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Scavenger receptor, Class B, Type I provides an alternative means for β -VLDL uptake independent of the LDL receptor in tissue culture

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Abstract

Recent evidence suggests that scavenger receptor, class B, type I (SR-BI) plays a physiological role in VLDL metabolism. SR-BI was reported to mediate β -VLDL uptake; however, cellular details of this process are not well characterized. In the present study we show that SR-BI delivers cholesterol derived from β -VLDL to LDL receptor negative SR-BI over-expressing Chinese Hamster Ovarian cells (IdIA7-SRBI). Cell association of β -VLDL was ~3 times higher after SR-BI over-expression, which was competed by β -VLDL, but only to a lesser extent by HDL and LDL. Almost all of the associated β -VLDL was located intracellularly, and therefore could not be released by a 50-fold excess of unlabeled β -VLDL. β -VLDL was degraded at a rate of 6 ng β -VLDL/mg cell protein and hour. In contrast to IdIA7 cells, β -VLDL association was competed by LDL in cells with a functional LDL receptor like CHO and HepG2 cells, indicating a strong impact of the LDL receptor in β -VLDL uptake. β -VLDL degradation was similar to IdIA7-SRBI cells. When β -VLDL uptake was followed using fluorescence microscopy, β -VLDL showed a different uptake pattern in SR-BI over-expressing cells, IdIA7-SRBI, compared to LDL receptor containing cells, CHO and HepG2.

Keywords

SR-BI; β -VLDL; LDL receptor; Chylomicrons; Cholesterol; Chinese Hamster Ovarian Cells; HepG2

1. Introduction

Scavenger receptor, class B, type I (SR-BI) is a cell-surface glycoprotein shown to bind a broad spectrum of ligands. In addition to high- and low-density lipoproteins (HDL and LDL) [1-4], it binds very low-density lipoprotein (VLDL), modified LDL, maleylated serum albumin and anionic phospholipids [5], advanced glycation end-products [6], even apoptotic

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbaliip.2009.11.005.

cells [7] and hepatitis C virus [8,9]. Recently, SR-BI was implicated to play a role in VLDL uptake [10,11] and chylomicron metabolism [12,13].

SR-BI is highly expressed in liver and steroidogenic tissues [2,14,15], where it mediates selective uptake of cholesteryl esters (CE) from lipoproteins, a process in which lipoprotein-derived CE are transferred to cells without degradation of lipoprotein particles [2,3,16,17]. Its tissue expression pattern as well as regulation by hormones is consistent with SR-BI being the well-characterized HDL receptor mediating selective CE uptake [14]. Inactivation of SR-BI in mice increases plasma HDL cholesterol levels [18]. Conversely, HDL cholesterol and apolipoprotein AI (apoAI) levels are decreased by hepatic SR-BI over-expression [19,20].

Its binding to a broad spectrum of ligands may be facilitated by two different binding sites at the extracellular domain of SR-BI, a binding site for HDL and an LDL/lipid binding site [21]. Twenty years ago Adelman and St Clair [22] showed the existence of two cellular binding sites for β -VLDL with different potentials for promoting cholesterol accumulation in pigeon macrophages: a high-affinity binding site, which shows many characteristics of an LDL receptor (LDLR), and a second binding site, which is relatively nonspecific regarding the lipoprotein class. This second binding site exhibits many characteristics of a scavenger receptor, class B.

A role of SR-BI in the clearance of remnant lipoproteins was described in LDLR or apoE knockout mice [23]. Furthermore, altered SR-BI expression affects plasma levels of apoB-containing lipoproteins [24]. However, it still remains to be elucidated to what extent SR-BI affects VLDL levels. To gain more insight into the role of SR-BI in β -VLDL metabolism we analyzed β -VLDL uptake in LDLR negative SR-BI over-expressing (IdIA7-SRBI) and LDLR expressing cell lines (CHO and HepG2). Our data indicate that SR-BI over-expression facilitates uptake and catabolism of β -VLDL. In HepG2 cells SR-BI, however, provides only an alternative way to supply hepatocytes with β -VLDL, whereas the LDLR mediates most β -VLDL uptake. Taken together SR-BI can mediate β -VLDL uptake and might play an important role for β -VLDL clearance in cases where the LDL receptor pathway is defective.

