Hepatic lipase mediates an increase in selective uptake of HDL-associated cholesteryl esters by cells in culture independent from SR-BI

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Abstract **Scavenger receptor class B type I (SR-BI) mediates the selective uptake of HDL cholesteryl esters (CEs) by the liver. Hepatic lipase (HL) promotes this lipid uptake independent from lipolysis. The role of SR-BI in this HL-mediated increase in selective CE uptake was explored. Baby hamster kidney (BHK) cells were transfected with the SR-BI cDNA yielding cells with SR-BI expression, whereas no SR-BI was detected in control cells. These cells were incubated in medium containing ^{125}I [^{3}H]cholesteryl oleyl ether-labeled HDL_3 (d = 1.125–1.21 g/ml) and HL was absent or present. Tetrahydrolipstatin (THL) blocked lipolysis. In control BHK cells and in BHK cells with SR-BI, HDL_3 selective CE uptake (^{3}H-^{125}I) was detectable and SR-BI promoted this uptake. In both cell types, HL mediated an increase in selective CE uptake from HDL_3. Quantitatively, this HL effect was similar in control BHK cells and in BHK cells with SR-BI. These results suggest that HL promotes selective uptake independent from SR-BI. To investigate the role of cell surface proteoglycans on the HL-mediated HDL_3 uptake, proteoglycan deficiency was induced by heparinase digestion. Proteoglycan deficiency decreased the HL-mediated promotion of selective CE uptake. In summary, the stimulating HL effect on HDL selective CE uptake is independent from SR-BI and lipolysis. Proteoglycans are a requisite for the HL action on selective uptake. Results suggest that (a) pathway(s) distinct from SR-BI mediate(s) selective CE uptake from HDL.—**Brundert, M., J. Heeren, H. Greten, and F. Rinninger. **Hepatic lipase mediates an increase in selective uptake of HDL-associated cholesteryl esters by cells in culture independent from SR-BI. *J. Lipid Res.* 2003.** 44: **1020–1032.**

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HDL-associated cholesteryl esters (CEs) are taken up by hepatocytes and steroidogenic cells selectively, i.e., independent from HDL holoparticle internalization (1). Scavenger receptor class B type I (SR-BI), an HDL receptor protein, plays a central role in this pathway in vivo (2). The physiologic function of SR-BI in HDL metabolism is illustrated in genetically modified mice that have no SR-BI expression or a reduced SR-BI expression in the liver (3, 4). SR-BI deficiency in these rodents increases plasma HDL cholesterol and decreases HDL selective CE uptake by the liver (3, 4).

Besides cell membrane receptors, lipoprotein metabolism in plasma is modified by lipolytic enzymes. Lipoprotein lipase (LPL) is abundant in muscle and adipose tissue and hydrolyzes chylomicron- and VLDL-associated triglycerides to provide fatty acids to tissues as an energy source (5). Hepatic lipase (HL) is related to LPL with respect to structure and function. This enzyme is synthesized by hepatocytes (6) and is attached to the vascular endothelium of adrenals, ovaries, and the sinusoids of the liver (7, 8). One metabolic function of HL is the hydrolysis of HDL-associated phospholipids and triglycerides (9).

Distinct from lipolysis, both LPL and HL have been implicated in lipoprotein holo-particle metabolism. LPL promotes the uptake of apolipoprotein B (apoB)-containing lipoproteins independent from lipolysis in cultured cells (10, 11). With respect to the mechanism of this effect, LPL binds to cell surface proteoglycans and associates with lipoproteins as well (5, 12). Presumably, this "bridging" concentrates the lipoprotein particles on the cell surface and thereby facilitates their uptake. This internalization may be mediated by cell surface heparan sulfate proteoglycans (13) and/or by lipoprotein receptors (14, 15).

Analogously, HL stimulates the cellular uptake of holo-lipoprotein particles. Experiments with cultured cells showed that this enzyme mediates the uptake of apoB-

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Abbreviations: ^{125}I-TC, radioiodinated tyramine cellobiose ligand; [^{3}H]CEt, [^{3}H]cholesteryl oleyl ether; BHK, baby hamster kidney; CE, cholesteryl ester; HDL, high density lipoprotein (d = 1.063–1.21 g/ml); HDL_3, high density lipoprotein$_3$ (d = 1.125–1.21 g/ml); HEK 293, human embryonal kidney 293; HL, hepatic lipase; LPDS, lipoprotein-deficient serum; LPL, lipoprotein lipase; SR-BI, scavenger receptor class B type I; THL, tetrahydrolipstatin.

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containing triglyceride-rich lipoproteins (13, 15, 16). In this mechanism, cell surface proteoglycans and cellular receptors may be involved as well; similarly, this was proposed for LPL (5). Again, this bridging function of HL is independent from lipolysis (13, 15, 16).

Besides holo-particle internalization, both LPL and HL promote the selective lipid uptake from lipoproteins. Initial evidence established that these enzymes facilitate the selective CE uptake from HDL by perfused rat liver (17, 18), as well as by hepatic and extrahepatic cells in culture (13, 19, 20, 21, 22). This lipase-mediated increase in selective CE uptake is independent from lipolysis but dependent on cell surface proteoglycans (20, 21, 22). Besides HDL, recent investigations provided evidence that LPL mediates an increase in selective CE uptake from LDL as well (23). In summary, these observations show that LPL and HL can modulate the selective lipid uptake pathway.

The LPL- and HL-mediated promotion of selective CE uptake raises the question of whether SR-BI is involved in the mechanism of these lipase actions. Recently the role of SR-BI in the lipase-promoted increase in selective CE uptake was explored (23, 24, 25). In transfected cells with stable SR-BI expression and in SR-BI-deficient cells, the LPL-mediated increase in selective CE uptake from LDL and HDL was independent from SR-BI (23, 24). In contrast, transient expression of HL and SR-BI in human embryonal kidney 293 (HEK 293) cells synergistically facilitated the selective CE uptake from HDL (25). Taken together, the role of SR-BI in the lipase-mediated increase in selective CE uptake from lipoproteins is controversial at present.

In this study, the question was addressed whether SR-BI plays a role in the mechanism of the HL-mediated increase in HDL selective CE uptake. Baby hamster kidney (BHK) cells with no detectable, or with substantial, SR-BI expression were the dominant experimental model (24). In addition, SR-BI-deficient HEK 293 cells were used (23, 25). HDL_3 (d = 1.125–1.21 g/ml) was radiolabeled in the protein and lipid moieties (26, 27), and human HL was prepared from plasma or from cell culture media (21, 28). Experiments show that HL stimulates HDL_3-selective CE uptake quantitatively to the same extent independent from the cellular SR-BI status, and this effect is independent from lipolysis. In agreement with these results, immunofluorescence studies revealed that HL binding to the cell surface was not affected by the presence or absence of SR-BI (29, 30). Similarly, there was only a minor colocalization of SR-BI and HL in both types of BHK cells. In summary, it is suggested that HL mediates an increase in HDL-selective CE uptake by a mechanism(s) distinct from SR-BI.

MATERIALS AND METHODS

Preparation of unlabeled HDL_3, apoA-I, and lipoprotein-deficient serum

Human HDL_3 (d = 1.125–1.21 g/ml) was isolated by ultracentrifugation from plasma of healthy donors (27). Heparin-Sepharose (Amersham Pharmacia) affinity chromatography was used to remove any HDL_3 particles containing apoE (27). Human apoA-I was prepared as described previously (27). Lipoprotein-deficient serum (LPDS) was isolated by ultracentrifugation as the d > 1.25 g/ml fraction of human plasma (22).

Preparation of doubly radiolabeled HDL_3

ApoA-I was traced with the radioiodinated tyramine cellobiose ligand (^{125}I-TC-apoA-I) (26). Na^{125}I and [1-α, 2-α (n)-^{3}H]cholesteryl oleyl ether ([^{3}H]CEt) were supplied by Amersham Pharmacia. Doubly radiolabeled HDL_3 was prepared as described (22, 26, 27). ^{125}I-TC-apoA-I and [^{3}H]CEt were incorporated into apoE-deficient HDL_3 by exchange. After uptake by cells, both HDL_3 tracers are intracellularly trapped (26).

Preparation of HL from human HuH7 hepatoma cell culture media

Differentiated HuH7 hepatoma cells secrete HL but no LPL in the culture medium (31). These cells were grown in DMEM (Life Technologies) containing FBS (10% v/v, Life Technologies), penicillin (100 IU/ml, Life Technologies), and streptomycin (100 μg/ml, Life Technologies). For HL preparation, these cells were cultured in Triple Flasks (500 qcm, Nunc) in DMEM, which was supplemented additionally with heparin (5 U/ml, Roche) and L-(1-tosylamido-2-phenyl)ethyl chloromethyl ketone (10 μg/ml, Roche) (21). HL secretion into the media was monitored by enzyme activity determinations as outlined below.

To prepare HL, the HuH7 cell-derived medium was loaded on heparin-sepharose columns (Amersham Pharmacia) after addition of sodium chloride (0.4 M, final concentration). HL was eluted from this column with buffer (pH 7.4) containing NaCl (1 M), Tris (10 mM), and glycerol (10% v/v). These lipase preparations are referred to as "partially purified HL."

Preparation of HL from human postheparin plasma

Highly purified human HL was prepared from postheparin plasma as described (28). This material was a kind gift from Dr. Gunilla Olivecrona, University of Umea, Umea, Sweden.

HL activity and mass determinations

HL lipolytic activity was measured using a gum arabic-stabilized [^{14}C]trioleyl glycerol emulsion as substrate (21). Briefly, this emulsion was prepared with unlabeled triolein (50 μg, Fluka) and [^{14}C]trioleyl glycerol (2.5 μCi, Amersham Pharmacia), followed by sonication in buffer containing Tris (0.2 M), lysophosphatidylcholine (0.2% w/v, Sigma), and BSA (5 mg/ml, Sigma). To each sample containing HL activity, 0.1 ml of this mixture was added, and an incubation (28°C, 30 min) followed. This reaction was stopped by the addition of chloroform-heptane-methanol (75:60:85, v/v/v). After centrifugation of the samples (20 min), the upper phases were transferred to a vial containing scintillation fluid.

HL mass was analyzed by ELISA (32).

