SR-BII, an Isoform of the Scavenger Receptor BI Containing an Alternate Cytoplasmic Tail, Mediates Lipid Transfer between High Density Lipoprotein and Cells*

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The scavenger receptor class B, type I (SR-BI), binds high density lipoprotein (HDL) and mediates selective uptake of cholesteryl ester from HDL and HDL-dependent cholesterol efflux from cells. We recently identified a new mRNA variant that differs from the previously characterized form in that the encoded C-terminal cytoplasmic domain is almost completely different. In the present study, we demonstrate that the mRNAs for mouse SR-BI and SR-BII (previously termed SR-BL2) are the alternatively spliced products of a single gene. The translation products predicted from human, bovine, mouse, hamster, and rat cDNAs exhibit a high degree of sequence similarity within the SR-BII C-terminal domain (62–67% identity when compared with the human sequence), suggesting that this variant is biologically important. SR-BII protein represents approximately 12% of the total immunodetectable SR-BI/S protein in mouse liver. Subcellular fractionation of transfected Chinese hamster ovary cells showed that SR-BII, like SR-BI, is enriched in caveolae, indicating that the altered cytoplasmic tail does not affect targeting of the receptor. SR-BII mediated both selective cellular uptake of cholesteryl ether from HDL as well as HDL-dependent cholesterol efflux from cells, although with approximately 4-fold lower efficiency than SR-BI. In vivo studies using adenoviral vectors showed that SR-BII was relatively less efficient than SR-BI in reducing plasma HDL cholesterol. These studies show that SR-BII, an HDL receptor isoform containing a distinctly different cytoplasmic tail, mediates selective lipid transfer between HDL and cells, but with a lower efficiency than the previously characterized variant.

The delivery of HDL1-cholesteryl ester to cells is achieved by a process that is fundamentally distinct from the well-characterized endocytic pathway mediated by the LDL receptor. LDL receptor-mediated endocytosis results in the uptake of the LDL particle and its receptor. In contrast, HDL-derived lipid is delivered to the cell by a poorly understood mechanism that does not involve internalization of apoAI or apoAII, the two major apolipoproteins of HDL (reviewed in Johnson et al. (1)). In rodents, this process of “selective cholesterol uptake” represents the major mechanism in which HDL-cholesteryl esters are delivered to the liver. The selective transport of lipids from HDL is also the major pathway by which cholesterol is provided to steroidogenic cells in rodents (2, 3).

Although selective cholesterol uptake is poorly understood, a candidate cell-surface receptor for this process has been identified (4, 5). This receptor, most commonly referred to as the scavenger receptor class B, type I (SR-BI), was originally identified through its similarity to CD-36 (6) and through its ability to bind modified LDL (7). However, unlike other characterized receptors for modified lipoproteins, SR-BI also exhibited high affinity binding of native LDL and HDL and was subsequently shown to mediate selective uptake of lipid from HDL (4). Recent studies of mice containing a targeted null mutation in the SR-BI gene provide compelling evidence that SR-BI plays an important role in HDL metabolism in vivo (8). Loss of SR-BI expression resulted in a significant increase in plasma cholesterol levels and decreased adrenal gland cholesterol content.

We recently discovered a variant of SR-BI that is expressed in mice and humans (9). This variant, previously termed SR-BI.2, is termed SR-BII in this paper to conform to the nomenclature recommended for scavenger receptors (10). cDNAs encoding the SR-BI and SR-BII variants are identical except for the region encoding the C-terminal cytoplasmic domain, suggesting that alternative splicing of a single transcript yields two distinct mRNAs. Nuclease protection assays have shown that the SR-BII transcript is relatively abundant in tissues known to express SR-BI, representing about 30, 40, and 80% of total SR-BI mRNA in mouse adrenal glands, liver, and testes, respectively (9). The occurrence of alternative forms has complicated the quantitation of SR-BI and SR-BII mRNAs since conventional Northern blotting did not distinguish between the two forms. Total SR-BI/SII mRNA is expressed at high levels in adrenal glands, ovary, and liver, tissues shown to be the principal sites of selective cholesterol uptake (7, 11). Total SR-BI/SII mRNA expression in adrenals is up-regulated both in apoAI-deficient mice and hepatic lipase-deficient mice (12) as well as lecithin:cholesterol acyltransferase-deficient mice (13), indicating feedback regulation in response to changes in cellular cholesterol stores. In addition, SR-BI/SII mRNA expression is increased in plasma membrane; IM, intracellular membranes; CM, caveolae membranes.