2. Materials and methods

2.1. Cell lines and tissue culture

Chinese hamster ovary (CHO) cells lacking LDL receptor activity (IdIA7 cells) and over-expressing SR-BI (IdIA7-SRBI cells) [3] and CHO-K1 cells were maintained in medium A [1:1 mixture of DMEM and Ham's F-12] supplemented with 5% fetal calf serum (FCS) and for IdIA7-SRBI cells with 0.25 mg/ml G418 sulfate (Gibco BRL, Austria). HepG2 cells were maintained in MEM with 10% FCS, MEM-non essential amino acids and 1 mmol/l sodium pyruvate (PAA, Austria) (for comparison of the expression levels of SR-BI, LRP1 and VLDL-R and LDL degradation in these cell lines see Supplemental Figure 1). On day 2, cells were washed twice with phosphate-buffered saline (PBS) and fed medium A containing 5% calf lipoprotein-deficient serum (cLPDS).

2.2. β -VLDL preparation and iodination

β -VLDL was isolated from New Zealand White rabbits fed a 2% cholesterol–10% corn oil diet for 1–2 weeks [25]. Blood was collected from the ear vein after animals had been fasted overnight. β -VLDL ($d < 1.006$) was isolated by ultracentrifugation [26] and iodinated with sodium [125]-iodide (Hartmann Analytic, Germany) using the Pierce IODO-BEADS reagent kit. The labeling procedure normally resulted in a specific activity of ~1000 cpm/ng. For the experiments, β -VLDL was diluted ~1:5 with unlabeled lipoprotein.

2.3. Cell association and uptake of [125 I]- β -VLDL

On day 3, cells were incubated with [125 I]- β -VLDL in medium A with 2 mg/ml fatty-acid-free BSA (faf-BSA) for 1 h. A 40-fold excess of unlabeled β -VLDL was added to every fourth data point. Media were recovered and cell monolayers were washed twice with cold 50 mmol/l Tris-HCl (pH=7.4), 0.9% NaCl and 0.2% BSA and twice without BSA. Cells were lysed with 0.1 mol/l NaOH. Radioactivity was determined using a gamma counter. Specific cell association was calculated by subtracting the amount of radioactivity detected with a 40-fold excess from total activity measured. For uptake experiments another set of cells was washed after association and incubated at 0 °C for 2 h with a 50-fold excess of unlabeled lipoprotein. Protein was quantitated using the Bradford Method (Biorad, Austria) [27].

2.4. Degradation of [125 I]- β -VLDL

Media were collected and proteins precipitated using 50% TCA. The supernatant was oxidized with 30% hydrogen peroxide, extracted with chloroform and counted to determine the amount of acid-soluble material formed by the cells [28].

2.5. Fluorescence microscopy

The apolipoprotein part of β -VLDL was covalently labeled with Alexa Fluor 568 or 488 (Molecular Probes, OR) and the lipid part with the fluorescent phospholipid DiI: labeling included incubation of β -VLDL in human LPDS with 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) overnight at 37 °C followed by ultracentrifugation [29]. For lipid droplet staining cells were incubated with 1 μ g/ml Bodipy 558/568 C₁₂ for 10 min, washed and incubated in MEM for 30 min. Cells were fixed with 4% formaldehyde for 30 min at RT, mounted with Fluoprep (Biomérieux, France) and visualized with a confocal microscope Zeiss LSM 510 (Zeiss, Germany).

2.6. Statistical analysis

Statistical analysis was performed using GraphPad Prism v4.00. Two-sided *t*-test or ANOVA was used to compare two or more groups, respectively.

