Scavenger receptor class B, type I (SR-BI) is a cell-surface glycoprotein that mediates selective uptake of high density lipoprotein cholesteryl ester (CE) without the concomitant uptake and degradation of the particle. We have investigated the endocytic and selective uptake of low density lipoprotein (LDL)-CE by SR-BI using COS-7 cells transiently transfected with mouse SR-BI. Analysis of lipoprotein uptake data showed a concentration-dependent LDL-CE-selective uptake when doubly labeled LDL particles were incubated with SR-BI-expressing COS-7 cells. In contrast to vector-transfected cells, SR-BI-expressing COS-7 cells showed marked increases in LDL cell association and CE uptake by the selective uptake pathway, but only a modest increase in CE uptake by the endocytic pathway. SR-BI-mediated LDL-CE-selective uptake exceeded LDL endocytic uptake by 50–100-fold. SR-BI-mediated LDL-CE-selective uptake was not inhibited by the proteoglycan synthesis inhibitor, p-nitrophenyl-β-D-xylopyranoside or by the sulfation inhibitor sodium chlorate, indicating that SR-BI-mediated LDL-CE uptake occurs independently of LDL interaction with cell-surface proteoglycan. Analyses with subclones of Y1 adrenocortical cells showed that LDL-CE-selective uptake was proportional to the level of SR-BI expression. Furthermore, antibody directed to the extracellular domain of SR-BI blocked LDL-CE-selective uptake in adrenocortical cells. Thus, in cells that normally express SR-BI and in transfected COS-7 cells SR-BI mediates the efficient uptake of LDL-CE via the selective uptake mechanism. These results suggest that SR-BI may influence the metabolism of apoB-containing lipoproteins in vivo by mediating LDL-CE uptake into SR-BI-expressing cells.

Scavenger receptor class B, type I (SR-BI) is a cell-surface glycoprotein of molecular mass ~82 kDa that binds HDL, LDL, modified LDL, and VLDL (1–4). In transfected cells, SR-BI mediates the selective uptake of HDL-CE (1), a process in which HDL-CE is transferred into the cell without the concomitant uptake and degradation of the HDL particle (5). Immunoblot analysis of SR-BI in rodents indicates that it is expressed most abundantly in the liver and in steroidogenic cells of the adrenal gland and ovary (1, 6, 7), where the selective uptake of HDL-CE is greatest. In humans, SR-BI (also referred to as Cla-I (4)) shows a similar tissue distribution (8, 9). Direct evidence for SR-BI function is provided by studies in which antibody to the extracellular domain of mouse (m) SR-BI blocked HDL-CE-selective uptake and the delivery of HDL cholesterol to the steroidogenic pathway in cultured murine adrenocortical cells (10). In addition, inactivation of the SR-BI gene in mice increased plasma HDL cholesterol levels and reduced neutral lipid stores in the adrenal glands (11). Similarly, mice carrying an induced SR-BI mutation that reduced hepatic SR-BI expression levels by 50% showed a similar reduction in hepatic HDL-CE-selective uptake (12). These studies with reduced SR-BI expression are complemented by studies in which hepatic SR-BI is overexpressed by either adenovirus vector (13) or via a transgene (14). In these studies HDL cholesterol and apoAI levels were greatly decreased by SR-BI overexpression. Taken together, these observations indicate that SR-BI plays a key role in mediating HDL-CE-selective uptake in the liver and in steroidogenic cells and in determining the plasma levels of HDL cholesterol in mice. Although it is clear that SR-BI binds native LDL (2) and VLDL (3), the role of SR-BI in the metabolism of apoB-containing particles is poorly understood. The cellular metabolism of LDL particles occurs primarily via the LDL receptor and other members of the LDL receptor family (15–17) which process LDL via endocytic uptake and lysosomal degradation (15, 16). Several recent in vivo studies suggest that SR-BI may also process LDL and other apoB-containing particles. Transgenic mice overexpressing SR-BI in the liver show reduced levels of plasma apoB (14, 18) and LDL-CE (14) on both chow and high fat diets and an accelerated plasma clearance of LDL apoB (18). In addition, the sizes of the LDL/intermediate density lipoprotein particles in the transgenic mice are reduced, a result consistent with the selective removal of LDL core CE by hepatic SR-BI (18). Furthermore, in one study with transgenic mice, aortic root lesions were reduced by 80% in heterozygous LDL receptor-deficient mice overexpressing hepatic SR-BI when fed a high fat-, cholesterol-, and bile salt-containing diet (19). In addition, mice deficient in both SR-BI and apoE show accelerated atherosclerotic lesion development in the aortic root region and an increased accumulation of VLDL-sized particles (20). These data indicate that hepatic SR-BI can influence, directly or indirectly, the metabolism of apoB-containing particles and the development of arterial lesions.

