Endocytosis is not required for the selective lipid uptake mediated by murine SR-BI

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Abstract

The scavenger receptor class B, type I (SR-BI) mediates the cellular selective uptake of cholesteryl esters and other lipids from high-density lipoproteins (HDL) and low-density lipoproteins (LDL). This process, unlike classical receptor-mediated endocytosis, does not result in lipoprotein degradation. Instead, the lipid depleted particles are released into the medium. Here we show that selective lipid uptake mediated by murine SR-BI can be uncoupled from the endocytosis of HDL or LDL particles. We found that blocking selective lipid uptake by incubating cells with the small chemical inhibitors BLT-1 or BLT-4 did not affect endocytosis of HDL. Similarly, blocking endocytosis by hyperosmotic sucrose or K$^+$ depletion did not prevent selective lipid uptake from HDL or LDL. These findings suggest that mSR-BI-mediated selective uptake occurs at the cell surface upon the association of lipoproteins with mSR-BI and does not require endocytosis of HDL or LDL particles.
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1. Introduction

The high-density lipoprotein (HDL) receptor SR-BI (scavenger receptor class B, type I) plays an important role in the metabolic control of HDL [1]. SR-BI mediates the selective uptake of cholesteryl esters [2] and other lipids [3–6] from HDL particles by cells, and it also facilitates the transfer of cholesterol from cells to HDL or to other acceptors present in the extracellular environment [7–9]. In addition, SR-BI binds to [10] and mediates selective lipid uptake from LDL [11–14], although to a lesser extent than from HDL. Results from in vitro experiments indicate that liposomes containing SR-BI and no other proteins are sufficient to sustain the selective transfer of cholesteryl esters from HDL particles to these liposomes [15].

Intracellular lipoprotein degradation is not required for selective lipid uptake in cells expressing SR-BI, because subsequent to lipid depletion most or all the HDL [2,16–18] or LDL particles [19–21] are released to the extracellular space. Although this is consistent with lipid transfer occurring at the cell surface, it does not rule out the possibility that lipid transfer might occur in a specialized endosomal compartment and thus depend on the internalization of HDL or LDL complexed to SR-BI. The model of endocytosis-independent selective lipid uptake is supported by the in vitro liposome reconstitution experiments [15] and electron microscopic images showing little or no intracellular HDL in cultured murine adrenal cells [22] or in steroidogenic tissues [23] that naturally exhibit high levels of SR-BI-mediated selective uptake. It is also supported by the observation that the transfer of fluorescently labeled-cholesterol from HDL to cells expressing SR-BI was not affected by ATP depletion, which blocked HDL internalization [24]. The alternative model of endocytosis-dependent

selective lipid uptake is supported by reports showing that the extent of HDL cycling between the cell surface and intracellular compartments is directly correlated with the efficacy of selective uptake [25–27].

To distinguish between these two models, we took advantage of distinct treatments that prevent either cellular endocytosis or SR-BI-mediated selective lipid transport to ask if the blockage of one pathway affects the other. We found that exposure of cells expressing murine SR-BI (mSR-BI) to BLT-1 or BLT-4, two potent small chemical inhibitors of SR-BI-dependent selective lipid uptake [28], did not interfere with the low level of internalization of HDL mediated by mSR-BI. The transient treatment of cells to block endocytosis with hyperosmotic sucrose [29,30] or the depletion of intracellular potassium [30,31] prevented the low levels of mSR-BI-mediated internalization of HDL and LDL, but did not inhibit mSR-BI-mediated selective uptake from these lipoproteins. Taken together, these results show that lipoprotein internalization is not a requisite for efficient mSR-BI-mediated lipid transport.

2. Materials and methods

2.1. Chemicals

Stock solutions of BLT-1 (Chembridge Corp.) and BLT-4 [32] were prepared in 100% DMSO and diluted into the appropriate assay media immediately prior to use. The final concentration of DMSO in the media was 0.5% (v/v). The control assay media contained 0.5% DMSO without BLT.

2.2. Lipoproteins

Human HDL and LDL were isolated and labeled with Alexa Fluor 568 (Molecular Probes) (Alexa-HDL and Alexa-LDL), ^{125}I (^{125}I-HDL), 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindodicarbocyanine 4-chlorobenzenesulfonate (DiD, Molecular Probes) (DiD-HDL and DiD-LDL), and [^{3}H]cholesteryl oleyl ether ([^{3}H]CEt, [^{3}H]CEt-HDL and [^{3}H]CEt-LDL) as described [2,10,33–35]. LDL was labeled with [^{3}H]CEt according to the [^{3}H]CEt-HDL labeling procedure [2].

2.3. Cells

LDL receptor deficient Chinese hamster ovary cells that express low levels of endogenous SR-BI (ldlA-7, [36]) and ldlA-7 cells that were stably transfected to express high levels of murine SR-BI (ldlA[mSR-BI]) [2] were grown in Ham's F12 culture medium supplemented with 5% (v/v) fetal bovine serum (FBS), 100 μg/ml each of penicillin and streptomycin and 500 μg/ml G418 (for ldlA[mSR-BI] cells). Y1-BS1 adrenal cells were grown in an RMPI culture medium (Gibco BRL) supplemented with 10% fetal bovine

serum and 100 μg/ml each of penicillin and streptomycin. The expression of endogenous SR-BI was induced by treating the Y1-BS1 cells for 24 h in the same medium supplemented with 0.1 μM adrenocorticotropic hormone (ACTH, Sigma) [37].

