Scavenger Receptor BI Transfers Major Lipoprotein-associated Phospholipids into the Cells*

Received for publication, May 11, 2000, and in revised form, July 26, 2000
Published, JBC Papers in Press, August 9, 2000, DOI 10.1074/jbc.M004031200

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The phospholipids of lipoproteins can be transferred to cells by an endocytosis-independent uptake pathway. We analyzed the role of scavenger receptor BI (SR-BI) for the selective cellular phospholipid import. Human monocytes rapidly acquired the pyrene (py)-labeled phospholipids sphingomyelin (SM), phosphatidylcholine, and phosphatidylethanolamine from different donors (low and high density lipoproteins [LDL, HDL], lipid vesicles). The anti-SR-BI antibody directed against the extracellular loop of the membrane protein lowered the cellular import of the phospholipids by 40–80%. The phospholipid transfer from the lipid vesicles into the monocytes was suppressed by LDL, HDL, and apoprotein A1. Transfection of BHK cells with the cDNA for human SR-BI enhanced the cellular import of the vesicle-derived py-phospholipids by 5–6-fold. In the case of the LDL donors, transfer of py-SM to the transfected cells was stimulated to a greater extent than the uptake of the other py-phospholipids. Similar differences were not observed when the vesicles and HDL were used as phospholipid donors. The concentration of LDL required for the half-maximal phospholipid import was close to the previously reported apparent dissociation constant for LDL binding to SR-BI. The low activation energy of the SR-BI-mediated py-phospholipid import indicated that the transfer occurs entirely in a hydrophobic environment. Disruption of cell membrane caveolae by cycloheximide treatment reduced the SR-BI-catalyzed incorporation of py-SM, suggesting that intact caveolae are necessary for the phospholipid uptake. In conclusion, SR-BI mediates the selective import of the major lipoprotein-associated phospholipids into the cells, the transfer efficiency being dependent on the structure of the donor lipoprotein.

Phospholipids are known to play essential roles in a multitude of cellular processes such as, for example, in intracellular signal transduction (1) and as cofactors of enzyme complexes mediating the proteolytic activation of coagulation proteins (2). Phospholipids are asymmetrically distributed over the two halves of cellular membranes, the asymmetry being generated by proteins catalyzing the transbilayer movement of the lipid molecules (3). Furthermore, there is also lateral separation of the phospholipids within the single monolayers. Indeed, the plasma membranes of many cells contain clusters of sphingolipids, cholesterol, and specific proteins known as caveolae and lipid rafts (4, 5). In order to maintain the physiologic intramembrane and intramonomer composition of the phospholipids, the influx and efflux of the phospholipids from the plasma membranes must be tightly regulated.

The cellular uptake of phospholipids from extracellular donors is part of this regulatory system. In humans, plasma lipoproteins are a major extracellular source for phospholipids. By means of the endocytosis of low density lipoproteins (LDL), cells also incorporate phospholipids. The capacity of different blood components to take up LDL particles by receptor-mediated endocytosis exhibits a great range of variation. For example, platelets and red blood cells barely acquire the lipoproteins through the endocytotic pathway as they lack the classic LDL receptor. Furthermore, other particles such as the high density lipoproteins (HDL), which carry equally high amounts of phospholipids as the LDL in the human plasma compartment, are in general only weakly incorporated via endocytosis.

Previous data indicate a further pathway of cellular phospholipid uptake, the selective (endocytosis-independent) import of lipoprotein-derived phospholipids into the cells.

The rapid phospholipid incorporation mediated by this pathway was shown to supply platelets and monocytes with highly polyunsaturated fatty acids such as arachidonic acid necessary for the production of the autocrine and paracrine eicosanoids (6, 7). Furthermore, following platelet activation, the stimulation of the import of specific phospholipids (8) was shown to enhance the procoagulant activities of the platelet surface. The selective phospholipid import was thus shown to play a major role in the cellular uptake of essential fatty acids and to modulate intra- and extracellular signal transduction pathways. The mechanisms mediating the selective phospholipid uptake are mostly unknown. Scavenger receptor BI (SR-BI), a cell membrane protein previously shown to mediate the selective uptake of cholesterol (9, 10), could also be involved in the cellular incorporation of the phospholipids (11, 12). In the present investigation, we studied the role of SR-BI for the selective phospholipid import into the cells. We find that SR-BI is able to form a hydrophobic environment, which allows the rapid extraction of the phospholipids from the lipoprotein donors and