Inhibition of the lipolytic activity of HL

Tetrahydrolipstatin (THL; Orlistat(R), Roche), a covalent inhibitor of the catalytically active site of all mammalian lipases, was used to inhibit the lipolytic activity of HL (33, 34). Media for the uptake assays with cells containing radiolabeled HDL_3 and HL were prepared, and THL (final concentration 50 μg/ml) dissolved in ethanol (final concentration 0.1% v/v) was added if HL was absent or present. The lipolytic activity of HL is completely blocked under these conditions, as demonstrated earlier (16, 21, 22) and as shown below.

Cloning of SR-BI and transfection of BHK cells

BHK cells were transfected with the plasmid pBK-CMV (control, vector) or pBK-CMV-hSR-BI containing the full-length human

SR-BI cDNA (24). The respective cells stably express SR-BI as demonstrated previously (24).

Culture of BHK cells

BHK cells were cultured (37°C) in DMEM containing FBS (5% v/v), penicillin (100 IU/ml), streptomycin (100 μg/ml), glutamine (2 mM, Life Technologies), and G418 sulfate (0.8 mg/ml, Life Technologies). For lipoprotein uptake experiments, both types of BHK cells (500,000 per well) were plated in wells (35 mm, multiwell tissue culture plates, Nunc). Twenty-four hours or 48 h after plating, when the cells were near confluency, the cells were washed (PBS, two times) and the culture medium was replaced by DMEM supplemented with LPDS (5% v/v), antibiotics (see above), and G418 sulfate (0.8 mg/ml). The preincubations and the uptake assays as outlined below followed after culture (37°C, 20 h) in this medium.

Culture of HEK 293 cells

HEK 293 cells were cultured (37°C) in DMEM supplemented with FBS (10% v/v), glutamine (2 mM), and antibiotics (see above). For uptake experiments, HEK 293 cells were plated in 35 mm culture wells (Nunc). Twenty hours before the HDL_3 uptake assays, confluent cells were washed (PBS, two times) and the culture medium was replaced by DMEM containing LPDS (5% v/v) and antibiotics (see above).

Preincubation of the cells

Before initiating the lipoprotein uptake assays, BHK cells were preincubated in serum-free and lipoprotein-free media (27). These preincubations were performed to allow internalization or dissociation of membrane-associated serum or protein components originating from culture in the presence of LPDS, from BSA, or from cell secretion. After aspiration of the culture medium, the cells were washed with PBS (one time). Preincubation was then performed (37°C, 30 min) in DMEM containing BSA (5 mg/ml) and antibiotics (see above).

Uptake of doubly radiolabeled HDL_3 by cells in culture

To investigate HDL_3 uptake, BHK cells or HEK 293 cells were incubated (37°C) in DMEM containing BSA (5 mg/ml) and doubly radiolabeled HDL_3; HL was absent or present as indicated in the respective legends (27). The catalytic activity of HL was blocked with THL in these assays as described above. After incubation for the indicated time periods, the medium was aspirated and the cells were washed (PBS, four times). Then DMEM containing BSA (5 mg/ml) and unlabeled HDL_3 (100 μg protein/ml) was added for a chase incubation (37°C, 2.0 h) to remove reversibly cell-associated tracers (35). After this chase period, the medium was aspirated and the cells were washed again (PBS, one time). The cells then were released from the wells with trypsin-EDTA solution (trypsin 0.5 g/l, EDTA 0.2 g/l, 0.5 ml/well; Life Technologies). Trypsin activity was quenched with PBS containing excess BSA (50 mg/ml). The cell suspensions were transferred to tubes with a PBS (4°C) rinse of the wells. The cells were pelleted by centrifugation (2,000 *g*, 4°C, 15 min) followed by aspiration of the supernatant. The cell pellet was thereafter resuspended in PBS (4°C, 5.0 ml) followed by centrifugation (2,000 *g*). The final cell pellet was dissolved in NaOH solution (0.1 N, 1.0 ml) and sonicated, and aliquots were used for protein determination, direct ^{125}I radioassay, and ^{3}H radioassay after lipid extraction (24, 27). In the case of HEK cells, no chase incubation was performed.

Immunoblot analysis and antibodies

An anti-SR-BI antiserum was prepared by immunization of rabbits with a recombinant human SR-BI fragment (amino acids 495 to 509) (36). Two anti-HL antisera were generated in rabbits by immunization with human HL fusion proteins [amino acids 8 to 174 (N-terminus) or amino acids 148 to 327 (middle)]. These antisera were a kind gift of Dr. Hans Will, University of Hamburg. These anti-HL antisera were mixed (50:50, v/v) and used in immunoblots. The anti-HL antiserum (goat), which was applied for immunofluorescence, was a donation from Hans Jansen, University of Rotterdam, The Netherlands (32).

For immunoblot analysis, postnuclear supernatants were prepared from BHK cells or from HEK 293 cells (24, 37). Samples containing the indicated amount of protein were reduced with 2-mercaptoethanol in gel loading buffer. HL preparations were dissolved in gel loading buffer. All samples were fractionated by SDS-PAGE and the proteins transferred to nitrocellulose membranes. Finally, the membranes were incubated in buffer containing the respective antiserum. The primary antibody was detected using a peroxidase-conjugated goat anti-rabbit IgG (1:5,000; Amersham Pharmacia). Antibody binding was visualized by enhanced chemiluminescence detection (Amersham Pharmacia) and autoradiography. Ponceau S staining of membranes detected molecular mass standards (Amersham Pharmacia).

Indirect immunofluorescence

Both types of BHK cells were cultured (48 h) on glass cover slips. Subconfluent cells were incubated (4°C, 60 min) in DMEM containing BSA (2 mg/ml) and apoE-deficient HDL_3 (40 μg protein/ml); highly purified HL (1 μg protein/ml) was absent or present as indicated. Afterwards, nonspecific surface-associated materials were removed by washing with PBS (four times). Indirect immunofluorescence was performed exactly as described (29, 30) using antibodies against human SR-BI (rabbit, dilution 1:100) (36) and against human HL (goat, dilution 1:500) (32). To visualize the primary antibodies, immune-absorbed goat anti-rabbit (Cy2) or donkey (Cy3) anti-goat $F(ab')_2$ fragments (Dianova, Hamburg, Germany) were used. Finally, cells were washed with PBS containing nucleus stain Hoechst 33342 (Sigma) and embedded in Mowiol (Calbiochem). For confocal scanning microscopy, an inverted Leica TCS microscope (Leica, Heidelberg, Germany) was used.

Miscellaneous

Heparinase I (EC 4.2.2.7, from Flavobacterium Heparinum) and heparinase III (EC 4.2.2.8, from Flavobacterium Heparinum) were obtained from Sigma (13). Cholesterol, phospholipids, and triglycerides were determined with enzymatic assays (Roche). Nonesterified fatty acids were analyzed with a colorimetric method (Wako).

Statistics and calculations

Data are expressed as means ± SEM. Significance of differences was examined using Student's *t*-test.

For cellular uptake of doubly radiolabeled HDL_3, uptake of each tracer is shown in terms of apparent lipoprotein particle uptake, expressed as lipoprotein protein (1, 27). This is done to compare the uptake of both tracers on a common basis. Outlined in this way, uptake of HDL_3 holo-particles is represented by equal uptakes of both tracers. In the figures, ^{125}I represents apparent lipoprotein particle uptake according to the protein tracer (^{125}I-TC-apoA-I) [^{3}H]CEt that, due to the CE tracer and [^{3}H]CEt−^{125}I, shows the difference in apparent HDL_3 particle uptake that is apparent selective CE uptake.

RESULTS

Stable cell clones were derived from BHK cells by transfection either with an expression plasmid containing the

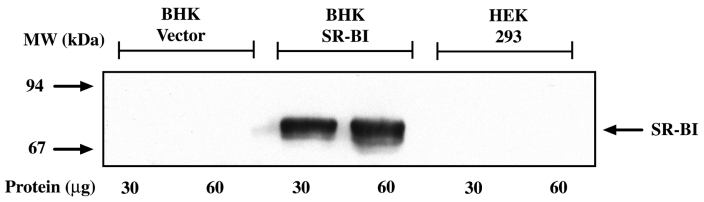

Fig. 1. Immunoblot analysis for scavenger receptor class B type I (SR-BI) in baby hamster kidney (BHK) cells and in human embryonal kidney 293 (HEK 293) cells. Control BHK cells (vector) or BHK cells with SR-BI expression and HEK 293 cells were cultured as described in Materials and Methods. Postnuclear supernatants were prepared from these cells and thereafter 30 μg or 60 μg protein (per lane) were fractionated by SDS-PAGE (10%). Finally, the proteins were transferred to a nitrocellulose membrane. This membrane was immunoblotted with an anti-SR-BI antiserum (human, dilution 1:1,000). IgG binding was visualized as outlined in Materials and Methods. Three similar blots with BHK cells and one with HEK 293 cells yielded qualitatively identical results. MW, molecular weight.

human SR-BI cDNA (pBK-CMV-hSR-BI) or with the control vector (pBK-CMV) (24). In addition, as an established SR-BI-deficient cell model, HEK 293 cells were used in this study (23, 24, 25). SR-BI expression was explored in postnuclear supernatants that were prepared from BHK or HEK 293 cells. Finally, immunoblots were performed using a human SR-BI-specific antiserum (36) (**Fig. 1**). In control BHK cells (vector), no signal corresponding to SR-BI could be visualized, whereas a strong band was apparent in SR-BI-transfected cells (Fig. 1). In HEK 293 cells, no SR-BI signal was visible in this analysis (Fig. 1). To verify that the human SR-BI-specific antiserum recognizes this protein in baby hamster tissue as well, a control immunoblot was performed. In this case, purified membranes were isolated from adult baby hamster adrenal glands. This immunoblot showed that the human SR-BI-specific antiserum recognized this HDL receptor protein from baby hamster tissue as well (data not shown). With respect to HDL_3 metabolism, the expression of SR-BI in BHK cells induces a substantial increase in HDL_3-selective CE uptake as presented below (24).