2.4. General assay conditions

Prior to the assay, cells were grown for 2 days on glass coverslips (for microscopy experiments) or in regular 24-well plates (for assays using radiolabeled lipoproteins). On the day of the assay, cells were washed twice in the appropriate culture medium supplemented with 0.5% bovine serum albumin and 25 mM HEPES/KOH, pH 7.4 but without serum, antibiotics or G418 (assay medium). For experiments involving DiD-labeled lipoproteins, we used Leibowitz medium (Gibco BRL) containing 0.5% bovine serum albumin (BSA) and 25 mM HEPES/KOH, pH 7.4. For control experiments, we used assay medium supplemented 0.5% DMSO.

2.5. Hypertonic sucrose treatment

Endocytosis was blocked by incubation with hypertonic sucrose [29,30]. Briefly, cells were incubated at 37 °C for 30 min with 0.45 M sucrose in the appropriate assay medium. Alexa-568, DiD, ^{125}I- or [^{3}H]CEt-labeled HDL or LDL was then added for an additional incubation of 1 h at the same temperature. During the last 20 min of the incubation, 50 μg/ml of Alexa488-labeled human holotransferrin (Molecular Probes) were added.

2.6. Potassium depletion

Endocytosis was blocked by potassium depletion [30,31]. Briefly, cells were washed twice with PBS/1 mM MgCl$_2$/0.1 mM CaCl$_2$ (PBS+) followed by one wash with hypotonic medium (PBS+/H$_2$O (1:3 v/v)) followed by a brief 5–7 min incubation with the hypotonic medium. The cells were then washed 5–8 times in a medium containing 150 mM NaCl/1 mM MgCl$_2$/1 mM CaCl$_2$/20 mM HEPES/KOH pH 7.4/0.5% DMSO/0.5% BSA followed by a 30 min incubation in the same medium. Alexa-568, DiD or ^{125}I-labeled HDL or LDL was then added for an additional incubation of 1 h at the same temperature. During the last 20 min of the incubation, 50 μg/ml of Alexa488-labeled human holotransferrin (Molecular Probes) was added.

2.7. Preparation of cells for fluorescence microscopy

After exposure to Alexa-HDL or -LDL, cells were chilled on ice for 10 min, washed three times with PBS+ and then fixed for 1 h on ice with 4% (w/v) paraformaldehyde (PFA) dissolved in PBS+(PBS-PFA). The PFA was quenched by the addition of 50 mM NH$_4$Cl for 5 min at room temperature (RT). Following a wash with PBS+, the surfaces of the cells

were stained at RT for 30 min with Alexa647-labeled Concanavalin A (100 μg/ml, Molecular Probes) in PBS+ containing 5% (w/v) BSA (PBS-BSA). The cells were then washed in PBS-BSA, fixed again in PBS-PFA for 15 min at RT and quenched as described above. We used the lipophylic dye DiD instead of the more commonly used dye DiI to avoid the excitation of DiI by the 488 nm spectral laser line required to excite Alexa-488. After the exposure of cells to DiD-labeled HDL or LDL, the medium containing the fluorescent lipoproteins was removed and each coverslip was placed in a live-cell imaging open perfusion chamber at 37 °C containing hypertonic sucrose or buffer A (for K+ depletion) to maintain the endocytic block. Cells were imaged with a spinning disk confocal microscope under the control of SlideBook 4 (Intelligent Imaging Innovations). Three-dimensional image stacks were recorded by sequential acquisition of optical sections along the z-axis with steps of 0.25 μm.

2.8. Cellular binding, uptake, and degradation of radiolabeled HDL and LDL

Assays of the uptake of [3H]CEt from [3H]CEt-HDL or [3H]CEt-LDL, and the cellular degradation and binding of 125I-HDL and 125I-LDL (all at 10 μg protein/ml) were performed in ldlA[mSR-BI] cells and control untransfected ldlA-7 cells as described [2]. The amounts of cell-associated [3H]cholesteryl ether are expressed as the corresponding amounts of protein (ng) in the [3H]CEt-labeled lipoprotein to permit a direct comparison of the relative amounts of 125I-HDL or 125I-LDL binding and [3H]CEt uptake [38]. Statistical analysis was performed with Graphpad Prism 3 software from Graphpad Software, Inc. (San Diego).

2.9. Flow cytometric analysis of mSR-BI surface expression

The surface expression of mSR-BI in ldlA[mSR-BI] and ldlA7 cells was determined using the polyclonal anti-SR-BI antibody KKB1 [33] as described [28].

3. Results

3.1. The inhibition of mSR-BI-mediated selective lipid uptake by BLTs does not prevent the endocytosis of HDL

It was previously shown that the expression of SR-BI results in the specific binding of HDL to the cell surface [2] and in the internalization of relatively little HDL [25–27,39]. These observations were confirmed by experiments using three-dimensional confocal fluorescence microscopy. In these experiments, stably transfected cells expressing high levels of mSR-BI (ldlA[mSR-BI] cells, [2]) were exposed for 1 h at 37 °C to HDL fluorescently labeled on its protein moiety with Alexa-568 (Alexa-HDL). Control untransfected ldlA-7 cells that express very little mSR-BI

[36] exhibited little binding or endocytosis of the fluorescently labeled lipoprotein (Fig. 1A, circumscribed areas demarcate the inside of the cell). The colocalization of Alexa-HDL with the fluorescently labeled plasma membrane marker Concanavalin A demonstrated that in ldlA[mSR-BI] cells the majority of the HDL was bound at the cell surface (Fig. 1D, F). Only a small portion of the Alexa-HDL was found in endocytic compartments, visualized by colocalization with Alexa 488-transferrin internalized by receptor-mediated endocytosis (Fig. 1E) [40].