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* This work was supported by grants from the Deutsche Forschungsgemeinschaft (to B. E.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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¶ The abbreviations used are: LDL, low density lipoprotein(s); HDL, high density lipoprotein(s); PC, phosphatidylcholine; PE, phosphatidylethanolamine; SM, sphingomyelin; apoA, apoprotein A; apoB, apoprotein B; BHK, baby hamster kidney cells; DPBS, Dulbecco’s phosphate-buffered saline; EMEM, Earle’s modified Eagle’s medium; BBMV, brush border membrane vesicle(s); BSA, bovine serum albumin; py, pyrene; SR-BI, scavenger receptor BI.
their subsequent insertion into the cellular aceta membranones, the transfer of sphingomyelin (SM) from LDL to the cells being catalyzed with particular efficiency.

**EXPERIMENTAL PROCEDURES**

**Materials**—1-Palmitoyl-2-lyrpyridenedecanoyl-sn-3-glycerophosphorycholine (py-PC), 1-palmitoyl-2-lyrpyridenedecanoyl-sn-3-glycerophosphoryethanolamine (py-PE), and (N-lyrpyridenedecanoyl)-sphingomyelin (py-SM) were from Sigma and from Molecular Probes. [1,25]Choline]SM and [1,25]Choline]PC were purchased from PerkinElmer Life Sciences. Egg phosphatidylcholine (egg PC) and bovine serum albumin (BSA) were from Sigma and 2-hydroxypropyl-β-cyclodextrin was obtained from ICN. The microbeads conjugated with the anti-CD14 antibodies and the positive selection column were purchased from Miltenyi Biotec. Earle’s modified Eagle’s medium (EMEM) and Dulbecco’s phosphate-buffered saline (DBPS) were from Pan Biotech and BioWhittaker. Genetin and Opti-MEM were from Life Technologies, Inc., and isotonic Ficoll was from Amersham Pharmacia Biotec. The polyclonal anti-human SR-BI antibody against the extracellular loop of human SR-BI was raised in goat as described previously (12) and purified using a Sepharse G column (MabTrap®GII, Amersham Pharmacia Biotech). Apoprotein A-I (apoA) was prepared as described previously (12).

**Isolation of Blood Cells**—Monocytes were isolated by buoyant density centrifugation of peripheral blood mononuclear cells (PBMC) from heparinized human blood. The buffy coats were incubated with microbeads coupled to anti-human CD14 antibodies and then suspended in phosphate-buffered saline supplemented with 0.15% EDTA and 0.1% BSA (pH 7.4, washing buffer). After centrifugation at 1200 × g for 30 min, the cells were resuspended in a buffer containing 138 mM NaCl, 2.7 mM KCl, 12 mM NaHCO₃, 0.4 mM NaH₂PO₄, 1 mM MgCl₂, 5 mM D-glucose, 5 mM HEPES, and 2 mM CaCl₂ (pH 7.35, resuspension buffer). For the isolation of platelets,uffy coats were prepared from citrated whole blood. The buffy coats were incubated with microbeads conjugated to anti-human CD14 antibodies. Theuffy coats were underlayered with low endotoxin Ficoll (density, 1.077). After centrifugation for 25 min at 500 × g, the interphase was collected and suspended in phosphate-buffered saline containing 0.13% EDTA and 0.15% BSA (pH 7.4, washing buffer). After centrifugation at 200 × g, the pellet was resuspended in washing buffer and again centrifuged at 1200 × g. The cell pellet obtained after the last centrifugation step containing the peripheral blood mononuclear cells was suspended in phosphate-buffered saline supplemented with 0.15% EDTA and 0.5% BSA (pH 7.4, antibody buffer). The peripheral blood mononuclear cell suspension was incubated at 8°C for 15 min with microbeads conjugated with anti-human CD14 antibodies and thereafter passed over a positive selection column. After removal of the magnetic beads, the monocytes were eluted from the column with a phosphate-buffered antibody buffer. The suspension was again centrifuged at 1200 × g and then resuspended in a buffer containing 138 mM NaCl, 2.7 mM KCl, 12 mM NaHCO₃, 0.4 mM NaH₂PO₄, 1 mM MgCl₂, 5 mM D-glucose, 5 mM HEPES, and 2 mM CaCl₂ (pH 7.35, resuspension buffer). For the isolation of the neutrophils, buffy coats were prepared from the citrated blood. The buffy coats were incubated with microbeads coupled to anti-neutrophil (neut) antibodies for 15 min at 4°C. The buffy bead suspensions were applied onto the positive selection column and neutrophils recovered by elution with the antibody buffer. The purity of the neutrophil suspensions was 95%. Platelets were isolated as described before (8).