To investigate the effect of HL on HDL_3 metabolism, HL was prepared. In one case, these HL preparations originated from tissue culture media of human HuH7 hepatoma cells, and these partially purified HL preparations were devoid of LPL (21, 31). Alternately, in many cases, highly purified HL preparations were used that were isolated from human plasma (28). To explore the purity of these HL proteins, immunoblots were performed. In this analysis of both HL preparations, the anti-HL antibody recognized only one band at the appropriate molecular weight (**Fig. 2**). This immunoreactivity suggests that both HL preparations indeed contain the native enzyme. To block HL-mediated lipolysis during the uptake assays for HDL_3, THL, an active site inhibitor of lipases (33, 34), was added to the medium of the cells. Previous experiments showed that lipolysis is completely inhibited under these experimental conditions (16, 21, 22). However, to investigate under the conditions of this study whether the lipolytic action of HL was indeed blocked by THL, HDL_3 was incubated (37°C, 4.0 h) in medium containing this enzyme or not; THL was absent or present (**Table 1**). After this incubation, HDL_3 was reisolated and the chemical composition was analyzed. Under these conditions, HL and THL had no significant effect on protein, phospholipid, triglyceride, or cholesterol content of the respective HDL_3 (Table 1). This experiment yields no evidence for HL-mediated lipolysis under the experimental conditions of this study. Besides the chemical composition of HDL_3, the lipase-mediated release of nonesterified fatty acids was investigated. HDL_3 was incubated (37°C, 4.0 h) under the same conditions as shown in Table 1. Afterwards, fatty acids in the medium were determined with a colorimetric assay. HL did not increase the fatty acid concentration in the medium (data not shown). These results also argue against lipolysis under these conditions.

To investigate the role of HL and SR-BI in cellular lipoprotein metabolism, apoE-deficient HDL_3 was labeled with ^{125}I-TC-apoA-I and [^{3}H]CEt in the protein and lipid moieties (27). The apoE deficiency of this preparation precludes recognition by cellular apoB and apoE (LDL) receptors. Cells were incubated in medium containing this radiolabeled HDL_3. After a chase incubation that removes reversibly cell-associated HDL_3 tracers (35), cellu-

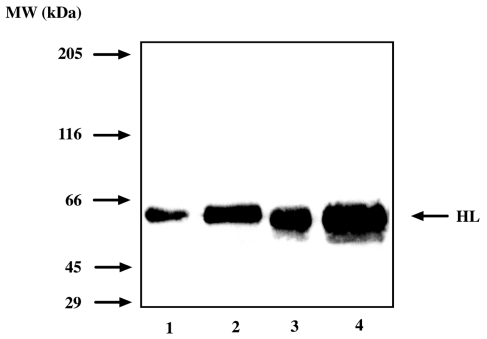

Fig. 2. Immunoblot analysis of human hepatic lipase (HL) preparations. Highly purified (lane 1, 40 ng protein; lane 2, 100 ng protein) or partially purified (lane 3, 100 ng protein; lane 4, 300 ng protein) HLs were loaded on SDS-PAGE (10%). After electrophoresis, the proteins were transferred to a nitrocellulose membrane. This membrane was immunoblotted with an anti-HL antiserum (dilution 1:1,000). IgG binding was visualized as outlined in Materials and Methods. One independent immunoblot yielded qualitatively identical results.

TABLE 1. Hepatic lipase, tetrahydrolipstatin, and the composition of HDL_3

	Incubation Conditions		
	−HL −THL	+HL −THL	+HL +THL
	% of total mass		
Protein	63.6 ± 4.8	66.1 ± 0.8	70.9 ± 3.8
Phospholipid	6.5 ± 0.5	6.9 ± 0.7	8.1 ± 0.4
Triglyceride	7.8 ± 1.4	5.3 ± 0.7	4.4 ± 0.8
Cholesterol	22.3 ± 2.8	21.7 ± 0.7	16.5 ± 4.4

HL, hepatic lipase; THL, tetrahydrolipstatin. Apolipoprotein E-deficient HDL_3 (80 μg protein/ml) was incubated (37°C, 4.0 h) in medium containing BSA (1 mg/ml), and highly purified HL (200 ng/ml) was absent or present as shown. THL (50 μg/ml) was added to the medium or not as indicated. Afterwards, HDL_3 was reisolated by ultracentrifugation at d = 1.21 g/ml followed by extensive dialysis (PBS). Finally, protein, phospholipid, triglyceride, and cholesterol were analyzed as described in Materials and Methods. Values are means ± SEM of four independent determinations. *P* values were between 0.18 and 0.86 according to Student's *t*-test.

lar tracer content and apparent HDL_3 particle uptake, according to ^{125}I-TC-apoA-I or [^{3}H]CEt, were analyzed (27).

The uptake of radiolabeled HDL_3 by BHK cells is shown in **Fig. 3**. Control BHK cells (vector, Fig. 3, left) or BHK cells with SR-BI expression (Fig. 3, right) were incubated (37°C, 4.0 h) in parallel in medium containing doubly radiolabeled HDL_3; highly purified human HL was absent or present during these incubations, as indicated in the abscissae. In the absence of HL in both types of BHK cells, apparent HDL_3 particle uptake according to [^{3}H]CEt was in excess of that due to ^{125}I-TC-apoA-I (Fig. 3, lower panels). The difference in uptake ([^{3}H]CEt-^{125}I-TC-apoA-I) yields apparent selective CE uptake from HDL_3 (1), and this rate is greater for BHK cells with SR-BI expression compared with control BHK cells (vector). In the experiment shown in Fig. 3, in control BHK cells (vector), apparent HDL_3-selective CE uptake was 216.9 ± 4.0 ng protein/mg cell protein (mean ± SEM, n = 4 incubations); in BHK cells with SR-BI expression, the respective value was 5,330.1 ± 54.7. SR-BI expression thus yields an increase in apparent HDL_3-selective CE uptake of 2,457% (control corresponds to 100%). Highly purified HL in the assay medium of both types of BHK cells containing labeled HDL_3 had virtually no effect on uptake of ^{125}I-TC-apoA-I (Fig. 3, lower panels). However, HL increased the uptake of HDL_3-associated [^{3}H]CEt dose dependently. As a result, apparent HDL_3 selective CE uptake ([^{3}H]CEt-^{125}I-TC-apoA-I) increased due to HL in a concentration-dependent manner in both types of BHK cells (Fig. 3, lower panels).

The difference in HDL_3-selective CE uptake in the absence and the presence of HL was calculated (Fig. 3, top panels). In control BHK cells (vector), HL (400 ng protein/ml) induced an absolute increase in apparent HDL_3 selective CE uptake of 1,293.7 ng protein/mg cell protein (mean, n = 2 incubations) (Fig. 3, left, top panel). In BHK cells with SR-BI expression, the same HL concentration promoted an absolute increase in apparent HDL_3-selective CE uptake of 1,356.3 ng protein/mg cell protein (mean, n = 2 incubations) (Fig. 3, right, top panel). Thus, quantitatively, HL stimulated the selective uptake of HDL_3-associated CE to a very similar extent in control BHK cells (vector) and in BHK cells with SR-BI expression.

Partially purified HL preparations instead of highly purified HL (see Materials and Methods) were used in two independent and very similar experiments (shown in Fig. 3) to stimulate HDL_3 selective CE uptake by both types of BHK cells. In this case, HL concentrations up to 600 ng HL protein/ml were applied (data not shown). These HL preparations also dose-dependently stimulated the selective CE uptake from HDL_3, and quantitatively this effect was similar in control BHK cells (vector) and in BHK cells with SR-BI expression. Taken together, partially and highly purified HL (Fig. 3) yielded qualitatively identical results with respect to cellular HDL_3 metabolism.

A dose-response curve for the uptake of radiolabeled HDL_3 by control BHK cells (vector) or by BHK cells with SR-BI expression is shown in **Fig. 4** (bottom panels). Both cell types were incubated in parallel in medium that contained increasing concentrations of doubly radiolabeled HDL_3, and partially purified HL was absent or present. Finally, cellular tracer content and apparent selective CE uptake ([^{3}H]CEt-^{125}I-TC-apoA-I) were analyzed. Increasing concentrations of radiolabeled HDL_3 yielded a dose-dependent increase in apparent selective CE uptake from HDL_3, and this was true for both types of BHK cells (Fig. 4, lower panels). HL stimulated apparent selective CE uptake by control BHK cells (vector) and by BHK cells with SR-BI expression (Fig. 4, lower panels). The absolute increase in apparent HDL_3-selective CE uptake induced by HL is shown in Fig. 4 (top panels) as well. Quantitatively, this HL-mediated increase in selective CE uptake was similar in control BHK cells (vector, Fig. 4, top, left panel) and in BHK cells with SR-BI expression (Fig. 4, top, right panel), and this HL effect was observed in each HDL_3 concentration examined. In three independent, very similar experiments (shown in Fig. 4), very low concentrations of radiolabeled HDL_3 (i.e., 5, 10, or 20 μg protein/ml) were used. At these low HDL_3 concentrations, the stimulatory effect of HL on selective CE uptake from HDL_3 quantitatively was very similar in both types of BHK cells (data not shown).

To investigate the effect of HL on the kinetics of selective CE uptake from HDL_3, control BHK cells (vector) or BHK cells with SR-BI expression incubated (37°C) for 30, 120, or 240 min in medium containing doubly radiolabeled HDL_3 and partially purified HL were absent or present (**Fig. 5**, lower panels). Finally, apparent HDL_3 selective CE uptake ([^{3}H]CEt)-^{125}I-TC-apoA-I) was analyzed. In the absence of HL, apparent selective CE uptake by both types of BHK cells was evident after 30 min of incubation, and this uptake increased in a time-dependent manner at fairly linear rates up to 240 min (Fig. 5, lower graphs). Throughout the entire time course, apparent HDL_3-selective CE uptake was higher in BHK cells with SR-BI expression compared with control cells (vector, Fig. 5, lower graphs). In parallel, both types of BHK cells were