We have previously identified a set of small molecule inhibitors named BLTs that block SR-BI-mediated selective
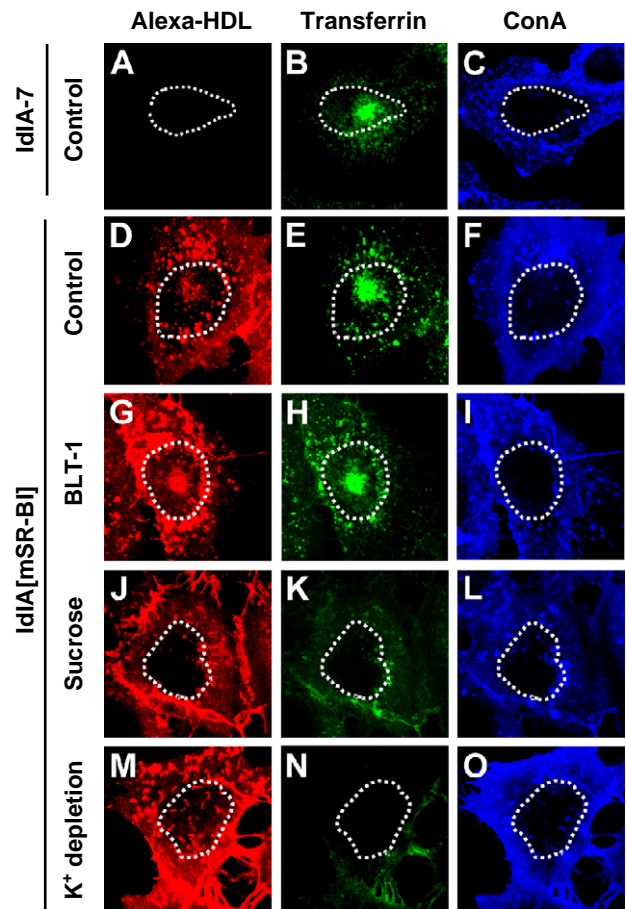

Fig. 1. Effects of BLT-1, hypertonic sucrose treatment and cellular potassium depletion on mSR-BI-mediated endocytosis of HDL. ldlA[mSR-BI] cells and ldlA-7 cells were incubated for 30 min with the control medium (A–F), 1 μM BLT-1 (G–I), hypertonic sucrose medium (J–L) or medium lacking potassium (M–O). The cells were then incubated for another 60 min with Alexa568-HDL (10 μg protein/ml) followed by the addition of Alexa488-transferrin (TF) (50 μg/ml) during the last 20 min of incubation. All the incubations were performed at 37 °C. Cells were transferred to 4 °C, fixed and incubated with Alexa647-Concanavalin A (ConA) to label the external cell surfaces. The cells were imaged using three-dimensional fluorescence confocal microscopy and representative confocal sections are shown. The white lines circumscribe the cell interior. Similar results were obtained using unlabeled HDL visualized with the polyclonal antibody 5F6 (specific for the major HDL protein apoA-I, University of Ottawa Heart Institute) in PFA fixed cells permeabilized with 0.1% saponin (not shown).

lipid uptake from HDL but do not block the entry of transferrin, EGFF or cholera toxin [28]. To test whether BLTs block selective uptake by preventing HDL internalization, we incubated the mSR-BI expressing cells with BLT-1, which is the most potent inhibitor. We found, as expected [28], a 83% decrease in the selective uptake of [^{3}H]cholesteryl ether ([^{3}H]CEt) from [^{3}H]CEt-HDL and a two-fold increase in cell-associated ^{125}I-HDL (not shown). Under these conditions in which selective lipid transfer was blocked, we observed an increase in the amount of Alexa-HDL bound at the cell surface, and a small increase in the amount of internalized Alexa-HDL (Fig. 1G). Similar results were obtained when cells were treated with 50 μM BLT-4 (not shown). These results suggest that the inhibition of selective lipid uptake by the BLTs is not a consequence of a block in the SR-BI-mediated endocytosis of the HDL particles.

