Hepatic lipase promotes the selective uptake of high density lipoprotein-cholesteryl esters via the scavenger receptor B1

Gilles Lambert,[1,*] Michael B. Chase,* Klaus Dugi,* Andre Bensadoun,[†] H. Bryan Brewer, Jr.,* and Silvia Santamarina-Fojo*

Molecular Disease Branch,* National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892, and Division of Nutritional Sciences,[†] Cornell University, Ithaca, NY 14852

Abstract **Hepatic lipase (HL) plays a major role in high-density lipoprotein (HDL) metabolism both as a lipolytic enzyme and as a ligand. To investigate whether HL enhances the uptake of HDL-cholesteryl ester (CE) via the newly described scavenger receptor BI (SR-BI), we measured the effects of expressing HL and SR-BI on HDL-cell association as well as uptake of ^{125}I-labeled apoA-I and [^{3}H]CE-HDL, by embryonal kidney 293 cells. As expected, HDL cell association and CE selective uptake were increased in SR-BI transfected cells by 2- and 4-fold, respectively, compared to controls ($P < 0.001$). Cells transfected with HL alone or in combination with SR-BI expressed similar amounts of HL, 20% of which was bound to cell surface proteoglycans. HL alone increased HDL cell association by 2-fold but had no effect on HDL-CE uptake in 293 cells. However, in cells expressing SR-BI, HL further enhanced the selective uptake of CE from HDL by 3-fold ($P < 0.001$). To determine whether the lipolytic and/or ligand function of HL are required in this process, we generated a catalytically inactive form of HL (HL-145G). Cells co-transfected with HL-145G and SR-BI increased their HDL cell association and HDL-CE selective uptake by 1.4-fold compared to cells expressing SR-BI only ($P < 0.03$). Heparin abolished the effect of HL-145G on SR-BI-mediated HDL-CE selective uptake. Thus, the enhanced uptake of HDL-CE by HL is mediated by both its ligand role, which requires interaction with proteoglycans, and by lipolysis with subsequent HDL particle remodeling. These results establish HL as a major modulator of SR-BI mediated selective uptake of HDL-CE.**—Lambert, G., M. B. Chase, K. Dugi, A. Bensadoun, H. B. Brewer, Jr., and S. Santamarina-Fojo. **Hepatic lipase promotes the selective uptake of high density lipoprotein-cholesteryl esters via the scavenger receptor B1.** *J. Lipid Res.* **1999.** 40: **1294–1303.**

Supplementary key words SR-BI • HDL • cholesterol • HEK 293 cells

Several lines of evidence that include epidemiological data, transgenic animal studies (1–6) and, more recently, prospective human clinical trials (7), indicate that increased plasma high density lipoprotein (HDL) levels protect against the development of atherosclerosis. One of several proposed functions of HDL as an anti-atherogenic lipoprotein is reverse cholesterol transport, a process by which cholesterol is transported from peripheral cells to the liver for excretion (8–11). HDL-cholesteryl esters (CE) may be transferred directly to the liver via whole particle and/or selective CE uptake (12, 13). Alternatively, CE may be transferred from HDL to apolipoprotein (apo) B-containing lipoproteins as a result of the activity of the cholesteryl ester transfer protein (14). Factors that alter the plasma concentrations of HDL are likely to modulate the process of HDL-mediated reverse cholesterol transport.

Hepatic lipase (HL) is a 66 kDa lipolytic enzyme synthesized primarily by the liver and found attached to the vascular endothelium of the liver, ovaries, and adrenals via heparan sulfate proteoglycans (15–18). HL hydrolyzes triglycerides and phospholipids present primarily on chylomicron remnants, intermediate density lipoproteins (IDL), and HDL (19, 20). Several lines of evidence support an important role for HL in HDL metabolism. Overexpression of HL using either recombinant adenovirus (21–24) or genetic manipulation significantly decreases plasma HDL concentrations in several animal models (25–27). Conversely, HL deficiency in humans (28, 29) as well as HL deficiency in rodents induced by infusion of anti-HL antibodies (19, 30–32) or by targeted disruption

Abbreviations: HDL, high-density lipoproteins; CE, cholesteryl ester; apo, apolipoprotein; HL, hepatic lipase; IDL, intermediate-density lipoproteins; VLDL, very low density lipoproteins; LDL, low-density lipoproteins; SR-BI, scavenger receptor class B type 1; CLA-1, collagen type 1 CD36 receptor and rat lysosomal integral membrane protein II analogous-1; DMEM, Dulbecco's modified Eagle's medium; PCR, polymerase chain reaction; CAT, chloramphenicol acetyl transferase; CMV, cytomegalovirus; PBS, phosphate-buffered saline; LPDS, lipoprotein-deficient serum; ELISA, enzyme-linked immunosorbent assay; ^{3}H-CE, [1α,2α(n)-^{3}H]cholesteryl oleate; TCA, trichloracetic acid; LPL, lipoprotein lipase; LRP, LDL receptor related protein; CHO, Chinese hamster ovary.

[1] To whom correspondence should be addressed at: Molecular Disease Branch, NHLBI-NIH, Bldg. 10, Rm. 7N115, 10 Center Drive, MSC 1666, Bethesda, MD 20892.

of the HL gene (33), lead to increased plasma HDL concentrations.

In addition to its traditional role as a lipolytic enzyme, recent studies have implicated HL in other aspects of cellular lipid and/or lipoprotein metabolism. HL appears to serve as a ligand that mediates the interactions of lipoproteins with cell surface proteoglycans and receptors, facilitating the cellular uptake of lipoproteins and/or lipoprotein lipids. HL enhances the binding and uptake of chylomicrons, chylomicron remnants, β-very low density lipoproteins (β-VLDL), and low density lipoproteins (LDL) by different cell types in vitro (34–41), a process that appears to require proteoglycans (35, 42, 43). Although most of these studies have focussed on the HL-mediated cellular binding and uptake of apoB-containing lipoproteins, several have suggested a similar role for HL in HDL metabolism. HL has been shown to increase HDL cholesterol uptake in perfused rat livers (31, 44) and in cultured cells (37, 38, 43, 45, 46), but the metabolic pathway(s) by which HL enhances the lipid and/or whole particle uptake of HDL remains to be established.

Recently, Acton et al. (47) described a new receptor, the scavenger receptor class B type I (SR-BI), which mediates the selective uptake of CE from HDL. The human homologue of mouse SR-BI is the collagen type I CD36 receptor and rat lysosomal integral membrane protein II analogous-1 (CLA-1), hereafter designated as human SR-BI (48). In vivo, this receptor is expressed mostly in the liver and steroidogenic tissues (47–50). Overexpression of SR-BI in transgenic mice (51, 52) or using recombinant adenovirus (53) reduces HDL-cholesterol levels and increases biliary cholesterol secretion, consistent with enhanced reverse cholesterol transport. Conversely, SR-BI knockout mice (54, 55) have increased plasma HDL-cholesterol concentrations. Apolipoproteins A-I, A-II, and C-III are able to mediate the binding of reconstituted HDL to SR-BI (56). A potential interaction between SR-BI and HL has been suggested by a recent study demonstrating that despite increased adrenal SR-BI, female mice with HL deficiency have decreased cholesterol adrenal stores (57). Thus, HL may be required for optimal SR-BI-mediated selective uptake of cholesterol by the adrenals.

In the present study we investigate the potential role of HL in mediating the cellular uptake of HDL-CE via the newly described SR-BI, by expressing human HL and SR-BI in embryonal kidney 293 cells. To distinguish the contribution of the lipolytic and non-lipolytic function of HL in this process, catalytically active as well as inactive HL was co-expressed with SR-BI. Our findings demonstrate that: *1*) in the absence of SR-BI, HL does not promote cellular cholesterol uptake in 293 cells, *2*) HL enhances the SR-BI-mediated selective uptake of cholesteryl esters from HDL by 3-fold, and *3*) the process involves both the lipolytic and non-lipolytic function of the enzyme. This study provides, for the first time, direct evidence supporting a synergistic role for HL and SR-BI in mediating the selective uptake of cholesteryl esters from HDL.