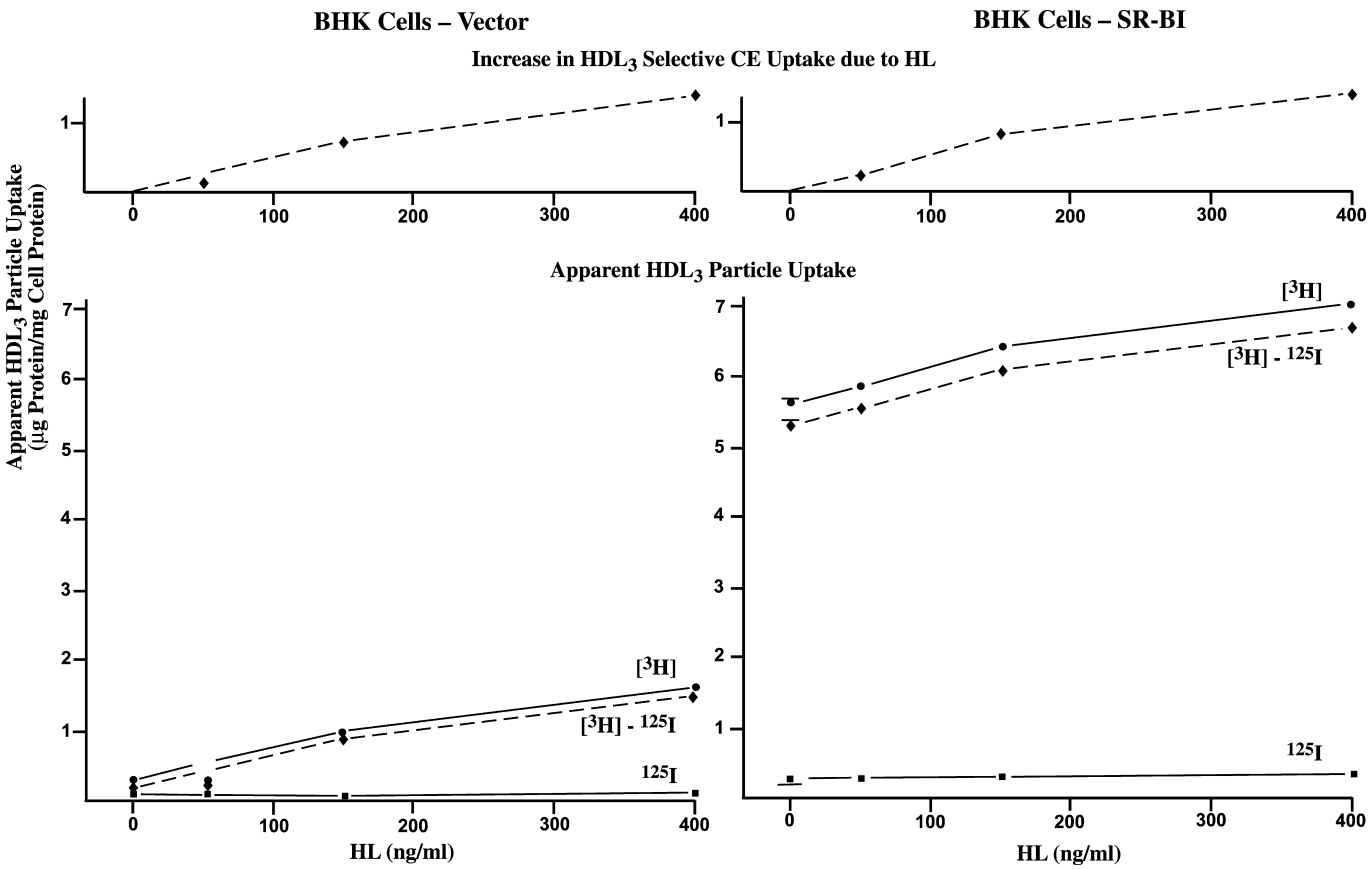

Fig. 3. HL dose-response curve on uptake of doubly radiolabeled HDL_3 by BHK cells. Control BHK cells (vector, left panels) or BHK cells with SR-BI expression (right panels) were incubated (37°C, 4.0 h) in parallel in DMEM containing doubly radiolabeled HDL_3 (40 µg HDL_3 protein/ml), and highly purified HL was absent or present as indicated in the abscissa. After a "chase" incubation, cellular tracer content and apparent HDL_3 particle uptake were analyzed. In the bottom panels, ^{125}I represents apparent HDL_3 particle uptake according to radioiodinated tyramine cellobiose ligand (^{125}I-TC)-apoA-I, ^{3}H demonstrates apparent particle uptake due to [^{3}H]cholesteryl oleyl ether ([^{3}H]CEt), and ^{3}H-^{125}I shows the difference, i.e., apparent HDL_3-selective cholesteryl ester (CE) uptake. The difference in apparent HDL_3-selective CE uptake between the absence and the presence of HL was calculated. This HL-induced absolute increase in apparent HDL_3 selective CE uptake is shown in the top panels. Values are means ± SEM of four incubations (without HL) or means of two incubations (with HL); where no error bars are shown, the SEM was smaller than the respective points. One independent experiment using highly purified HL and two independent experiments using partially purified HL yielded qualitatively identical results.

incubated in the additional presence of partially purified HL in the medium. HL significantly increased apparent selective CE uptake ([^{3}H]CEt-^{125}I-TC-apoA-I) from HDL_3 by both types of BHK cells, and again this effect was evident throughout the entire time course. The absolute increase in apparent selective CE uptake, which was mediated by HL, was calculated (Fig. 5, top panels). Quantitatively, this HL-induced increase in apparent HDL_3-selective CE uptake was very similar in control BHK cells (vector, Fig. 5, top, left panel) and in BHK cells with SR-BI expression (Fig. 5, top, right panel).

The experiments presented above suggest that the stimulatory effect of HL on HDL_3-selective CE uptake is independent from SR-BI. A well-established SR-BI-deficient cell model is HEK 293 cells (23, 24, 25). These cells do not express SR-BI (Fig. 1). To explore the effect of HL on HDL_3 metabolism in HEK 293 cells, these cells were incubated (37°C, 4.0 h) in a medium containing several distinct concentrations of doubly radiolabeled HDL_3, and partially purified HL was absent (**Fig. 6**, left panel) or present (Fig. 6, right panel). After this incubation, cellular tracer content was determined. In the absence of HL, apparent HDL_3 particle uptake according to ^{125}I-TC-apoA-I or [^{3}H]CEt increased dose dependently. This yielded an HDL_3 dose-dependent increase in apparent selective CE uptake ([^{3}H]CEt-^{125}I-TC-apoA-I) by HEK 293 cells. Addition of HL to the incubation medium containing radiolabeled HDL_3 had virtually no effect on uptake of ^{125}I-TC-apoA-I by HEK 293 cells (Fig. 6, right panel). However, HL induced an increase in [^{3}H]CEt uptake. As a result, HL addition yielded a dose-dependent increase in apparent cellular HDL_3-selective CE uptake ([^{3}H]CEt-^{125}I-TC-apoA-I) (Fig. 6, right panel). These experiments with HEK 293 cells also suggest that the HL-mediated increase in HDL_3 selective CE uptake is independent from SR-BI.

All experiments presented above suggest that the HL-mediated stimulation of HDL_3-selective CE uptake is independent from SR-BI. To evaluate this proposal with an alternative methodological approach, this issue was addressed by an immunofluorescence analysis (29, 30) using

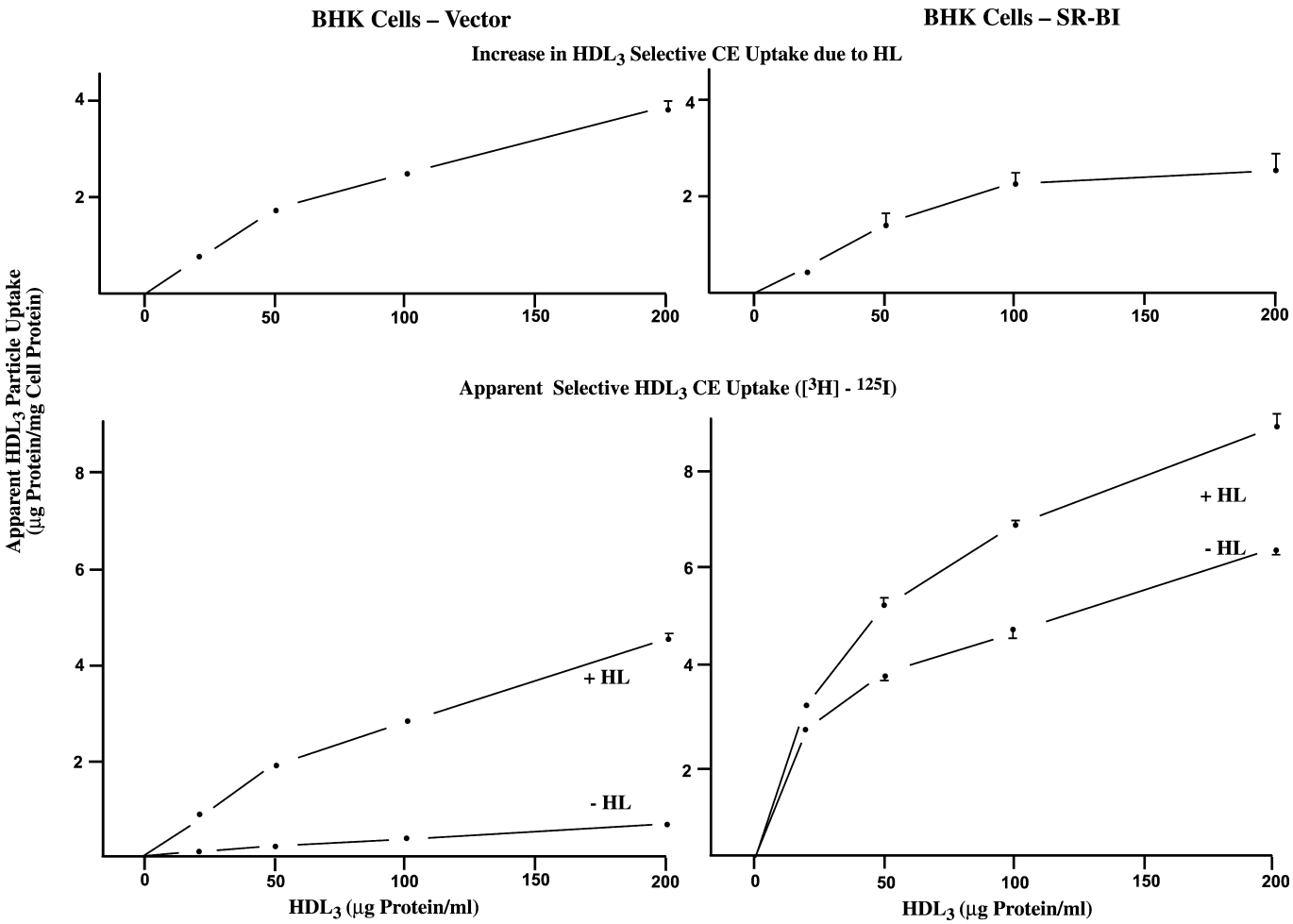

Fig. 4. Dose-response curve for the uptake of doubly radiolabeled HDL_3 by BHK cells and the effect of HL. Control BHK cells (vector, left panels) or BHK cells with SR-BI expression (right panels) incubated (37°C, 4.0 h) in parallel in DMEM containing doubly radiolabeled HDL_3, and the respective HDL_3 concentrations are indicated in the abscissa; partially purified HL (200 ng protein/ml) was absent or present as shown. After a chase incubation, cellular tracer content and apparent HDL_3 selective CE uptake ([^{3}H]CEt-^{125}I-TC-apoA-I) were analyzed (bottom panels). The difference in apparent HDL_3 selective CE uptake between the absence and the presence of HL was calculated, and this HL-induced absolute increase in selective CE uptake is shown in the top panels. Values are means ± SEM of three incubations; where no error bars are shown, SEM was smaller than the respective points. One independent experiment yielded qualitatively identical results.

specific antibodies directed against SR-BI or HL (32, 36). Control BHK cells (vector, **Fig. 7A**) or BHK cells with SR-BI expression (Fig. 7B) were incubated (4°C, 60 min) in medium that contained apoE-deficient HDL_3, and highly purified HL was absent or present. Immunofluorescence analysis followed (see Materials and Methods) (29, 30). In control BHK cells (vector), SR-BI immunofluorescence (green) was very low in cells incubated without or with HL (Fig. 7A). In contrast to control BHK cells (vector), substantial amounts of SR-BI protein were visualized in BHK cells with SR-BI expression (Fig. 7B, green fluorescence). Incubation of both types of BHK cells in medium containing no HL yielded no signal for this enzyme in the immunofluorescence analysis (red fluorescence, Figs. 7A, B). However, if the cells were incubated in the presence of highly purified HL, a signal for this enzyme (red fluorescence) was present in control BHK cells (vector, Fig. 7A) and in BHK cells with SR-BI expression (Fig. 7B). These figures show that HL binds to the surface of control BHK cells (vector) and to the surface of BHK cells with SR-BI expression without any difference in localization and binding capacity.