3. Results

3.1. SR-BI over-expression increases β -VLDL uptake

SR-BI ablation was shown to influence β -VLDL uptake *in vivo* [11]. To determine the ability of SR-BI to transport lipids from β -VLDL particles to cells independently of the LDLR, we used CHO cells expressing no functional LDLR but over-expressing SR-BI (IdIA7-SRBI, for expression levels see Fig. S1, Supplemental Material). First, we assessed if cell association of β -VLDL was increased. IdIA7 and IdIA7-SRBI cells were incubated with increasing concentrations of [125 I]- β -VLDL for 1 h (Fig. 1A). IdIA7 cells showed an increase in β -VLDL cell association. SR-BI over-expression resulted in a higher dose response. To determine if β -VLDL association was due to specific binding we used an excess of unlabeled β -VLDL, and to analyze if β -VLDL binding could be competed by other lipoproteins we added unlabeled HDL or LDL (Fig. 1B). [125 I]- β -VLDL cell association (10 μ g/ml) was dramatically decreased by the addition of a 10-fold excess of unlabeled β -VLDL, but was much less affected by a 10-fold excess of HDL or LDL. CHO-K1 cells showed a ~2-fold higher β -VLDL association compared to IdIA7-SRBI cells indicating that the LDLR plays an important role in β -VLDL uptake. β -VLDL association could be competed with a 10-fold excess of LDL and β -VLDL, but not with HDL in CHO-K1 cells thus demonstrating the prominent role of the LDLR in β -VLDL uptake in genetically not altered CHO cells.

To investigate if cell association leads to a concomitant uptake of β -VLDL, surface bound [125 I]- β -VLDL was displaced by a 50-fold excess of unlabeled VLDL at 4 °C for 2 h. The amount of radioactivity remained unchanged when surface bound β -VLDL was displaced by excess VLDL in ldlA7-SRBI cells (see Table 1, Supplemental Material). To check if internalized β -VLDL is released to the media, a parallel set of cells was further incubated at 37 °C with a 20-fold excess of β -VLDL for 30 min. β -VLDL in the cell lysate did not change significantly indicating that internalized β -VLDL was not released. No specific release of surface bound β -VLDL was seen with 10, 20 or 40 U/ml heparin in ldlA7-SRBI cells at 4 °C for 20 and 60 min, indicating that binding of β -VLDL is heparin resistant (data not shown). Next, we assessed if β -VLDL could compete for the association of HDL or LDL (Fig. 1C). ldlA7-SRBI cells were incubated with 10 μ g/ml [125 I]-HDL or [125 I]-LDL for 1 h and a 10-fold excess of β -VLDL. While HDL association was not diminished by excess β -VLDL, LDL association was decreased, indicating that β -VLDL and LDL binding to SR-BI might occur at a similar position. Finally we assessed if any selective lipid uptake occurs in these cell lines; using double labeled β -VLDL particles (3 H cholesteryl linoleate labeled 125 I- β -VLDL) no selective uptake could be detected (data not shown).

3.2. β -VLDL delivered via SR-BI increases the cellular lipid pool

To determine whether incubation with β -VLDL results in an increase in the cellular cholesterol content, ldlA7 and ldlA7-SRBI cells were incubated with β -VLDL over night and then the cholesterol and CE content was measured (see Fig. S2, Supplemental Material). After β -VLDL feeding total cholesterol (TC) increased in ldlA7 cells and to a higher extent in ldlA7-SRBI cells. This increase was due to both an increase in the CE content as well as in free cholesterol (FC). To determine if SR-BI expression increases the delivery of sterols to the endoplasmic reticulum (ER) for cholesterol esterification, cells were incubated with increasing amounts of β -VLDL for 5 h, followed by 2 h incubation with [3 H]-oleate and CE formation was quantitated by TLC. A small increase in cholesteryl esterification was seen in the ldlA7 cells, while addition of β -VLDL to ldlA7-SRBI cells resulted in a dramatic increase in CE formation (see Fig. S3, Supplemental Material). Next, triglycerides and CE were estimated by Oil Red O staining and the cellular triglyceride content was quantitated by gas chromatography (see Fig. S4, Supplemental Material). In ldlA7 cells there was an increase both in Oil Red O staining and triglyceride content which was more pronounced in ldlA7-SRBI cells after β -VLDL incubation. Furthermore we analyzed the formation of lipid droplets in these cells using Bodipy 558/568 C₁₂ (Fig. 1D). Hardly any lipid droplets were observed in control cells, whereas β -VLDL treatment induced the formation of intensively stained lipid droplets. This effect was more pronounced in ldlA7-SRBI cells.

