The Class B, Type I Scavenger Receptor Promotes the Selective Uptake of High Density Lipoprotein Cholesterol Ethers into Caveolae*

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The uptake of cholesterol esters from high density lipoproteins (HDLs) is characterized by the initial movement of cholesterol esters into a reversible plasma membrane pool. Cholesterol esters are subsequently internalized to a nonreversible pool. Unlike the uptake of cholesterol from low density lipoproteins, cholesterol ester uptake from HDL does not involve the internalization and degradation of the particle and is therefore termed selective. The class B, type I scavenger receptor (SR-BI) has been identified as an HDL receptor and shown to mediate selective cholesterol ester uptake. SR-BI is localized to cholesterol- and sphingomyelin-rich microdomains called caveolae. Caveolae are directly involved in cholesterol trafficking. Therefore, we tested the hypothesis that caveolae are acceptors for HDL-derived cholesterol ether (CE). Our studies demonstrate that in Chinese hamster ovary cells expressing SR-BI, >80% of the plasma membrane associated CE is present in caveolae after 7.5 min of selective cholesterol ether uptake. We also show that excess, unlabelled HDL can extract the radiolabelled CE from caveolae, demonstrating that caveolae constitute a reversible plasma membrane pool of CE. Furthermore, 50% of the caveolae-associated CE can be chased into a nonreversible pool. We conclude that caveolae are acceptors for HDL-derived cholesterol ethers, and that caveolae constitute a reversible, plasma membrane pool of cholesterol ethers.

Plasma levels of high density lipoprotein (HDL)1 cholesterol are negatively correlated with the risk of developing atherosclerosis, the leading cause of death in western, industrialized countries (1, 2). The role of HDL in cholesterol metabolism includes the delivery of cholesterol esters to steroidogenic tissues (3, 4) and the transfer of cholesterol from peripheral tissues to the liver in a process termed reverse cholesterol transport (5, 6). Reverse cholesterol transport requires the extraction of cholesterol from extrahepatic cells by HDL and the subsequent delivery of cholesterol esters to hepatocytes.

The mechanism for the delivery of cholesterol esters from HDL to cells is described as selective uptake, because the uptake of cholesterol ester is independent of HDL internalization (7, 8). Selective uptake of cholesterol ester from HDL is characterized by the initial movement of cholesterol ester into a reversible, plasma membrane pool and the subsequent internalization to a nonreversible, intracellular pool (9, 10).

The mechanisms of cholesterol and cholesterol ester exchange between the cell surface and HDL are not well understood. Receptor-independent and receptor-dependent hypotheses have been proposed to explain the transfer of cholesterol and cholesterol ester between the cell surface and HDL (6). In the receptor-independent model, diffusion is thought to account for both the uptake of cholesterol esters and the efflux of free cholesterol. In contrast, HDL-binding proteins, such as class B, type I scavenger receptor (SR-BI) and class B, type II scavenger receptor, can mediate the selective uptake of cholesterol esters from HDL (11–13).

SR-BI, appears to be a physiological HDL receptor. Several studies support this assertion. First, SR-BI binds HDL and mediates the selective uptake of cholesterol esters (11, 13, 14). Second, SR-BI mediates the efflux of cholesterol from cells to HDL (15, 16). Third, the expression of SR-BI is greatest in tissues that selectively take up cholesterol esters from HDL, including liver, adrenal, testis, and ovary (11, 14). In mice, overexpression of SR-BI in hepatic tissue causes a dramatic decline in plasma HDL and an increase in biliary cholesterol (17). Finally, disruption of the SR-BI gene leads to an increase in plasma cholesterol concentrations in mice (18). Collectively, these data indicate that SR-BI is an HDL receptor involved in cholesterol homeostasis.

SR-BI has been localized to caveolae (13, 19), which are cholesterol- and sphingomyelin-rich microdomains in the plasma membrane (20–22). Caveolae appear to be directly involved in cellular cholesterol homeostasis. Newly synthesized cholesterol translocates from the endoplasmic reticulum (ER) to caveolae before diffusing into the bulk plasma membrane (23). Caveolae mediate the efflux of free cholesterol derived either from de novo synthesis or low density lipoproteins (24).

In addition, caveola morphology is dependent on the presence of free cholesterol. Depletion of membrane cholesterol causes invaginated caveolae to flatten within the plane of the membrane (25, 26). Caveolin is a cholesterol-binding protein associated with the caveola coat (21, 27). The subcellular distribution of caveolin is, in part, controlled by caveola cholesterol. Oxidation of caveola cholesterol by cholesterol oxidase causes caveolin to translocate to the ER (22). Thus, both caveola proteins and morphology are dependent on cholesterol.