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‡The abbreviations used are: HDL, high density lipoprotein; LDL, low density lipoprotein; SR-BI, scavenger receptor class B, type I; CHO, Chinese hamster ovary; BSA, bovine serum albumin; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PAG, polyacrylamide gel electrophoresis; bp, base pair; kb, kilobase pair; FPLC, fast protein liquid chromatography; PNS, post-nuclear supernatants; PM,

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duced in rat ovary in response to gonadotropin (14) and, in bovine ovary, during granulosa cell luteinization (15).

To date, studies of protein expression have been limited to the SR-BI isoform since the antisera used would not recognize SR-BII. SR-BI protein expression in steroidogenic tissues and liver was shown to be regulated by physiological conditions that alter cholesterol metabolism and cell requirements for cholesterol (11, 16). In addition to mediating the selective uptake of cholesteryl ester from HDL, SR-BI also stimulates HDL-dependent cellular cholesterol efflux (17). Adenovirus-mediated overexpression of SR-BI in the livers of mice resulted in a dramatic decrease in plasma HDL levels and increased cholesterol secretion into the bile (18). Thus, in vitro and in vivo data provide strong evidence that SR-BI plays a key role in HDL metabolism. The role of SR-BII, however, is not yet clear and the presence of markedly different C-terminal cytoplasmic tails raises questions regarding the relative functional activities and physiological significance of the two isoforms.

CHO cells transfected with murine SR-BI cDNA acquired the ability to take up fluorescent lipid from HDL, suggesting that this variant may serve as a functional HDL receptor (9). In the present study, we show that SR-BII mRNA results from alternative splicing of SR-BI precursor transcripts and demonstrate that this isoform mediates selective transfer of lipid between HDL and cells.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—Ida cells (clone 7), an LDL receptor-deficient CHO line (provided by M. Krieger), were cultured in Ham’s F-12 medium containing 5% (v/v) fetal bovine serum, 2 mM glutamine, 50 units/ml penicillin, and 50 μg/ml streptomycin. For the production of stable transfectants, mouse SR-BI and SR-BII coding sequences (9) were cloned into the expression vector pCMV5 (19) and transfected in CHO Ida cells using an MBS transfection kit (Stratagene, La Jolla, CA). Expressing lines were isolated and maintained in media containing 0.5 mg/ml G418. Human HeLa, HepG2, and THP-1 cells were obtained from the supplier.

**Generation of Antibodies and Immunoblot Analysis**—To produce rabbit antisera specific for the two receptor isoforms, we synthesized peptide epitopes containing the intronic sequences of SR-BI exon-intron boundaries were 5’-CACCCCAGTG (forward primer exF1) and 5’-9CCCAGGTTCTTCACTACGCG (reverse primer exR2). DNA fragments based on SR-BI cDNA sequences were used as primers to amplify corresponding genomic sequences from the genomic clone. Sequences of primers used to amplify a 2.9-kb genomic fragment were 5’-CCCCAGTTCTTCTACTAGCGG (forward primer exF1) and 5’-CCCCATCTTATCCTGGGAGCCC (reverse primer exR1). To amplify a 2.9-kb fragment, inF1 (5’-GACCACAGCTCACCCAGTG) and exR2 (5’-GTTCTCAGATGACTAGG) were used for PCR. Sequences of other intronic primers used to confirm the structure and sequence of SR-BI exon-intron boundaries were 5’-GAGGAGGATTGAGAACAAGAG (inR1), 5’-AGGATCCCGTGGTCTTAG (inF2), and 5’-CATCCAGAAGTGGTATTAG (inR2). DNA fragments generated by PCR were sequenced by using an AmpliCycle® sequencing kit (Perkin-Elmer).

**RNA Preparation and S1 Nuclease Analysis**—Total RNA was isolated from human cell lines using the TRIzol® reagent (Life Technologies, Inc.). S1 nuclease analyses were performed as described previously (9) using 50 μg of total RNA and a 291-bp fragment isolated from a human SR-BI cDNA clone. The probe was 3’-end-labeled on the anti-sense strand using the Klenow enzyme and (α-32P)dCTP. For each cell line, three separate preparations of RNA were assayed in duplicate.