3.3. Fate of holo β -VLDL after uptake via SR-BI

To determine if β -VLDL taken up by cells gets dismantled, we analyzed specific degradation of [125 I]- β -VLDL particles (10 μ g/ml) during the 1 h association experiment. Both ldlA7 and ldlA7-SRBI cells exhibited a small amount of specific β -VLDL degradation (6 ± 3 ng β -VLDL/mg cell protein and hour, $n=3$). A similar amount of degradation was seen in CHO-K1 cells (see also Supplemental Table 1). To more conclusively answer to what extent β -VLDL is degraded an association and decay experiment was performed (Fig. 1E–F). Both ldlA7 and ldlA7-SRBI cells were incubated with 10 μ g/ml [125 I]- β -VLDL for 1 h, then cells were washed and kept in media for up to 24 h. There was an exponential decrease of radioactivity indicating β -VLDL degradation (Fig. 1E). Simultaneously there was an increase in TCA soluble radioactivity in the media (Fig. 1F). After 24 h half of the radioactivity taken up was degraded indicating that dismantling of the particle is slow. However, degradation of β -VLDL was clearly detected.

3.4. β -VLDL uptake and utilization in the human liver cell line HepG2

To determine β -VLDL uptake and utilization in a more physiological setting we used the liver cell line HepG2 that expresses the LDLR and also SR-BI, but to a lesser extent (see Supplemental Figure 1). After 1 h of incubation with [125 I]- β -VLDL (10 μ g/ml) HepG2 cells displayed a cell association of 82 ± 23 ng β -VLDL/mg cell protein (Fig. 2A). This is much lower than in IdIA7-SRBI cells. A 10-fold excess of HDL did not compete for β -VLDL association, indicating a limited role of SR-BI in β -VLDL uptake. A 10-fold excess of LDL and β -VLDL blocked [125 I]- β -VLDL cell association (Fig. 2A) demonstrating that uptake of β -VLDL is mediated preferentially by the LDLR. We assessed competition of [125 I]-HDL or [125 I]-LDL with β -VLDL. Both HDL and LDL association was competed by β -VLDL (Fig. 2B). This association and competition pattern of HepG2 cells is similar to CHO cells, but different from IdIA7-SRBI cells. These data indicate differences between β -VLDL uptake mediated by SR-BI in IdIA7-SRBI cells and by the LDLR-family in HepG2 cells. siRNA down-regulation of SR-BI, leading to ~50% decrease of SR-BI protein, had no effect on β -VLDL association in HepG2 cells (see Fig. S5, Supplemental Material).

Next, we checked if β -VLDL particles associated were internalized and metabolized by HepG2 cells. First, we measured TCA resistant radioactivity of β -VLDL in the association media. Like in IdIA7-SRBI cells β -VLDL degradation was low but measurable (5 ± 4 ng β -VLDL/mg cell protein, see Table S1). Subsequently we analyzed if β -VLDL was located intracellularly. Therefore cells were incubated for 1 h with [125 I]- β -VLDL (10 μ g/ml), washed and kept at 4 °C for 2 h in the presence of a 50-fold excess of β -VLDL. Afterwards the amount of β -VLDL found in the cell lysate was unchanged (see Table S1, Supplemental Material). To clearly show β -VLDL uptake, we performed an association and decay experiment. Cells were incubated with [125 I]- β -VLDL (10 μ g/ml) for 1 h and radioactivity retained in the cell lysate and released to the media was analyzed (Fig. 2C). There was a time-dependent decrease in radioactivity in the cell lysate and an increase in TCA-resistant radioactivity in the media of HepG2 cells. After 24 h ~2/3 of [125 I]- β -VLDL was metabolized.