EXPERIMENTAL PROCEDURES

Cells

Human embryonic kidney 293 cells (CRL-1573) were obtained from the American Type Culture Collection (Rockville, MD). Cells were grown in 175 cm^2 flasks (Costar, Cambridge, MA) and maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum, penicillin/streptomycin/glutamine (Gibco BRL, Gaithersburg, MD) at 37°C in humidified 95% air/5% CO_2.

Generation of cDNA expression vectors

The expression vector, pcEXV-3 containing the human SR-BI cDNA (a generous gift from Dr. Miguel A. Vega) (58) was used as template to amplify SR-BI cDNA by polymerase chain reaction (PCR). Specific 5′ and 3′ oligonucleotides containing Not I restriction sites (5′-TGCGAGCCGCGGGGCGGCCGCAGGCG-3′ and 5′-CTGTGGGGCTGGGCGGCCGCCTGCTGGGAG-3′) were synthesized on a 380B DNA synthesizer (Applied Biosystems, Foster City, CA). Human HL and chloramphenicol acetyl transferase (CAT) cDNAs were obtained as previously described (59). Catalytically inactive HL-145G cDNA containing a substitution of the active serine 145 by glycine (AGT to GGT) was synthesized by the overlap extension PCR using pCMV-HL as template (60). PCR was performed in an automated DNA thermal cycler (Perkin-Elmer, Norwalk, CT) using DNA polymerase from *Pyrococcus furiosus* (Stratagene, La Jolla, CA) for 30 cycles with 1 min denaturation at 95°C, 1 min annealing at 50°C, and 2 min extension at 72°C in 1× buffer #2 (Stratagene), 200 μm each dNTP (Boehringer Mannheim, Indianapolis, IN), and 0.5 μm each primer. The cDNAs were subcloned into the unique Not I site of the pCMV-β vector (Clontech, Palo Alto, CA) containing the cytomegalovirus (CMV) immediate early promoter and the polyadenylation site of simian virus 40, by replacing the β-galactosidase cDNA. Plasmid DNA was isolated by minipreparation (Promega Wizard Kit, Madison, WI) and the cDNA sequence was verified by sequence analysis using the dideoxynucleotide chain termination method (61). The recombinant vectors were amplified and purified by double CsCl banding.

In vitro expression of SR-BI, HL, and HL-145G in 293 cells

Costar 6-well plates were seeded with 5×10^5 cells per well 48 h prior to transfection with 1 μg of pCMV-SR-BI and 2 μg of pCMV-HL or pCMV-HL-145G using SuperFect (Qiagen, Santa Clarita, CA). pCMV-CAT was utilized as control as well as to bring the total amount of transfected DNA to 3 μg per well. Twenty four hours after transfection, the cells were rinsed twice with 1× phosphate-buffered saline (PBS) and the medium was replaced with DMEM, penicillin/streptomycin/glutamine, containing 10% lipoprotein-deficient serum (LPDS) obtained by ultracentrifugation of fetal calf serum for 72 h at 60,000 rpm (d 1.25 g/ml). Three to five separate transfection experiments were performed with each experimental condition in hexaduplicates.

Immunoblot analysis of SR-BI expression

Cells were harvested in 0.5 ml of a 0.01 m NaOH, 0.1% SDS solution and immediately frozen at −70°C. Cellular proteins (10 μg) were analyzed on Nu-PAGE 4–12% Bis-Tris gels in MES-SDS buffer (Novex, San Diego, CA) under reducing conditions. Proteins were transferred onto an Immobilon membrane (Millipore, Bedford, MA), probed with polyclonal anti-human SR-BI-IgG (a generous gift from Dr. Susan Acton) using Vectastain PK6101 (Vector, Burlingame, CA) and SR-BI expression was quantified on a Personal Densitometer SI (Molecular Dynamics, Sunnyvale, CA).

Determination of HL activity and concentration

Media was harvested 48 h after transfection and supplemented with glycerol to a final concentration of 30% (v/v). Aliquots of media were stored at −70°C. HL activity was assayed in triplicate using ^{14}C-labeled triolein substrate in 1 M NaCl (62). HL concentration was determined in triplicate by a sandwich enzyme-linked immunosorbent assay (ELISA) (63).

Preparation and labeling of HDL

Human HDL (d 1.063–1.21 g/ml) was labeled with [1α, 2α(n)-^{3}H]cholesteryl oleate (^{3}H-CE) (Amersham, Arlington Heights, IL). HDL was diluted in 1× PBS containing 0.01% Na-EDTA to a final protein concentration of 2.5 mg/ml. The ^{3}H-CE was dissolved in ethanol at 1 μg/μl (~3.5 μCi/μl); 20 μl was added to 1 ml of the HDL solution. The mixture was gently stirred at room temperature for 30 min, centrifuged (40,000 rpm) at 4°C for 18 h (d 1.08) to separate the unincorporated label, and dialyzed against 1× PBS. The purity of labeled HDL preparations was evaluated after gel filtration on two Superose 6 columns in series (absorbance at 280 nm) and aliquots were counted by liquid scintillation detection. The specific activity of the ^{3}H-CE labeled HDL preparations was 17 dpm/ng CE.

Lyophilized human apoA-I (100 μg) was solubilized in a buffer of 6 M guanidine/1 M glycine (pH 8.5) and iodinated with one mCi of ^{125}I (NEN, Boston, MA) by a modification of the iodine monochloride method described by McFarlane (64). ^{125}I-labeled apoA-I was added to the 2 mg HDL solution and dialyzed against 1× PBS to remove free iodine. The specific activity of ^{125}I-labeled apoA-I-labeled HDL was 740 dpm/ng apoA-I. The HDL apoA-I/CE mass ratio was 2.7 ± 0.1 in all the HDL preparations used.

Determination of HDL cell association, selective CE uptake, and apolipoprotein degradation

Twenty-four hours after transfection, the media were replaced with DMEM containing 10% LPDS as described above and 48 h after transfection 10 μg/ml of ^{125}I-labeled apoA-I/[^{3}H]CE-labeled HDL was added to the cells. After a 5-h incubation, the cells were washed three times with PBS (pH 7.4), lysed with 0.5 ml of 0.01 M NaOH and homogenized for 3 sec on a XL2020 sonicator (Misonix, Farmingdale, NY). An aliquot (180 μl) of the cell lysate as well as media were precipitated with 20 μl of 100% trichloracetic acid (TCA) as described (65, 66). TCA-insoluble radioactivity in the cells is assumed to represent ^{125}I-labeled apoA-I internalized and non-degraded or bound to the cell surface whereas TCA-soluble ^{125}I radioactivity in both cells and media represents apoA-I that has undergone internalization and degradation (23, 50). CE was extracted with hexane–isopropanol 3:2 (v/v) and ^{3}H radioactivity was measured by liquid scintillation spectrometry. Cellular proteins were measured using the BCA protein assay (Pierce, Rockford, IL). HDL-CE selective uptake (expressed as ng HDL-CE/mg cell protein) was calculated by subtracting the CE associated with both TCA-insoluble counts in the cells (^{125}I-labeled apoA-I cell associated, undegraded) and TCA-soluble counts in cells and media (^{125}I-labeled apoA-I degraded) from the total [^{3}H]CE uptake (23, 50, 65, 66). The selective uptake of HDL-CE in cells expressing SR-BI, SR-BI + HL, and SR-BI + HL-145G was normalized for SR-BI expression in each experiment as previously described.

Treatment of cultured cells with heparin

In separate experiments, 1 ml of cultured media was harvested 48 h after transfection, the cells were placed on ice for 20 min with 20 U/ml of heparin (Sigma, St Louis, MO), and the remaining 1 ml media was harvested. To determine the amount of HL free in the media and bound to the cell membranes, HL mass was measured by ELISA (63) on both pre- and post-heparin media. To measure the effects of heparin on CE cellular uptake, transfected cells were treated for 20 min with heparin (20 U/ml) before addition of [^{3}H]CE-labeled HDL. The experiment was then performed as previously described.

Statistical analysis

Data are expressed as mean ± SEM. The statistical significance of the differences of the mean between two groups is evaluated using the Student's *t*-test.

