Mechanism of Scavenger Receptor Class B Type I-mediated Selective Uptake of Cholesteryl Esters from High Density Lipoprotein to Adrenal Cells*

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Despite extensive studies and characterizations of the high density lipoprotein-cholesterol ester (HDL-CE)-selective uptake pathway, the mechanisms by which the hydrophobic CE molecules are transferred from the HDL particle to the plasma membrane have remained elusive, until the discovery that scavenger receptor BI (SR-BI) plays an important role. To elucidate the molecular mechanism, we examined the quantitative relationships between the binding of HDL and the selective uptake of its CE in the murine adrenal Y1-B51 cell line. A comparison of concentration dependences shows that half-maximal high affinity cell association of HDL occurs at 8.7 ± 4.7 μg/ml and the Km of HDL-CE-selective uptake is 4.5 ± 1.5 μg/ml. These values are similar, and there is a very high correlation between these two processes (r² = 0.98), suggesting that they are linked. An examination of lipid uptake from reconstituted HDL particles of defined composition and size shows that there is a non-stoichiometric uptake of HDL lipid components, with CE being prefered over the major HDL phospholipids, phosphatidylcholine and sphingomyelin. Comparison of the rates of selective uptake of different classes of phospholipid in this system gives the ranking: phosphatidylserine > phosphatidylcholine ~ phosphatidylinositol > sphingomyelin. The rate of CE-selective uptake from donor particles is proportional to the amount of CE initially present in the particles, suggesting a mechanism in which CE moves down its concentration gradient from HDL particles docked on SR-BI into the cell plasma membrane. The activation energy for CE uptake from either HDL₃ or reconstituted HDL is about 9 kcal/mol, indicating that HDL-CE uptake occurs via a non-aqueous pathway. HDL binding to SR-BI allows access of CE molecules to a “channel” formed by the receptor from which water is excluded and along which HDL-CE molecules move down their concentration gradient into the cell plasma membrane.

In humans, peripheral cells receive exogenous cholesterol from low density lipoprotein (LDL) particles, which are incorporated into the cells by receptor-mediated endocytosis (1). The cholesteryl ester (CE) in the LDL particles is hydrolyzed to free cholesterol in lysosomes. Since non-steriodogenic cells cannot catabolize cholesterol, homeostasis is maintained by efflux of free cholesterol to extracellular high density lipoprotein (HDL) particles (2). The concentration gradient required for net efflux of cell cholesterol mass is maintained by the action of lecinthin-cholesterol acyltransferase, which converts free cholesterol in HDL to CE. This CE is then returned to the liver by the reverse cholesterol transport pathway (3). In addition to this flux of HDL-CE to the liver, where it can be converted into bile acids, HDL-CE is used by steriodogenic cells such as adrenal, ovary, and testis for production of steroid hormones (4). In rodents, HDL cholesterol is taken up by adrenal cells in vitro (5) and in vivo (6, 7) in preference to LDL cholesterol. The HDL-dependant increase in corticosterone production in cultured rat adrenocortical cells greatly exceeds what can be accounted for by HDL particle uptake and degradation by the cell, suggesting that HDL-CE is taken up by the cell without concomitant uptake of the HDL particle (8). Subsequent studies in vivo and in cell culture have shown directly that HDL-CE is taken up by adrenal cells without HDL particle uptake and degradation (9–12).

This HDL-CE-selective uptake pathway is distinct from the LDL receptor-mediated pathway wherein LDL binds to the receptor, is internalized in coated pits, and is directed to lysosomes for whole particle degradation (13). In contrast, HDL-CE is preferentially taken up by a variety of tissues and cell types by a non-endocytic mechanism without either degradation of apolipoproteins or whole particle uptake (10). CE from HDL is transferred to cell plasma membranes by a passive process that is dependent on the cholesterol content of the membrane (14, 15). Thereafter, CE molecules are irreversibly transferred into the cell interior (16) and are hydrolyzed by a non-lysosomal mechanism (17, 18). When reconstituted HDL particles containing different apolipoproteins were studied in cultured adrenal cells, the HDL-CE-selective uptake process showed only a modest preference for apolipoprotein (apo) A-I (19) although apoA-I appears to be essential in vivo (20). Although the HDL-
CE-selective uptake pathway has been extensively studied and characterized, understanding of the mechanisms by which the hydrophobic CE molecules are transferred from the HDL core to the plasma membrane has remained elusive.