The in vivo studies noted above raise important questions about the role of SR-BI in the metabolism of apoB-containing lipoproteins.
lipoproteins. First, are the observed effects indirect via changes in HDL metabolism or direct via processing of LDL particles by SR-BI? Second, if SR-BI can process LDL particles, does it do so by the selective uptake pathway or does SR-BI mediate LDL-CE uptake by endocytic mechanisms? Third, how does the efficiency of SR-BI-mediated CE uptake compare for LDL and HDL particles? The aim of this study was to determine directly whether mSR-BI processes LDL particles by the selective and endocytic uptake pathways. The results showed that mSR-BI mediates the efficient uptake of LDL-CE via the selective uptake mechanism in cells that normally express mSR-BI and in transfected COS-7 cells. These findings suggest that mSR-BI may influence the metabolism of apoB-containing lipoproteins in vivo by mediating LDL-CE uptake into SR-BI-expressing cells.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Transfections—**COS-7 cells were maintained in Dulbecco's modified Eagle's medium, 10% calf serum, 2 mM l-glutamine, 50 units/ml penicillin, 50 μg/ml streptomycin, and 1 mM sodium pyruvate (complete medium). At day 0, cells were seeded at a density of 2 × 10⁶ in a 10-cm dish in 10 ml of fresh medium and incubated until approximately 80–90% confluence (~24 h). On day 1 transfections were performed with Fugene 6 (Roche Molecular Biochemicals) as directed by the manufacturer using pSG5 vector (Strategene) and SR-BI-expressing pSG5 vector, pSG5(mSR-BI) (21). On day 2, cells were trypsinized, resuspended in a total volume of 6 ml with fresh media, and plated 1 ml to each well of a 6-well plate. On day 3, COS-7 cells were incubated with doubly labeled LDL or HDL particles for the indicated times after which cells were washed three times with phosphate-buffered saline containing 0.1% bovine serum albumin and once with phosphate-buffered saline. Cells were lysed with 1.5 ml 0.1 N NaOH, and the lysate was processed to determine trichloroacetic acid-soluble and -insoluble 125I radioactivity and organic solvent-extractable 3H radioactivity as described (22) and cell protein (23). Trichloroacetic acid-insoluble 125I radioactivity represents cell-associated apolipoprotein which is the sum of cell surface-bound apolipoprotein and endocytosed apolipoprotein which is not yet degraded. Trichloroacetic acid-soluble 125I radioactivity represents endocytosed and degraded apolipoprotein that is trapped in lysosomes due to the dilauroylphosphatidyl ethanolamine label. The sum of the 125I-degraded and 125I cell-associated degraded apolipoprotein expressed as CE equivalents was subtracted from the CE measured as extractable 3H radioactivity to get the selective uptake of LDL-CE and HDL-CE (22, 24). Values are expressed as nanograms of cholesterol/mg of cell protein. The LDL concentration dependence for each of these parameters was modeled by a simple binding isotherm composed of a high affinity saturable process and a low affinity non-saturable process (25).