RESULTS

Expression of SR-BI in 293 cells

Figure 1 illustrates the expression of SR-BI and HDL-CE uptake in 293 cells transfected with increasing amounts of

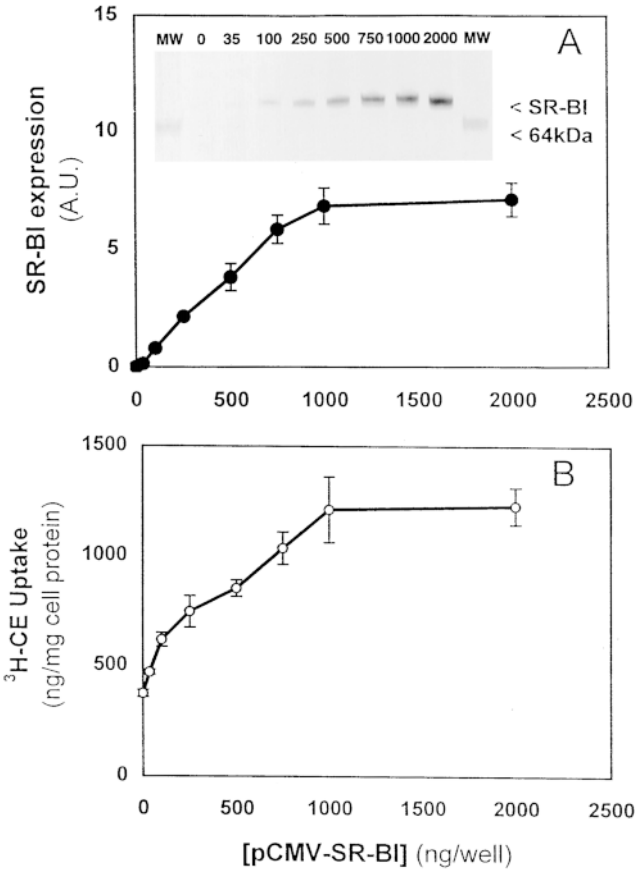

Fig. 1. Human SR-BI expression promotes a dose-dependent uptake of CE from HDL in human embryonic kidney 293 cells. 293 Cells were transfected with increasing amounts of pCMV-SR-BI. One day after transfection, the cells were placed in LPDS-containing media for 24 h. Ten μg of HDL (labeled with [^{3}H]CE) per ml of media was subsequently added to the cells. After a 5-h incubation, the media were removed, the cells were washed, and [^{3}H]CE was extracted with hexane/isopropanol. The cells were harvested in a 0.01 M NaOH, 0.1% SDS solution. Cellular proteins were resolved by PAGE under reducing conditions, transferred onto a nitrocellulose membrane, and probed with polyclonal anti-human SR-BI-IgG (panel A, inset). Relative SR-BI expression was quantified by laser densitometry in 3 independent experiments (panel A) and the ^{3}H extracted radioactivity was measured by liquid scintillation spectrometry to determine the cellular uptake of CE from HDL (panel B), as described in the experimental procedures.

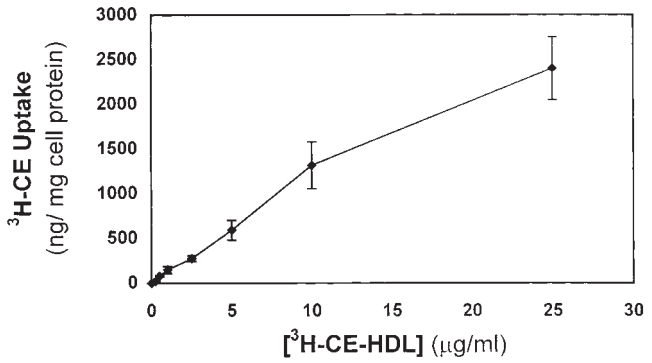

Fig. 2. Concentration dependence of HDL-CE uptake by 293 cells expressing SR-BI. 293 Cells were transfected with 1 μg of pCMV-SR-BI. One day after transfection, the cells were placed in LPDS-containing media for 24 h. Subsequently, increasing amounts of HDL (labeled with [^{3}H]CE) were added to the cells. After a 5-h incubation, the media were removed, the cells were washed, and [^{3}H]CE was extracted with hexane/isopropanol. The cells were harvested in a 0.01 m NaOH, 0.1% SDS solution for protein determination. The ^{3}H extracted radioactivity was measured by liquid scintillation spectrometry. The cellular uptake of CE from HDL was calculated as described in Experimental Procedures, and plotted as a function of the HDL concentration. Data represent the mean ± SEM of 4 independent experiments.

pCMV-SR-BI. SR-BI was quantified by immunoblot analysis of transfected cell extracts (inset, Fig. 1A) followed by densitometric scanning. Peak expression of the transgene was reached 48 h after transfection (data not shown). Non-transfected 293 cells did not express SR-BI. Transfection of 293 cells with increasing amounts of pCMV-SR-BI resulted in a dose-dependent increase of cellular SR-BI protein, which reached a plateau with 1 μg of plasmid (Fig. 1A). The uptake of ^{3}H-CE from HDL by 293 cells transfected with increasing amounts of pCMV-SR-BI was then quantitated. The amount of CE uptake paralleled the level of expression of SR-BI from 374 up to 1229 ng CE/mg of cell protein (Fig. 1B). Maximal SR-BI expression and HDL-CE uptake were achieved by transfecting 1 μg pCMV-SR-BI per well. Using these conditions, transfected 293 cells were then incubated with increasing amounts of ^{3}H-CE-labeled HDL. The cellular uptake of CE increased linearly with the HDL concentration (**Fig. 2**), indicating that under our experimental conditions, the amount of HDL was the limiting factor for SR-BI-mediated uptake of CE. All subsequent experiments were performed by transfecting 1 μg of pCMV-SR-BI DNA per well and adding HDL at a concentration of 10 μg/ml to the media.

Cell association of HDL by 293 cells transfected with HL and SR-BI plasmids

To examine the effects of HL and SR-BI on HDL cell association, 293 cells were transfected with pCMV-HL either alone or in combination with pCMV-SR-BI. HL expression was quantified by determination of both HL concentration by ELISA and HL activity by the triolein assay in the transfection media (**Table 1**). Cells transfected with pCMV-HL alone or together with pCMV-SR-BI expressed similar levels of an enzymatically active lipase with a specific activity of 0.6 nmol/min per ng (Table 1). In contrast, HL was not detectable in the media of cells transfected with either pCMV-CAT or pCMV-SR-BI establishing that 293 cells did not express the lipolytic enzyme.

Treatment of the transfected cells with 20 U/ml of heparin released human HL into the media, indicating that a portion of HL was associated with 293 cell surface proteoglycans. The distribution of free (media) and surface-bound (heparin-releasable) HL was determined by ELISA to be 80% and 20%, respectively (data not shown). No significant difference between cells expressing either HL alone or HL and SR-BI was evident.

To determine whether HL mediates the binding and uptake of HDL, transfected 293 cells were incubated for 5 h with ^{125}I-labeled apoA-I HDL and HDL-cell association was determined. As shown in **Fig. 3A**, the amount of HDL bound and internalized by cells transfected with either pCMV-HL or pCMV-SR-BI was approximately 2-fold greater than control pCMV-CAT-transfected cells. Co-expression of HL and SR-BI led to an additive increase in cell association (3-fold compared to controls). Thus, HL further enhances the association of HDL by 293 cells expressing SR-BI.