The recent demonstration that scavenger receptor BI (SR-BI) mediates the selective uptake of HDL-CE in transfected cells provides an important link between the selective uptake pathway and a specific cell surface receptor (21). These studies showed that expression of SR-BI in Chinese hamster ovary (CHO) cells resulted in HDL binding and the selective uptake of a fluorescent lipid probe and CE (21). This report provided strong evidence that a cell surface receptor may mediate the uptake of HDL-CE into cells; similar results have been reported with the human homologue, CLA-1 (22). In both mice (21) and rats (23), SR-BI expression occurs predominantly in tissues exhibiting high levels of HDL-CE-selective uptake, namely steroidogenic cells and the liver (9, 24). SR-BI expression is induced by adrenocorticotropic hormone (ACTH) in mouse adrenal gland in vivo (25) and in Y1-B51 (25) and Y1 (26) adrenocortical cells in culture. Direct evidence for SR-BI function in steroidogenic cells is provided by recent results showing that antibody to the extracellular domain of SR-BI blocks HDL-CE-selective uptake and the delivery of HDL cholesterol to the steroidogenic pathway in cultured adrenocortical cells (27). In addition, inactivation of the SR-BI gene by a targeted mutation in mice results in a dramatic reduction in neutral lipid stores in adrenal glands (28). These mice also show increased plasma levels of large CE-rich HDL particles. Furthermore, adenovirus-mediated overexpression of SR-BI in the liver results in a marked reduction of HDL cholesterol in plasma and enhanced biliary secretion of cholesterol (29). Taken together, these observations provide strong evidence that SR-BI plays an important role in mediating HDL-CE-selective uptake in steroidogenic cells and the liver.

Our goal was to define the molecular mechanisms of SR-BI-mediated HDL-CE-selective uptake. In order to achieve this, we examined the relationship between the binding of HDL, and the selective uptake of its CE in the Y1-B51 cell; the quantitative data show a tight correlation among these parameters. The stoichiometry and temperature dependence of CE uptake from HDL and recombinant HDL particles of defined composition support a model in which HDL binding to SR-BI results in the formation of a non-aqueous pathway through which HDL-CE molecules move down their concentration gradient into the adrenocortical cell membrane. By obtaining novel quantitative data with the Y1-B51 cell line with which Pittman and colleagues (9–11, 14–16, 18, 19, 24) made extensive kinetic measurements to establish the phenomenon of HDL-CE-selective uptake, it has been possible to build on their work and develop the first model of the mechanism by which SR-BI facilitates HDL-CE-selective uptake in this physiologically relevant system.

**EXPERIMENTAL PROCEDURES**

Preparation of 125I-Apolipoprotein- and [3H]Cholesteryl Hexadecyl Ether-labeled Reconstituted HDL Particles—Briefly, human apoA-I phospholipid (PL) discoidal complexes were prepared by adding 5.5 mg of total lipid composed of 1-palmitoyl,2-oleoyl-phosphatidylcholine (POPC, 98% grade, Avanti Polar Lipids) and cholesteryl oleate oleate to aqueous solutions in chloroform to a glass tube and mixing them so that an initial mole ratio of 99:5:0.5, 99:1:0.1, or 97:3 PL-to-CE was achieved. Thereafter, 40 μCi of [3H]CHE was added as a trace label to monitor CE transfer. This mixture of lipid and radiolabeled markers was dried into a film under a stream of nitrogen, then dialyzed overnight against saline buffer containing 97 mM potassium iodide, then with saline buffer without potassium iodide. Specific activities were typically 300–1000 dpm/ng of protein for [3H]-labeled HDL and 5–25 dpm/ng of CE for [3H]CHE-HDL.