To show that cholesterol of β -VLDL particles reaches the intracellular pool we measured FC and CE in HepG2 cells (see Fig. S2, Supplemental Material). Both the CE and TC content increased after incubation with β -VLDL overnight. Furthermore there was a small increase in cellular lipid content indicated by Oil Red O retention and triglyceride mass (see Fig. S4, Supplemental Material). Concomitantly lipid droplets increased in size and number after β -VLDL treatment in HepG2 cells stained with Bodipy 558/568 C₁₂ (Fig. 2D). The size and numbers of the lipid droplets in HepG2 cells was increased compared to IdIA7-SRBI cells.

3.5. Visualization of β -VLDL uptake in the presence and absence of the LDL receptor

To clearly delineate if there are differences in uptake routes of β -VLDL via SR-BI and LDLR, we fluorescently labeled the protein moiety of β -VLDL particles using Alexa 488 and visualized its uptake (Fig. 3). In IdIA7 cells there was a small but distinct staining at the cell surface and intracellularly (Fig. 3A). This staining increased dramatically in IdIA7-SRBI cells (Fig. 3A). Besides significant staining at the cell surface β -VLDL particles were found in the perinuclear area. The intensity of staining varied; there were areas of distinct bright staining but also diffuse staining. In contrary, HepG2 cells incubated with fluorescently labeled β -VLDL showed a punctuate/vesicular staining pattern (Fig. 3A), resembling LDLR mediated vesicular uptake, with a light lining of the cell surface. A similar staining pattern was seen in CHO-K1 cells. Furthermore, we followed uptake of β -VLDL-Alexa 488 over time (Fig. 3B). While in IdIA7-SRBI cells an intense staining of the cell membrane appeared at early time points almost no membrane staining was detectable in HepG2 cells. By contrast, in HepG2 cells punctuate intracellular staining was observed at early time points.

These data indicate that β -VLDL is handled differently if taken up preferentially via SR-BI or LDL receptor family. Next, we compared the fate of the lipid component of β -VLDL (labeled with DiI) in these cells (Fig. 4). DiI- β -VLDL uptake was considerably increased by SR-BI over-expression in IdIA7 cells (Fig. 4A). The staining pattern was comparable to Alexa β -VLDL (compare Fig. 3A). HepG2 and CHO-K1 cells showed a more punctuate staining pattern without staining of the plasma membrane. Already at early time points IdIA7-SRBI cells exhibited accumulation of fluorescent lipid derived from DiI-labeled β -VLDL at the cell membrane (Fig. 4B). Incubation with DiI- β -VLDL for 15 or 30 min resulted in an intense staining of IdIA7-SRBI cells mostly at the cell surface. There was a diffuse staining on the cell surface, particularly at cell-cell interfaces. Fluorescent lipid accumulated adjacent to the nucleus especially at later time points (middle). Contrary, in HepG2 cells there was a more punctuate/vesicular staining seen after DiI- β -VLDL treatment already at early time points. Within time these vesicle increased in size, similar to β -VLDL-Alexa 488.

Finally, we analyzed if there was a colocalization of SR-BI and β -VLDL in IdIA7-SRBI-GFP cells (Fig. 5). SR-BI-GFP signal was found at the cell surface and intracellularly exhibiting a punctuate staining pattern (middle). Similarly, β -VLDL visualized by covalent linkage with Alexa 568 showed cell surface staining and punctuate intracellular staining pattern, with intense staining at the perinuclear area (left). There was colocalization (right) of SR-BI and β -VLDL staining both at the cell surface (panel A) and intracellularly (see circles in panel B and inset).