SR-BI mediates the selective uptake of cholesterol esters from HDL and is localized to caveolae (13, 19). Therefore, we hypothesized that caveolae are acceptors for HDL-derived cholesterol ethers. We further speculated that caveolae constitute...
a reversible pool of cholesterol ethers within the plasma membrane. The present study demonstrates that caveolae are acceptors for HDL-derived cholesterol ethers, and that caveolae constitute a reversible plasma membrane pool of cholesterol ethers.

**EXPERIMENTAL PROCEDURES**

**Materials**—Ham's F-12 medium, Geneticin (G418 sulfate), fetal calf serum, 1-glutamine, trypsin-EDTA, penicillin-streptomycin, and Opti-Prep were purchased from Life Technologies, Inc. Percol, polyvinylidene difluoride membrane and Tween 20 were purchased from Sigma. Bradford reagent was purchased from Bio-Rad. Rabbit IgG directed against caveolin-1 was obtained from Transduction Laboratories (Lexington, KY). Mouse IgG directed against the human transferrin receptor was supplied by Zymed Laboratories Inc. (San Francisco, CA). Horseradish peroxidase-conjugated IgGs were supplied by Cappel (West Chester, PA). Super Signal chemiluminescent substrate was purchased from Pierce. 1,2-3H-Cholesteryl-oleyl ether (47 Ci/ mmol) was supplied by Amersham Pharmacia Biotech. [125I]-Na (1 mCi/ ml) was purchased from DuPont NEN.

**Buffers**—Sample buffer (5 ×) consisted of 0.31 mM Tris, pH 6.8, 2.5% (w/v) SDS, 50% (v/v) glycerol, and 0.125% (w/v) bromphenol blue. Tris-buffered saline consisted of 20 mM Tris, pH 7.6, and 137 mM NaCl. BLOTTO consisted of Tris-buffered saline plus 0.5% Tween 20 and 5% dry milk. Wash buffer consisted of Tris-buffered saline plus 0.5% Tween 20 and 0.2% dry milk. Tris-saline consisted of 50 mM Tris, pH 7.4, and 150 mM NaCl.

**Cell Culture**—Ida-7 (a low density lipoprotein receptor-negative Chinese hamster ovary (CHO) cell line) cells were generously provided by Dr. Monty Kreiger (Massachusetts Institute of Technology, Cambridge, MA). CHO lines were cultured in Ham's F-12 medium containing 5% fetal bovine serum, 2 mM l-glutamine, 100 units/ml penicillin, and 100 µg/ml streptomycin. The human SR-BI (hSR-BI) cDNA was cloned into the pCMV5 expression vector (28) and transfected into Ida-7 cells with the MBS transfection kit (Stratagene, La Jolla, CA). Lines expressing hSR-BI were selected and maintained in medium containing 0.5 mg/ml G418 sulfate. For the analysis of cell associated cholesterol ether (CE), cells were seeded (2.5 × 10^6 cells/well) in six-well plates on day 0. Cells were fed on day 1, and uptake into reversible and nonreversible pools was determined at confluence on day 2. For cell fractionation studies, cells were seeded (5 × 10^5 cells/plate) in 10-cm dishes on day 0. Cells were confluent on days 4–5.

**Lipoprotein Isolation and Radiolabeling**—HDL (d 1.063–1.21 g/ml) was isolated from fresh human plasma by density gradient ultracentrifugation as described previously (29). The HDL2 subfraction (d 1.13–1.18 g/ml) was isolated from other HDL subfractions using a density gradient fractionator (ISCO). 1,2-3H-Cholesteryl-oleyl ether was incorporated into HDL2 as described previously (30). The specific activity of [3H]CE-HDL ranged from 32 to 35 dpm/ng of cholesterol. HDL2 apolipoproteins were iodinated by the iodine monochloride method (31) to a specific activity of 400–600 cpm/ng of protein.