Selective uptake of HDL-CE by 293 cells expressing HL and SR-BI

To examine the effect of expressing HL on SR-BI-mediated selective uptake of HDL-CE by transfected 293 cells, the uptake of ^{125}I-labeled apoA-I/^{3}H-CE-labeled HDL was measured (Fig. 3B). Compared to control pCMV-CAT-transfected cells, SR-BI expression resulted in a 5-fold increase in HDL-CE selective uptake. Despite enhanced HDL cell association, the expression of HL alone did not increase the uptake of ^{3}H-CE HDL by 293 cells. However,

TABLE 1. Hepatic lipase expression in transfected 293 cells

Plasmids	HL Mass	HL Activity	Specific Activity
	ng/mg cell protein	*nmol FFA/min/mg cell protein*	*nmol FFA/min/ng HL protein*
pCMV-CAT	ND	ND	—
pCMV-SR-BI	ND	ND	—
pCMV-HL	7.6 ± 1.5	5.2 ± 0.8	0.68
pCMV-SR-BI + pCMV-HL	8.2 ± 1.4	4.4 ± 1.2	0.54
pCMV-HL-145G	9.6 ± 2.0	ND	—
pCMV-SR-BI + pCMV-HL-145G	8.4 ± 3.6	ND	—

Data are presented as mean ± SEM (n = 6); ND, not detectable.

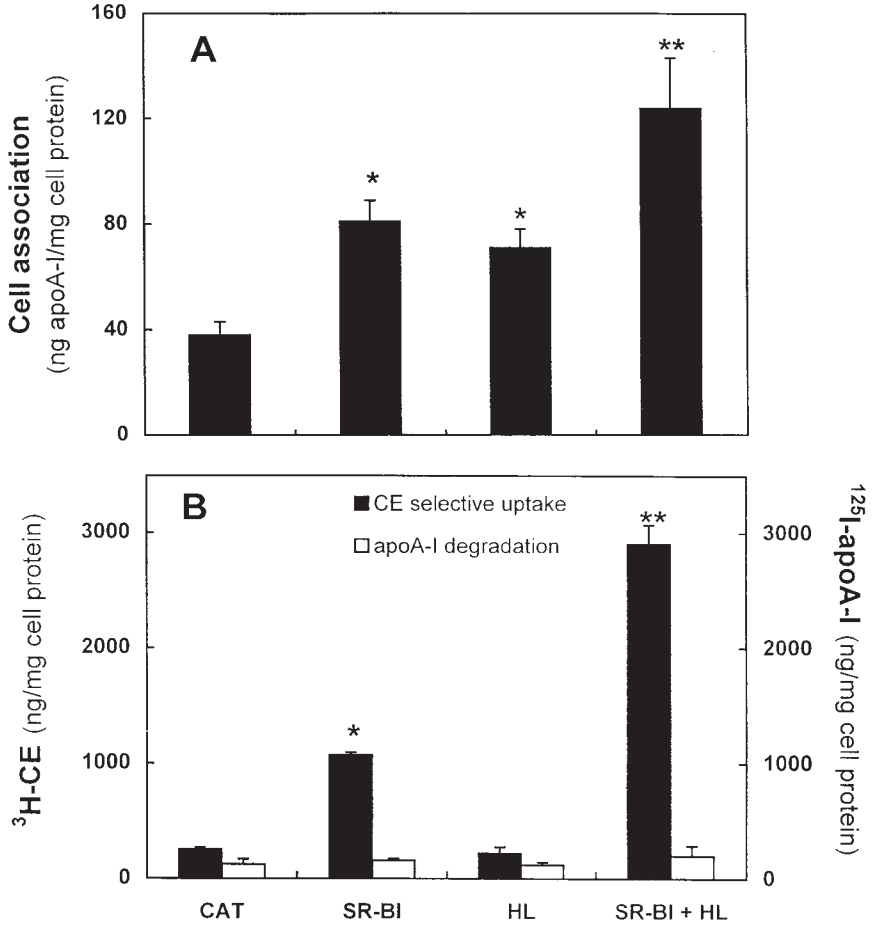

Fig. 3. HDL cell association, HDL-CE selective uptake, and degradation of HDL by 293 cells expressing SR-BI and HL. Cells were transfected with 1 μg of pCMV-SR-BI and 2 μg of pCMV-HL either alone or in combination. One day after transfection, the cells were placed in LPDS-containing media for 24 h. Subsequently, 10 μg of HDL (labeled with ^{125}I-labeled apoA-I and [^{3}H]CE) per ml of media was added to the cells. After a 5-h incubation at 37°C, the media were removed and the cells were washed with PBS and harvested. The protein concentration and SR-BI expression level were determined. Cell extracts and media aliquots were precipitated with TCA. Cell association (panel A) was determined by quantitating the TCA-insoluble radioactivity in the cells, which represents ^{125}I-labeled apoA-I internalized and non-degraded or bound to the cell surface. ApoA-I degradation (panel B) was determined by quantitating the TCA-soluble ^{125}I radioactivity in both cells and media, which represents apoA-I that has undergone internalization and degradation. CE was extracted with hexane/isopropanol and ^{3}H radioactivity was measured. HDL-CE selective uptake (panel B) was calculated by subtracting the CE associated with both TCA-insoluble counts in the cells (^{125}I-labeled apoA-I cell associated) and TCA-soluble counts in both cells and media (^{125}I-labeled apoA-I degraded) from the total [^{3}H]CE uptake. Data represent the mean ± SEM of 3 independent experiments in hexaduplicate; * $P < 0.001$ (compared to control CAT); ** $P < 0.001$(compared to SR-BI).

in the presence of SR-BI, HL further enhanced the selective uptake of CE from HDL by 15-fold compared to controls (Fig. 3B, black bars). In contrast, expression of HL and SR-BI, either alone or in combination, did not significantly alter the degradation of ^{125}I-labeled apoA-I HDL (Fig. 3B; white bars). Thus, HL enhances the selective uptake of HDL-CE in 293 cells via SR-BI.

Cell association of HDL by cells transfected with catalytically inactive HL-145G and SR-BI plasmids

To determine whether the HL-enhanced selective uptake of HDL-CE via SR-BI requires the lipolytic and/or ligand functions of the enzyme, we generated a catalytically inactive form of HL (HL-145G) and transfected 293 cells with pCMV-HL-145G. As shown in Table 1, the concentration of HL-145G in the media of cells transfected with pCMV-HL-145G alone or in combination with pCMV-SR-BI was similar to that of native HL. As expected, HL enzymatic activity could not be detected in the media of 293 cells expressing the mutant HL-145G. Like native HL, treatment of the transfected cells with heparin released HL-145G into the media, indicating that a portion of HL was associated with cell surface proteoglycans. The distribution of free (media) and surface-bound (heparin-releasable) HL was determined by ELISA to be 80 and 20%, respectively (data not shown). No significant differ-