Merged images revealed only a rare appearance of yellow spots in control BHK cells (vector, Fig. 7A) and in BHK cells with SR-BI expression (Fig. 7B). This suggests only a minor colocalization of SR-BI and HL (~20%). This immunofluorescence analysis proposes that SR-BI and HL mediate selective CE uptake from HDL_3 at distinct sites of the plasma membrane.

Cell surface proteoglycan deficiency diminishes the lipase-mediated increase in selective CE uptake from HDL and LDL (13, 21, 22). Therefore, the question was addressed of whether the effect of HL on HDL_3-selective CE uptake is dependent on cell surface proteoglycans in control BHK cells (vector) and in BHK cells with SR-BI expression. Both types of BHK cells were incubated in

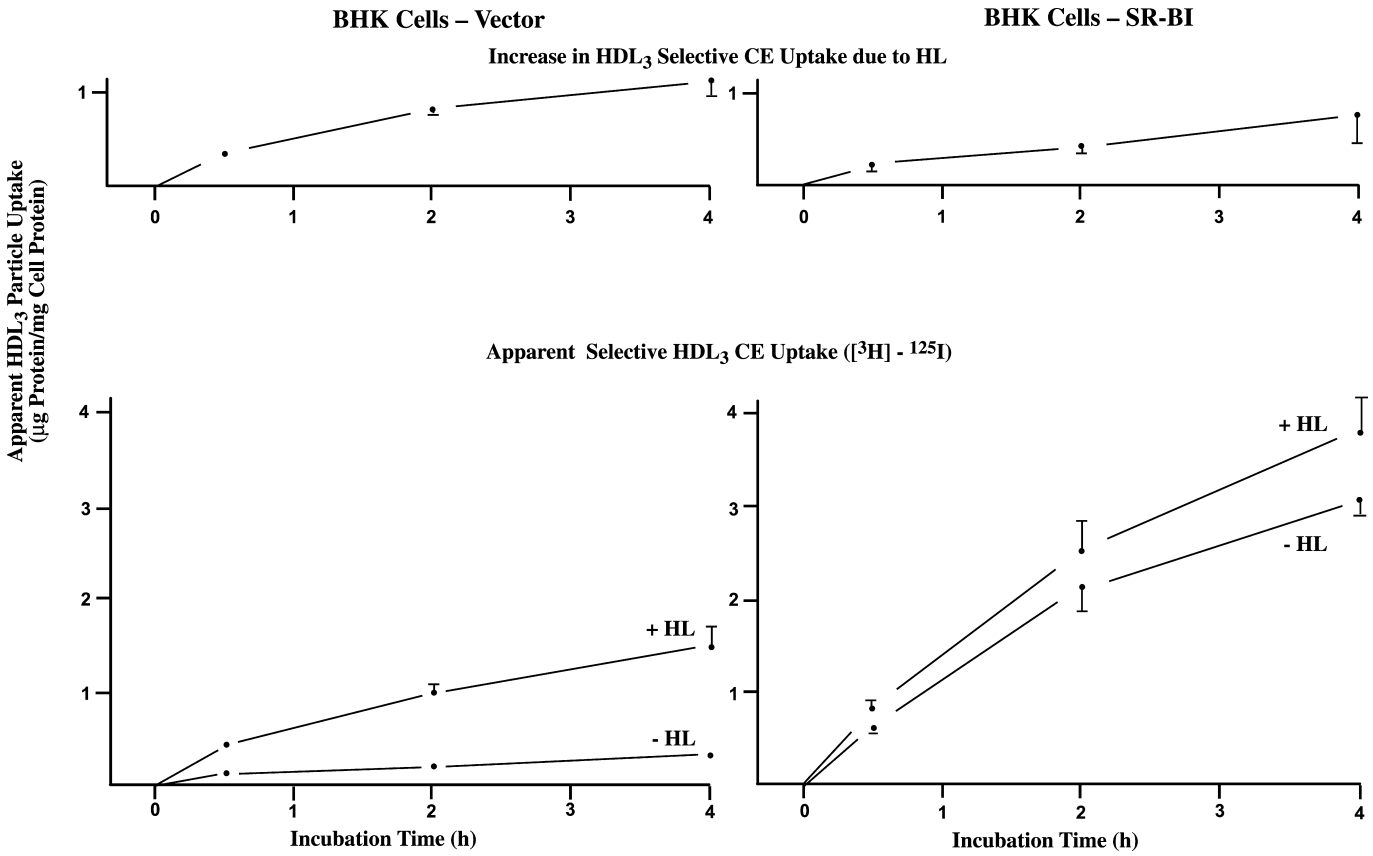

Fig. 5. Kinetics for the uptake of doubly radiolabeled HDL_3 by BHK cells and the effect of HL. Control BHK cells (vector, left panels) or BHK cells with SR-BI expression (right panels) incubated (37°C, 0.5, 2.0 or 4.0 h) in parallel in DMEM containing doubly radiolabeled HDL_3 (40 μg HDL_3 protein/ml); partially purified HL (200 ng protein/ml) was absent or present as indicated. After a chase incubation, cellular tracer content and apparent HDL_3-selective CE uptake ([^{3}H]CEt-^{125}I-TC-apoA-I, bottom panels) were analyzed. The difference in apparent HDL_3-selective CE uptake between the absence and the presence of HL was calculated, and this HL-induced absolute increase in selective CE uptake is shown in the top panels. Values are means ± SEM of three incubations. Where no error bars are shown, SEM was smaller than the respective points. One independent experiment using partially purified HL yielded qualitatively identical results.

parallel in the absence or presence of heparinase I and heparinase III in the medium to deplete the cells from surface proteoglycans (**Fig. 8**). Previous experiments established that the cells are depleted from proteoglycans under these conditions (22). Afterwards followed an incubation of both types of BHK cells in a medium that contained doubly radiolabeled HDL_3, and partially purified HL was absent or present. Apparent HDL_3 selective CE uptake ([^{3}H]CEt-^{125}I-TC-apoA-I) was analyzed. In control BHK cells (vector) or in BHK cells with SR-BI expression, digestion with heparinases per se had no effect on apparent HDL_3-selective CE uptake (Fig. 8). As expected, HL stimulated apparent selective CE uptake from HDL_3 in control BHK cells (vector) or in BHK cells with SR-BI expression. However, preincubation of the cells with heparinases decreased the stimulatory HL action on apparent HDL_3-selective CE uptake ([^{3}H]CEt-^{125}I-TC-apoA-I) significantly, and this was observed in control BHK cells (vector) and in BHK cells with SR-BI expression (Fig. 8). Thus, independent of the cellular SR-BI status, the HL-mediated stimulation of HDL_3-selective CE uptake is dependent from cell surface proteoglycans.

DISCUSSION

Biochemical experiments of this study provide evidence that both partially purified and highly purified HL preparations promote the selective CE uptake from HDL_3 in cultured cells. This HL-stimulated uptake of HDL_3 was explored in the presence of THL, a compound that blocks HL-mediated lipolysis (33, 34). Under the conditions of this investigation, there was no HL-induced lipolysis of HDL_3 detectable, and this is in agreement with previous studies (21, 22). Due to these observations, the stimulatory effect of HL on selective CE uptake is independent from lipolysis. Quantitatively, the HL-mediated increase in selective CE uptake from HDL_3 is very similar in control BHK cells (vector) and in BHK cells with SR-BI expression. This HL effect is time-, HDL_3-, and HL concentration-dependent in both types of BHK cells. In agreement with these cells, this lipase-stimulated selective CE uptake from HDL_3 is observed in SR-BI-deficient HEK 293 cells (23, 24, 25). Taken together, these results suggest that the HL-mediated increase in HDL_3-selective CE uptake is independent from SR-BI and independent from lipolysis. This conclusion is reinforced by an immunofluorescence