**Uptake and Ligand Binding Assays**—The selective uptake of cholesterol ether from HDL into cells was determined using nonhydrolyzable [3H]CE (9). Confluent Ida-7 and hSR-BI (Ida-7 cells expressing hSR-BI) cells were rinsed twice with PBS (37 °C). Ham's F-12 medium containing 5% human lipoprotein-deficient serum and 10 µg/ml heparin was added to the cells for the indicated times. After incubation, uptake was terminated by aspirating the medium and washing the cell monolayers four times with Tris-saline (4 °C). The cells were dissolved in 1 N NaOH, and the amount of radiation was determined by scintillation counting. To determine the quantity of CE within the reversible plasma membrane pool, medium (efflux medium) containing 100 µg/ml unlabeled HDL was added for 120 min at 37 °C (9). The efflux medium was collected, and [3H]CE was quantified by liquid scintillation counting. Radiolabel present in the efflux medium was designated the reversible pool (9). Cells were then dissolved in 1 N NaOH. Total cellular protein was determined by Bradford assay, and the radiolabel remaining was designated the nonreversible pool (9). [125I]-HDL binding experiments were conducted in the same manner as the uptake assays.

To compare cell-associated [125I]-HDL with [3H]CE-HDL, [3H]CE uptake was measured as apparent HDL protein uptake, assuming that [3H]CE uptake resulted from whole HDL particle uptake as described by Knetch and Pittman (9).

**SDS-Polyacrylamide Gel Electrophoresis and Immunoblotting**—Cellular fractions were dissolved in 0.015% (w/v) deoxycholate, concentrated by precipitation with 7% (w/v) trichloroacetic acid, and washed in acetone (32). Pellets were suspended in 1 × sample buffer plus 1.2% (v/v) β-mercaptoethanol and heated to 95 °C for 5 min immediately before loading. Proteins were separated on a 12.5% polyacrylamide gel at 50 mA (constant current) and subsequently transferred to a polyvinylidene difluoride membrane at 50 V (constant voltage) for 2 h. Membranes were blocked with blotting buffer for 60 min at 22 °C. Primary antibodies were diluted in blotting buffer and incubated with blocked membranes for 60 min at 22 °C. Membranes were washed four times for 10 min in wash buffer. Horseradish peroxidase-conjugated IgGs directed against the appropriate host IgG were diluted and incubated with membranes as described for primary antibodies. Membranes were washed four times for 10 min in wash buffer and visualized using chemiluminescence.

**Isolation of Caveolae**—Caveolae were isolated as described previously (32, 33). This method generates a highly purified fraction of caveolae that is enriched in caveolin and free of bulk plasma membrane markers such as transferrin receptor and integrins (32). Twenty to 30 µg of purified caveolae are generally isolated from CHO cells with this method. The yield of caveolae was estimated by immunoblot analysis. Two, 5 and 10% of the caveola and plasma membrane fractions were compared. The signal intensities varied linearly with the percent of each fraction loaded. The estimated yield of plasma membrane caveolae in the present study was 50% ± 8%.

**Statistical Analysis**—Least squares analysis of variance was used to evaluate the data with respect to cell fraction, time, and their interaction using the analysis of variance procedure of STATISTICA (Statsoft, Tulsa, OK). When appropriate, fractions were compared within a given time using the Tukey's honestly significant difference test. Means were considered different at p < 0.01.
CE Uptake in Caveolae

**RESULTS**

**SR-BI-dependent CE Uptake into Reversible and Nonreversible Pools**—Selective uptake of HDL cholesterol ethers involves the initial movement of CE into a reversible plasma membrane pool and the subsequent movement into an internal, nonreversible pool (9). We determined whether SR-BI mediates the selective uptake of CE into a reversible pool. SR-BI-negative CHO cells (ldlA-7) and ldlA-7 cells expressing hSR-BI were incubated in the presence of $^{[3]H}$CE-HDL (10 μg/ml) for various times at 37 °C. The cells were washed to remove unbound $^{[3]H}$CE-HDL and then incubated with excess (100 μg/ml) unlabeled HDL (eflux medium) for 120 min at 37 °C. The $^{[3]H}$CE in the eflux medium was designated the reversible pool (9). The remaining, cell-associated CE was designated the nonreversible pool. Fig. 1A indicates that after 7.5 min the reversible CE pool was 5.0-fold greater in hSR-BI cells than in ldlA-7 cells ($p < 0.01$; ○ versus □). Similarly, the nonreversible CE pool was 8.4-fold greater in hSR-BI cells than in ldlA-7 cells ($p < 0.01$; Fig. 1A, ○ versus □). The nonreversible CE pool increased linearly ($p < 0.01$) in hSR-BI cells. In contrast, the reversible CE pool did not increase (104 ± 11 ng of CE/mg of cell protein) between 30 and 60 min.

