistent with previous reports that HL promotes the uptake of HDL cholesteryl esters in perfused rat liver (34, 35) and in HL-deficient mice receiving HL adenoviral constructs (92). Furthermore, the selective uptake of HDL-derived cholesteryl esters by cultured cells has been increased by treating HDL with HL (36, 37) and by transflecting cells with cell surface-anchored HL (51).

There are several similarities between HL- and apoE-mediated enhancement of HDL catabolism. First, apoE mediates the enhanced binding of HDL at 4 °C and uptake of HDL at 37 °C by binding to HSPG and/or the LRP. The fact that HDL plus any of the three isoforms of apoE bound similarly to the cells supports this concept because all three apoE isoforms have similar binding affinities for HSPG and the LRP (93).2 The LRP pathway contributing to apoE-enriched HDL uptake was recently demonstrated in cultured neuronal cells (20). Second, most of the apoE-mediated cell association can be blocked by heparinase, whereas only about 40% is blocked by the 39-kDa protein, indicating that a significant portion of apoE-mediated enhancement of HDL uptake occurs via an HSPG-dependent, LRP-independent pathway. It remains to be determined whether HL and apoE mediate uptake via the same HSPG-dependent pathway. The fact that apoE did not promote an additional uptake of HDL in the presence of already enhanced uptake mediated by HL suggests that apoE and HL may compete for either HDL particles or HSPG binding sites on the cell surface.

Our results also show important differences between HL- and apoE-mediated HDL catabolism. Although exogenous apoE enhanced the uptake of HDL particles, it had little or no effect on the selective uptake of 3H-CLE. Therefore, the apoE-enhanced binding and uptake of HDL are not associated with enhanced selective uptake of HDL cholesteryl esters. In contrast, HL enhanced both the direct uptake of the HDL particles and the selective uptake of 3H-CLE (Fig. 7). These results suggest that the enhanced selective uptake of HDL cholesteryl esters requires both HL-mediated binding and some degree of HL catalytic activity. In support of this concept, blocking HL-mediated enhancement with heparinase or using medium containing HL-CAT reduced the selective uptake of HDL cholesteryl esters. Furthermore, the enhanced direct or selective uptake involving HL is mediated primarily by the cell surface HSPG-dependent pathway.

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