Murine Y1-BS1 adenocarcinoma cells were maintained as described (7). Y1-BS1 cells were treated for 24 h with 100 μM cortrosyn (Organon), a synthetic ACTH1–24 analogue to induce mSR-BI expression prior to incubation with labeled HDL and LDL (10). Antibody inhibition of LDL-CE uptake was performed by incubating Y1-BS1 cells in serum-free Ham's F-10 medium containing 6 mg/ml non-immune IgG or anti-mSR-BI IgG for 1 h prior to addition of labeled LDL (10).

**Isolation of Y1 Subclones and Determination of Cellular SR-BI Levels**—Subclones of the Y1 parent line obtained from ATCC were isolated by limited dilution cloning. Cell lines were maintained as described above, stimulated with ACTH, and post-nuclear supernatants were isolated as described previously (7). Proteins (20 μg) were resolved on an SDS-8% polyacrylamide gel, transferred to a nitrocellulose membrane, and probed with a rabbit polyclonal IgG (2 μg/ml) for the extracellular domain of SR-BI (10) as described (7). Anti-SR-B1 was detected by incubation with 125I-labeled goat anti-rabbit IgG (NEN Life Science Products, 106 dpm/ml) and quantified with a Molecular Dynamics PhosphorImager. Each subclone was analyzed at least twice in triplicate.

**Preparation of Labeled Lipoproteins,** 125I-Dilactitol Tyramine-[3H]Cholesteryl Oleoyl Ether hHDL (125I-[3H]hHDL) and 125I-Dilactitol Tyramine-[3H]Cholesteryl Oleoyl Ether hLDL (125I-[3H]hLDL)—Human (h) HDL (1.125 g/ml < ρ < 1.210 g/ml) and human LDL (1.019 g/ml < ρ < 1.063 g/ml) were doubly labeled with 125I-dilactitol tyramine and [3H]cholesteryl oleoyl ether as described (24). The specific activity of the 125I-[3H]hHDL ranged from 46 to 70 dpm/ng protein for 125I and from 6 to 28 dpm/ng protein for [3H]. The specific activity of the 125I-[3H]hLDL ranged from 25 to 75 dpm/ng protein for 125I and from 3 to 30 dpm/ng protein for [3H]. For 125I-[3H]LDL rabbit βNLDL was prepared as described (26) except all protease inhibitors were omitted. βNLDL was isolated by centrifugation with Na2CO3 modified ICI method (27). Specific activities were 600–1000 dpm/ng protein.

**RESULTS**

**SR-BI-mediated LDL-CE-selective Uptake**—In order to determine whether mSR-BI mediates the selective uptake of LDL-CE, COS-7 cells transfected with vector or with vector expressing mSR-BI were incubated for 4 h with 125I-[3H]hHDL after which LDL cell association, LDL-CE-selective uptake, and LDL-CE endocytic uptake (Fig. 1) were measured. As shown in Fig. 1A, mSR-BI-expressing cells showed a concentration-dependent increase in the cell association of LDL that was approximately 2.5-fold greater than vector-transfected cells. mSR-BI-dependent cell association of LDL showed a high affinity component with an apparent Kd of 14 μg/ml LDL protein, which is similar to that reported for the 4 °C binding of LDL to hamster SR-BI (Kd = 5 μg/ml) (2). Measurement of LDL-CE-selective uptake showed a similar 2.5-fold increase in mSR-BI-expressing cells in comparison to vector-transfected cells (Fig. 1B). LDL-CE-selective uptake showed both low and high efficiency components. The high efficiency component showed an apparent Kd of 9.6 μg/ml. Within the concentration range of LDL examined, the low efficiency component showed a linear increase in LDL-CE-selective uptake as a function of LDL concentration (Fig. 1B) with a slope that was considerably greater than that seen in vector-transfected cells. Thus, the low efficiency component of LDL-CE-selective uptake, as well as the high efficiency component, is dependent on mSR-BI expression. SR-BI expression also increased the endocytic uptake of LDL-CE (Fig. 1C), but the increase was significantly less than that seen for LDL-CE-selective uptake. Importantly, the amount of SR-BI-dependent LDL-CE-selective uptake exceeded LDL endocytic uptake by approximately 50-fold in this experiment and by as much as 100-fold in other experiments (data not shown). These data indicate that SR-BI-mediated cell-surface binding of LDL leads to efficient CE uptake by the selective uptake pathway but only a marginal increase by endocytic uptake.