3.2. The inhibition of HDL endocytosis does not prevent mSR-BI-mediated selective lipid uptake

The second approach we used to determine if lipoprotein internalization is required for mSR-BI-mediated selective lipid uptake was to examine the effects on selective lipid uptake of an acute block of endocytosis. A brief exposure of ldlA[mSR-BI] cells to hyperosmotic sucrose [29,30] or transient depletion of intracellular K$^+$ [30,31] results in the expected strong inhibition of receptor-mediated endocytosis of Alexa-488 transferrin (Fig. 1K, N, compared to B, E and H). Similarly, these perturbations interfered with the uptake of Alexa-HDL particles (Fig. 1J, M, compared to D and G). In contrast, the mSR-BI-dependent selective uptake of the fluorescent lipid probe DiD from DiD-labeled HDL particles to the cells was not affected (Fig. 2). Similar results (not shown) were obtained using ACTH-treated Y1-BS1 murine adrenal cortical cells [37] that normally express mSR-BI. Combined, these qualitative results suggest that blocking the internalization of HDL particles does not affect noticeably mSR-BI-mediated selective lipid uptake. Independent support for these observations was obtained by following the effect of hypertonic sucrose treatment on the cell association and degradation of ^{125}I-HDL, and on the selective uptake of [^{3}H]CEt from [^{3}H]CEt-HDL (Fig. 3A–C). These experiments showed that in ldlA[mSR-BI] cells under normal conditions most of the cell-associated ^{125}I-HDL, which represents both surface bound and internalized lipoprotein, was dependent on mSR-BI expression as there was little association in control ldlA-7 cells. Hypertonic sucrose treatment decreased this association by ~30% (Fig. 3A). In addition, the low level of intracellular ^{125}I-HDL proteolysis, as measured by the generation of ^{125}I-labeled acid soluble degradation products released in to the medium, was also reduced when the ldlA[mSR-BI] cells were treated with hyperosmotic sucrose (Fig. 3B). This was presumably due to the inhibition of HDL internalization, since the amount of HDL at the cell surface as determined by
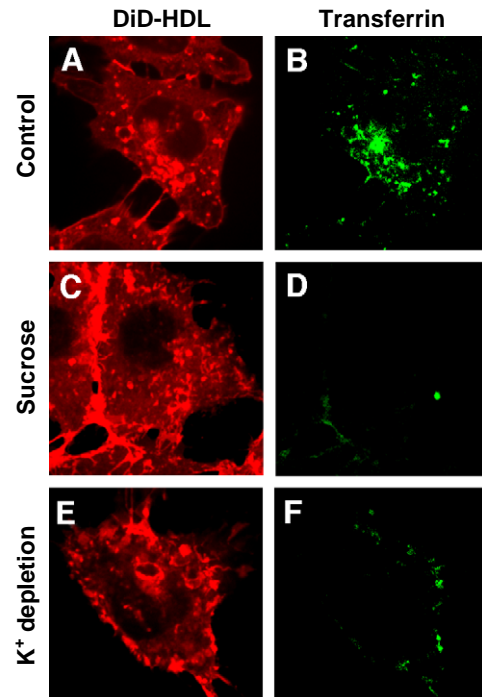

Fig. 2. Effect of hypertonic sucrose treatment and cellular potassium depletion on mSR-BI-mediated selective uptake of DiD from DiD-HDL. ldlA[mSR-BI] cells were incubated for 30 min with the medium only (A, B), hypertonic sucrose medium (C, D) or potassium-controlled medium (E, F) followed by the addition of DiD-labeled HDL (DiD-HDL) (80 μg protein/ml final) for another 60 min. Alexa488-transferrin (TF) was added during the last 20 min of incubation. All incubations were carried at 37 °C. Cells were washed at room temperature and imaged without fixation by confocal fluorescence microscopy. Representative sections are shown.

fluorescence microscopy of Alexa-HDL did not change (Fig. 1), and the level of SR-BI at the cell surface as was not altered (determined by flow cytometry, not shown). Taken together, these results demonstrate that hyperosmotic sucrose prevents the internalization of HDL mediated by mSR-BI. Under these conditions of endocytic blockade, however, the selective uptake of [^{3}H]CEt from [^{3}H]CEt-HDL was not affected (Fig. 3C). Thus, mSR-BI-mediated selective lipid uptake from HDL can occur independently of HDL endocytosis and degradation.