**Cell Culture**—Baby hamster kidney (BHK) cells were maintained in a 37°C humidified 95% air, 5% CO₂ incubator in medium A (EMEM with glutamine supplemented with nonessential amino acids, 0.8 mg/ml Geneticin, and 10% heat-inactivated BSA). BHK cells were usually cultured for 48 h in medium A until they were subconfluent. After two washing steps with DPBS, the cells were refed with Opti-MEM supplemented with nonessential amino acids) and maintained for another 24 h. The cells were gently dissolved from the culture dish by a 5-min incubation at 37°C with 1 ml of a 2% EDTA buffer containing 0.5% BSA. The cells were rinsed from the dish with the resuspension buffer, centrifuged at 1200 × g, and suspended in resuspension buffer.

**Cloning of Human SR-BI and Stable Transfections**—Total RNA from HepG2 cells was used for reverse transcriptase-based polymerase chain reaction. One microgram of total RNA was subjected to reverse transcriptase-based polymerase chain reaction according to the SuperScript one-step kit protocol (Life Technologies, Inc.). The sequences of the primers used in the amplification of the human cDNA were 5′-CCCAAGCCGCCGAGACATGG (forward primer) and 5′-TACAGTTTTGCTTCCTGTCG (reverse primer). The main amplification product was a fragment of 15146 base pairs comprising the complete coding region plus 15 and 1 base pair of the 5′ and the 3′-untranslated region, respectively. The sequence was verified by cycle sequencing with the ABI PRISM BigDye terminator cycle sequencing kit (PE Biosystems, Foster City, CA). The cDNA fragment was cloned into the mammalian expression vector pBK-CMV (Stratagene, La Jolla, CA). The cDNA fragment was cloned into the mammalian expression vector pBK-CMV (Stratagene, La Jolla, CA). The cDNA fragment was cloned into the mammalian expression vector pBK-CMV (Stratagene, La Jolla, CA). The cDNA fragment was cloned into the mammalian expression vector pBK-CMV (Stratagene, La Jolla, CA). The cDNA fragment was cloned into the mammalian expression vector pBK-CMV (Stratagene, La Jolla, CA). The cDNA fragment was cloned into the mammalian expression vector pBK-CMV (Stratagene, La Jolla, CA). The cDNA fragment was cloned into the mammalian expression vector pBK-CMV (Stratagene, La Jolla, CA).

**RESULTS**

SR-BI Mediates Phospholipid Import into Monocytes—We first tested whether SR-BI, a mediator of the selective sterol uptake, was present in the cellular membranes of the platelets. Previous work indicated that platelets take up phospholipids nearly exclusively by the endocytosis-independent, protein-mediated pathway (see Introduction). Western blot analysis of the solubilized platelets using the affinity-purified goat anti-human SR-BI antibody gave no immunoreactive band (Fig. 1A). In contrast, a single band with an apparent molecular mass of 82
SR-BI Mediates Phospholipid Import

**TABLE I**

| Lipoprotein | py-phospholipid transfer | % of py-phospholipid in donor |
|-------------|--------------------------|-------------------------------|
| LDL         |                          |                               |
| Control     | 0.40 ± 0.11              | 0.53 ± 0.17                   | 0.90 ± 0.38                   |
| + Anti-SR-BI| 0.24 ± 0.09              | 0.28 ± 0.06                   | 0.50 ± 0.22                   |
| + Isotype antibody | 1.01 ± 0.08  |                               |
| HDL         |                          |                               |
| Control     | 0.31 ± 0.09              | 0.32 ± 0.11                   | 0.18 ± 0.08                   |
| + Anti-SR-BI| 0.20 ± 0.07              |                               |

LDL and HDL particles enriched with the indicated py-phospholipids were incubated for 10 min at 37 °C with the monocytes (10^6) in the absence or presence of the anti-SR-BI antibody (0.1 mg/ml). The lipoproteins were added at 20 μg of protein/ml corresponding to 23 nmol (LDL) and 26 nmol (HDL) of total phospholipids. Table represents means ± S.D. from three to five experiments.