To examine the relationship between SR-BI-dependent binding and LDL-CE-selective uptake, competition experiments were performed by preincubating transfected COS-7 cells with increasing concentrations of unlabeled LDL prior to addition of labeled LDL. Fig. 2A shows that unlabeled LDL competed effectively at low concentrations (~50 μg/ml) for the cell association of 125I-LDL to both vector-transfected and SR-BI-expressing cells. However, the mSR-BI-dependent component of the LDL cell association showed a more sluggish or gradual competition than occurs with the vector-transfected cells. This is illustrated in Fig. 2B which shows the mSR-BI-dependent component (mSR-BI IgG minus the vector control at each LDL concentration) of competition to vector-transfected cells. The competition curve for the mSR-BI-dependent LDL cell association is indicative of a mixture of low and high affinity components and showed only 70% competition at the highest concentration of competitor tested (300 μg/ml = 30-fold excess compared with radiolabeled LDL). This pattern is even more pronounced for the selective uptake of LDL-CE (Fig. 2C).
concentrations of 125I,3H-hLDL for 4 h at 37°C. Cells were processed to mSR-BI or pSG5 vector pressing COS-7 cells. COS-7 cells transiently transfected with pSG5 select CE uptake, and endocytic CE uptake in mSR-BI-expressing COS-7 cells. LDL-[3H]CE-selective uptake by vector-transfected cells is distinct from that mediated by SR-BI. Note also that we have been unable to detect SR-BI expression in control or vector-transfected COS-7 cells confirming that the basal level of LDL-CE-selective uptake is not due to SR-BI.

Similar experiments were carried out to test HDL as a competitor for SR-BI-mediated LDL-CE-selective uptake. Fig. 3 shows that HDL competed modestly for LDL cell association in vector-transfected or mSR-BI-expressing cells (Fig. 3A). HDL competed for LDL-CE-selective uptake in SR-BI-expressing cells over a wide concentration range but showed no competition for LDL-CE-selective uptake in vector-transfected cells (Fig. 3B). The mSR-BI-dependent component of LDL-[3H]CE-selective uptake was competed by HDL to a maximum of 65% competition at 300 μg/ml unlabeled HDL. Comparison of LDL and HDL as competitors for the mSR-BI-dependent component of LDL-CE-selective uptake (Fig. 4) showed that both lipoproteins competed gradually over a broad concentration range and were similarly effective on a protein basis.

The Role of Cell-surface Proteoglycans in SR-BI-mediated LDL-CE-selective Uptake—The interaction of apoB-containing lipoproteins with cell-surface proteoglycans is believed to play a major role in concentrating LDL within the space of Disse in the liver (28), in facilitating endocytic uptake of remnant particles by the hepatocyte low density lipoprotein receptor-related protein (29), and in direct proteoglycan-mediated LDL endocytosis (30). To investigate the possible significance of cell-surface proteoglycans for selective uptake of LDL-CE, two approaches were taken. In the first, cells were pretreated with 30 mM NaClO3 for 20 h to inhibit sulfation of heparan sulfate and chondroitin sulfate proteoglycans prior to measurement of LDL-CE-selective uptake. As shown in Fig. 5A, NaClO3 treatment reduced LDL-CE-selective uptake by 50% in vector-transfected cells but only by 20% in mSR-BI-expressing cells. After correction for the absolute level of inhibition in the vector-transfected cells, the SR-BI-dependent component of LDL-CE-selective uptake was inhibited by only 15%. In the second approach, COS-7 cells were pretreated with 2 mM p-nitrophenyl-β-D-xylopyranoside for 20 h to inhibit the addition of glycosaminoglycan side chains during proteoglycan biosynthesis. p-Nitrophenyl-β-D-xylopyranoside is a potent inhibitor of chon-
droitin sulfate proteoglycan expression but has little effect on heparan sulfate proteoglycans (31). As shown in Fig. 5B, p-nitrophenyl-β-D-xlyopyranoside failed to inhibit LDL-CE-selective uptake in vector-transfected or mSR-BI-expressing COS-7 cells. To ensure that these inhibitors had effectively reduced cell-surface proteoglycans, untransfected COS-7 cells were pretreated with inhibitors as above and then incubated at 4 °C with rabbit 125I-βVLDL since previous studies showed that the cell-surface binding of βVLDL is mediated by proteoglycans (29). The results showed that the binding of 125I-βVLDL was reduced by 41 and 55% by NaClO₃ and p-nitrophenyl-β-D-xlyopyranoside, respectively, indicating that the inhibitors were effective in reducing proteoglycan biosynthesis (data not shown). We conclude that mSR-BI-mediated selective uptake of LDL-CE occurs independently of cell-surface proteoglycan.