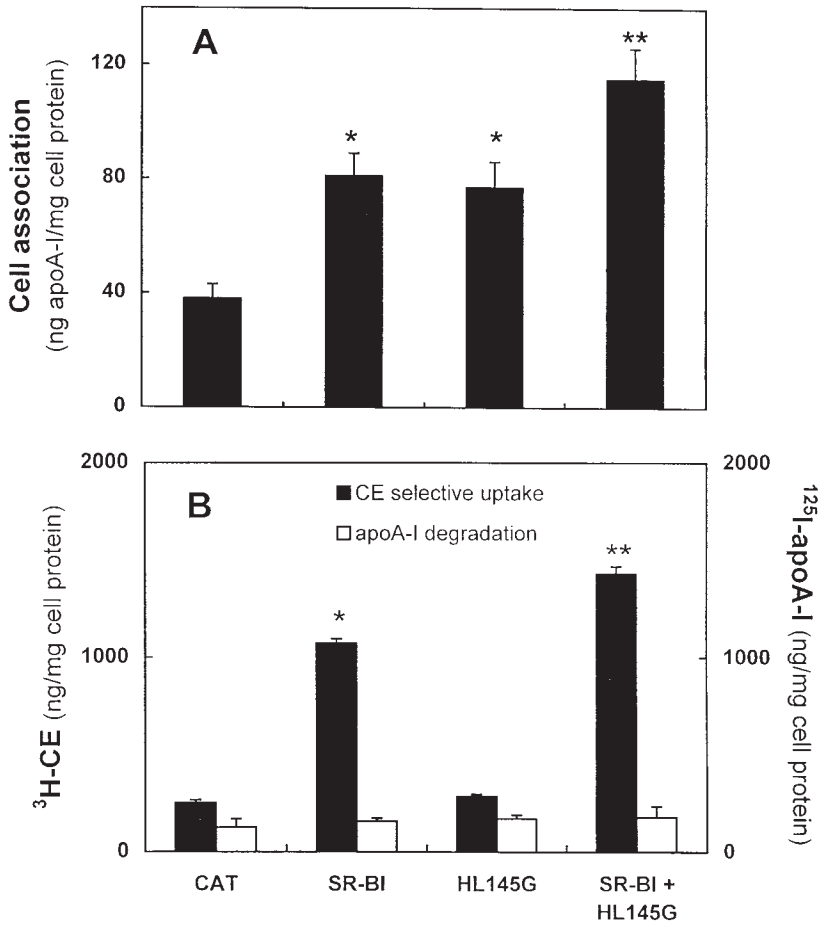

Fig. 4. HDL cell association, HDL-CE selective uptake, and HDL-apoA-I degradation by 293 cells expressing SR-BI and the catalytically inactive HL145G. Cells were transfected with 1 μg of pCMV-SR-BI and 2 μg of pCMV-HL145G either alone or in combination. The HDL cell association (panel A), the CE selective uptake, and apoA-I degradation (panel B) were determined as indicated in the legend of Fig. 3. Data represent the mean ± SEM of 3 independent experiments in hexaduplicate; * $P < 0.001$ (compared to control CAT); ** $P < 0.03$ (compared to SR-BI).

ence between cells expressing either HL alone or HL and SR-BI was evident.

To determine whether the catalytically inactive HL-145G mediates the binding and uptake of HDL, transfected 293 cells were incubated for 5 h with ^{125}I-labeled apoA-I HDL and cell association was determined. As shown in **Fig. 4A**, the amount of HDL bound by cells transfected either with pCMV-HL-145G or pCMV-SR-BI was approximately 2-fold greater than control pCMV-CAT-transfected cells. Co-expression of HL and SR-BI led to an additive increase in cell association (3-fold compared to controls). Thus, like native HL, HL-145G further enhances the association of HDL by 293 cells expressing SR-BI.

Selective uptake of HDL-CE by 293 cells expressing catalytically inactive HL-145G and SR-BI

To examine whether HL-mediated enhanced selective uptake of CE via SR-BI requires lipolysis and/or involves the ligand-binding function of the enzyme, we evaluated the effect of expressing HL-145G on ^{125}I-labeled apoA-I/^{3}H-CE labeled HDL uptake (Fig. 4B). Like native HL, the expression of catalytically inactive HL-145G alone did not increase the selective uptake of ^{3}H-CE HDL by 293 cells. However, in the presence of SR-BI, HL-145G further enhanced the selective uptake of CE from HDL by 7-fold compared to controls (Fig 4B; black bars). Expression of HL-145G and SR-BI, either alone or in combination did not alter the degradation of ^{125}I-labeled apoA-I HDL (Fig. 4B; white bars). Even in the absence of lipolysis, HL-145G further enhanced the selective uptake of HDL-CE in 293 cells via SR-BI, suggesting that the ligand-binding function of the enzyme mediated the effect.

However, maximal uptake of HDL-CE requires co-expression of SR-BI with the lipolytically active enzyme. As shown in **Fig. 5**, at every concentration of HL, the uptake of ^{3}H-CE HDL was significantly greater by cells expressing the native enzyme than by those expressing HL-145G, suggesting that HL-mediated lipolysis and lipoprotein processing play a major role in facilitating the uptake of HDL-CE via SR-BI.

The effect of HL on SR-BI mediated-HDL-CE uptake may require proteoglycans which facilitate the interaction of lipoproteins and HL with cell surface receptors. Heparin displaces HL from the cell surface and thus disrupts this interaction. To evaluate the role of proteoglycans in facilitating the HL-enhanced SR-BI-mediated selective uptake of HDL-CE, we measured the HDL-CE uptake by 293 cells expressing CAT, SR-BI, SR-BI+HL, and SR-BI + HL-145G in the presence of 20 U/ml of heparin (**Fig. 6**). Heparin did not significantly alter the CE uptake in cells expressing SR-BI alone. However, heparin treatment abolished the enhanced uptake of HDL-CE mediated by the catalytically inactive HL-145G and reduced the effect mediated by the native HL by 35% ($P < 0.01$). Thus, the release

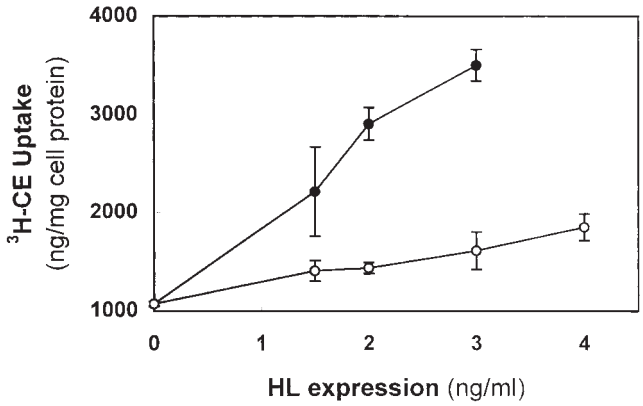

Fig. 5. Uptake of HDL-CE by 293 cells expressing SR-BI + HL (closed circles) or SR-BI + HL145G (open circles). Cells were transfected with 1 μg of pCMV-SR-BI and 2 μg of pCMV-HL (or HL145G) either alone or in combination. The uptake of HDL-CE was measured as described in Experimental Procedures. The level of SR-BI expression was quantified by laser densitometry scanning of immunoblots for each single experiment. The uptake of HDL-CE transfected cells, normalized for SR-BI expression, was determined. The HL/HL145G expression, quantitated by ELISA, ranged from 1.5 to 4 ng/ml. The uptake of [^{3}H]CE-HDL is expressed as a function of HL/HL145G expression. Data represent the mean ± SEM of 3 independent experiments.

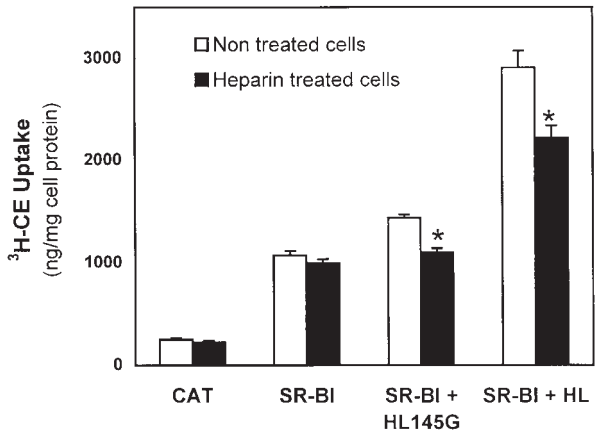

Fig. 6. Effect of heparin on the uptake of [^{3}H]CE from HDL by 293 cells expressing SR-BI and HL/HL145G. Cells were transfected with 1 μg of pCMV-SR-BI and 2 μg of pCMV-HL (or HL145G) either alone or in combination. One day after transfection cells were placed in LPDS-containing media for 24 h. Heparin (20 U/ml) was added to the media 20 min before addition of the [^{3}H]CE-labeled HDL, and the cells were incubated for 5 h at 37°C. The cellular uptake of CE from HDL was calculated as described in Experimental Procedures. Data represent the mean ± SEM of 3 independent experiments; $*P < 0.01$ (compared to non-treated cells).

of HL from the cell surface prevents the enhanced CE uptake mediated by the ligand-binding function of HL, but does not abolish the effect mediated by lipolysis.