To ensure that the reversible CE pool reached equilibrium, SR-BI-dependent uptake was examined for up to 4 h. SR-BI-specific uptake was calculated by subtracting the values obtained for ldlA-7 cells from those obtained for hSR-BI cells (11). Fig. 1B shows that SR-BI-dependent uptake of CE into the nonreversible (□) pool increased linearly ($p < 0.01$) and did not saturate under these conditions. In contrast, the reversible (Fig. 1B, ○) CE pool reached equilibrium by 60 min and remained constant for up to 4 h.

We next determined whether the reversible CE pool was integral to the membrane or caused by displacement of bound $^{[3]H}$CE-HDL with unlabeled HDL. Cells expressing hSR-BI were incubated with $^{[3]H}$CE-HDL and then processed as described for Fig. 1. $^{125}$I-labeled HDL allows the tracking of the lipoprotein, whereas $^{[3]H}$CE tracks cholesterol ethers (11). The selective uptake of $^{[3]H}$CE was expressed as apparent particle uptake (see “Experimental Procedures”) into the reversible and nonreversible CE pools (9). As shown in Fig. 2, after 7.5 min the uptake of $^{[3]H}$CE into the nonreversible (□ and □) CE pool was 4-fold greater than that observed for $^{125}$I-labeled HDL (54 ± 12.3 versus 12 ± 1.3 ng/mg of cell protein). Likewise, the $^{[3]H}$CE present in the reversible (Fig. 2, ○ and ■) CE pool was 20-fold greater than the HDL protein (68.1 ± 8.2 versus 3.6 ± 0.8 ng/mg of cell protein). These data demonstrate that SR-BI mediates the selective uptake of HDL CE into both reversible and nonreversible pools.

**Caveolae Are HDL Cholesterol Ether Acceptors**—Confocal microscopy experiments have demonstrated that the majority of SR-BI expressed in CHO cells co-localizes with caveolin, a marker protein for caveolae (19). We therefore hypothesized that caveolae are acceptors for HDL-derived cholesterol ethers. To test this hypothesis, hSR-BI cells were incubated with $^{[3]H}$CE-HDL for various times and then fractionated into cytosol, total plasma membranes (which includes caveola membranes), and intracellular membranes (e.g. ER and Golgi). Isolation of caveola membrane (CM) from the plasma membrane (PM) showed that >80% of the $^{[3]H}$CE within the plasma membrane was associated with caveola at 7.5 min (Fig. 3A), indicating that initially, <20% of selective uptake occurred outside of caveola. Fig. 3B shows that at 7.5 min the specific activity of $^{[3]H}$CE (ng of $^{[3]H}$CE/mg of total protein in the subcellular fraction) was 12-fold greater in the caveolae fraction in the plasma membrane fraction (173 ± 79 versus 142 ± 49 ng/mg of protein). Similar to the reversible pool of CE, the amount of $^{[3]H}$CE associated with caveola saturated by 30 min and remained constant for 4 h (data not shown). However, the amount of $^{[3]H}$CE associated with the plasma membrane

![Fig. 2](image2.png)

**Fig. 2.** SR-BI-mediated selective uptake of $[^{3}H]$cholesterol ether into a reversible pool is not attributable to exchange of bound HDL. hSR-BI cells were incubated with $[^{[3]H]}$CE-HDL (10 μg/ml, ○ and ■) or $^{125}$I-HDL (10 μg/ml, □ and □) for up to 60 min (37 °C) and chased with unlabeled HDL (100 μg/ml). The amount of each label present in the reversible (○ and ■) and nonreversible (□ and □) pools was quantified. $[^{3}H]$CE present in the reversible and nonreversible pools is expressed as apparent particle uptake (HDL protein). Values are mean ± S.D.; $n = 3$.

![Fig. 3](image3.png)

**Fig. 3.** Selective uptake of labeled $[^{3}H]$cholesterol ether into the plasma membrane occurs via caveolae. hSR-BI cells were incubated with $[^{[3]H]}$CE-HDL (10 μg/ml, 37 °C), washed, and processed to isolate caveolae. A, $[^{3}H]$CE present in the total plasma membrane (●) and in caveolae (○). B, Concentration (ng of $[^{3}H]$CE/mg of protein) of $[^{3}H]$CE in caveolae (○) and total plasma membrane (●) fractions. Representative data are from four independent experiments. Values are mean ± S.D.; $n = 2$. 

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and intracellular membranes increased throughout the incubation period (data not shown).