**Subcellular Fractionation and Isolation of Caveolae**—Plasma membrane (PM), intracellular membranes (IM), and caveolar membranes (CM) were isolated according to the following modifications (22, 23) with the following modifications. The top 5 ml of the first Opti-Prep gradient was collected, transferred to a new SW41 centrifuge tube, and adjusted to 23% (v/v) Opti-Prep and centrifuged at 105,000 g for 3 h. At 50,000 × g for 90 min at 4 °C. A distinct opaque band was present at both interfaces. The band at the 5–15% interface was collected and designated caveolae membranes. We typically obtained 10–20 μg of protein in this fraction.

**Isolation and Labeling of Lipoproteins**—HDL (d = 1.063 to 1.21 g/ml) was isolated from fresh human plasma by density gradient ultracentrifugation as described previously (24). The HDL3 subfraction (d = 1.13 to 1.18 g/ml) was separated from other HDL subfractions using a density gradient fractionator (ISCO). All isolated fractions were dialyzed against 150 mM NaCl, 0.01% EDTA, sterile-filtered, and stored under nitrogen at 4 °C. Protein concentrations were determined by the method of Lowry et al. (25), and cholesterol concentrations were determined enzymatically (Wako Chemicals). HDL3 apolipoproteins were iodinated by the iodine monochloride method (26) to a specific activity of 400–700 cpm/mg protein. HDL3-associating photoreactive oleyl ester was traced with a nonhydrolyzable, intracellularly trapped [1α,2α-3H]cholesterol oleoyl ether prepared according to the methods of Gwynne and Mahaffee (3).

**Ligand Binding and Uptake Assays**—Binding and uptake assays were carried out essentially as described by Acton et al. (4, 7). Transfected CHO cells and control CHO Ida cells were seeded in 6-well plates 48 h prior to assay (2.5 × 10^5 cells per well). Cell association assays were performed at 37 °C in Ham’s F-12 containing 100 units/ml penicillin, 100 units/ml streptomycin, 2 mM glutamine, 0.5% fatty acid-free BSA, and 10% FCS. Assay conditions were those recommended by the manufacturers, with all solutions prepared fresh daily. Ligand binding and uptake were determined using [3H]cholesterol and [3H]AcLDL at 4 °C. In uptake assays, tissue membranes were prepared from fresh tissues homogenized with 20-s pulses in a Polytron homogenizer (Brinkman Instruments, Westbury, NY) in buffer A (150 mM NaCl, 1 mM CaCl2, 10 mM Tris-HCl, pH 7.4, containing 0.5 μM leupentin and 1 mM phenylmethylsulfonyl fluoride). Homogenates were centrifuged at 50,000 × g for 15 min and the resulting supernatant was resuspended in 6 ml of buffer A by flushing 10 times through a 22-gauge needle. The final membrane pellet was collected by re-centrifugation at 100,000 × g for 1 h, solubilized, and separated by non-reducing SDS-PAGE. Antibody binding was visualized by chemiluminescence detection (ECL, Amersham Pharmacia Biotech) and autoradiography. For some experiments, immunoreactivity was quantitated by densitometry (Personal Densitometer SI, Molecular Dynamics, Sunnyvale, CA). The isoform-specific anti-photoreptide antibodies were used to determine the relative levels of SR-BI and SR-BII protein in mouse tissues. For these experiments, the relative levels of the two isoforms were estimated by determining the immunoreactivities obtained with the two tail-specific antibodies relative to standard amounts of each isoform. Standards for each isoform, prepared from transfected CHO cells and normalized to each other using anti-RED1 reactivity, which cross-reacts with both forms, were run on the same blot.