3.3. Uncoupling of mSR-BI-mediated LDL endocytosis and selective lipid uptake

Next, we asked if mSR-BI-mediated selective uptake from LDL requires endocytosis of LDL particles. As shown in Fig. 4A, there was virtually no surface binding or internalization of Alexa568-LDL by control ldlA-7 cells, which express essentially no SR-BI [2] or LDL receptor [36]. In contrast, the expression of mSR-BI in ldlA[mSR-BI] cells led to the intracellular accumulation of LDL labeled on its protein moiety with Alexa568 (Alexa-LDL) in an endosomal compartment (Fig. 4D), identified by the colocalization with Alexa488-transferrin internalized through the clathrin-mediated pathway (Fig. 4E). Further-
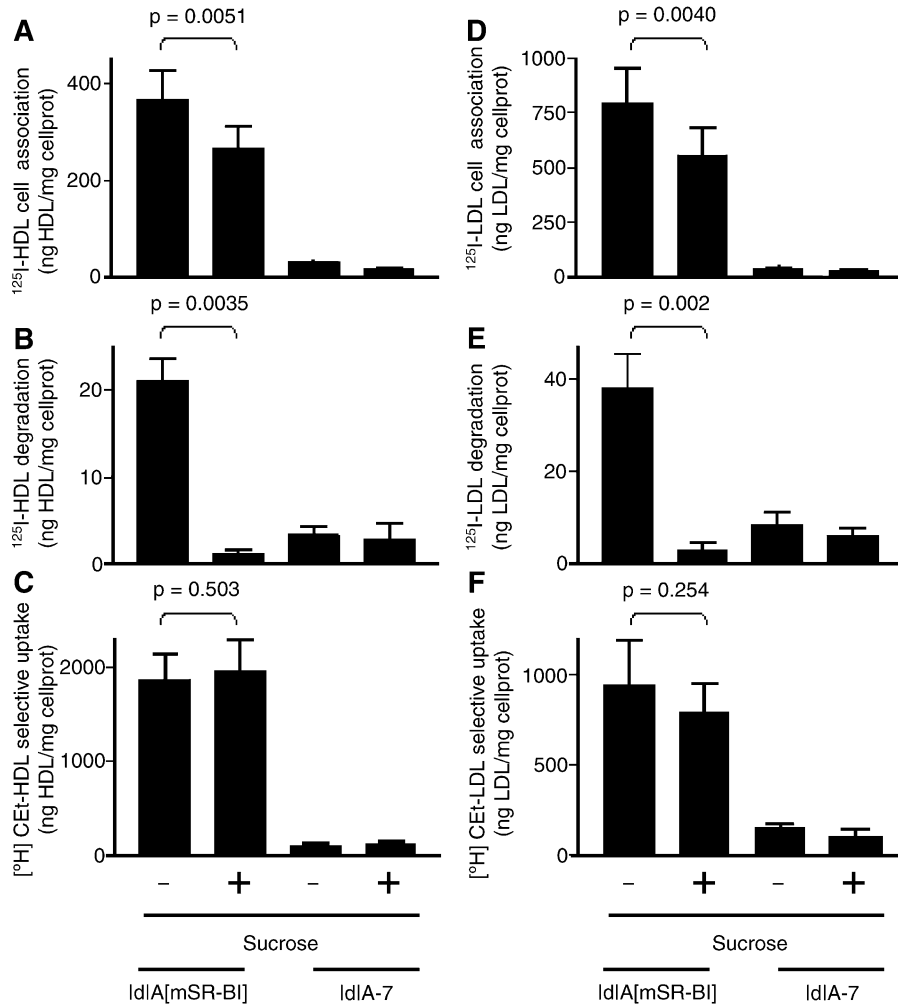
Fig. 3. Effects of endocytic block by hypertonic sucrose on cell association and degradation of ^{125}I-HDL and ^{125}I-LDL and on selective uptake of [^{3}H]CEt from [^{3}H]CEt-HDL or [^{3}H]CEt-LDL. Cells (ldlA[mSR-BI] or ldlA-7) were incubated for 30 min with the control medium (−) or hypertonic sucrose medium (+) followed by the addition for 60 min of ^{125}I-HDL, ^{125}I-LDL, [^{3}H]CEt-HDL, or [^{3}H]CEt-LDL (10 μg protein/ml final) in the presence or absence of a 40-fold excess of the respective unlabeled lipoproteins. The cells were washed and lysed to determine the total amounts of ^{125}I-HDL (A) or ^{125}I-LDL (D) associated with the cells (this value represents lipoprotein both bound at the cell surface and internalized). Media were harvested and used to determine the extent of ^{125}I-HDL (B) or ^{125}I-LDL (E) degradation. The selective transfer of [^{3}H]CEt from the [^{3}H]CEt-HDL (C) or [^{3}H]CEt-LDL (F) to the cells was calculated by subtracting the contributing amounts of [^{3}H]CEt contained in the lipoproteins associated with the cells. The mean values and standard errors determined in 6 independent experiments are shown. *P* values are shown for ldlA[mSR-BI] samples only.

more, the incubation of ldlA[mSR-BI] cells with ^{125}I-LDL results in a low level of lipoprotein degradation not observed in the untransfected control ldlA-7 cells (Fig. 3E). Thus, mSR-BI mediates a low level of endocytosis and degradation of LDL.

As was the case for HDL, hyperosmotic treatment of ldlA[mSR-BI] cells also inhibited the mSR-BI-mediated internalization (Fig. 4G) and degradation of LDL (Fig. 3E) but did not interfere with mSR-BI-dependent selective uptake of [^{3}H]CEt from [^{3}H]CEt-LDL (Fig. 3F) or of DiD from DiD-LDL (Fig. 5). As expected, ldlA-7 cells did not support selective uptake from [^{3}H]Cet-LDL (Fig. 3F) or DiD-LDL (not shown), nor cell association and degradation of ^{125}I-LDL (Fig. 3D, E). Based on these observations, we suggest that the mSR-BI-mediated selective lipid uptake from LDL also does not require lipoprotein endocytosis.

4. Discussion

In vitro and in vivo studies have established that the HDL receptor SR-BI plays a key role in mediating the physiologically relevant cellular selective uptake of lipid from lipoproteins, and thus controls the metabolism of HDL [1]. The detailed molecular mechanisms underlying selective lipid uptake mediated by mSR-BI remain to be established. One area of controversy regarding the mechanism of mSR-BI-mediated selective lipid uptake has been the role of receptor-ligand endocytosis. In the current study we have shown that the inhibition of cellular lipoprotein endocytosis by either hypertonic sucrose treatment or K$^+$ depletion does not prevent the mSR-BI-mediated selective uptake of lipids (cholesterol ethers or the lipophilic dye DiD) from either HDL or LDL. These results support our previous conclusion

that mSR-BI-mediated HDL binding and selective lipid uptake are intrinsic properties of the receptor that do not require the intervention of other proteins or traffic to specific intracellular compartments [15]. Furthermore, we found that the specific inhibition of mSR-BI-dependent lipid transport by the small molecules BLT-1 and BLT-4 does not involve the inhibition of HDL particle internalization mediated by mSR-BI. Our results are consistent with previous electron microscopic morphological studies that support the model of HDL particle internalization independent SR-BI-mediated lipid transport [22,23].