Preparation of 125I-Apolipoprotein- and [3H]Cholesteryl Hexadecyl Ether-labeled Reconstituted HDL Particles—Briefly, human apoA-I phospholipid (PL) discoidal complexes were prepared by adding 5.5 mg of total lipid composed of 1-palmitoyl,2-oleoyl-phosphatidylcholine (POPC, 98% grade, Avanti Polar Lipids) and cholesteryl oleate oleate to aqueous solutions in chloroform to a glass tube and mixing them so that an initial mole ratio of 99:5:0.5, 99:1:0.1, or 97:3 PL-to-CE was achieved. Thereafter, 40 μCi of [3H]CHE was added as a trace label to monitor CE transfer. This mixture of lipid and radiolabeled markers was dried into a film under a stream of nitrogen, then dialyzed overnight against saline buffer containing 97 mM potassium iodide, then with saline buffer without potassium iodide. Specific activities were typically 300–1000 dpm/ng of protein for [3H]-labeled HDL and 5–25 dpm/ng of CE for [3H]CHE-HDL.

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Where necessary, preparative thin layer chromatography was used to isolate radiolabeled PL of > 99.9% purity. No detectable decomposition occurred after 2-h incubations of the rHDL particles with cells. In control experiments, the uptake of [14C]DPPC and [3H]DPPC was identical.

Determination of HDL Cell Association and HDL-CE-selective Uptake—Mouse Y1 and Y1-BS1 adrenal cells (27) were grown in Ham’s F-10 medium supplemented with 12.5% horse serum and 5% heat-inactivated fetal bovine serum (FBS) and 50 μg of Gentamycin/ml of medium (complete medium) in a humidified incubator equilibrated to 5% CO2, 95% air at 37 °C unless otherwise stated. For experiments, cells were seeded at 1.5 × 10^5 cells/well in 6-well plates or at 0.5 × 10^6 cells/well in 12-well plates. After 48 h, medium was replaced with 1.5 ml of serum-free medium containing either 100 nM ACTH (Sigma) or Cortrosyn, a synthetic 1-24 ACTH analogue (Organon), to up-regulate SR-BI expression (25). After 24 h, wells containing cells and empty wells that served as “no cell” controls were washed with serum-free medium, and serum-free medium containing 100 nM ACTH (or Cortrosyn) plus either dual-labeled HDL or dual-labeled discoidal rHDL particles was added, and wells were incubated for 2 h at 37 °C. Time-course experiments were carried out over a 4-h period. To eliminate loss of HDL by binding to plastic, the polypropylene test tubes used to prepare serum-free test medium were initially rinsed with a 10% bovine serum albumin solution, exhaustively washed with phosphate-buffered saline (PBS), then allowed to dry to produce both unlabeled and dual-labeled HDL or dual-labeled discoidal rHDL particles into them. All incubations were performed in a humidified incubator equilibrated to 5% CO2, 95% air at 37 °C unless otherwise stated.

At the end of the incubation period, medium was removed and placed in glass tubes; 250 μl of a 50% trichloroacetic acid solution was added, and 125I-degradation products released from the cells were measured as monoidotyrosine by the method described by Goldstein et al. (13). Cells were washed four times with 2 ml of ice-cold PBS containing 0.1% bovine serum albumin and then with a final wash of 4 ml of PBS. Cells were lysed with 1.25 ml of 0.1 M NaOH, and aliquots of the lysates were taken for: 1) hepane extraction to determine cell-associated HDL-CE (27), 2) trichloroacetic acid precipitation to distinguish between HDL-cell association (trichloroacetic acid-insoluble) and internalized and degraded apolipoprotein (trichloroacetic acid supernatant), and 3) protein determination by a modified Lowry (34) to measure cellular protein mass. This analysis assumes that cell-associated HDL is intact and trichloroacetic acid-precipitable, whereas internalized apolipoprotein is degraded and either remains in the cells or is released from the cells as 125I-degradation products.