**Metabolic Labeling and Immunoprecipitation**—Cells were plated into 6-well dishes 48 h prior to labeling (200,000 cells/well). For labeling experiments, cells were washed with PBS and incubated for 30 min in 2 ml of cysteine- and methionine-free Dulbecco’s modified Eagle’s medium (ICN) supplemented with 2 mM glutamine and 2.5 mg/ml lipid-deficient human serum, and then incubated in 0.5 ml of the same media containing 75 μCi/ml Tran3H-label (ICN). Fatty acid labeling was performed essentially as described (21). Labeled cells were collected in 140 μl of ice-cold buffer B (10 mM Hepes, pH 7.4, 200 mM NaCl, 2 mM CaCl2, 2.5 mM MgCl2, 0.1 mM leupentin, 0.1 mM phenylmethylsulfonyl fluoride, 1% (v/v) Triton X-100). Clarified lysates were incubated with 4 μl of anti-RED1 antibody at 4 °C for 90 min on a rotary shaker. Samples were then incubated for 2 h at 4 °C with 45 μl of a 1:1 slurry of protein A-Sepharose CL-4B beads (Sigma) in PBS. The beads were washed 4 times in IP buffer wash (0.1% Trit, pH 8, 2 mM phenylmethylsulfonyl fluoride, 0.5% w/v SDS, 1% (v/v) Triton X-100, 1% (v/v) deoxycholate). For some experiments, the immune complexes were then incubated in either 1% Trit, pH 7.0, or 1% hydroxyamine HCl, pH 7.0, for 1 h at room temperature. Prior to electrophoresis the beads were boiled for 5 min in SDS sample buffer (57 mM Tris-HCl, pH 6.8, 1% (v/v) SDS, 0.005% bromphenol blue, 10% (v/v) glycerol). After electrophoresis on SDS-PAGE (5–20% acrylamide gradient), gels were soaked in 1 mM sodium metabisulfite, dried, and subjected to autoradiography.

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Fig. 1. A, schematic diagram showing the structure of a portion of the murine gene encoding SR-BII. Partial sequences of exons and introns are shown, with exonic sequences boxed. FspI and BamHI recognition sites present in SR-BI cDNA and the location of oligonucleotide primers used for PCR amplifications and cycle sequencing are indicated. Alternative mRNA splicing (boxed) giving rise to SR-BI and SR-BII transcripts is shown above and below the diagram, respectively. B, comparison of the predicted amino acid sequences of human, bovine, rat, mouse, and hamster SR-BII comprising the C-terminal end of the cytoplasmic tail unique to SR-BII. Sequences identical to the human sequence are shown by dashes. The symbols * and △ indicate translation stop codons and the end of published sequences, respectively.

three times with Tris-HCl buffer, pH 7.4, containing 150 mM NaCl and 0.5% fatty acid-free BSA, followed by two washes with Tris-HCl, 150 mM NaCl, pH 7.4. All washes were performed at 4 °C with pre-chilled solutions. Cells were solubilized in 0.1 N NaOH for 20 min at room temperature prior to protein and radioactivity quantitation. 125I-Labeled HDL binding assays at 4 °C were performed similarly, except that cells were incubated for 5 h with varying concentrations of 125I-labeled HDL in Ham's F-12 containing 0.2% BSA to allow for the equilibration of cellular cholesterol pools. Cells were then washed and incubated at 37 °C for the indicated times in efflux media (Ham's F-12, 0.2% fatty acid-free BSA, followed by two washes with Tris-HCl, 150 mM NaCl and 0.01% sodium azide). The cholesterol content of individual FPLC fractions (0.5 ml) was determined enzymatically (Wako Chemicals). Plasma HDL cholesterol was measured enzymatically after precipitation of LDL and VLDL by heparin and manganese.

**RESULTS**

The sequences of cDNAs encoding SR-BI and SR-BII suggested that these two forms result from alternative processing of a single precursor transcript such that a 129-nucleotide sequence present in the SR-BI mRNA is removed to yield SR-BII (9). To define the nature of the alternative splicing, a BALB/c genomic clone which spanned the sequence of interest was isolated and analyzed by PCR and cycle sequencing. Amplifications with primers exF1 and exR1 (Fig. 1A) yielded a 2.9-kb product whose 5'- and 3'-terminal sequences coincided with SR-BI cDNA sequences. Furthermore, nucleotide sequencing delineated an exon-intron boundary precisely at the 5' end of the 129-bp sequence which is absent from SR-BII. A similar analysis using inF1 and exR2 as PCR primers identified a 3.6-kb intron located at the 3' end of the 129-bp sequence. These data reveal that SR-BII mRNA arises from an "exon skipping" mechanism whereby a 129-nucleotide exon included in SR-BI transcripts is spliced out.