**SR-BI-mediated LDL-CE-selective Uptake in Y1-BS1 Adrenocortical Cells**

To explore the physiological significance of the SR-BI-mediated LDL-CE-selective uptake as seen in transfected COS-7 cells, we examined the Y1-BS1 adrenocortical cell, which naturally expresses mSR-BI and in which mSR-BI has been shown to mediate HDL-CE-selective uptake (10). Fig. 6 shows the cell association (Fig. 6A), selective uptake (Fig. 6B), and endocytic uptake (Fig. 6C) of LDL-CE in Y1-BS1 cells as a function of LDL concentration using double-labeled 125I,3H-hLDL particles. As was seen with the transfected COS-7 cells, these parameters show LDL concentration dependencies indicative of both high and low affinity (or efficiency) components. The high affinity component for cell association showed an apparent $K_d = 6.3 \mu$g/ml, and the high efficiency component for selective uptake showed an apparent $K_m = 3.2 \mu$g/ml, values similar to those seen with the transfected COS-7 cells. Within the LDL concentration range examined, the low affinity components for cell association (Fig. 6A), selective uptake (Fig. 6B), and endocytic uptake (Fig. 6C) behaved in a linear fashion.

In order to test the relationship between mSR-BI expression levels and LDL-CE-selective uptake in Y1 cells, subclones of the parent Y1 cell line were isolated by limited dilution cloning. Measurements of mSR-BI levels and LDL-CE-selective uptake activity were made for 7 subclones and for the Y1-BS1 line. Fig. 7A shows the LDL-CE-selective uptake activities for these clones plotted versus the mSR-BI levels, which were expressed relative to that in the Y1-BS1 set as 100%. Correlation analysis
shows a strong relationship ($r^2 = 0.82$, $p = 0.002$) between the LDL-CE-selective uptake activity and the expression level of mSR-BI. These data suggest that the level of LDL-CE-selective uptake is largely determined by the level of mSR-BI expression in adrenocortical cells. A similar degree of correlation ($r^2 = 0.86$, $p = 0.001$) was seen between mSR-BI levels and the HDL-CE-selective uptake activities among the Y1 subclones (Fig. 7B).

As a further test of the role of mSR-BI in LDL-CE-selective uptake in Y1-BS1 adrenocortical cells, we used antibody raised against the extracellular domain of mSR-BI to block LDL-CE-selective uptake. This antibody has been shown previously to block the selective uptake of HDL-CE in Y1-BS1 cells (10). In comparison to control IgG, anti-mSR-BI inhibited the cell association of LDL by 40% at either of two LDL concentrations (Fig. 8A). Anti-mSR-BI inhibited LDL-CE-selective uptake by 30% at 6 µg/ml LDL and by 75% at 3 µg/ml LDL (Fig. 8B). Thus, at an LDL concentration near the apparent high efficiency $K_m$ for LDL-CE-selective uptake, the major fraction of LDL-CE-selective uptake in Y1-BS1 adrenocortical cells was inhibited by anti-mSR-BI antibody.