The extent of HDL-whole particle association with cells was traced by the 125I-labeled apolipoprotein tracer, and the total uptake of HDL-CE was traced by [3H]CHE. The calculation of cell-associated HDL and HDL-CE-selective uptake was carried out as follows. 125I and 3H radioactivity obtained from y and scintillation counting were adjusted for the concentration dependences for HDL cell association and HDL-CE-selective uptake. In order to test the relationship between HDL particle binding and HDL-CE-selective uptake, we examined the concentration dependences for HDL cell association and HDL-CE-selective uptake. The data in Fig. 1 for HDL cell association represent the average from six separate experiments in which triplicate wells were incubated for 1 h. These experiments include data using single-labeled 125I-HDL and dual-labeled HDL as these particles exhibited similar extents of cell association. For selective uptake measurements, data from 2-h incubations were used to enhance the sensitivity. As shown in Fig. 1, both processes showed a concentration dependence indicative of high and low affinity components. The high affinity component for each process was resolved as described under “Experimental Procedures.” These results are consistent with those reported by Temel et al. (27). From nonlinear regression analysis, half-maximal high affinity cell association of HDL occurred at 8.7 ± 4.1 μg/ml and the Km of HDL-CE-selective uptake was 4.5 ± 1.5 μg/ml, as traced by [3H]CHE. These values are similar, suggesting that the two processes are linked and, indeed, there is a very high correlation between high affinity HDL-cell association and high affinity HDL-CE-selective uptake (Fig. 2). A similar correlation is also observed if total HDL-cell association and total HDL-CE-selective uptake are plotted (data not shown).

HDL binding isotherms were analyzed as follows. The total cell-associated 125I-HDL as a function of HDL concentration was analyzed by nonlinear regression (Prism) and tested for best fit to equations for three models, which describe: 1) high affinity and saturable binding to a single site (SR-BI), 2) high affinity and saturable binding to 2 sites (SR-BI being one of them), or 3) high affinity and saturable binding to one site (SR-BI) plus low affinity and nonsaturable binding to other sites. The best fit was found for the model with high affinity binding to a specific site (SR-BI) plus low affinity binding to other sites. The equation is given below,

\[ B_{\text{total}} = \frac{B_{\text{max,cell}}}[\text{HDL}] + C[\text{HDL}] \]  

(Eq. 1)

where \( B_{\text{total}} \) is the measured amount of HDL bound, \( B_{\text{max,cell}} \) is the amount of HDL bound at saturating concentrations of HDL, \( K_{\text{d,app,cell}} \) is the apparent high affinity \( K_{d} \) and \( C \) is the slope of the low affinity nonsaturable process. \( B_{\text{total}} \) was resolved into high and low affinity components by determining \( B \) and subtracting \( C \) (HDL) from \( B_{\text{total}} \) to generate the binding isotherm for the high affinity HDL interaction. The same equation (in which the high affinity term is analogous to the Michaelis-Menten equation) was also used to estimate \( K_{\text{HA}} \), the apparent \( K_{d} \) for the rate of CE-selective uptake. In some experiments, specific cell-associated 125I-HDL was calculated after subtracting values for bound HDL obtained in the presence of a 40-fold excess of unlabeled HDL from total cell-associated HDL (cf. Ref. 21). In this case, the binding isotherm was subjected to Scatchard analysis to estimate \( B_{\text{max}} \) and \( K_{\text{HA}} \).

Temperature Dependence of HDL-CE-selective Uptake—For temperature dependence studies, cells were grown in six-well plates and treated either ACTH or Cortrosyn as described under “Experimental Procedures.” On the day of the experiment, cells were washed with 2 ml of Ham’s F-10 medium supplemented with 100 mM HEPES (pH 7.4). Plates were equilibrated in water baths of the appropriate temperature for at least 5 min, and the test medium, equilibrated at the same temperature, was added. Visual examinations of the cells prior to, during, and after the 2-h incubation period showed no obvious cell toxicity. Uptake studies were done at the following temperatures (°C): 4, 15, 23, and 37. The activation energy for HDL-CE-selective uptake was calculated from the Arrhenius equation.