The 129-bp alternatively spliced exon contains a translation stop codon (TAG) at its 3' end (Fig. 1A). Alternative splicing, therefore, shifts the open reading frame such that a 3'-untranslated sequence of the SR-BI transcript becomes a coding sequence in the SR-BII transcript. As described previously for mouse and human SR-BII, the predicted translation products extend 39 amino acids beyond the splice junction, with 67% of these being identical between mice and humans. These 39 amino acids unique to SR-BII comprise the C-terminal end of a predicted 44-amino acid cytoplasmic tail (SR-BI has a 47-amino acid C-terminal cytoplasmic tail). To determine whether other species have the potential to encode SR-BII, we compared the 3'-untranslated regions of human, bovine, mouse, and hamster SR-BI cDNAs (GenBank accession numbers Z22555, AF019384, U37799, and U11453, respectively) and rat SR-BI cDNA (14). The length of the open reading frame corresponding to SR-BII is the same in human, bovine, rat, and mouse cDNAs (the available sequence for hamster does not extend far enough to identify the predicted SR-BI translation stop codon). Furthermore, the nucleotide sequences coding for SR-BII are highly conserved in this region (75% identity when compared with the...
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human sequence). In contrast, the sequences immediately downstream of the predicted SR-BII stop codon are not at all similar. The predicted SR-BII translation product for each of these species shows a high degree of sequence similarity that ranges from 62 to 67% identity when compared with the human sequence (Fig. 1B), suggesting that SR-BII homologues exist in each of these species.

We have previously shown that SR-BII mRNA is expressed in significant amounts in the mouse, representing about 80% of total SR-BII mRNA in testes (9). To measure the relative abundance of SR-BII in human cells, S1 nuclease protection experiments (see below) by immunoblotting known dilutions of transfected cell lysates with anti-RED1 (not shown). By using the tail-specific antibodies, immunoblot analysis was carried out on membrane fractions isolated from mouse liver, adrenal, and testes (Fig. 2B). Anti-BII491 detected a polypeptide in each of the tissues which migrated at the same apparent molecular weight as SR-BII expressed by transfected CHO cells, albeit at lower levels than SR-BI. The identities of the other anti-BII491 immunoreactive bands are not known. Taking into account the relative immunoreactive signals and the amount of protein loaded in each lane (5 times more for the anti-BII491 blots), SR-BII was found to represent approximately 12% of the total immunodetectable SR-BII in mouse liver and lesser amounts, approximately 5%, of total SR-BII in mouse testes and adrenal glands.

The presence of distinctly different cytoplasmic tails might be expected to result in differential trafficking of SR-BI and SR-BII. SR-BII has recently been shown to be concentrated in caveola, a plasma membrane microdomain enriched in cholesterol and glycolipids (21). To compare the subcellular localization of SR-BI and SR-BII, intracellular membrane (IM) and plasma membrane (PM) fractions were isolated from post-nuclear supernatants (PNS) of CHO cells transfected with SR-BI or SR-BII. Immunoblot analysis of cell equivalent portions of the PNS, IM, and PM showed that the bulk of this endogenous SR-BI/II in mouse liver and lesser amounts, approximately 5%, of total SR-BII/II in mouse testes and adrenal glands.

To distinguish between SR-BI or SR-BII protein, rabbit antisera were prepared using peptides corresponding to a unique region of the C-terminal tail of the two mouse isoforms (anti-BI495 and anti-BII491). The specificity of these antisera is demonstrated in Fig. 2A, where the SR-BI and SR-BII isoforms, expressed in transfected CHO cells, were recognized only by their corresponding antisera. An antiserum (anti-RED1) raised against a portion of the extracellular domain shared by both SR-BI and SR-BII recognized both isoforms. Based on the relative signals produced by each of the isoform-specific antisera and the signals produced by anti-RED1 (compare lane 2 of the anti-BI495 blot and lane 3 of the anti-BII491 blot with lanes 2 and 3 of the anti-RED1 blot), we conclude that the immunoreactivity of anti-BI495 and anti-BII491 with their respective isoform is similar. The relative amounts of SR-BI and SR-BII in the two transfected cell lines were determined in quantitation experiments (see below) by immunoblotting known dilutions of transfected cell lysates with anti-RED1 (not shown). By using the tail-specific antibodies, immunoblot analysis was carried out on membrane fractions isolated from mouse liver, adrenal, and testes (Fig. 2B). Anti-BII491 detected a polypeptide in each of the tissues which migrated at the same apparent molecular weight as SR-BII expressed by transfected CHO cells, albeit at lower levels than SR-BI. The identities of the other anti-BII491 immunoreactive bands are not known. Taking into account the relative immunoreactive signals and the amount of protein loaded in each lane (5 times more for the anti-BII491 blots), SR-BII was found to represent approximately 12% of the total immunodetectable SR-BII/II in mouse liver and lesser amounts, approximately 5%, of total SR-BII/II in mouse testes and adrenal glands.