DISCUSSION

Previous studies have demonstrated that HL plays a major role in HDL metabolism. HL-mediated lipolysis may account for a significant amount of the effect of HL on HDL metabolism. Like lipoprotein lipase (LPL) (67–71), HL may also serve as a ligand that mediates the interaction of lipoproteins with cell surface proteoglycans and/or receptors, facilitating the uptake of lipoproteins and/or lipoprotein lipids (34–40, 42). HL has been shown to increase HDL cholesterol uptake in perfused rat livers (31, 44) and in cultured cells (37, 38, 43, 45, 46). More recently, expression of a catalytically inactive HL has shown that in the absence of lipolysis, HL will still reduce the circulating plasma levels of HDL in HL knockout mice (72), suggesting an important in vivo physiologic role for the ligand function of HL. Despite these advances, the mechanism(s) by which HL enhances the lipid and/or whole lipoprotein particle uptake of HDL remains to be established. One potential pathway may involve the newly described scavenger receptor SR-BI that mediates the selective uptake of CE from HDL (47, 48). It is expressed primarily in liver and steroidogenic tissues where HL is also localized (73). Recent studies show that although SR-BI is up-regulated in the adrenals of HL knockout female mice (57), adrenal cholesterol stores are reduced, suggesting that HL may be necessary for optimal SR-BI-mediated HDL-CE uptake.

In the present study we examined the potential role of HL in enhancing the uptake of HDL-CE via SR-BI by expressing SR-BI, native HL, and catalytically inactive HL-145G in human embryonal kidney 293 cells. These cells provide an ideal system to address this question as they do not express HL or SR-BI endogenously. In addition, they display minimal background CE selective uptake as well as HDL degradation. This latter may reflect uptake mediated by cell surface proteoglycans and/or the LDL receptor related protein (LRP), as previously reported (38). Thus, any changes in HDL-CE selective uptake can be attributed to newly expressed HL and SR-BI. The selective uptake of HDL-CE by 293 cells expressing human SR-BI (~1200 ng/mg of cell protein) reaches levels comparable to those previously reported in Chinese hamster ovary (CHO) cells stably transfected with mouse SR-BI (47), as well as in Y1-BS1 adrenal cells (50). In addition, the total HL secreted by the transfected 293 cells (8 ng of HL/mg) is similar to that observed in previous studies (36, 37). The expressed lipase was enzymatically active with a specific activity of 0.6 nmol/min per ng and 20% bound to the cell surface. Previous studies have emphasized the importance of HL-cell binding for cellular uptake of lipoproteins (36, 37).

Our study demonstrates that separate expression of HL, HL-145G, and SR-BI led to a similar, approximately 2-fold increase in HDL cell association, confirming previous data showing that HL enhances the cellular binding of HDL, LDL, β-VLDL, and chylomicron remnants (36–39, 41). When HL and SR-BI were co-expressed in 293 cells, an additive increase in HDL cell association was observed, suggesting that the interaction of HDL with SR-BI and HL can occur, at least initially, on separate sites at the cell sur-

face. The binding of HDL to HL associated to the cell surface via proteoglycans may approximate the HDL to the membrane-bound SR-BI, and ultimately facilitate their interaction. A similar mechanism has been proposed for HL (35) and LPL (68) in mediating the interaction of apoB-containing lipoproteins with LRP. Alternatively, HL may bind directly to SR-BI, thereby facilitating the interaction between HDL and SR-BI. Not surprisingly, the HDL cell association was similar in cells expressing native or catalytically inactive HL-145G. These findings are consistent with studies showing that the lipolytic activity of LPL is not necessary for enhanced cellular binding and uptake of apoB-containing lipoproteins (74).

Despite increased HDL cell association induced by HL, expression of the lipase alone did not enhance the uptake of CE from HDL in 293 cells. Compared with controls, the selective uptake of HDL-CE by cells expressing either HL or HL-145G remained unchanged. Previous studies have demonstrated that the selective uptake of HDL-CE in hepatoma cell lines (38, 43, 45) or CHO cells (37, 43) is enhanced by expression of HL alone. However, these cell lines are derived from tissues known to synthesize SR-BI, i.e., liver and ovaries, respectively (47, 48). SR-BI was first cloned from a CHO cell library (75). Our data clearly demonstrate that HL was able to increase the uptake of CE from HDL in 293 cells only in the presence of SR-BI. Like SR-BI, HL enhanced the selective uptake of HDL-CE, rather than the whole particle uptake, providing additional evidence that HL is acting via the selective-uptake receptor.

To further investigate the mechanism by which HL enhances SR-BI-mediated HDL-CE uptake, we studied the effect of expressing the catalytically inactive HL-145G in 293 cells. In the absence of lipolysis, HL-145G is able to enhance the uptake of HDL-CE via SR-BI by 1.4-fold, supporting a role for the ligand-binding function of HL independent of lipolysis in this process. However, at every level of expression studied, HL-145G was less effective than the active enzyme in enhancing the selective uptake of HDL-CE via SR-BI indicating that lipolysis was necessary for maximal HDL-CE uptake. It does not appear that HL-mediated lipolysis enhanced the accumulation of HDL at the cell surface as HL-145G had the same effect on HDL cell association as the native HL. Previous studies have shown that HDL treated with HL have an enhanced ability to deliver cholesterol to perfused rat livers (44) and hepatoma cells (43, 45). HDL remodeled by secreted phospholipase-A2 have an increased capacity to deliver CE to SR-BI stably expressing CHO cells (76). It is possible that the hydrolysis of phospholipids from the HDL surface by HL may enhance the interaction of HDL apolipoproteins with membrane sites or/and facilitate the release of CE from the HDL core for cellular uptake, as previously suggested (45, 46, 77). Thus, HL-mediated HDL lipolysis and subsequent particle remodeling may increase access to the CE core, further enhancing the uptake of CE from HDL. Our findings that the lipolytic function of the enzyme is necessary for maximal stimulation of SR-BI-mediated HDL-CE selective uptake is consistent with data published by Ji et al. (38) suggesting that HL catalytic activity is essential for HDL uptake mediated by LRP and proteoglycans in McArdle cells. We have also recently shown that expression of catalytically inactive HL-145G in HL-knockout mice reduces their HDL-cholesterol by approximately half that of the native enzyme (72). This contrasts, however, with previous data suggesting that the HL-enhanced selective uptake of HDL-CE in CHO and Hep3B cells is independent of lipolysis as it is not altered by inhibition of the HL activity (37, 43). The different findings in these combined studies may reflect the different cell systems as well as the mode of HL inhibition used. Interestingly, the lipolytic activity of LPL is not required for lipoprotein binding and uptake by LRP (74).

The present study also demonstrates that HL bound to the cell surface plays a major role in facilitating the enhanced selective uptake of HDL-CE via SR-BI. Thus, addition of heparin displaced HL-145G from the cell surface into the media and totally abolished the increase in CE selective uptake mediated by the catalytically inactive enzyme. These findings suggest that proteoglycans are required for the HL-145G-mediated effect on CE selective uptake via SR-BI. Our results are in agreement with previous data suggesting that HL-mediated uptake of HDL-CE decreases in proteoglycan-deficient CHO cells (43) and that proteoglycans are required to enhance the uptake of HDL-CE via both an LRP-dependent and -independent pathway in McArdle cells (38). However, our study also demonstrates that the displacement of the native HL by heparin reduced the SR-BI-mediated, HL-enhanced HDL-CE uptake by 35%. Thus, the remodeling of HDL by HL, whether the enzyme is bound to the cell surface or not, enhances the selective uptake mediated by SR-BI of CE from HDL.

In conclusion, our study provides evidence to support an important role for the two major functions of HL in enhancing the selective uptake of HDL-CE via SR-BI. First, the ligand-binding function of the enzyme, which requires interaction of the lipase with cell-surface proteoglycans and is independent of lipolysis, may enhance the interaction of HDL with SR-BI, thus facilitating uptake. Second, HL-mediated HDL lipolysis and particle remodeling may increase access to core CE and/or HDL-apolipoproteins, thereby facilitating the selective uptake of HDL-CE via SR-BI. This study provides, for the first time, direct evidence for a role of HL in mediating the selective uptake of CE from HDL via SR-BI, adding new insights into the mechanism by which SR-BI and HL function in cellular cholesterol metabolism.

This work was supported in part by the National Institutes of Health grant HL 14990. We thank Marie Kindt for her excellent technical assistance and Judy Burk for helping in the preparation of the manuscript.

Manuscript received 24 February 1999 and in revised form 19 March 1999.

REFERENCES

1. Gordon, D. J., and B. M. Rifkind. 1989. High-density lipoprotein: the clinical implications of recent studies. *N. Engl. J. Med.* **321:** 1311–1316.