Caveola membranes were analyzed by immunoblotting (Fig. 4A). The caveola marker protein caveolin was present in the total PM fraction and was highly enriched in the CM of the plasma membrane. SR-BI was also highly enriched in the caveola fraction. The noncaveola proteins transferrin receptor and clathrin were detected in the plasma membrane fraction but not in the caveola fraction. The relative distribution of total protein and SR-BI are shown in Fig. 4B. Although the caveola fraction contained only 1.6% of the starting postnuclear supernatant protein, it contained 60 ± 4% of the total plasma membrane SR-BI as determined by immunoblot analysis of hSR-BI in the plasma membrane and caveola membrane fractions (see “Experimental Procedures”).

**Cavola-associated CE Constitutes a Reversible CE Pool**—SR-BI-dependent selective uptake of HDL cholesterol ether involves uptake into a reversible plasma membrane pool (Fig. 1). If the reversible pool corresponds to the caveola compartment, then incubation with excess unlabeled HDL should result in an efflux of caveola-associated CE to HDL. To test this, hSR-BI cells were incubated with [3H]CE-HDL (10 μg/ml, 37 °C, 60 min). The cells were then washed and incubated with unlabeled HDL (100 μg/ml, 37 °C) for 0, 60, or 120 min. Plasma membranes were isolated, and caveolae were prepared as described. As shown in Fig. 5A, the [3H]CE associated with caveolae declined during the efflux period, whereas the [3H]CE in the plasma membrane remained constant, indicating that caveola [3H]CE constituted the reversible pool of CE.

Knetch and Pittman (9) demonstrated that isolated plasma membranes are capable of selective uptake of HDL CE. They also established that the reversible pool of HDL-derived cholesterol ethers can be effluxed from isolated plasma membranes (9). To determine whether efflux of caveola HDL CE occurred from isolated plasma membranes, the membranes were labeled in vitro with [3H]CE-HDL for 60 min at 37 °C. Efflux was then determined after 0, 60, or 120 min of incubation with excess unlabeled HDL (100 μg/ml). Caveolae were isolated as in previous experiments. Interestingly, the incorporation of [3H]CE into isolated plasma membranes appears to be greater in vitro than in intact cells (Fig. 5, A versus B). Consistent with efflux from whole cells, [3H]CE declined in the caveola fraction during incubation with unlabeled HDL (Fig. 5B). In contrast, [3H]CE associated with the plasma membrane fraction did not change during the efflux period.

**The Reversible Caveola Pool of CE Is Internalized to a Nonreversible Pool**—We next determined whether the SR-BI-dependent reversible pool proceeded to a nonreversible pool. Cells were incubated for 30 min with [3H]CE-HDL and then washed...
and incubated in medium only (no HDL) for up to 3 h. Efflux medium (100 μg/ml HDL) was then added for 120 min, and the reversible and nonreversible pools were determined. Fig. 6A shows that the nonreversible pool tended to increase with time, whereas the reversible pool decreased with time (p < 0.01, linear). The [3H]CE associated with the SR-BI-dependent reversible pool moved to a nonreversible pool.

If caveolae are the SR-BI-dependent reversible pool, then [3H]CE associated with caveolae should decline during a chase period. To test this, hSR-BI cells were incubated with [3H]CE-HDL (100 μg/ml) for 30 min. Cells were washed and incubated in the absence of HDL for up to 3 h. A, after the incubation, the [3H]CE in the reversible pool (○) was quantified by incubating cells in the presence of unlabeled HDL (100 μg/ml) for 120 min and counting radiolabeled CE in the medium. The labeled CE that remained cell associated was designated the nonreversible pool (●). B, after the incubation, caveolae were prepared as described. The amount of [3H]CE present in each fraction was quantified by scintillation counting. ▲, total plasma membranes; ●, caveola membranes; ○, intracellular membranes. Representative data are from two independent experiments. Values are mean ± S.D.; n = 2.

**DISCUSSION**

The present study demonstrates that cholesterol ether from HDL is initially transferred to caveolae and that the transfer of HDL cholesterol ether to caveolae requires the expression of SR-BI. We previously demonstrated that the majority of murine SR-BI co-localizes with caveolin, a caveola marker protein (13, 19). In the present study, ~60% of total plasma membrane SR-BI co-purifies with caveolin. The yield of SR-BI in the caveola fraction is similar to the yield of caveola based on immunoblots of caveolin (60%; Fig. 4; versus 53%; “Experimental Procedures”). These results suggest that the incomplete recovery of SR-BI is attributable to the incomplete recovery of caveolae, because caveolin present in the plasma membrane is predominantly associated with caveola membranes, as shown by Anderson and colleagues by immunoelectron microscopy (21). However, some fraction of SR-BI (potentially up to 40%) may not be associated with caveola. Importantly, >80% of the [3H]CE is associated with caveolae by 7.5 min of uptake. This strongly suggests that caveolae are involved in the initial uptake of CE. The absolute dependence of selective CE uptake on caveolae will require additional studies.