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cytic receptor was present in the PM and not detected in the CM (data not shown).

Like many proteins concentrated in caveolae, SR-BI is palmitoylated, most likely on one or two cysteine residues located on the short C-terminal cytoplasmic tail of the receptor (Cys<sup>462</sup> and Cys<sup>470</sup>) (21). Since one of these cysteine residues (Cys<sup>462</sup>) is conserved in SR-BII, we determined whether this isofrom is similarly modified. CHO cells stably transfected with either mouse SR-BI or SR-BII cDNA were metabolically labeled with [3H]palmitate or [35S]methionine/cysteine for 2 h, and cell lysates were immunoprecipitated using anti-RED1. Both cell lines incorporated label from [3H]palmitate (Fig. 4, lanes 1 and 3). Treatment with 1 M hydroxylamine resulted in the loss of the radiolabel derived from [3H]palmitate (lanes 2 and 4) but not [35S]methionine/cysteine (not shown). The observed sensitivity to hydroxylamine is consistent with the presence of a thioester linkage, which is characteristic of protein palmitoylation (32).

CHO cells transfected with SR-BII cDNA were previously reported to acquire the ability to take up fluorescent lipid from HDL (9). To determine directly the HDL-binding properties of mouse SR-BII, equilibrium binding analyses using transfected CHO ldlA cells was performed. Receptor-specific binding was defined as the difference between total HDL binding to transfected cells and to control non-transfected ldlA cells. Cells expressing SR-BII were found to exhibit specific, high affinity, saturable binding of [125I]-labeled HDL at 4 °C (apparent K<sub>d</sub> = 28 μg/ml) comparable to the binding exhibited by SR-BI-transfected cells (K<sub>d</sub> = 22 μg/ml; data not shown).

The ability of SR-BII to bind [125I]-HDL with high affinity was not surprising, given the fact that the extracellular domain of this isoform is identical to that of SR-BI. To assess the impact of the distinct C-terminal cytoplasmic tail on the ability of SR-BII to mediate selective lipid uptake from HDL, transfected CHO cells were incubated with either [125I]-HDL or HDL labeled with [3H]cholesteryl ether. Maximum association of [125I]-HDL was achieved after 1–2 h (Fig. 5A). Maximal cell association was about 5 times greater for SR-BII than SR-BI expressing cells, and this was attributed to the greater expression of receptor in the SR-BII cells. Quantitative immunoblot analysis of the transfected cells using anti-RED1 showed that SR-BII levels were approximately 6-fold higher than SR-BI (see also Fig. 2). No [125I]-labeled degradation products were observed for either of the cell lines during the incubation period indicating that the ligand was not internalized and degraded by a process analogous to receptor-mediated endocytosis. Incubation of both cell lines with [3H]cholesteryl ether-labeled HDL resulted in the association of increasing amounts of [3H]label during the 3-h incubation period (Fig. 5B). In comparison to [125I]-HDL, significantly greater amounts of [3H]cholesteryl ether were cell-associated (note difference in scales of y axis), indicating selective uptake of cholesteryl ether from HDL by both receptor isoforms. Interestingly, both of the transfected lines accumulated similar amounts of cell-associated cholesteryl ether (approximately 30% of added ligand after 2 h), despite the fact that the SR-BII-expressing cells showed a higher level of cell-associated [125I]-HDL.

The ability of SR-BII to mediate HDL-dependent free cholesteryl efflux from cells was also assessed. As shown in Fig. 6, SR-BII in transfected cells promoted cholesteryl efflux from cells, which was dependent on HDL in the media. Efflux was significantly lower in untransfected ldlA cells. The rate of cholesteryl efflux from SR-BII-expressing cells was similar to that from the CHO-SR-BI cells. To determine whether SR-BII functions as an HDL receptor in vivo, we tested the effect of high level hepatic expression of SR-BII on plasma HDL cholesterol levels in mice. For these experiments, we constructed recombinant adenoviruses (AdSR-BI and AdSR-BII) containing mouse SR-BI or SR-BII coding sequences under the control of the strong, constitutive cytomegalovirus immediate-early enhancer promoter. Injection of 5 × 10<sup>10</sup> particles of AdSR-BI resulted in a drop in plasma HDL cholesterol to undetectable levels 3 days after treatment (Fig. 7A), in agreement with a previous report that overexpression of SR-BI by a similar expression vector results in a dramatic

| radiolabel | [3H] Palmitate |
|-----------|--------------|
| cell line | SR-BI | SR-BII |
| Hydroxylamine | - | + | + |