4. Discussion

The present study was designed to analyze β -VLDL uptake in two different settings: in LDL receptor negative SR-BI over-expressing CHO cells and in a liver cell line, HepG2, expressing the LDL receptor family and at much lower levels SR-BI. SR-BI over-expression in IdIA7 cells resulted in a large increase in β -VLDL cell association (Fig. 1); β -VLDL was internalized and degraded and lipids were delivered to intracellular compartments in IdIA7-SRBI cells. HepG2 cells showed lower β -VLDL uptake, and, like in IdIA7-SRBI cells, the particles were degraded over time (Fig. 2). In IdIA7-SRBI cells HDL and LDL competed β -VLDL uptake slightly, whereas in HepG2 and CHO-K1 cells only LDL competed β -VLDL. These data indicate that IdIA7-SRBI and HepG2/CHO-K1 cells use other pathways to internalize β -VLDL, one mediated preferentially by SR-BI and the other one mainly by the LDL receptor family. These data were substantiated by fluorescence microscopy: In IdIA7-SRBI cells a high degree of membrane staining and a diffuse intracellular staining pattern was seen. SR-BI and β -VLDL were found at the same areas of the plasma membrane and at distinct perinuclear compartments. In HepG2 cells and also in CHO-K1 cells most of the β -VLDL was detected in intracellular compartments. Thus, both cell lines exhibited very different uptake pathways, one representing LDLR mediated endocytosis in the liver, the other one representing SR-BI as an alternative way to utilize β -VLDL in the absence of the LDLR.

Several *in vivo* studies suggest that SR-BI processes LDL and other apoB-containing particles thus playing a role in their clearance. First, transgenic mice over-expressing SR-BI in liver show reduced levels of plasma apoB [19,30] and LDL-CE [19] and accelerated plasma clearance of apoB [30]. Second, heterozygous LDLR-deficient mice over-expressing hepatic SR-BI, when fed a high fat diet, exhibit a reduction of atherosclerotic lesions in the aortic root [24]. Third, mice deficient in both SR-BI and apoE show accelerated atherosclerotic lesion development and increased accumulation of VLDL-sized particles [31]. In addition, LDLR^{-/-} mice still show a substantial CE uptake in peritoneal macrophages [10] suggesting an involvement of SR-BI. In this work we present evidence

that SR-BI mediates uptake and subsequent degradation of β -VLDL. Taken together these data indicate that SR-BI can influence, directly or indirectly, the metabolism of apoB-containing particles.

In our study β -VLDL taken up by HepG2 cells exhibited a vesicular staining pattern both when labeling the lipid or apolipoprotein part of β -VLDL. Besides the LDL receptor family several other receptors and uptake mechanisms have been described for VLDL or chylomicron catabolism. Recently, GPI-anchored HDL binding protein 1 (GPIHBP1) was described to be a chylomicron receptor. GPIHBP1-deficient mice accumulate chylomicrons in their plasma [32]. GPIHBP1, however, does not seem to be expressed in our cell culture system, at least no specific signals were detected using a GPIHBP1 antibody [32] and RT-PCR (Stangl and Röhl unpublished data). Therefore it seems unlikely that GPIHBP1 provides an alternative receptor for β -VLDL uptake in our experimental settings. In addition remnant uptake is mediated by multiple interactions of apoE and lipases with heparin sulfate proteoglycans (HSPG) [33]. In our study association of [125 I]- β -VLDL was increased with SR-BI over-expression, but no β -VLDL was released by heparin, indicating differences in binding of SR-BI to β -VLDL and hence for binding of lipases. Therefore, HSPG seem not to be involved in β -VLDL uptake via SR-BI.

It is well described that there is virtually no specific degradation of HDL in IdIA7-SRBI cells while there is a small but detectable degradation of LDL [3,16,17]. This indicates that SR-BI mediates not only selective lipid uptake, but can deliver LDL to cells for degradation. Recently, Van Eck et al. [11] elucidated the role of SR-BI in VLDL metabolism *in vivo*. [125 I]- β -VLDL association was 1.6 fold higher in SR-BI^{+/+} hepatocytes compared with SR-BI^{-/-} hepatocytes, however no specific accumulation of degradation products was observed. Here we reported that there was indeed β -VLDL degradation in both IdIA7-SRBI and HepG2 cells; however degradation was relatively low compared to background levels. We could clearly detect degradation products in the media as well as observe a decay of radioactivity intracellularly. Despite the different receptors and uptake pathways involved β -VLDL degradation was similar in HepG2 and IdIA7-SRBI cells. The amount of radioactivity released to the media is relatively low, at least lower than LDL degradation. Our data are in accordance with previous reports [34] demonstrating a low rate of protein degradation and CE hydrolysis of β -VLDL in macrophages. Moreover, in HepG2 cells most of the β -VLDL was detected in intracellular compartments, probably endosomes which is in line with findings in macrophages [34]. Similarly, Jaeckle et al. [35] showed that β -VLDL and LDL appear at similar subcellular fractions representing an endosomal compartment of rat liver homogenates.