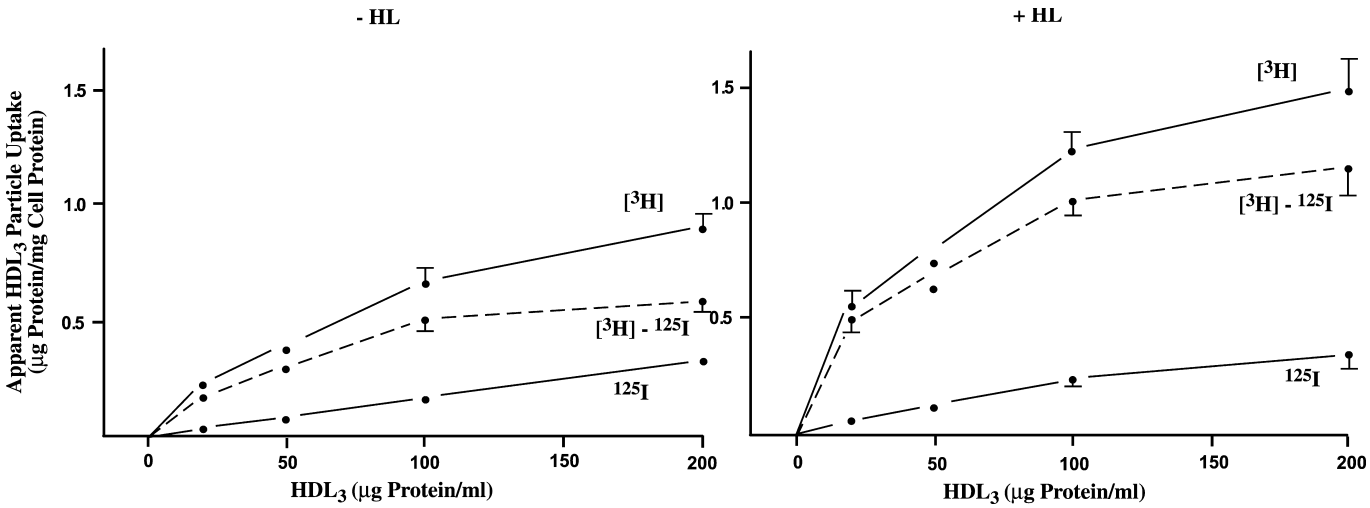

Fig. 6. Dose-response curve for the uptake of doubly radiolabeled HDL_3 by HEK 293 cells and the effect of HL. HEK 293 cells incubated (37°C, 4.0 h) in DMEM containing doubly radiolabeled HDL_3 and the respective HDL_3 concentrations are indicated in the abscissa; partially purified HL (200 ng protein/ml) was absent (left panel) or present (right panel) as shown. After this incubation, cellular tracer content was analyzed. ^{125}I represents apparent HDL_3 particle uptake according to ^{125}I-TC-apoA-I; ^{3}H demonstrates apparent particle uptake due to [^{3}H]CEt, and ^{3}H-^{125}I shows the difference, i.e., apparent selective CE uptake. Values are means ± SEM of three incubations. Where no error bars are shown, the respective bars were smaller than the points. One similar experiment using highly purified HL yielded qualitatively identical results.

analysis. Images showed similar binding capacities for HL in both types of BHK cells, and only a minor colocalization of HL and SR-BI. This suggests that SR-BI expression does not alter cellular binding of HL, and subsequently that the HL effect on selective CE uptake is mainly independent from SR-BI. It should be kept in mind that a complete separation of both proteins at the plasma membrane cannot be expected in this analysis due to the limited resolution of fluorescence microscopy. Heparinase-induced proteoglycan deficiency of the cell surface reduced the HL-mediated increase in HDL_3-selective CE uptake, and this suggests that these molecules play a role in the HL-promoted increase in selective lipid uptake (13, 21). This lipase-induced and proteoglycan-mediated HDL_3 tethering to the cell membrane most likely plays a role in the mechanism of the HL-induced increase in selective CE uptake (13).

The HL-mediated and SR-BI-independent increase in HDL_3 selective CE uptake shown here is in disagreement with earlier experiments (25). Transient expression of SR-BI and catalytically active or inactive HL in HEK 293 cells suggested a synergistic role for these molecules in the mechanism of the lipase-induced increase in HDL selective CE uptake (25). A possible explanation for this discrepancy in results may be methodological differences between these studies (25). Here, cells stably expressed SR-BI, and exogenous HL was added to the assay medium containing labeled HDL_3. In contrast, the earlier investigators (25) used a transient transfection approach for HL and SR-BI to investigate the role of these molecules in HDL metabolism.

Besides HL, another member of the lipase gene family, LPL, mediates a lipolysis-independent increase in HDL_3-selective CE uptake in vitro (20, 22). Recently, evidence was presented that the mechanism of this LPL-mediated increase in HDL selective CE uptake is independent from SR-BI (24). Besides HDL, LPL induces an enhancement in LDL selective CE uptake as well, and this effect was again independent from SR-BI (23). In summary, both HL and LPL increase selective CE uptake from HDL and LDL, and the receptor protein SR-BI does not play a role in the mechanism. This conclusion is true for the majority of studies presented so far.

Which is (are) the specific molecular mechanism(s) for the SR-BI-independent increase in HDL-selective CE uptake mediated by lipases? LPL and HL bind to cell surface proteoglycans and lipoproteins, and these interactions closely associate these particles with cell membranes (5, 11, 12, 13, 16). For holo-lipoprotein uptake, presumably this "bridging" facilitates particle internalization by cells (5, 10, 16, 38). Analogously, the results of this and of previous studies (22, 23, 24) are in line with the hypothesis that HL and LPL "anchor" lipoproteins in close association with the plasma membrane. This bridging may facilitate the selective lipid transfer from lipoproteins into cells. This model is in agreement with recent results on the molecular mechanism of the selective CE uptake pathway (39, 40). In transfected cells expressing SR-BI or CD36, a class B scavenger receptor that is closely related to SR-BI, both SR-BI and CD36 bind HDL with high affinity (40). This HDL binding to SR-BI or CD36 mediates the lipid transfer from this lipoprotein to cells, and this presumably constitutes one component in the molecular mechanism of the selective CE pathway. Analogously, in Y1-BS1 adrenocortical cells, a tight correlation between HDL binding and HDL selective CE uptake has been established (39). These observations are in line with the above-presented hypothesis, i.e., the HL- or LPL-induced

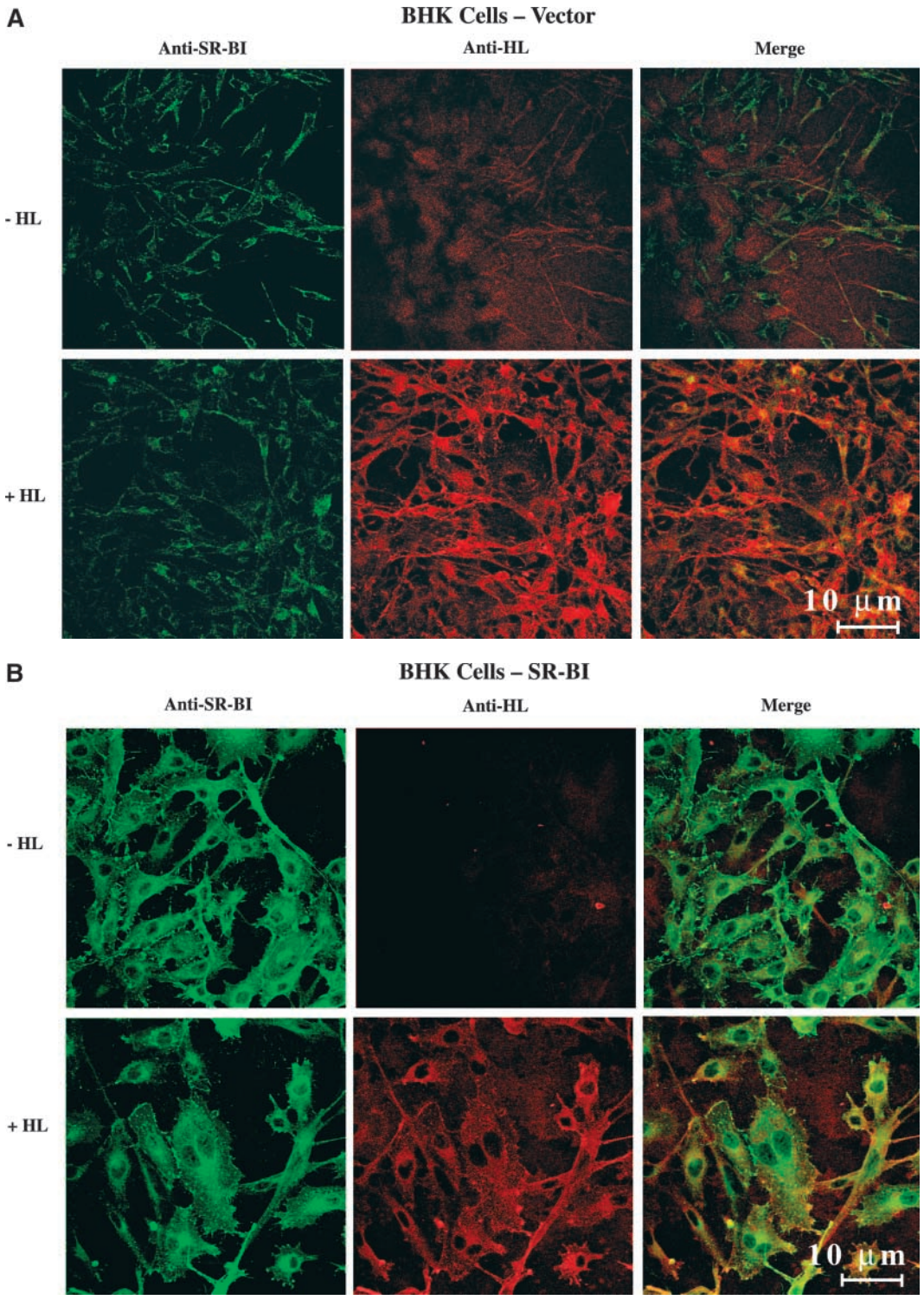

Fig. 7. Localization of SR-BI and HL in BHK cells by immunofluorescence analysis. Control BHK cells (A) or BHK cells with SR-BI expression (B) were grown on glass coverslips. These cells were incubated (4°C, 60 min) in DMEM containing apoE-deficient HDL_3 (40 μg protein/ml), and highly purified HL (1 μg protein/ml) was absent or present as indicated. Thereafter, nonspecific cell-associated materials were removed by washing (PBS, four times). After fixation and permeabilization, cells were immune double-labeled with an anti-SR-BI antibody (dilution 1:100, visualized in green) and an anti-HL antibody (dilution 1:500, visualized in red) as described in Materials and Methods. Confocal images from each fluorochrome were recorded and superimposed to reach yellow colocalization (merge). Images correspond to confocal projections.