**Legend**

- LDL: Low-density lipoprotein
- HDL: High-density lipoprotein
- py-phospholipid transfer: Incorporation of py-phospholipids from lipoprotein donors to the monocytes
- % of py-phospholipid in donor: Percentage of py-phospholipid transfer from lipoprotein donors

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**FIG. 1**

A. Western blot analysis for the detection of SR-BI in human blood cells. Lane 1, rabbit BBMV; lane 2, human monocytes; lane 3, human platelets; lane 4, human neutrophils; lane 5, Caco-2 cells. The affinity-purified goat anti-human SR-BI was used as the primary antibody. In each lane 50 μg of protein were applied. The arrow on the left marks the position of the 84 kDa marker protein. BBMV were prepared according to Ref. 12. B, py-phospholipid transfer from LDL to the monocytes. LDL particles (20 μg/ml) enriched with the py-phospholipids py-PC, py-PE, and py-SM were incubated for the indicated time periods at 37 °C with the isolated monocytes (10^6). Figure shows means ± S.D. from three to five independent experiments.

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kDa was observed in the solubilized monocytes and neutrophils. Caco-2 cells and brush border membrane vesicles (BBMV) were used as a positive control yielding a single band at 84 kDa previously assigned to SR-BI (12). The differences in the molecular weight of SR-BI between the cell types are likely due to different cell specific glycosylation patterns. Thus, although the protein could not be detected in the platelets, monocytes were found to contain SR-BI, in accordance with previous data (14). We therefore used the monocytes to evaluate the participation of SR-BI in the selective cellular uptake of the phospholipids.

SR-BI is known to bind LDL particles with high affinity (15). LDL (20 μg protein/ml) enriched with py-labeled phospholipids (PC, PE, or SM) were incubated with the isolated monocytes, and the selective import of the phospholipids was measured under on line conditions. The incorporation of the py-phospholipids steeply increased within the first 10 min of incubation (Fig. 1B), a slower uptake rate being observed at longer incubation periods (up to 60 min; data not shown). The amount of py-SM transferred from LDL to the monocytes within 10 min was 2.1- and 1.7-fold higher than the one of py-PC and py-PE, respectively (Fig. 1B). In the presence of the anti-SR-BI-antibody (0.1 mg/ml), the incorporation of the py-phospholipids was reduced by 40% (py-PC), 47% (py-PE), and 44% (py-SM) (Table I). The isotype-matched control antibody had no effect on the transfer of py-SM from the LDL particles to the monocytes. The transfer of the py-phospholipids from HDL to the monocytes was analyzed at total protein and phospholipid concentrations similar to those in the experiments with the LDL donors (see legend to Table I). The import of HDL originating py-PC and py-SM into the monocytes was lowered by 36% and 44%, respectively (Table I). Together, the data suggested that the translocation of the phospholipids from the lipoproteins to the monocytes was mediated partly by SR-BI.

In order to evaluate whether the apoprotein components of the lipoproteins were necessary for the phospholipid uptake catalyzed by SR-BI, egg PC vesicles were used as phospholipid donors instead of the lipoproteins. The monocytes rapidly acquired the py-phospholipids from the vesicle donors (data not shown). The amount of py-PC, py-PE, and py-SM being transferred from the vesicles to the monocytes within a 10-min incubation interval was reduced by 70%, 77%, and 62% in the presence of the anti-SR-BI antibody (Fig. 2). Accordingly, the transfer of the phospholipids from the vesicles to the monocytes was mainly catalyzed by SR-BI.