2. Buring, J. E., G. T. O'Connor, S. Z. Goldhaber, B. Rosner, P. N. Herbert, C. B. Blum, J. L. Breslow, and C. H. Hennekens. 1992. Decreased HDL_2 and HDL_3 cholesterol, apoA-I and apoA-II, and increased risk of myocardial infarction. *Circulation*. **85:** 22–29.
3. Miller, N. E. 1987. Associations of high-density lipoprotein subclasses and apolipoproteins with ischemic heart disease and coronary atherosclerosis. *Am. Heart J.* **113:** 589–597.
4. Rubin, E. M., R. M. Krauss, E. A. Spangler, J. G. Verstuyft, and S. M. Clift. 1991. Inhibition of early atherogenesis in transgenic mice by human apolipoprotein AI. *Nature*. **353:** 265–267.
5. Duverger, N., H. Kruth, F. Emmanuel, J. M. Caillaud, C. Viglietta, G. Castro, A. Tailleux, C. Fievet, J. C. Fruchart, L. M. Houdebine, and P. Denefle. 1996. Inhibition of atherosclerosis development in cholesterol-fed human apolipoprotein A-I-transgenic rabbits. *Circulation*. **94:** 713–717.
6. Badimon, J. J., L. Badimon, and V. Fuster. 1990. Regression of atherosclerotic lesions by high density lipoprotein plasma fraction in the cholesterol-fed rabbit. *J. Clin. Invest.* **85:** 1234–1241.
7. Davenport, J., D. Collins, V. Babikian, L. M. Brass, H. Rubins, and S. Robins. 1999. Stroke events and risk factors in the veterans affairs HDL intervention trial (VA-HIT). *Stroke.* **30:** P21.
8. Glomset, J. A. 1968. The plasma lecithin:cholesterol acyltransferase reaction. *J. Lipid Res.* **9:** 155–167.
9. Fielding, C. J., and P. E. Fielding. 1995. Molecular physiology of reverse cholesterol transport. *J. Lipid Res.* **36:** 211–228.
10. Barter, P. J., and K-A. Rye. 1996. Molecular mechanisms of reverse cholesterol transport. *Curr. Opin. Lipidol.* **7:** 82–87.
11. Tall, A. R. 1998. An overview of reverse cholesterol transport. *Eur. Heart J.* **19:** A31–A35.
12. Eisenberg, S., Y. Oschry, and J. Zimmerman. 1984. Intravascular metabolism of the cholesteryl ester moiety of rat plasma lipoproteins. *J. Lipid Res.* **25:** 121–128.
13. Glass, C., R. C. Pittman, D. B. Weinstein, and D. Steinberg. 1983. Dissociation of tissue uptake of cholesterol ester from that of apoprotein A-I of rat plasma high density lipoprotein: selective delivery of cholesterol ester to liver, adrenal, and gonad. *Proc. Natl. Acad. Sci. USA.* **80:** 5435–5439.
14. Tall, A. R. 1993. Plasma cholesteryl ester transfer protein. *J. Lipid Res.* **34:** 1255–1274.
15. Jansen, H., T. J. van Berkel, and W. C. Hulsmann. 1978. Binding of liver lipase to parenchymal and non-parenchymal rat liver cells. *Biochem. Biophys. Res. Commun.* **85:** 148–152.
16. Kuusi, T., E. A. Nikkla, I. Virtanen, and P. K. Kinnunen. 1979. Localization of the heparin-releasable lipase in situ in the rat liver. *Biochem. J.* **181:** 245–256.
17. Jansen, H., C. Kalkman, J. C. Birkenhager, and W. C. Hulsmann. 1980. Demonstration of a heparin-releasable liver-lipase-like activity in rat adrenals. *FEBS Lett.* **112:** 30–34.
18. Doolittle, M. H., H. Wong, R. C. Davis, and M. C. Schotz. 1987. Synthesis of hepatic lipase in liver and extrahepatic tissues. *J. Lipid Res.* **28:** 1326–1334.
19. Grosser, J., O. Schrecker, and H. Greten. 1981. Function of hepatic triglyceride lipase in lipoprotein metabolism. *J. Lipid Res.* **22:** 437–442.
20. Bensadoun, A., and D. E. Berryman. 1996. Genetics and molecular biology of hepatic lipase. *Curr. Opin. Lipidol.* **7:** 77–81.
21. Applebaum-Bowden, D., J. Kobayashi, V. S. Kashyap, D. R. Brown, A. M. Berard, S. Meyn, C. Parrott, N. Maeda, R. D. Shamburek, H. B. Brewer, Jr., and S. Santamarina-Fojo. 1996. Hepatic lipase gene therapy in hepatic lipase-deficient mice: adenovirus-mediated replacement of a lipolytic enzyme to the vascular endothelium. *J. Clin. Invest.* **97:** 799–805.
22. Dugi, K. A., B. L. Vaisman, N. Sakai, C. L. Knapper, S. M. Meyn, H. B. Brewer, Jr., and S. Santamarina-Fojo. 1997. Adenovirus-mediated expression of hepatic lipase in LCAT transgenic mice. *J. Lipid Res.* **38:** 1822–1832.
23. Amar, M. J. A., K. A. Dugi, C. C. Haudenschild, R. D. Shamburek, B. Foeger, M. Chase, A. Bensadoun, R. F. Hoyt, Jr., H. B. Brewer, Jr., and S. Santamarina-Fojo. 1998. Hepatic lipase facilitates the selective uptake of cholesteryl esters from remnant lipoproteins in apoE-deficient mice. *J. Lipid Res.* **39:** 1–7.
24. Kobayashi, J., D. Applebaum-Bowden, K. A. Dugi, D. Brown, V. Kashyap, C. Parrott, N. Maeda, H. B. Brewer, Jr., and S. Santamarina-Fojo. 1996. Analysis of protein structure–function in vivo: adenovirus-mediated transfer of lipase mutants in hepatic lipase deficient mice. *J. Biol. Chem.* **271:** 26296–26301.
25. 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.
26. 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.
27. 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 decreases apolipoprotein B-containing and high density lipoproteins. *J. Biol. Chem.* **273:** 1896–1903.
28. Breckenridge, W. C., J. A. Little, P. Alaupovic, C. S. Wang, A. Kuksis, G. Kakis, F. Lindgren, and G. Gardiner. 1982. Lipoprotein abnormalities associated with a familial deficiency of hepatic lipase. *Atherosclerosis.* **45:** 161–179.
29. Santamarina-Fojo, S., C. Haudenschild, and M. Amar. 1998. The role of hepatic lipase in lipoprotein metabolism and atherosclerosis. *Curr. Opin. Lipidol.* **9:** 211–219.
30. Kuusi, T., P. K. Kinnunen, and E. A. Nikkilä. 1979. Hepatic endothelial lipase antiserum influences rat plasma low and high density lipoproteins in vivo. *FEBS Lett.* **104:** 384–388.
31. 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.
32. Goldberg, I. J., N. A. Le, J. R. Paterniti, Jr., H. N. Ginsberg, F. T. Lindgren, and W. V. Brown. 1982. Lipoprotein metabolism during acute inhibition of hepatic triglyceride lipase in the cynomolgus monkey. *J. Clin. Invest.* **70:** 1184–1192.
33. 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.
34. Shafi, S., S. E. Brady, A. Bensadoun, and R. J. Havel. 1994. Role of hepatic lipase in the uptake and processing of chylomicron remnants in rat liver. *J. Lipid Res.* **35:** 709–720.
35. 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 β-VLDL into cells via the LDL receptor-related protein (LRP). *J. Lipid Res.* **37:** 926–936.
36. Ji, Z-S., S. J. Lauer, S. Fazio, A. Bensadoun, J. M. Taylor, and R. W. Mahley. 1994. Enhanced binding and uptake of remnant lipoproteins by hepatic lipase-secreting hepatoma cells in culture. *J. Biol. Chem.* **269:** 13429–13436.
37. Komaromy, M., S. Azhar, and A. D. Cooper. 1996. Chinese hamster ovary cells expressing a cell surface-anchored form of hepatic lipase. *J. Biol. Chem.* **271:** 16906–1914.
38. Ji, Z-S., 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.
39. Diard, P., M-I. Malewiak, D. Lagrange, and S. Griglio. 1994. Hepatic lipase may act as a ligand in the uptake of artificial chylomicron remnant-like particles by isolated rat hepatocytes. *Biochem. J.* **299:** 889–894.
40. Choi, S. Y., M. C. Komaromy, J. Chen, L. G. Fong, and A. D. Cooper. 1994. Acceleration of uptake of LDL but not chylomicrons or chylomicron remnants by cells that secrete apoE and hepatic lipase. *J. Lipid Res.* **35:** 848–859.
41. Donner, C., C. Sungshin, M. Komaromy, and A. D. Cooper. 1998. Accelerated lipoprotein uptake by transplantable hepatomas that express hepatic lipase. *J. Lipid Res.* **39:** 1805–1815.
42. 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.
43. 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 Hep3B hepatoma cells in culture. *Atherosclerosis.* **141:** 273–285.