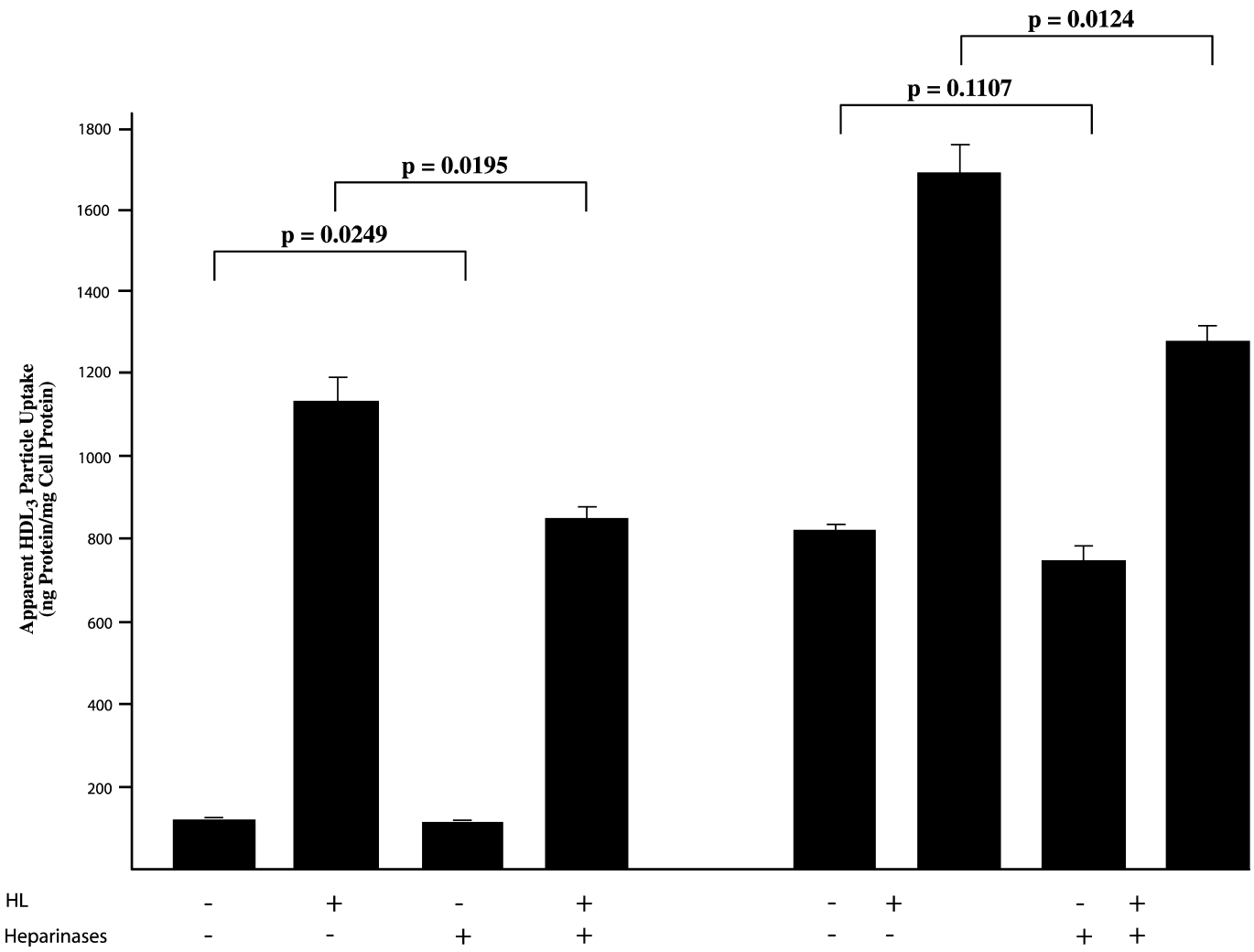

Fig. 8. Effect of preincubation with heparinases on the HL-mediated increase in selective CE uptake from doubly radiolabeled HDL_3 by BHK cells. After preincubation (37°C, 30 min, see Materials and Methods), control BHK cells (vector, left panels) or BHK cells with SR-BI expression (right panels) were incubated (37°C, 120 min) in parallel in DMEM that contained heparinase I (8.7 U/ml) and heparinase III (2.6 U/ml), or not, as indicated in the abscissa. Afterwards, this medium was aspirated, and both groups of cells were washed (PBS), followed by an incubation (37°C, 30 min) in DMEM containing doubly radiolabeled HDL_3 (40 μg protein/ml); partially purified HL (360 ng protein/ml) was absent or present during this incubation as indicated in the abscissa. Finally, cellular tracer content and apparent HDL_3-selective CE uptake ([^{3}H]CEt-^{125}I-TC-apoA-I) were analyzed. Values are means ± SEM of four (without HL) or three (with HL) incubations. *P* values for the unpaired two-tailed Student's *t*-test are given. One independent experiment using partially purified HL yielded qualitatively identical results.

close association between HDL and the plasma membrane facilitates the selective CE transfer from lipoproteins into cells.

By which mechanism(s) are the hydrophobic CE molecules finally transferred from the neutral lipid core of the HDL particle and into the cell following the lipase-mediated HDL tethering to the plasma membrane? The molecular mechanism(s) of this lipid transfer is not defined at present. Pittman and coworkers (41) explored the CE transfer from HDL particles to membranes. Experiments using model membranes, purified plasma membranes, native radiolabeled HDL, and recombinant HDL suggested that CE molecules are transferred to membranes in a collision-mediated process. The latter mechanism involves lipid-lipid interactions independent of membrane proteins (41). According to an alternative model, SR-BI forms a nonaqueous "channel" that mediates the CE movement from a lipoprotein into the plasma membrane (39). However in view of the results of this study, it seems unlikely that this "channel" contributes to the lipase-mediated HDL CE transfer. Taken together, future experiments have to define the molecular mechanism(s) that mediate(s) the lipase-induced increase in selective CE transfer from lipoproteins to cells. Besides lipid-lipid interactions,

a plasma membrane protein distinct from SR-BI may play a role in this lipid transfer pathway.

The interaction between HDL, SR-BI, and HL in lipid delivery to cells was investigated in this study in vitro. What are the physiological implications of these results for the HDL-mediated reverse cholesterol transport to the liver in vivo? Relevant in this context are studies with genetically modified animals. SR-BI and HL play physiologic roles in reverse cholesterol transport to the liver in rodents (2–4, 42). Mice with a genetically induced deficiency of HL (43) or SR-BI (3, 4) have an increase in HDL cholesterol and a decrease in selective CE uptake by the liver. In contrast, hepatic overexpression of HL (42, 44, 45) or SR-BI (46) in mice or rabbits decreases this protective HDL lipoprotein fraction in plasma. In murine adrenal glands, an induced HL deficiency yields an increase in SR-BI expression (47). These studies show that, in vivo, both HL and SR-BI have a substantial impact on HDL metabolism. On the other hand, it is generally accepted that this lipoprotein fraction plays a central role in reverse cholesterol transport. This pathway presumably transports excess cholesterol from peripheral cells to the liver and protects from atherosclerosis. The in vitro investigations presented here explain one potential mechanism by which HL modifies HDL metabolism in vivo (48).

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REFERENCES

1. Pittman, R. C., T. P. Knecht, M. S. Rosenbaum, and C. A. Taylor, Jr. 1987. A nonendocytotic mechanism for the selective uptake of high density lipoprotein-associated cholesterol esters. *J. Biol. Chem.* **262:** 2443–2450.
2. Acton, S., A. Rigotti, K. T. Landschulz, S. Xu, H. H. Hobbs, and M. Krieger. 1996. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science.* **271:** 518–520.
3. Rigotti, A., B. L. Trigatti, M. Penman, H. Rayburn, J. Herz, and M. Krieger. 1997. A targeted mutation in the murine gene encoding the high density lipoprotein (HDL) receptor scavenger receptor class B type I reveals its key role in HDL metabolism. *Proc. Natl. Acad. Sci. USA.* **94:** 12610–12615.
4. Varban, M. L., F. Rinninger, N. Wang, V. Fairchild-Huntress, J. D. Dunmore, Q. Fang, M. L. Gosselin, K. L. Dixon, J. D. Deeds, S. L. Acton, A. R. Tall, and D. Huszar. 1998. Targeted mutation reveals a central role for SR-BI in hepatic selective uptake of high density lipoprotein cholesterol. *Proc. Natl. Acad. Sci. USA.* **95:** 4619–4624.
5. Goldberg, I. J. 1996. Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. *J. Lipid Res.* **37:** 693–707.
6. Jansen, H., C. Kalkman, A. J. Zonnevled, and W. C. Hülsmann. 1979. Secretion of triacylglycerol hydrolase activity by isolated parenchymal rat liver cells. *FEBS Lett.* **98:** 299–302
7. Kuusi, T., E. A. Nikkilä, I. Virtanen, and P. K. J. Kinnunen. 1979. Localization of the heparin-releasable lipase in situ in the rat liver. *Biochem. J.* **181:** 245–246.
8. Sanan, D. A., J. Fan, A. Bensadoun, and J. M. Taylor. 1997. Hepatic lipase is abundant on both hepatocyte and endothelial cell surfaces in the liver. *J. Lipid Res.* **38:** 1002–1013.
9. Bengtsson, G., and T. Olivecrona. 1980. The hepatic heparin releasable lipase binds to high density lipoproteins. *FEBS Lett.* **119:** 290–292.
10. Beisiegel, U., W. Weber, and G. Bengtsson-Olivecrona. 1991. Lipoprotein lipase enhances the binding of chylomicrons to low density lipoprotein receptor-related protein. *Proc. Natl. Acad. Sci. USA.* **88:** 8342–8346.
11. Eisenberg, S., E. Sehayek, T. Olivecrona, and I. Vlodavsky. 1992. Lipoprotein lipase enhances binding of lipoproteins to heparan sulfate on cell surfaces and extracellular matrix. *J. Clin. Invest.* **90:** 2013–2021.
12. Rumsey, S. C., J. C. Obunike, Y. Arad, R. J. Deckelbaum, and I. J. Goldberg. 1992. Lipoprotein lipase-mediated uptake and degradation of low density lipoproteins by fibroblasts and macrophages. *J. Clin. Invest.* **90:** 1504–1512.
13. Ji, Z-H., H. L. Dichek, R. D. Miranda, and R. W. Mahley. 1997. Heparan sulfate proteoglycans participate in hepatic lipase- and apolipoprotein E-mediated binding and uptake of plasma lipoproteins, including high density lipoproteins. *J. Biol. Chem.* **272:** 31285–31292.
14. Chappell, D. A., G. L. Fry, M. A. Waknitz, P. H. Iverius, S. E. Williams, and D. K. Strickland. 1992. The low density lipoprotein receptor-related protein/alpha2-macroglobulin receptor binds and mediates catabolism of bovine milk lipoprotein lipase. *J. Biol. Chem.* **267:** 25764–25767.
15. Kounnas, M. Z., D. A. Chappell, H. Wong, W. S. Argraves, and D. K. Strickland. 1995. The cellular internalization and degradation of hepatic lipase is mediated by low density lipoprotein receptor-related protein and requires cell surface proteoglycans. *J. Biol. Chem.* **270:** 9307–9312.
16. Krapp, A., S. Ahle, S. Kersting, Y. Hua, K. Kneser, M. Nielsen, J. Gliemann, and U. Beisiegel. 1996. Hepatic lipase mediates the uptake of chylomicrons and beta-VLDL into cells via the LDL receptor-related protein (LRP). *J. Lipid Res.* **37:** 926–936.
17. Kadowaki, H., G. M. Patton, and S. J. Robins. 1992. Metabolism of high density lipoprotein lipids by the rat liver: evidence for participation of hepatic lipase in the uptake of cholesteryl ester. *J. Lipid Res.* **33:** 1689–1698.
18. Marques-Vidal, P., C. Azéma, X. Collet, C. Vieu, H. Chap, and B. Perret. 1994. Hepatic lipase promotes the uptake of HDL esterified cholesterol by the perfused rat liver: a study using reconstituted HDL particles of defined phospholipid composition. *J. Lipid Res.* **35:** 373–384.
19. Bamberger, M., J. M. Glick, and G. H. Rothblat. 1983. Hepatic lipase stimulates the uptake of high density lipoprotein cholesterol by hepatoma cells. *J. Lipid Res.* **24:** 869–876.
20. Panzenboeck, U., A. Wintersberger, S. Levak-Frank, R. Zimmermann, R. Zechner, G. M. Kostner, E. Malle, and W. Sattler. 1997. Implications of endogenous and exogenous lipoprotein lipase for the selective uptake of HDL_3-associated cholesteryl esters by mouse peritoneal macrophages. *J. Lipid Res.* **38:** 239–253.
21. Rinninger, F., W. A. Mann, T. Kaiser, S. Ahle, N. Meyer, and H. Greten. 1998. Hepatic lipase mediates an increase in selective uptake of high-density lipoprotein-associated cholesteryl esters by human Hep 3B hepatoma cells in culture. *Atherosclerosis.* **141:** 273–285.
22. Rinninger, F., T. Kaiser, W. A. Mann, N. Meyer, H. Greten, and U. Beisiegel. 1998. Lipoprotein lipase mediates an increase in the selective uptake of high density lipoprotein-associated cholesteryl esters by hepatic cells in culture. *J. Lipid Res.* **39:** 1335–1348.
23. Seo, T., M. Al-Haideri, E. Treskova, T. S. Worgall, Y. Kako, I. J. Golderg, and R. J. Deckelbaum. 2000. Lipoprotein lipase-mediated selective uptake from low density lipoprotein requires cell surface proteoglycans and is independent of scavenger receptor class B type 1. *J. Biol. Chem.* **275:** 30355–30362.
24. Rinninger, F., M. Brundert, I. Brosch, N. Donarski, R. M. Budzinski, and H. Greten. 2001. Lipoprotein lipase mediates an increase in selective uptake of HDL-associated cholesteryl esters by cells in culture independent of scavenger receptor BI. *J. Lipid Res.* **42:** 1740–1751.
25. Lambert, G., M. B. Chase, K. Dugi, A. Bensadoun, H. B. Brewer, Jr., and S. Santamarina-Fojo. 1999. Hepatic lipase promotes the selective uptake of high density lipoprotein-cholesteryl esters via the scavenger receptor B1. *J. Lipid Res.* **40:** 1294–1303.
26. Pittman, R. C., and C. A. Taylor. 1986. Methods for assessment of