Taken together we have shown that SR-BI mediates β -VLDL uptake besides the well-established LDL receptor mediated endocytosis; this might play a physiological role in case of LDL receptor deficiency or physiological down-regulation by nutrients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. [125 I]- β -VLDL uptake and degradation in ldlA7, ldlA7-SRBI and CHO cells. Cells were grown as described in the methods section. On day 3, cells were fed media A containing faf-BSA and the indicated concentrations of [125 I]- β -VLDL (A). After 1 h cells were washed and lysed. Specific radioactivity associated with cells was estimated by subtracting unspecific binding using a 40-fold excess of unlabeled β -VLDL from total cell association. (B) Cells were incubated with 10 μ g/ml [125 I]- β -VLDL with and without a 10-fold excess of unlabeled HDL, LDL or β -VLDL or (C) 10 μ g/ml [125 I]-HDL or [125 I]-LDL and a 10-fold excess of β -VLDL. (D) Analysis of lipid droplet formation using Bodipy 558/568 C₁₂ in ldlA7 and ldlA7-SRBI cells after incubation with 10 μ g/ml β -VLDL over night. (E) β -VLDL uptake and degradation: cells were incubated with 10 μ g/ml [125 I]- β -VLDL for 1 h; cells were washed and kept in media for up to 24 h. Cells were lysed and analyzed at the indicated time points (E-F). The media was analyzed for specific TCA resistant radioactivity as described in the Materials and methods section (F). Data represent the mean $\pm$ SD of three experiments. ** p <0.01.

Fig. 2.

Characterization of β -VLDL uptake in HepG2 cells. (A) On day 3, cells were incubated with 10 μ g/ml [125 I]- β -VLDL with and without a 10-fold excess of unlabeled HDL, LDL or β -VLDL. After 1 h cells were washed, lysed and analyzed as described above. Data represent mean $\pm$ SD of four experiments. (B) Cells were incubated with 10 μ g/ml [125 I]-HDL or [125 I]-LDL and a 10-fold excess of β -VLDL. (C) After 1 h association cells were washed and kept in media for up to 24 h. Cells were lysed and analyzed at the indicated time points. (D) Visualization of lipid droplet formation by Bodipy 558/568 C₁₂ after incubation with 10 μ g/ml β -VLDL over night. Cells were visualized as described in Materials and methods. * p <0.05, ** p <0.01.

Fig. 3. Visualization of Alexa 488 labeled β -VLDL uptake in ldlA7, ldlA7-SRBI, CHO-K1 and HepG2 cells (A). After overnight cholesterol depletion cells were incubated with 20 $\mu\text{g/ml}$ β -VLDL-Alexa 488 for 1 h and fixed and imaged as described. (B) Time course of β -VLDL uptake in HepG2 and ldlA7-SRBI cells; Bar=10 μm (A) and 5 μm (B). Note the different staining patterns between ldlA7-SRBI and HepG2 cells.

Fig. 4. Cellular distribution of fluorescent lipid - DiI - from β -VLDL delivered to ldlA7, ldlA7-SRBI, CHO-K1 and HepG2 cells. Cells were incubated with 100 μ g/ml DiI-labeled β -VLDL for 1 h (A) or for the indicated times (B). Cells were washed, fixed and analyzed on a fluorescence microscope with a TRITC-filter, Bar=10 μ m (A) and 5 μ m (B). Note that the exposure time was increased at shorter time points (15 and 30 min) for better visualization.

Fig. 5. Colocalization of SR-BI and β -VLDL during the uptake process. IdIA7-SRBI-GFP cells were seeded on chamber slides. After overnight cholesterol depletion cells were treated with 50 μ g/ml β -VLDL-Alexa 568 for 1 h and fixed and imaged as described. Note that colocalization can be observed on the plasma membrane (A) as well as in the perinuclear area (B). Bar=2 μ m.