RESULTS

Relationship between HDL Binding and HDL-CE-selective Uptake—In order to test the relationship between HDL particle binding and HDL-CE-selective uptake, we examined the concentration dependences for HDL cell association and HDL-CE-selective uptake. The data in Fig. 1 for HDL cell association represent the average from six separate experiments in which triplicate wells were incubated for 1 h. These experiments include data using single-labeled 125I-HDL and dual-labeled HDL as these particles exhibited similar extents of cell association. For selective uptake measurements, data from 2-h incubations were used to enhance the sensitivity. As shown in Fig. 1, both processes showed a concentration dependence indicative of high and low affinity components. The high affinity component for each process was resolved as described under “Experimental Procedures.” These results are consistent with those reported by Temel et al. (27). From nonlinear regression analysis, half-maximal high affinity cell association of HDL occurred at 8.7 ± 4.1 μg/ml and the \( K_{m} \) of HDL-CE-selective uptake was 4.5 ± 1.5 μg/ml, as traced by [3H]CHE. These values are similar, suggesting that the two processes are linked and, indeed, there is a very high correlation between high affinity HDL-cell association and high affinity HDL-CE-selective uptake (Fig. 2). A similar correlation is also observed if total HDL-cell association and total HDL-CE-selective uptake are plotted (data not shown). When high affinity cell-associated 125I-HDL was determined by subtracting values obtained in the presence of a 40-fold excess of unlabeled HDL, half-maximal cell association of HDL occurred at 98 ± 14 μg/ml and the \( K_{m} \) of HDL-CE-selective uptake was 3.0 ± 0.5 μg/ml. These \( K_{m} \) values are similar to those reported by Pittman et al. (28). The use of excess unlabeled ligand often causes problems in estimating the slope of the low affinity component so that \( K_{d} \) is overestimated (35). Therefore, we believe the \( K_{m} \) and \( K_{d} \) values derived by fitting the data to a two-site model by nonlinear regression are probably more accurate. In selected experiments, the uptake of [3H]CHE and [3H]cholesteryl oleate was compared and found to be similar (178 ± 27 and 187 ± 54.
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It is well established that, during CE-selective uptake from donor particles, CE is taken up into cells without the uptake and lysosomal degradation of the apolipoproteins (see Ref. 36 for review). Much less is known about the uptake of the other lipid components of the donor particles. To examine the selectivity of SR-BI-mediated uptake for CE, the rates of PL and CE uptake from rHDL particles into cells were compared. Discoidal rHDL was radio-labeled with \(^{[3]H}\)CHE to track CE uptake and \(^{14}C\)DPPC to monitor PC uptake. Table I summarizes the composition of the particles used, the rates of CE and PC total cell association, the percentage of total and selective uptake of CE and PC and the relative fractional selective uptake of CE and PC. Note that the absolute rates of PC uptake are 25-fold higher than those for CE; however, the percentage of uptake of PC from rHDL as tracked by the DPPC label is about 4-fold lower than the percentage of uptake of CE. These results indicate that there is a non-stoichiometric uptake of HDL lipid components with CE being preferred over PC. In parallel experiments using \(^{[3]H}\)CHE/\(^{125}I\)-rHDL, we determined that 12% of the total CE uptake was due to rHDL cell association plus endocytosis and degradation and 88% was due to selective uptake. These values were used to calculate the rate constant (percentage of selective uptake per 2 h) for selective uptake of CE and PC. The ratio of the CE and PC rate constants is about 6/1, indicating that the selective uptake mechanism mediated by SR-BI discriminates for CE over PC (about 55% of total PC uptake was due to selective uptake). The kinetic data summarized in Table II further demonstrate that the structure of the transferring lipid molecule affects the rate of selective uptake into the Y1-BS1 cells. In particular, the rate of uptake of PL molecules depends upon the structure of their polar group. Thus, the rate constant for PS-selective uptake is about 8 times that of DPPC (Table II), which is similar to the ratio observed for CE (Table I). In contrast, the rate constant for PI-selective uptake is similar to that for DPPC whereas the rate constant for SM-selective uptake is much smaller (Table II); in fact, only about 10% of the total SM uptake is due to the selective pathway. It is important to note that the rates of radiolabeled PL-selective uptake were all measured using a common rHDL particle (containing \(\leq 1.5\) mol% of PL radiolabel) so that there are no confounding issues of either variations of particle size and charge, or differences in rHDL-cell interactions.