We next analyzed whether the lipoproteins competed with the lipid vesicles for the interaction with SR-BI. Unlabeled LDL and HDL were added to the vesicle-monocyte mixtures. The transfer of py-labeled PC, PE, and SM from the vesicles to the monocytes was lowered by 75–93% in the presence of the LDL particles (at 20 μg of protein/ml; Fig. 3A). At comparable protein concentrations, the addition of HDL decreased the py-phospholipid transfer between the vesicles and the cells by 53–61%. ApoAI (10 μg/ml), the main apoprotein of the HDL particles (which is not present in LDL), was previously shown to reduce the selective sterol uptake via SR-BI (12). ApoAI diminished the transfer of the py-phospholipids by 84% (py-PC), 75% (py-PE), and 53% (py-SM). The concentration dependence of the influence of apoAI on the exchange of py-SM between the lipid vesicles and the monocytes is shown in Fig. 3B. Elevating the apoAI concentration from 0 to 10 μg/ml increasingly reduced the py-SM transfer. At the concentration of 10 μg/ml, the py-SM import was maximally inhibited. The lipoprotein particles thus competed with the phospholipid vesicles for the interaction with SR-BI, the apoAI component being likely responsible for the inhibition elicited by HDL.

**SR-BI Particularly Enhances Uptake of LDL-derived SM**—To characterize in more detail the phospholipid import catalyzed by SR-BI, BHK cells were transfected with the cDNA for human SR-BI. In the wild type cells, no SR-BI mRNA...
expression could be observed (Fig. 4A). A strong band corresponding to the SR-BI transcript was found in the transfected cells. The mRNA expression level in the SR-BI-transfected cells was greater than the one of HepG2 cells, a cell line known to incorporate cholesterol in a selective manner through SR-BI (16). The translocations of py-PC, py-PE, and py-SM from egg PC vesicles to the BHK cells were analyzed within a 10-min incubation interval. The uptake of the phospholipids by the transfected cells was increased by 4.8- (py-PC), 5.6- (py-PE), and 5.2-fold (py-SM) compared with the wild type cells (Fig. 4B).

In Fig. 5, the lipoproteins were used as phospholipid donors instead of the lipid vesicles. The transfer of the phospholipids from the LDL particles (2 μg of protein/ml) into the BHK cells was analyzed at a total LDL phospholipid concentration comparable to the one of the donor vesicles employed in Fig. 4B. The SR-BI-transfected cells acquired 2.8- and 3.6-fold more py-PC and py-PE, respectively, than the wild type cells. The amount of py-SM taken up by the transfected cells was 7.5-fold higher than the amount of py-SM incorporated into the native cells (Fig. 5). At the LDL concentration of 20 μg/ml, the stimulation of py-SM import by SR-BI was again increased as compared with the acceleration of uptake of the glycerophospholipids. The transfer of the phospholipids from HDL particles (20 μg/ml) to the BHK cells was stimulated by 2.4- (py-PC), 3.2- (py-PE), and 2.8-fold (py-SM) after transfection with the SR-BI cDNA (Fig. 5). In further experiments, LDL particles (65 μg/ml) enriched with [14C]PC or [14C]PE were incubated with BHK cells (3 × 10⁶) for 10 min at 37°C. Thereafter, a 50-fold excess of unlabelled LDL was added in order to remove the lipoproteins bound to the extracellular surface of the cells. The cell-associated [14C]-SM thereafter determined was increased by 3.4 ± 1.1-fold in the SR-BI-transfected cells as compared with their wild type controls. Following incubation with [14C]-PC-LDL and removal of the bound LDL, the cell-associated [14C]-PC was 2.1 ± 0.6-fold higher in the transfected cells than in the wild type BHK cells (means ± S.D. of duplicate determinations from two to three independent experiments). The data with [14C]-labeled phospholipids thus essentially confirm the results obtained with the py-labeled phospholipids. Together, our observations suggest that SR-BI catalyzes the uptake of LDL-derived SM with particular efficiency.