During the preparation of this manuscript, two reports appeared supporting the proposal that SR-BI-mediated lipid transport is independent of lipoprotein internalization. In the first study, Maxfield and co-workers [24] reported that the intracellular depletion of ATP in HepG2 cells increased the efficiency of selective lipid uptake mediated by human SR-BI from the HDL of the fluorescent unesterified cholesterol analogue dehydroergesterol, even though the endocytic uptake of HDL particles was blocked. They noted, however, that the dehydroergesterol intracellular trafficking pathway involved a rapid non-vesicular mechanism that might differ from that of the selective uptake of HDL's cholesteryl esters (or cholesteryl ether analogues). In the second study, Eckhardt et al. [41] compared the selective uptake and endocytic activities of mSR-BI and a naturally occurring murine splice variant called SR-BII in non-polarized transfected cells. SR-BII and SR-BI differ only in their short 45–47 amino acid C-terminal cytoplasmic domains [42]. The steady state ratios of the levels of intracellular-to-cell surface receptor and
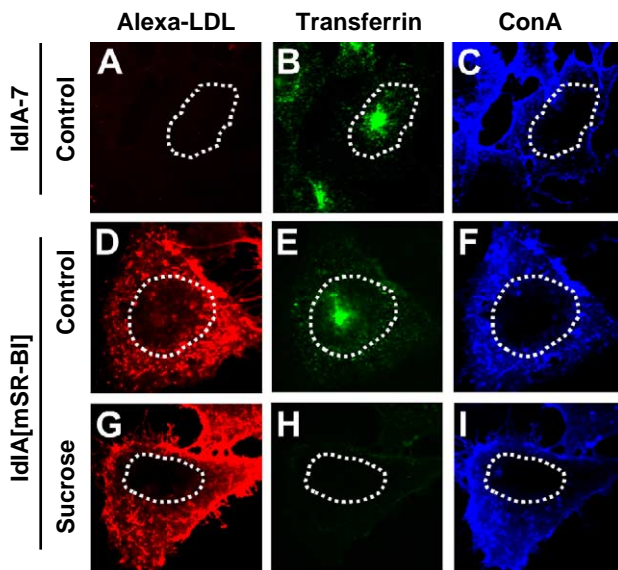
Fig. 4. Effect of hypertonic sucrose treatment on mSR-BI-mediated endocytosis of LDL. ldlA-7 and ldlA[mSR-BI] cells were treated with control medium (A–C) or medium containing hyperosmotic sucrose (D–F) as described in Fig. 1, but using Alexa568-labeled LDL (10 μg protein/ml final). Representative confocal sections are shown. The white lines circumscribe the cell interior.
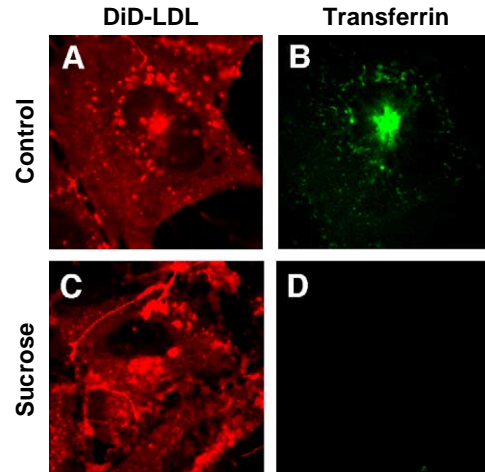
Fig. 5. Effect of hypertonic sucrose treatment on mSR-BI-mediated selective uptake of DiD from DiD-LDL. ldlA[mSR-BI] cells were treated with control medium (A, B) or medium containing hyperosmotic sucrose (C, D) as described in Fig. 1, but using DiD-labeled LDL (80 μg protein/ml final) (DiD-LDL). During the last 20 min of the incubation, Alexa488-transferrin was added. Cells were then washed and analyzed without fixation by three-dimensional fluorescence confocal microscopy. Representative confocal sections are shown.

associated HDL were substantially higher in the SR-BII expressing cells, possibly due to SR-BII's more rapid endocytosis (and/or slower recycling to the cell surface). Selective uptake by these receptors positively correlated with their levels of surface expression, but not with the levels of internalized HDL. Eckhardt et al. suggested that these data support the model that most of the selective uptake mediated by SR-BI occurred at the cell surface, although their study did not rule out the possible requirement for a rapid cycle of endocytosis and recycling that would result in a very small internal pool of SR-BI and its ligand that is able to efficiently support selective uptake. It remains possible that in some specialized cells (e.g. mammary epithelial cells [43]) mSR-BI-mediated endocytosis plays a role in lipid metabolism; however, in many, if not all cases, it clearly is not required for efficient selective uptake.

These reports, together with our study, demonstrate that the endocytosis of HDL or LDL is not required for mSR-BI-mediated selective uptake from these lipoproteins. Furthermore, our data strongly suggests that selective uptake occurs at the surface of the cell.

References

[1] M. Krieger, Charting the fate of the "good cholesterol": identification and characterization of the high-density lipoprotein receptor SR-BI, Annu. Rev. Biochem. 68 (1999) 523–558.

[2] S. Acton, A. Rigotti, K.T. Landschulz, S. Xu, H.H. Hobbs, M. Krieger, Identification of scavenger receptor SR-BI as a high density lipoprotein receptor, Science 271 (1996) 518–520.

[3] D. Goti, A. Hrzenjak, S. Levak-Frank, S. Frank, D.R. van der Westhuyzen, E. Malle, W. Sattler, Scavenger receptor class B, type I is expressed in porcine brain capillary endothelial cells and contributes to selective uptake of HDL-associated vitamin E, J. Neurochem. 76 (2001) 498–508.

[4] D.J. Greene, J.W. Skeggs, R.E. Morton, Elevated triglyceride content diminishes the capacity of high density lipoprotein to deliver cholesteryl esters via the scavenger receptor class B type I (SR-BI), J. Biol. Chem. 276 (2001) 4804–4811.

[5] P. Mardones, P. Strobel, S. Miranda, F. Leighton, V. Quinones, L. Amigo, J. Rozowski, M. Krieger, A. Rigotti, Alpha-tocopherol metabolism is abnormal in scavenger receptor class B type I (SR-BI)-deficient mice, J. Nutr. 132 (2002) 443–449.