Fig. 4. Incorporation of radiolabel from [3H]palmitate in SR-BI and SR-BII. CHO ldlA cells transfected with SR-BI or SR-BII cDNA were incubated in media containing [3H]palmitate. After labeling, cell lysates were immunoprecipitated using anti-RED1 and protein A-Sepharose beads as described under “Experimental Procedures.” The immune complexes were incubated in either 1 M hydroxylamine HCl, pH 7.0 (lanes denoted with +), or 1 M Tris, pH 7.0 (lanes denoted with −), prior to analysis by non-reducing SDS-PAGE and autoradiography. The autoradiogram shown was exposed for 2 weeks.

Fig. 5. Selective HDL cholesterol uptake mediated by SR-BI and SR-BII. A, cells were incubated at 37 °C with [125I]-HDL (10 μg of protein/ml) for the indicated times, and cell-associated label was quantified as described under “Experimental Procedures.” Values represent the mean of triplicate determinations and are expressed as a percentage of the total [125I]-HDL added. B, cells were incubated at 37 °C with [3H]cholesteryl ether-labeled HDL (2 μg of cholesterol/ml; approximately 9.2 μg of protein/ml) for the indicated times, and cell-associated label was quantified. Values represent the mean of triplicate determinations and are expressed as a percentage of total [3H](cholesteryl ether added. Similar results to those depicted in A and B were obtained in two additional experiments.
reduction of plasma HDL cholesterol in mice (18). Day 3 corresponded to the maximum level of expression in the treated animals (data not shown) and was previously shown for SR-BI to correspond to the time of maximal reduction of plasma HDL cholesterol (18). Injection of AdSR-BII (5 × 10^{10} particles) also produced a significant drop in cholesterol in the HDL fraction at 3 days after treatment, although this effect was less pronounced than that produced by AdSR-BI. Treatment with a control adenoviral vector containing no transgene (Adnull) had no effect on lipoprotein profiles compared with untreated mice. Plasma HDL cholesterol levels in the treated and control animals are shown in Fig. 7B. Plasma HDL cholesterol levels were significantly reduced by AdSR-BI (52% decrease), although to a lesser extent than for AdSR-BI (75% decrease). This difference between AdSR-BI and AdSR-BII is not simply due to lower hepatic SR-BII expression since immunoblotting indicated that the levels of SR-BII are approximately 3-fold higher than SR-BI in the livers of these mice (Fig. 7B).

**DISCUSSION**

SR-BI and SR-BII are two isoforms of a class B scavenger receptor distinguished by markedly different C-terminal cytoplasmic tail domains. SR-BI and SR-BII mRNAs are present in comparable amounts in mouse liver and adrenal glands, whereas in testes SR-BII mRNA is approximately 4-fold more abundant (9). In this report, we extend our analysis of SR-BII by defining the alternative splicing which gives rise to murine SR-BI and SR-BII mRNAs, measuring the relative abundance of SR-BI and SR-BII transcripts in human cells and identifying SR-BII protein in mouse tissues. In addition, the impact of the different cytoplasmic tail of SR-BII on the cellular localization and functional activity of the receptor is evaluated. These studies demonstrate that the two receptor isoforms, which contain distinct C-terminal cytoplasmic tails, both transfer lipid between HDL and cells although with different efficiencies.

Nucleotide sequencing of mouse genomic DNA determined that precursor mRNAs are alternatively spliced such that a 129-nucleotide exon is either included or omitted to yield SR-BI or SR-BII transcripts, respectively. The gene encoding mouse SR-BI/II resides on chromosome 5 (33). The mechanisms responsible for splice-site selection and the variation that yields different amounts of the two transcripts in different tissues are not yet understood. We investigated whether adrenocorticotropic hormone, which induces SR-BI gene expression in mouse adrenal cells both in vivo and in vitro (12, 16), preferentially up-regulates SR-BI or SR-BII transcripts in mouse adrenal Y1 cells. In two separate experiments, treatment with 100 nM adrenocorticotropic hormone for 8 h resulted in a 50–60% increase in the level of total SR-BI/II mRNA, whereas the relative amounts of SR-BI and SR-BII transcripts remained unchanged (data not shown). Further experiments are necessary to determine if splice-site selection is modified by development or other hormone-induced metabolic changes.