Dependence of Phospholipid Uptake on Donor Concentration, Temperature, and Cell Membrane Organization—The preferential stimulation of the incorporation of py-SM originating from LDL led us to analyze the concentration dependence of the transfer. The amount of py-SM transferred from LDL to the cells in a SR-BI-dependent way steeply increased between 0 and 10 μg of LDL protein/ml (Fig. 6). No further increase of the import was noted at the concentration of 20 μg/ml. The apparent Kₘ value for the SR-BI-mediated phospholipid import was at 4.4 μg of LDL protein/ml. The curve for the concentration dependence of the SR-BI-catalyzed transfer of py-PE from the lipoproteins to the BHK cells was less steep than the one for the translocation of py-SM (Fig. 6). The apparent Kₘ value for the SR-BI-promoted py-PE transfer amounted to 7.1 μg of LDL protein/ml. Taken together, the results confirm the high affinity of SR-BI toward LDL-associated SM.

In order to calculate the activation energy of the SR-BI-catalyzed transfer of py-SM, the temperature dependence of the phospholipid import was studied. Suspensions of BHK cells together with py-SM contained in either LDL particles (20 μg
of protein/ml) or lipid vesicles (1.9 μg of egg PC and 0.6 μg of py-phospholipid) were equilibrated at 4 °C, 15 °C, 22 °C, and 37 °C. Arrhenius plots were generated from the temperature dependence of the differences in the py-SM uptake between the transfected and the wild type cells. For the SR-BI-mediated import of LDL-derived py-SM, a value of 7.1 ± 0.9 kcal/mol was obtained. In the case of lipid vesicles as py-SM donors, the activation energy of the SR-BI-catalyzed transfer amounted to 8.2 ± 1.3 kcal/mol (means ± S.D. from three experiments). These values suggest that the SR-BI-mediated phospholipid translocations occur exclusively in a hydrophobic environment.

SR-BI is known to be mostly localized in caveolae, lateral domains of the cell membrane rich in sphingomyelin and cholesterol (17, 18). We analyzed whether the SR-BI-mediated import of SM was dependent on the integrity of the caveolae. BHK cells were treated with cyclodextrin, a procedure that is known to disrupt the sphingolipid rich domains of the plasma membrane by depleting the cells of cholesterol (19). The SR-BI-mediated transfer of py-SM from lipid vesicles to the wild type BHK cells remained unchanged after the cholesterol removal and subsequent reenrichment of the cells with the sterol (Table II). The import of the sphingolipid into the transfected BHK cells was lowered by 29% after the 30-min incubation with cyclodextrin. Refilling the cholesterol pool of the transfected cells reincreased the import of the py-labeled SM, the amount of the sphingolipid incorporated being similar to the one taken up by the control cells. In transfected cells that had been treated for 60 min with cyclodextrin, the py-SM import was reduced by 61%. Accordingly, intact caveolae were necessary for the SM uptake mediated by SR-BI.

**DISCUSSION**

LDL and HDL particles are the main carriers of phospholipids in the human plasma compartment. Structural models of the lipoproteins predict that the phospholipids are arranged in a monolayer that surrounds the core of the particles. Both in HDL and LDL, choline (PC, SM) and ethanolamine phospholipids (PE) form nearly 90% of total phospholipids. Although the importance of the lipoproteins for the cellular cholesterol homeostasis is well documented, much less is known about their relevance for the cellular phospholipid metabolism. By means of the cellular uptake and efflux of phospholipids, lipoproteins are able to exchange these lipid molecules with the

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**FIG. 4.** Transfection of BHK cells with the cDNA for human SR-BI enhances the cellular phospholipid import. A, overexpression of human SR-BI in stably transfected BHK cells. Northern blot analysis of total RNA (10 μg/lane) hybridized to a probe generated from full-length human SR-BI cDNA. Endogenous SR-BI of HepG2 cells is larger due to differences in 5’- and 3’-untranslated regions. B, wild type (open columns) and SR-BI-transfected (filled columns) BHK cells (5 × 10⁴) were incubated for 10 min at 37 °C with lipid vesicles enriched with the py-phospholipids (1.9 μg/ml egg PC and 0.6 μg/ml py-phospholipid). Figure shows mean values ± S.D. from five experiments.