We also demonstrated in the present study that cholesterol ether associated with caveolae constituted a reversible pool from which the sterol could efflux back to HDL. Furthermore, caveola-associated cholesterol ether was chased into an internal, nonreversible pool. These findings are consistent with earlier work by Pittman and Knetch (9), who used Hep G2 and adrenal cells to demonstrate that HDL cholesterol ether was taken up into a small, reversible plasma membrane pool before progressing to an internal, nonreversible pool. Both hepatocytes and adrenal cells abundantly express SR-BI, and it is therefore likely that the selective uptake observed in these earlier studies was mediated, at least in part, by SR-BI (11, 34).

We used subcellular fractionation and pulse-chase methods to demonstrate that cholesterol ether moved to an intracellular membrane compartment. We have not identified the intracellular membrane compartment. Once the cholesterol ether reached the intracellular membrane compartment, it could not be rapidly effluxed from these cells. These data show that cholesterol ether can be selectively internalized to an intracellular membrane compartment. Furthermore, these data suggest that internalization of caveola cholesterol ester does not require its hydrolysis to unesterified, free cholesterol.

How does cholesterol ether traffic between caveolae and an intracellular membrane compartment? Recent research on caveola function offers two possible mechanisms. First, Schnitzer et al. (35) has shown that endothelial cell caveolae contain vesicle-trafficking proteins such as SNAPS and SNAREs. Furthermore, this group has shown that endothelial caveolae can invaginate and detach from the plasma membrane proper. The detached caveolae are capable of translocating through the cytosol and fusing with other membranes. Cholesterol ethers could therefore be internalized and transported to intracellular membranes in this manner. However, the ability of caveolae to vesiculate is contentious and may not occur in other types of cells (26, 36, 37). This mechanism would also internalize entire HDL particles and therefore not be selective uptake. A second possible mechanism for sterol trafficking was described by Uittenbogaard et al. (33). We demonstrated that a protein chaperone complex consisting of caveolin, HSP56, cyclophilin 40, and cyclophilin-A transported newly synthesized cholesterol directly from the ER to caveolae. This type of mechanism might also permit the uptake of cholesterol ethers from caveolae without the internalization of SR-BI and HDL. However, the caveolin-chaperone complex has not yet been demonstrated to traffic from caveolae to the ER or to transport cholesterol ethers.

We have shown that both SR-BI and an isoform, class B, type II scavenger receptor, are localized to caveolae (38). In addition, another class B scavenger receptor, CD36, has also been localized to caveolae (39, 40). Both SR-BI (19) and CD36 (41) are palmitoylated. Protein acylation is one mechanism whereby proteins can be targeted to caveolae. Shenoy-Scaria et al. (42),
for example, demonstrated that dual acylation targeted nonreceptor tyrosine kinases to caveolae. Shaul et al. (43) demonstrated that endothelial nitric oxide synthase required acylation for caveolae association. The role of acylation in the targeting of SR-BI or CD36 to caveolae has not been tested. Recently, Reaven et al. (44) published electron micrographs showing the localization of SR-BI to microvillar channels in luteal cells. The relationship between microvillar channels and caveolae is unclear. Parton et al. (45, 46) demonstrated that caveolae can form deep invaginations into the cytosol in skeletal muscle myocytes. Microvillar channels may be caveolae or the functional equivalent in luteal cells. Alternatively, it is possible that SR-BI is not associated with caveolae in all types of cells.

SR-BI plays an important role in regulating cholesterol flow between lipoproteins and cell membranes. We suggest the following pathway for the selective uptake of HDL cholesterol ester: 1) HDL binds to SR-BI in caveolae; 2) CE is transferred to caveolae; and 3) caveola-associated CE translocates to an intracellular membrane compartment. However, our data do not rule out the possibility that caveola-associated CE moves into the bulk (noncaveola) plasma membrane before transport to an intracellular compartment. Additional work is required to elucidate these steps in CE transport and their potential regulatory mechanisms.

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