tissue sites of lipoprotein degradation. *Methods Enzymol.* **129:** 612–628.
27. Rinninger, F., M. Brundert, S. Jäckle, P. R. Galle, C. Busch, J. R. Izbicki, X. Rogiers, D. Henne-Bruns, B. Kremer, C. E. Broelsch, and H. Greten. 1994. Selective uptake of high-density lipoprotein-associated cholesteryl esters by human hepatocytes in primary culture. *Hepatology.* **19:** 1100–1114.
28. Zaidan, H., R. Dhanireddy, M. Hamosh, G. Bengtsson-Olivecrona, and P. Hamosh. 1985. Lipid clearing in premature infants during continous heparin infusion: role of circulating lipases. *Pediatr. Res.* **19:** 23–25.
29. Heeren, J., W. Weber, and U. Beisiegel. 1999. Intracellular processing of endocytosed triglyceride-rich lipoproteins comprises both recycling and degradation. *J. Cell Sci.* **112:** 349–359.
30. Heeren, J., T. Grewal, S. Jäckle, and U. Beisiegel. 2001. Recycling of apolipoprotein E and lipoprotein lipase through endosomal compartments in vivo. *J. Biol. Chem.* **276:** 42333–42338.
31. Nakabayashi, H., K. Taketa, K. Miyano, T. Yamane, and J. Sato. 1982. Growth of human hepatoma cell lines with differentiated functions in chemically defined medium. *Cancer Res.* **42:** 3858–3863.
32. Jansen, H., A. J. M. Verhoeven, L. Weeks, J. J. P. Kastelein, D. J. J. Halley, A. van den Ouweland, J. C. Seidell, and J. C. Birkenhäer. 1997. Common C-to-T substitution at position –480 of the hepatic lipase promoter is associated with a lowered lipase activity in coronary artery disease patients. *Arterioscler. Thromb. Vasc. Biol.* **17:** 2837–2842.
33. Hadvary, P., W. Sidler, W. Meister, W. Vetter, and H. Wolfer. 1991. The lipase inhibitor tetrahydrolipstatin binds covalently to the putative active site serine of pancreatic lipase. *J. Biol. Chem.* **266:** 2021–2027.
34. Lookene, A., N. Skottova, and G. Olivecrona. 1994. Interactions of lipoprotein lipase with the active-site inhibitor tetrahydrolipstatin (Orlistat(R)). *Eur. J. Biochem.* **222(2):** 395–403
35. Rinninger, F., S. Jaeckle, and R. C. Pittman. 1993. A pool of reversibly cell-associated cholesteryl esters involved in the selective uptake of cholesteryl esters from high-density lipoproteins by Hep G2 hepatoma cells. *Biochim. Biophys. Acta.* **1166:** 275–283.
36. Webb, N. R., P. M. Connell, G. A. Graf, E. J. Smart, W. J. S. de Villiers, F. C. de Beer, and D. van der Westhuyzen. 1998. SR-BII, an isoform of the scavenger receptor BI containing an alternate cytoplasmic tail, mediated lipid transfer between high density lipoprotein and cells. *J. Biol. Chem.* **273:** 15241–15248.
37. Rigotti, A., E. R. Edelman, P. Seifert, S. N. Iqbal, R. B. DeMattos, R. E. Temel, M. Krieger, and D. L. Williams. 1996. Regulation by adrenocorticotropic hormone of the in vivo expression of scavenger receptor class B type I (SR-BI), a high density lipoprotein receptor, in steroidogenic cells of the murine adrenal gland. *J. Biol. Chem.* **271:** 33545–33549.
38. Obunike, J. C., I. J. Edwards, S. C. Rumsey, L. K. Curtiss, W. D. Wagner, R. J. Deckelbaum, and I. J. Goldberg. 1994. Cellular differences in lipoprotein lipase-mediated uptake of low density lipoproteins. *J. Biol. Chem.* **269:** 13129–13135.
39. Rodriguza, W. V., S. T. Thuahnai, R. E. Temel, S. Lund-Katz, M. C. Phillips, and D. L. Williams. 1999. Mechanism of scavenger receptor class B type I-mediated selective uptake of cholesteryl esters from high density lipoprotein to adrenal cells. *J. Biol. Chem.* **274:** 20344–20350.
40. Connelly, M. A., S. M. Klein, S. Azhar, N. A. Abumrad, and D. L. Williams. 1999. Comparison of class B scavenger receptors, CD36 and scavenger receptor BI (SR-BI), shows that both receptors mediate high density lipoprotein-cholesteryl ester selective uptake but SR-BI exhibits a unique enhancement of cholesteryl ester uptake. *J. Biol. Chem.* **274:** 41–47.
41. Morrison, J. R., M. J. Silvestre, and R. C. Pittman. 1994. Cholesteryl ester transfer between high density lipoprotein and phospholipid bilayers. *J. Biol. Chem.* **269:** 13911–13918.
42. Dichek, H. L., W. Brecht, J. Fan, Z-S. Ji, S. P. A. McCormick, H. Akeefe, L. A. Conzo, D. A. Sanan, K. H. Weisgraber, S. G. Young, J. M. Taylor, and R. W. Mahley. 1998. Overexpression of hepatic lipase in transgenic mice decreaes apolipoprotein B-containing and high density lipoproteins. *J. Biol. Chem.* **273:** 1896–1903.
43. Homanics, G. E., H. V. de Silva, J. Osada, S. H. Zhang, H. Wong, J. Borensztajn, and N. Maeda. 1995. Mild dyslipidemia in mice following targeted inactivation of the hepatic lipase gene. *J. Biol. Chem.* **270:** 2974–2980.
44. Fan, J., J. Wang, A. Bensadoun, S. J. Lauer, Q. Dang, R. W. Mahley, and J. M. Taylor. 1994. Overexpression of hepatic lipase in transgenic rabbits leads to a marked reduction of plasma high density lipoproteins and intermediate density lipoproteins. *Proc. Natl. Acad. Sci. USA.* **91:** 8724–8728.
45. Busch, S. J., R. L. Barnhart, G. A. Martin, M. C. Fitzgerald, M. T. Yates, S. J. T. Mao, C. E. Thomas, and R. L. Jackson. 1994. Human hepatic triglyceride lipase expression reduces high density lipoprotein and aortic cholesterol in cholesterol-fed transgenic mice. *J. Biol. Chem.* **269:** 16376–16382.
46. Wang, N., T. Arai, Y. Ji, F. Rinninger, and A. R. Tall. 1998. Liver-specific overexpression of scavenger receptor BI decreases levels of very low density lipoprotein apoB, low density lipoprotein apoB, and high density lipoprotein in transgenic mice. *J. Biol. Chem.* **273:** 32920–32926.
47. Wang, N., W. Weng, J. L. Breslow, and A. R. Tall. 1996. Scavenger receptor BI (SR-BI) is up-regulated in adrenal gland in apolipoprotein A-I and hepatic lipase knock-out mice as a response to depletion of cholesterol stores. *J. Biol. Chem.* **271:** 21001–21004.
48. Thuren, T. 2000. Hepatic lipase and HDL metabolism. *Curr. Opin. Lipidol.* **11:** 277–283.