[6] S. Urban, S. Zieseniss, M. Werder, H. Hauser, R. Budzinski, B. Engelmann, Scavenger receptor BI transfers major lipoprotein-associated phospholipids into the cells, J. Biol. Chem. 275 (2000) 33409–33415.

[7] X. Gu, K. Kozarsky, M. Krieger, Scavenger receptor class B, type I-mediated [3H]cholesterol efflux to high and low density lipoproteins is dependent on lipoprotein binding to the receptor, J. Biol. Chem. 275 (2000) 29993–30001.

[8] Y. Ji, B. Jian, N. Wang, Y. Sun, M.L. Moya, M.C. Phillips, G.H. Rothblat, J.B. Swaney, A.R. Tall, Scavenger receptor BI promotes high density lipoprotein-mediated cellular cholesterol efflux, J. Biol. Chem. 272 (1997) 20982–20985.

[9] B. Jian, M. de la Llera-Moya, Y. Ji, N. Wang, M.C. Phillips, J.B. Swaney, A.R. Tall, G.H. Rothblat, Scavenger receptor class B type I as a mediator of cellular cholesterol efflux to lipoproteins and phospholipid acceptors, J. Biol. Chem. 273 (1998) 5599–5606.

[10] S.L. Acton, P.E. Scherer, H.F. Lodish, M. Krieger, Expression cloning of SR-BI, a CD36-related class B scavenger receptor, J. Biol. Chem. 269 (1994) 21003–21009.

[11] M.A. Connelly, S.M. Klein, S. Azhar, N.A. Abumrad, D.L. Williams, Comparison of class B scavenger receptors, CD36 and scavenger receptor BI (SR-BI), shows that both receptors mediate high density lipoprotein–cholesteryl ester selective uptake but SR-BI exhibits a unique enhancement of cholesteryl ester uptake, J. Biol. Chem. 274 (1999) 41–47.

[12] H. Stangl, G. Cao, K.L. Wyne, H.H. Hobbs, Scavenger receptor, class B, type I-dependent stimulation of cholesterol esterification by high density lipoproteins, low density lipoproteins, and nonlipoprotein cholesterol, J. Biol. Chem. 273 (1998) 31002–31008.

[13] H. Stangl, M. Hyatt, H.H. Hobbs, Transport of lipids from high and low density lipoproteins via scavenger receptor-BI, J. Biol. Chem. 274 (1999) 32692–32698.

[14] S. Swarnakar, R.E. Temel, M.A. Connelly, S. Azhar, D.L. Williams, Scavenger receptor class B, type I, mediates selective uptake of low density lipoprotein cholesteryl ester, J. Biol. Chem. 274 (1999) 29733–29739.

[15] B. Liu, M. Krieger, Highly purified scavenger receptor class B, type I reconstituted into phosphatidylcholine/cholesterol liposomes mediates high affinity high density lipoprotein binding and selective lipid uptake, J. Biol. Chem. 277 (2002) 34125–34135.

[16] C. Glass, R.C. Pittman, D.B. Weinstein, D. Steinberg, Dissociation of tissue uptake of cholesterol ester from that of apoprotein A-I of rat plasma high density lipoprotein: selective delivery of cholesterol ester to liver, adrenal, and gonad, Proc. Natl. Acad. Sci. U. S. A. 80 (1983) 5435–5439.

[17] C. Glass, R.C. Pittman, M. Civen, D. Steinberg, Uptake of high-density lipoprotein-associated apoprotein A-I and cholesterol esters by 16 tissues of the rat in vivo and by adrenal cells and hepatocytes in vitro, J. Biol. Chem. 260 (1985) 744–750.

[18] Y. Stein, Y. Dabach, G. Hollander, G. Halperin, O. Stein, Metabolism of HDL-cholesteryl ester in the rat, studied with a nonhydrolyzable analog, cholesteryl linoleyl ether, Biochim. Biophys. Acta 752 (1983) 98–105.

[19] S.R. Green, R.C. Pittman, Selective uptake of cholesteryl esters from low density lipoproteins in vitro and in vivo, J. Lipid Res. 32 (1991) 667–678.

[20] E. Reaven, Y.D. Chen, M. Spicher, S.F. Hwang, C.E. Mondon, S. Azhar, Uptake of low density lipoproteins by rat tissues. Special emphasis on the luteinized ovary, J. Clin. Invest. 77 (1986) 1971–1984.

[21] S. Azhar, A. Cooper, L. Tsai, W. Maffe, E. Reaven, Characterization of apoB, E receptor function in the luteinized ovary, J. Lipid Res. 29 (1988) 869–882.

[22] Y. Peng, W. Akmentin, M.A. Connelly, S. Lund-Katz, M.C. Phillips, D.L. Williams, Scavenger receptor BI (SR-BI) clustered on micro-villar extensions suggests that this plasma membrane domain is a way station for cholesterol trafficking between cells and high-density lipoprotein, Mol. Biol. Cell 15 (2004) 384–396.

[23] E. Reaven, Y.D. Chen, M. Spicher, S. Azhar, Morphological evidence that high density lipoproteins are not internalized by steroid-producing cells during in situ organ perfusion, J. Clin. Invest. 74 (1984) 1384–1397.

[24] D. Wustner, A. Mondal, A. Huang, F.R. Maxfield, Different transport routes for high density lipoprotein and its associated free sterol in polarized hepatic cells, J. Lipid Res. 45 (2004) 427–437.