In order to compare the efficiency of CE-selective uptake from LDL and HDL particles, the selective uptake data of Fig. 6 were expressed on the basis of the amount of cell-associated CE for LDL or HDL. Thus, this analysis shows the amount of CE uptake as a function of the steady state level of cell-associated lipoprotein during the uptake assay. Since the selective uptake and cell association measurements were done in replicate, the values for each individual measurement are shown plotted in Fig. 9. These data show that for a given amount of cell-associated lipoprotein CE, HDL delivers a much greater percent of its lipoprotein core as compared with LDL. Similar calculations were made in a variety of experiments with the Y1 BS-1 line with similar results. When these data were pooled with those shown in Fig. 9, the average delivery of HDL-CE was 7.4 ± 1.8 (S.D.)-fold greater than that of LDL-CE for an equivalent amount of cell-associated lipoprotein CE. Thus, the fractional delivery of HDL-CE was markedly greater than that...
of LDL-CE. When viewed on a molar basis, however, an LDL particle contains 43 times as many CE molecules per particle compared with an HDL₃ particle. Consequently, for an equivalent steady state number of lipoprotein particles bound to the cell surface, an LDL particle delivered 6.2 times as many CE molecules compared with an HDL particle. Similar values were obtained when LDL-CE and HDL-CE uptake were compared in SR-BI-expressing COS-7 cells. In this case the fractional uptake of HDL-CE was 6.1-fold greater than the uptake of LDL-CE (data not shown).

**DISCUSSION**

Although SR-BI has been characterized as an HDL receptor (1, 10), its ability to bind LDL particles suggested that it might also play a role in LDL metabolism. Several studies with transgenic animals have demonstrated that SR-BI can influence plasma LDL-CE and apoB levels in vivo (14, 18, 19), although it was not clear whether this occurred directly or was secondary to altered HDL metabolism. In the present study we have tested the ability of mSR-BI to mediate the selective and endocytic uptake of LDL-CE. The results show that in transfected COS-7 cells mSR-BI efficiently mediated LDL-CE-selective uptake with only a minimal increase in LDL endocytic uptake. SR-BI-mediated LDL-CE-selective uptake exceeded LDL endocytic uptake by 50–100-fold. In addition, the demonstration that mSR-BI mediates LDL-CE-selective uptake in Y1 adrenocortical cells shows that this activity of mSR-BI occurs in a physiological setting and is not the result of overexpressing this receptor in a transfected cell. These results suggest that at least some of changes in LDL metabolism observed in SR-BI transgenic animals may result from the ability of this receptor to mediate selective CE uptake from LDL particles. SR-BI-mediated changes in HDL metabolism might also indirectly alter the metabolism of apoB-containing particles in vivo. Interestingly, the SR-BI-expressing COS-7 cells showed very little SR-BI-dependent endocytosis of LDL, suggesting that the increased clearance of plasma apoB in SR-BI transgenic mice (18) may be an indirect result of SR-BI expression. Our results, which show directly that mSR-BI mediates LDL-CE-selective uptake, are in agreement with a recent report showing that mSR-BI expression in Chinese hamster ovary cells stimulates the esterification of cholesterol in response to LDL (32), a result consistent with mSR-BI mediating the uptake of either free cholesterol or cholesteryl ester, or both, from LDL particles. In the context of the transfected COS-7 cell, cell-surface proteoglycans do not appear to have a significant role in SR-BI-mediated LDL-CE-selective uptake.

The physiological significance of SR-BI-mediated LDL-CE metabolism in humans remains to be determined. LDL clearance in humans is primarily determined by the LDL receptor pathway that accounts for 67–80% of plasma LDL removal as judged by measurements of radioiodinated LDL clearance in normal individuals and patients with homozygous familial hypercholesterolemia (33). It is possible that SR-BI contributes to the fraction of LDL not removed by the LDL receptor pathway or removes some CE from particles in the VLDL-LDL cascade. Further studies will be required to test these possibilities.