To test the influence of different CE contents on HDL-CE-selective uptake, reconstituted discoidal particles were prepared with different ratios of CE to apoA-I. Table III summarizes the rates of CE-selective uptake to Y1-BS1 adrenal cells from rHDL particles containing 0.6 mol% CE and 1.3 mol% CE. The binding of both rHDL particles to the cells was similar (data not shown) but increasing the CE content of rHDL particles resulted in an increase in the rate of CE-selective uptake. This increase in uptake was proportional to the amount of CE initially present in the donor particles; the ratio of the CE contents of the two types of particles was 2.3 to 1, while the ratio of CE-selective uptake was 2.6 to 1 (Table III). It is interesting to note that the uptake of CE is much greater from spherical human HDL\(_{50}\) particles, which are relatively enriched in CE relative to discoidal rHDL. Apparently, this is in contrast to the results of Pittman and colleagues (19), who found that larger HDL (with more CE) gave less CE-selective uptake. The reason for this discrepancy is not entirely clear, but it may be due to effects of HDL particle size on binding to SR-BI.

The temperature dependence for the selective uptake process was measured using particles containing tracers for apolipoprotein and CE. For these experiments, cells were equilibrated at four temperatures between 4 and 37 °C, and selective CE uptake was determined in a 2-h assay. Fig. 3 illustrates a typical Arrhenius plot generated when the temperature dependence of HDL-CE-selective uptake was measured; it is apparent that the rate of HDL-CE-selective uptake is reduced by cooling the system. As shown in Table IV, the activation energies for the uptake of CE from HDL\(_{50}\) and discoidal rHDL into
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TABLE I

| Phospholipid | Percent total uptake | Percent selective uptake | Relative selective uptake |
|--------------|----------------------|--------------------------|--------------------------|
| Phosphatidylcholine (PC) | 1.4 ± 0.21 | 0.8 ± 0.1 | 1 |
| Phosphatidylethanolamine (PE) | 6.9 ± 0.67 | 6.3 ± 0.6 | 7.8 |
| Phosphatidylinositol (PI) | 1.3 ± 0.31 | 0.7 ± 0.2 | 0.9 |
| Sphingomyelin (SM) | 0.68 ± 0.09 | 0.07 ± 0.03 | 0.08 |

a The molar composition of the rHDL particle was 80 POPC:1 cholesteryl oleate:0.5 [14C]DPPC:0.005 [3H]CHE:1.0 apoA-I, and the concentration of the particles in the extracellular medium was 30 μg of protein/ml.

b All uptake values are expressed as pmol of CE per 2 h. Values are mean ± S.D. from two experiments each with triplicate wells and includes total cell-associated CE and PC. Percent uptake is of moles of CE (cholesteryl oleate + CHE) and PC (POPC + DPPC) from donor rHDL particles as traced by the radiolabeled lipids.

c Values are fraction of mass uptake based on 88% of CE mass taken up selectively, using [125I]-apoA-I/[3H]CHE HDL particles.

d Value is mean from two sets of experiments (n = 6 wells).

The Stoichiometry of Phosphatidylcholine and Cholesteryl oleate uptake from discoidal rHDL particles

| rHDL particle | Total CE uptake | Total PC uptake | Percent total CE uptake | Percent total PC uptake | Percent selective CE uptake | Percent selective PC uptake | Relative fractional selective uptake of CE to PC |
|---------------|----------------|----------------|-------------------------|------------------------|---------------------------|------------------------------|-----------------------------------------------|
| [14C]DPPC    | 60 ± 9         | 1589 ± 166     | 5.5 ± 0.8               | 1.5 ± 0.15             | 4.8 ± 0.1                 | 0.94 ± 0.09                   | 6.2 ± 1.9                                    |

These studies were carried out with the Y1-BS1 adrenocortical cell line to provide a physiological model for the selective uptake process. In addition, previous studies with Y1-BS1 cells have shown that SR-BI is the major component responsible for the cell association of HDL particles and for the delivery of HDL-CE to the steroidogenic pathway (27). These findings are consistent with the idea that HDL comes on and off SR-BI related to the off-rate of LDL from its receptor (Kd ≈ 2 nm apo B-100). The dissociation constant (Kd) is a function of both the on and off rates (Kd = koff/kon) of a ligand and its receptor. For an approximately spherical ligand, the Stokes-Einstein equation predicts that the diffusion coefficient is inversely proportional to particle radius, so that the diffusion-limited on-rate for HDL (~10 nm diameter) should be two times faster than that of LDL (~25 nm). Knowing the ratio of the HDL and LDL Kd values, it can be estimated that the off-rate of HDL from SR-BI is about 300 times higher than the off-rate of LDL from its receptor. These findings are consistent with the idea that HDL comes on and off SR-BI relatively readily and may explain, in part, why HDL particles are not endocytosed by SR-BI. These rapid on and off interactions would facilitate lipid delivery by releasing the HDL particle once the HDL core CE has been transferred to the membrane, thereby freeing SR-BI for another round of interaction.