[25] D.L. Silver, N. Wang, A.R. Tall, Defective HDL particle uptake in ob/ob hepatocytes causes decreased recycling, degradation, and selective lipid uptake, J. Clin. Invest. 105 (2000) 151–159.

[26] D.L. Silver, N. Wang, X. Xiao, A.R. Tall, High density lipoprotein (HDL) particle uptake mediated by scavenger receptor class B type 1 results in selective sorting of HDL cholesterol from protein and polarized cholesterol secretion, J. Biol. Chem. 276 (2001) 25287–25293.

[27] D. Rhainds, P. Bourgeois, G. Bourret, K. Huard, L. Falstrault, L. Brissette, Localization and regulation of SR-BI in membrane rafts of HepG2 cells, J. Cell. Sci. 117 (2004) 3095–3105.

[28] T.J. Nieland, M. Penman, L. Dori, M. Krieger, T. Kirchhausen, Discovery of chemical inhibitors of the selective transfer of lipids mediated by the HDL receptor SR-BI, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 15422–15427.

[29] G. Daukas, S.H. Zigmond, Inhibition of receptor-mediated but not fluid-phase endocytosis in polymorphonuclear leukocytes, J. Cell Biol. 101 (1985) 1673–1679.

[30] J.E. Heuser, R.G. Anderson, Hypertonic media inhibit receptor-mediated endocytosis by blocking clathrin-coated pit formation, J. Cell Biol. 108 (1989) 389–400.

[31] J.M. Larkin, M.S. Brown, J.L. Goldstein, R.G. Anderson, Depletion of intracellular potassium arrests coated pit formation and receptor-mediated endocytosis in fibroblasts, Cell 33 (1983) 273–285.

[32] T.J. Nieland, A. Chroni, M.L. Fitzgerald, Z. Maliga, V.I. Zannis, T. Kirchhausen, M. Krieger, Cross-inhibition of SR-BI- and ABCA1-mediated cholesterol transport by the small molecules BLT-4 and glyburide, J. Lipid Res. 45 (2004) 1256–1265.

[33] X. Gu, B. Trigatti, S. Xu, S. Acton, J. Babitt, M. Krieger, The efficient cellular uptake of high density lipoprotein lipids via scavenger receptor class B type I requires not only receptor-mediated surface binding but also receptor-specific lipid transfer mediated by its extracellular domain, (erratum appears in J. Biol. Chem. 1998 Dec 25; 273 (52): 35388.), J. Biol. Chem. 273 (1998) 26338–26348.

[34] X. Gu, R. Lawrence, M. Krieger, Dissociation of the high density lipoprotein and low density lipoprotein binding activities of murine scavenger receptor class B type I (mSR-BI) using retrovirus library-based activity dissection, J. Biol. Chem. 275 (2000) 9120–9130.

[35] R.E. Pitas, T.L. Innerarity, J.N. Weinstein, R.W. Mahley, Acetoacetylated lipoproteins used to distinguish fibroblasts from macrophages in vitro by fluorescence microscopy, Arteriosclerosis 1 (1981) 177–185.

[36] D.M. Kingsley, M. Krieger, Receptor-mediated endocytosis of low density lipoprotein: somatic cell mutants define multiple genes required for expression of surface-receptor activity, Proc. Natl. Acad. Sci. U. S. A. 81 (1984) 5454–5458.

[37] A. Rigotti, E.R. Edelman, P. Seifert, S.N. Iqbal, R.B. DeMattos, R.E. Temel, M. Krieger, D.L. Williams, Regulation by adrenocorticotropic hormone of the in vivo expression of scavenger receptor class B type I (SR-BI), a high density lipoprotein receptor, in steroidogenic cells of the murine adrenal gland, J. Biol. Chem. 271 (1996) 33545–33549.

[38] R.C. Pittman, T.P. Knecht, M.S. Rosenbaum, C.A. Taylor Jr., A nonendocytotic mechanism for the selective uptake of high density lipoprotein-associated cholesterol esters, J. Biol. Chem. 262 (1987) 2443–2450.

[39] T.G. Vishnyakova, A.V. Bocharov, I.N. Baranova, Z. Chen, A.T. Remaley, G. Csako, T.L. Eggerman, A.P. Patterson, Binding and internalization of lipopolysaccharide by Cla-1, a human orthologue of rodent scavenger receptor B1, J. Biol. Chem. 278 (2003) 22771–22780.

[40] D.J. Spiro, W. Boll, T. Kirchhausen, M. Wessling-Resnick, Wortmannin alters the transferrin receptor endocytic pathway in vivo and in vitro, Mol. Biol. Cell 7 (1996) 355–367.

[41] E.R. Eckhardt, L. Cai, B. Sun, N.R. Webb, D.R. van der Westhuyzen, High density lipoprotein uptake by scavenger receptor SR-BII, J. Biol. Chem. 279 (2004) 14372–14381.

[42] N.R. Webb, P.M. Connell, G.A. Graf, E.J. Smart, W.J. de Villiers, F.C. de Beer, D.R. van der Westhuyzen, SR-BII, an isoform of the scavenger receptor BI containing an alternate cytoplasmic tail, mediates lipid transfer between high density lipoprotein and cells, J. Biol. Chem. 273 (1998) 15241–15248.

[43] J. Monks, P.U. Huey, L. Hanson, R.H. Eckel, M.C. Neville, S. Gavigan, A lipoprotein-containing particle is transferred from the serum across the mammary epithelium into the milk of lactating mice, J. Lipid Res. 42 (2001) 686–696.