Once bound to mSR-BI, lipoprotein CE is transferred to the cell in a process that appears to occur with different efficiencies for HDL and LDL. In either Y1 cells or mSR-BI-expressing COS-7 cells, the fractional transfer of lipoprotein CE was approximately 6–7-fold greater for HDL than LDL (Fig. 9). Whether this reflects differences in how LDL and HDL particles interact with mSR-BI or is due to inherent differences in the transfer of CE from the particles is not known. Interestingly, because the CE core of the LDL particle is so much larger than that of an HDL₃, the absolute uptake of CE from the LDL particle is about 6-fold greater than from an HDL. Thus, in terms of the absolute delivery of CE molecules, mSR-BI-mediated selective uptake of LDL-CE has the potential to provide for substantial cholesterol delivery to SR-BI-expressing cells. In previous work Reaven and colleagues (22) showed that LDL was used efficiently by the selective uptake pathway of rat ovarian granulosa cells to support steroid production in vivo (34) and in cell culture. Since these cells have now been shown to express high levels of SR-BI (6, 35), it is likely that the ovarian uptake of LDL-CE is largely due to SR-BI.

The present data show that in addition to SR-BI-mediated LDL-CE-selective uptake, COS-7 cells show an SR-BI-independent pathway for LDL-CE-selective uptake. The experiments in Figs. 1B, 2B, and 3B show that vector-transfected cells exhibit LDL-CE-selective uptake at levels of 10–40% that seen in mSR-BI-expressing cells. We have been unable to detect SR-BI expression by COS-7 cells indicating that this level of LDL-CE-selective uptake is unlikely due to low levels of endogenous SR-BI in untransfected cells. Furthermore, the lipoprotein competition data showed that the LDL-CE-selective uptake in vector-transfected COS-7 cells could be competed by LDL (Fig. 2, C and D) but not by HDL (Fig. 3B). In contrast, the mSR-BI-dependent component of LDL-CE-selective uptake was competed equally well by LDL and HDL (Fig. 4). Thus, the LDL-CE-selective uptake seen in untransfected COS-7 cells is not due to SR-BI. The physiological significance of this SR-BI-independent pathway for LDL-CE-selective uptake is unknown at present.

An interesting feature of mSR-BI-mediated LDL-CE-selective uptake is that the LDL concentration dependence shows two components, one indicative of a high efficiency (or affinity) process and the other of a low efficiency process. This was seen in both mSR-BI-expressing COS-7 cells (Fig. 1B) and in Y1-BS1 adrenocortical cells (Fig. 6B). Consistent with the interpretation of multiple SR-BI-dependent components, competition of LDL-[³H]CE-selective uptake by unlabeled LDL occurred gradually over a broad LDL concentration range reaching a maximum of only 45% competition at 300 μg/ml unlabeled LDL (Fig. 2D). One interpretation of these data is that mSR-BI occurs in two forms that exhibit high and low efficiencies of LDL-CE-selective uptake, possibly depending on the state of receptor multimerization or on its localization to different domains of the plasma membrane. An alternative interpretation is that
mSR-BI expression alters the lipid domain organization of the plasma membrane in such a way as to promote low affinity LDL-cell membrane interactions that, while requiring mSR-BI expression, do not reflect direct mSR-BI-LDL binding and, thus, are not effectively competed by unlabeled LDL. Support for the idea that SR-BI expression alters membrane lipid organization is provided by recent studies showing that SR-BI expression in COS-7 cells increased the fraction of membrane-free cholesterol that was sensitive to oxidation by extracellular cholesterol oxidase (36). Although speculative, one possibility is that these changes reflect an altered plasma membrane domain with an increased capacity for free cholesterol flux and possibly for low affinity LDL interactions that lead to LDL-CE-selective uptake. We cannot distinguish these possibilities with the available evidence, but additional studies will serve to test these hypotheses.  

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Scavenger Receptor Class B, Type I, Mediates Selective Uptake of Low Density Lipoprotein Cholesteryl Ester
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