**FIG. 5.** SR-BI preferentially enhances the import of LDL-derived py-SM. Wild type and SR-BI-transfected BHK cells (5 × 10⁴) were incubated for 10 min at 37 °C with two different concentrations of LDL particles (2 μg of protein/ml, striped columns; 20 μg/ml, black columns) (A) and with HDL (20 μg of protein/ml) (B) enriched with the py-phospholipids. Figure shows mean values ± S.D. from three to five experiments.

**FIG. 6.** Concentration dependence of the py-phospholipid transfer between LDL and BHK cells. Wild type and SR-BI-transfected BHK cells (5 × 10⁴) were incubated for 10 min at 37 °C with different concentrations of LDL particles enriched with the indicated py-phospholipids. Data are representative of a total of three experiments.
SR-BI Mediates Phospholipid Import

Table II

Cyclodextrin treatment reversibly decreases cellular py-SM import mediated by SR-BI

| Wild type SR-BI transfected | % of import in untreated cells |
|-----------------------------|-------------------------------|
| Control cells               | 100                           |
| + 30-min cyclohextrin       | 104 ± 13                      |
| + 60-min cyclohextrin       | 104 ± 36                      |
| + 30-min cyclohextrin       | 91 ± 24                       |
| + 30-min cholesterol        | 100 ± 10                      |

Wild type and SR-BI-transfected BHK cells (5 × 10⁴) were incubated for 30 and 60 min at room temperature with 2% cyclodextrin (vol/vol) in order to disrupt the cells for 30 min. The results are expressed as the percentage of the phospholipid uptake by the untreated cells. The liposomes were incubated with the cells in an endocytosis-independent manner. SR-BI was previously shown to mediate the selective uptake of different cholesterol species into the cells (9, 10) and to promote the efflux of free and esterified cholesterol (11). In particular, SR-BI was found to catalyze the absorption of cholesterol in the small intestine (12) and to deliver the sterol to adrenal cells (20), where it might serve as the precursor for the synthesis of steroid hormones. The prominent role of SR-BI in the translocations of cholesterol led us to investigate its function for the selective phospholipid import. We first analyzed the effect of the anti-SR-BI antibody on the transfer of the major lipoprotein phospholipids into the monocytes, blood cells that constitutively express SR-BI. The antibody markedly reduced the cellular phospholipid import indicating that SR-BI catalyzed the greater part of the phospholipid uptake by the monocytes. SR-BI appeared to be absent from platelets, blood components that incorporate phospholipids predominantly by the endocytosis-independent pathway. The platelet phospholipid import was previously shown to be protein-mediated (8, 13). Platelets will thus provide a valuable model for studying the SR-BI-independent phospholipid uptake in future studies. In BHK cells transfected with the cDNA for human SR-BI, the selective incorporation of the phospholipids was strongly enhanced. These data confirmed that SR-BI promotes the phospholipid import into the cells. Thus, SR-BI represents the first membrane protein known to mediate the endocytosis-independent cellular uptake of phospholipids.

LDL and HDL particles were shown to serve as donors for the SR-BI-catalyzed cellular uptake of the phospholipids. The LDL concentration allowing half-maximal phospholipid import through SR-BI (Fig. 5) was in the same range as the Kₜ for the LDL binding to SR-BI (around 5 μg of protein/ml; Ref. 15). This suggests that the lipoprotein binding to SR-BI is essential for the delivery of the phospholipids. In addition, SR-BI was found to transfer phospholipids from pure vesicles into the cells. The latter finding indicates that SR-BI is in principle able to directly interact with the phospholipids of the donors, independent of the presence of the apoproteins. The lipoproteins inhibited the transfer of the phospholipids from the lipid vesicles to the cells. Apparently, the different phospholipids competed for the interaction with SR-BI. Together, these data suggest that the interaction of SR-BI with the phospholipids of the lipoprotein monolayer might be sufficient for the delivery of the phospholipids to the cells, as discussed previously (14). Nevertheless, apoAI alone also reduced the phospholipid exchange between the lipid vesicles and the cells. This apoprotein is likely to mediate the interaction of HDL with SR-BI. Thus, at least in the case of phospholipid transfer between the HDL particles and the cells, the apoprotein component might also be involved in the phospholipid exchange catalyzed by SR-BI. Overall, the results are in line with the view that SR-BI represents a multiligand protein with binding sites for both apoproteins and phospholipids. It remains to be investigated how the interplay between the different ligands precisely regulates the lipid uptake via SR-BI.