44. 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.
45. 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.
46. Collet, X., B. P. Perret, G. Simard, C. Vieu, and L. Douste-Blazy. 1990. Behaviour of phospholipase-modified HDL towards cultured hepatocytes. I. Enhanced transfers of HDL sterols and apoproteins. *Biochim. Biophys. Acta.* **1043:** 301–310.
47. 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.
48. Murao, K., V. Terpstra, S. R. Green, N. Kondratenko, D. Steinberg, and O. Quehenberger. 1997. Characterization of CLA-1, a human homologue of rodent scavenger receptor BI, as a receptor for high density lipoprotein and apoptotic thymocytes. *J. Biol. Chem.* **272:** 17551–17557.
49. Landschulz, K. T., R. K. Pathak, A. Rigotti, M. Krieger, and H. H. Hobbs. 1996. Regulation of scavenger receptor, class B, type I, a high density lipoprotein receptor, in liver and steroidogenic tissues of the rat. *J. Clin. Invest.* **98:** 984–995.
50. Temel, R. E., B. Trigatti, R. B. DeMattos, S. Azhar, M. Krieger, and D. L. Williams. 1997. Scavenger receptor class B, type I (SR-BI) is the major route for the delivery of high density lipoprotein cholesterol to the steroidogenic pathway in cultured mouse adrenocortical cells. *Proc. Natl. Acad. Sci. USA.* **94:** 13600–13605.
51. Wang, N., T. Arai, Y. Ji, F. Rinninger, A. Walsh, J. L. Breslow, and A. R. Tall. 1998. Transgenic mice with liver-specific overexpression of scavenger receptor BI show decrease VLDL and LDL apoB levels on chow and high fat diets, as well as reduced HDL levels. *Circulation.* **17:** I2(Abstract).
52. Sehayek, E., J. G. Ono, S. Shefer, L. B. Nguyen, N. Wang, A. K. Batta, G. Salen, J. D. Smith, A. R. Tall, and J. L. Breslow. 1998. Biliary cholesterol excretion: a novel mechanism that regulates dietary cholesterol absorption. *Proc. Natl. Acad. Sci. USA.* **95:** 10194–10199.
53. Kozarsky, K. F., M. H. Donahee, A. Rigotti, S. N. Iqbal, E. R. Edelman, and M. Krieger. 1997. Overexpression of the HDL receptor SR-BI alters plasma HDL and bile cholesterol levels. *Nature.* **387:** 414–417.
54. 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.
55. Varban, M. L., F. Rinninger, N. Wang, V. Fairchild-Huntress, J. H. 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.
56. Xu, S., M. Laccotripe, X. Huang, A. Rigotti, V. I. Zannis, and M. Krieger. 1997. Apolipoproteins of HDL can directly mediate binding to the scavenger receptor SR-BI, an HDL receptor that mediates selective lipid uptake. *J. Lipid Res.* **38:** 1289–1298.
57. 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: in vivo evidence that SR-BI is a functional high density lipoprotein receptor under feedback control. *J. Biol. Chem.* **271:** 21001–21004.
58. Calvo, D., and M. A. Vega. 1993. Identification, primary structure, and distribution of CLA-1, a novel member of the CD36/LIMPII gene family. *J. Biol. Chem.* **268:** 18929–18935.
59. Dichek, H. L., C. Parrott, R. Ronan, J. D. Brunzell, H. B. Brewer, Jr., and S. Santamarina-Fojo. 1993. Functional characterization of a chimeric protein genetically engineered from human lipoprotein lipase and human hepatic lipase. *J. Lipid Res.* **34:** 1393–1401.
60. Ho, S. N., H. D. Hunt, R. M. Horton, J. K. Pullen, and L. R. Pease. 1989. Site-directed mutagenesis by overlap extension using the polymerase chain reaction. *Gene.* **77:** 51–59.
61. Sanger, F., A. R. Coulson, B. G. Barrell, A. J. Smith, and B. A. Roe. 1980. Cloning in single-stranded bacteriophage as an aid to rapid DNA sequencing. *J. Mol. Biol.* **143:** 161–178.
62. Iverius, P-H., and J. D. Brunzell. 1985. Human adipose tissue lipoprotein lipase: changes with feeding and relation to postheparin plasma enzyme. *Am. J. Physiol.* **249:** E107–E114.
63. Bensadoun, A. 1996. Sandwich-immunoassay for measurement of human hepatic lipase. *Methods Enzymol.* **263**: 333–338.
64. McFarlane, A. S. 1958. Efficient trace-labeling of proteins with iodine. *Nature.* **182:** 53–57.
65. Azhar, S., L. Tsai, and E. Reaven. 1990. Uptake and utilization of lipoprotein cholesteryl esters by rat granulosa cells. *Biochim. Biophys. Acta.* **1047:** 148–160.
66. Azhar, S., D. Stewart, and E. Reaven. 1989. Utilization of cholesterol-rich lipoproteins by perfused rat adrenals. CA. *J. Lipid Res.* **30:** 1799–1810.
67. 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.
68. Beisiegel, U., and J. Heeren. 1997. Lipoprotein lipase (*EC* 3.1.1.34) targeting of lipoproteins to receptors. *Proc. Nutr. Soc.* **56:** 731–737.
69. 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.
70. 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.
71. Chappell, D. A., G. L. Fry, M. A. Waknitz, L. E. Muhonen, M. W. Pladet, P. H. Iverius, and D. K. Strickland. 1993. Lipoprotein lipase induces catabolism of normal triglyceride-rich lipoproteins via the low density lipoprotein receptor-related protein / $_2$-macroglobulin receptor in vitro. *J. Biol. Chem.* **268:** 14168–14175.
72. Dugi, K. A., C. L. Knapper, D. Applebaum-Bowden, A. Bensadoun, S. M. Meyn, T. T. Le, N. Maeda, H. B. Brewer, Jr., and S. Santamarina-Fojo. 1996. In vivo evidence for a nonlipolytic role of hepatic lipase in the metabolism of high density lipoprotein. *Circulation.* **94:** I–398(Abstract).
73. Jansen, H., and W. J. De Greef. 1981. Heparin-releasable lipase activity of rat adrenals, ovaries and testes. *Biochem. J.* **196:** 739–745.
74. 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.
75. Acton, S. L., P. E. Scherer, H. F. Lodish, and M. Krieger. 1994. Expression cloning of SR-BI, a CD36-related class B scavenger receptor. *J. Biol. Chem.* **269:** 21003–21009.
76. De Beer, F. C., P. M. Connell, M. C. De Beer, N. R. Webb, and D. R. van de Westhuyzen. 1998. SAA and sPLA$_2$ modification of HDL influences SR-BI mediated lipid flow. *Circulation.* **17:** I202(Abstract).
77. Guendouzi, K., X. Collet, B. Perret, H. Chap, and R. Barbaras. 1998. Remnant high density lipoprotein$_2$ particles produced by hepatic lipase display high-affinity binding and increased endocytosis into a human hepatoma cell line (HEPG$_2$). *Biochemistry.* **37:** 14974–14980.