The similarity of the Kd and Kma values for HDL binding and HDL-CE-selective uptake argues that the two processes are linked. A high degree of correlation between HDL binding and CE-selective uptake is seen over the entire range of HDL binding (Fig. 2), indicating a tight correspondence between HDL binding to SR-BI and the subsequent transfer of HDL-CE into the cell.

Mechanism of SR-BI-mediated Uptake of Lipids—To determine the mechanism of SR-BI-mediated CE uptake, the fractional uptake of different components of recombinant HDL particles was examined. There are three possible pathways by which lipid molecules in the HDL particles can become cell-associated: 1) binding and uptake of intact rHDL, 2) diffusion of individual lipid molecules from rHDL through the aqueous phase to the cell plasma membrane, and 3) the selective uptake pathway mediated by SR-BI. The first pathway leads to stoichiometric uptake of the constituents of the rHDL particle. Thus, any non-stoichiometric lipid uptake is due to the second and third pathways. The situation is relatively simple for CE uptake.
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TABLE III
Rate of CE selective uptake from rHDL and HDL

| RHDL particle | Molar composition PL:CE:apoA-I | Relative CE contents | CE uptake^a | Relative CE uptake^b |
|---------------|---------------------------------|----------------------|--------------|----------------------|
| Low mol% CE-rHDL | 84.0:6.1                        | 1                    | 7.1 ± 1      | 1                    |
| High mol% CE-rHDL | 82.1:3.1                        | 2.3                  | 18.3 ± 2     | 2.6                  |
| HDL\(_{36}\) | 12.8:1.1                        | 14.5                 | 320 ± 13     | 46                   |

^a Values are shown as mean ± S.E. (average of two different experiments with n = 3 triplicate determinations in each experiment).

^b Relative rate of CE uptake when compared to low mol% CE containing rHDL.

^c Data obtained from Shen et al. (43).

![Fig. 3](image.png)

**Fig. 3. Arrhenius plot of HDL-CE-selective uptake.** The temperature dependence of HDL-CE-selective uptake in ACTH-stimulated Y1-BS1 cells was measured using 30 μg of protein/ml of dual-labeled ([^1]H)CE and ^125^I-apoA-I) HDL particles after a 2-h incubation.

![Fig. 4](image.png)

**Fig. 4. Model of SR-BI-mediated selective uptake of CE from HDL.** This model proposes that SR-BI contains a non-aqueous channel, which excludes water, and serves as a conduit for hydrophobic CE molecules diffusing from bound HDL down their concentration gradient to the cell plasma membrane. The scheme depicts a channel formed by a single SR-BI molecule, but it is possible that self-association of SR-BI is required to create the channel.

**TABLE IV**
Temperature dependence of HDL-CE-selective uptake

| System                  | Activation energy (kcal/mol) | Relative CE uptake^a |
|-------------------------|------------------------------|----------------------|
| CE uptake from HDL\(_{36}\) to Y1-BS1 | 9 ± 1^a                     | 1                    |
| CE uptake from rHDL to Y1-BS1 | 8 ± 1.5^b                   |                      |
| HDL\(_{36}\) cholesterol transfer to LDL via the aqueous phase^c | 17 ± 1^c                  |                      |

^a Values are shown as mean ± S.D. (average of two different experiments; n = 3 triplicate determinations).

^b Values are shown as mean ± S.D. (n = 3 triplicate determinations).