The activation energies for the SR-BI-catalyzed py-SM transfer across the extracellular medium were calculated to amount to 7–8 kcal/mol. A similar value has previously been obtained for the activation energy of the SR-BI-mediated translocation of cholesterol esters (21). Importantly, the diffusion of SM through the aqueous phase is known to require activation energies of 21–25 kcal/mol (22). Thus, SR-BI is able to reduce the activation energy for the SM transfer between the two lipidic compartments by about two thirds. Our data suggest that the protein-mediated transfer of SM across the extracellular medium takes place in a hydrophobic environment. The SR-BI-mediated transfer process, which involves the extraction of the phospholipid from the donor (desorption) and the insertion of the phospholipid into the acceptor cells, thus occurs in a way that contact of the phospholipid with water is completely excluded.

When analyzing the transfer of the phospholipids from the HDL particles to the cells, we noticed that SR-BI catalyzed the incorporation of PE, PC, and SM with rather equal efficiency. The transfer of the vesicle-derived phospholipids was also stimulated to a similar degree by SR-BI. A different pattern was observed when LDL particles were employed as phospholipid donors. In this case, SM was more efficiently transferred to the cells by SR-BI as compared with PC and PE. Previous work indicates that SR-BI plays an important role in the metabolism of LDL and apoprotein B (apoB)-containing lipoproteins in general. Overexpression of SR-BI led to a marked reduction in the plasma contents of lipoproteins with apoB (23) and polymorphisms in the SR-BI gene were found to be associated with variations in the plasma LDL concentrations (24). Furthermore, SR-BI was found to bind LDL with high affinity (15) and to supply cells with cholesteryl esters (25). Earlier investigations show that the LDL-associated PC is mostly bound to apoB100, the major protein component of the lipoprotein. In contrast, the greater proportion of SM is able to move freely within the LDL phospholipid monolayer (26, 27). In the HDL particles, the movements of SM are most probably restricted due to the interaction of the sphingolipid with apoAI (28). In addition to other factors, these particular phospholipid-protein interactions may contribute to the impaired capacity of discoidal HDL to deliver SM (as compared with PC) via SR-BI (21). Taken together, it appears likely that the LDL-associated SM is more easily available for the interaction with SR-BI compared with the LDL bound PC. This most probably facilitates the desorption of the sphingolipid from the LDL monolayer by SR-BI. Overall, the results indicate that the structure of the donor particle plays a critical role in determining the selectivity of the SR-BI-mediated phospholipid uptake.

The phospholipids of the plasma membranes are not only asymmetrically distributed over the two leaflets, they are also laterally separated within the membranes. Sphingolipids such as SM are clustered within the cell membranes in caveolae and lipid rafts, together with cholesterol, caveolin-1, and other specific proteins (4, 5). When the caveolae were disrupted by treating them for different time periods with cyclodextrin, the SR-BI-mediated SM uptake was increasingly reduced. Thus, intact caveolae are required for the phospholipid uptake. On the other hand, the delivery of the phospholipids to the cells can occur by a selective uptake mechanism whereby phospholipids are transferred to the cells in an endocytosis-independent manner.
hand, SR-BI was previously shown to be localized in caveolae and to transfer its substrates into these membrane domains (18). Taken together, the results of the present and earlier investigations suggest that SR-BI promotes the uptake of the two major lipid components of the caveolae, namely SM and cholesterol.

In conclusion, SR-BI is shown to catalyze the transfer of the major lipoprotein-associated phospholipids into the cells. Lipoproteins and pure lipid vesicles can principally serve as phospholipid donors for the SR-BI-mediated phospholipid uptake. However, the preferential stimulation of the import of LDL-derived SM by SR-BI indicates that the monolayer structure of the donor lipoprotein is of relevance for the facilitation of the phospholipid transfer. Although intact sphingolipid-cholesterol-rich areas of the plasma membrane are required for the efficient insertion of the phospholipid into the cells, SR-BI is also likely to contribute to the maintenance of these domains by supplying their main lipid constituents.

Acknowledgments—We thank Dr. M. Phillips and Dr. F. Rinninger for helpful suggestions.

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