^c Data from Lund-Katz et al. (44).

because this very hydrophobic molecule does not transfer significantly by diffusion through the aqueous phase (38) so that the non-stoichiometric uptake of CE is entirely due to the SR-BI-mediated selective pathway. Uptake of PL molecules can occur by both aqueous diffusion and selective uptake pathways. Consequently, the assumption that non-stoichiometric uptake of PL is entirely due to SR-BI-mediated selective uptake leads to the PL rate constants in Tables I and II being upper limits for PL-selective uptake. However, the differences between the rate constants for PL-selective uptake (Table II) must arise from SR-BI-mediated uptake. This follows because the rate constants for the spontaneous transfer of PL molecules such as PC, PS, and SM from rHDL particles via aqueous diffusion are affected primarily by the hydrophobic content and not the nature of the polar headgroup (39, 40). With respect to the mechanism of HDL lipid-selective uptake, the marked differences in selective uptake between PL classes indicate that transient fusion of the HDL PL with the outer leaflet of the plasma membrane does not occur. If this were the case, it is difficult to envision how this process could give rise to the observed specificity of PL transfer.

The results in Table I show that the rate constant for selective uptake of CE is approximately 6-fold higher than that for PC. Thus, in Y1-BS1 adrenocortical cells, the SR-BI-mediated selective uptake process discriminates in favor of CE as compared with this class of PL. This result indicates that the selective uptake process does not arise simply from bringing the SR-BI-docked HDL particle close to the plasma membrane, but must involve subsequent steps that permit the selective removal of CE from the particle while leaving the PC behind. It is striking that the SR-BI-facilitated selective uptake seems not to discriminate between CE and PS molecules but does discriminate against PC, PI, and SM molecules relative to CE. The fact that the rate constants for selective uptake vary as shown in Tables I and II suggests that the putative non-aqueous channel created by SR-BI (see below and Fig. 4) can distinguish aspects of the structure of the transferring lipid molecules. The nonpolar CE molecule can transfer readily from the bound rHDL particle into the channel, whereas the more polar PC, PI, and SM molecules transfer less well. The charge on the PL molecule seems not to be critical, because PI and PS are both negatively charged at neutral pH but PS transfers as well as CE. Understanding of the molecular basis for this specificity requires more knowledge of the structure of SR-BI, but it is possible that the receptor contains a recognition site for PS that facilitates transfer of PS molecules out of the bound rHDL particles.

Comparison of reconstituted HDL particles containing different amounts of CE showed that CE-selective uptake increases proportionally with particle CE content (Table III). This was demonstrated by exposing cells to equivalent protein concentrations of donor rHDL which were virtually identical in composition except that one was 2.3-fold-enriched in CE versus the other. In this way, cells were exposed to identical numbers of rHDL particles. CE uptake was even greater from spherical HDL\(_{36}\) particles, which are even more enriched in CE, suggesting a mechanism in which CE moves down its concentration
gradient from the SR-BI-docked HDL particle to the plasma membrane. Since the capacity of the plasma membrane to accommodate CE is limited to 2–3 mol% with respect to the membrane PL, continued selective uptake must involve the removal of CE from the cytoplasmic side of the membrane either by a CE transfer mechanism or via CE hydrolysis. In the case of the adrenocortical cell, cholesterol utilization by the steroidogenic pathway would serve to drive this process.

The temperature dependence studies provided important quantitative information relative to the mechanism of the SR-BI-mediated uptake process, for which the rate-limiting step is presumably the selective transfer of CE molecules out of the HDL particle. The activation energy for the rate-limiting step in CE-selective uptake from either HDL or from reconstituted HDL (∼8–9 kcal/mol) is lower than the activation energy for the desorption of free cholesterol from HDL particles into the aqueous phase (Table IV). The aqueous solubility of CE is very low, and thus its movement into the cells via the aqueous phase would be extremely energetically unfavorable. It has been estimated that if CE transfers via the aqueous phase, then the activation energy associated with this process would be enormous and the process would require years (38). The finding that the activation energy for HDL-CE uptake is relatively low strongly suggests that HDL-CE uptake occurs via a non-aqueous pathway. We postulate that SR-BI binds HDL at the cell surface in such a way as to provide a “channel” from which water is excluded and along which CE molecules diffuse down the concentration gradient from HDL particles to the cell plasma membrane (Fig. 4). At this time, it is not clear whether one or several SR-BI molecules are involved in formation of the channel. This model is consistent with the results of a recent mutagenesis studies of SR-BI showing that its extracellular domain is responsible for facilitating transfer of lipid from bound HDL particles to the cell (41, 42).

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