A recent description of the human SR-BI gene (34), which maps to chromosome 12 (35), indicates that exon 12 corresponds exactly in size and position to the 129-nucleotide exon described in the mouse in this study. We have now identified and quantified the human homologue of SR-BI mRNA, demonstrating that alternative splicing of precursor transcripts occurs also in human cells. The analysis of the 3′ non-coding sequences of bovine, hamster, and rat SR-BI cDNAs suggested that other species might also have the potential to encode SR-BII protein. A high degree of nucleotide sequence similarity exists in the region immediately downstream of the SR-BI
transcription stop codon (approximately 75% identity when compared with the human sequence), including a completely conserved putative SR-BII stop codon. The translation product encoded by this region also exhibits extensive sequence similarity (67% identity when compared with the human sequence) and is distinguished by the presence of several conserved proline, serine, and threonine residues. Although the exonic organization of the bovine, hamster, and rat SR-BI genes has not been characterized, we predict on the basis of this sequence conservation that alternative splicing may well result in expression of SR-BII protein in each of these species. The possibility that the SR-BII C-terminal tail is phosphorylated on serine or threonine has not been investigated. An analysis of these sequences did not identify a phosphorylation site consensus motif that was conserved in the different species (36).

SR-BII protein was detected in mouse liver, testis, and adrenal glands using an antisera specific for this isoform. However, the level of SR-BII protein was low (5–12% of immunodetectable SR-BI) relative to the abundance of SR-BII transcripts in these tissues (30–80% of total SR-BI/II mRNA). A discrepancy between mRNA and protein levels has previously been reported for SR-BI (5). A possible reason for this discrepancy is that the SR-BII translation product is unstable in cells. However, the apparent half-lives of SR-BI and SR-BII in transfected CHO cells as determined by pulse-chase studies were similar (approximately 2 h), suggesting that protein instability is not responsible for the relatively low levels of SR-BII detected in mouse tissues (data not shown). Another possibility is that SR-BI transcripts are translated more efficiently. If this is the case, the structure of the mRNAs near the 3’ end of the SR-BI coding region might impact on translational efficiency since the transcripts otherwise are presumably identical. In the case of human cells, immunoblotting with anti-BII 491 did not detect SR-BII, but this could have been due to the sequence differences between human and mouse (only 10 out of 16 amino acid residues are identical in the region used to generate the mouse anti-peptide antibody).

The functional activities of mouse SR-BI and SR-BII were assessed in transfected CHO lines expressing each isoform. SR-BI and SR-BII both bind HDL with similar high affinity. SR-BII, as previously shown for SR-BI, mediates the selective uptake of cholesterol ester from HDL, whereas no whole particle uptake occurred, as judged by a lack of apolipoprotein uptake and degradation. Interestingly, [3H]cholesteryl ether uptake mediated by CHO cells expressing SR-BI or SR-BII was almost equivalent, even though the apparent half-lives of SR-BI and SR-BII in transfected CHO cells as determined by pulse-chase studies were similar (approximately 2 h), suggesting that protein instability is not responsible for the relatively low levels of SR-BII detected in mouse tissues (data not shown). Another possibility is that SR-BI transcripts are translated more efficiently. If this is the case, the structure of the mRNAs near the 3’ end of the SR-BI coding region might impact on translational efficiency since the transcripts otherwise are presumably identical. In the case of human cells, immunoblotting with anti-BII 491 did not detect SR-BII, but this could have been due to the sequence differences between human and mouse (only 10 out of 16 amino acid residues are identical in the region used to generate the mouse anti-peptide antibody).

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The mechanism by which SR-BI and SR-BII promote selective lipid transfer is not understood. Our studies demonstrate that two isoforms whose predicted extracellular domains are identical bind HDL with similar affinity but transfer lipid between HDL and cells with different efficiency. This indicates that the C-terminal intracellular domain is important for optimal cholesterol uptake/efflux. With the exception of 5 identical residues proximal to the predicted transmembrane domain, the sequence of the 47-amino acid SR-BI and 44-amino acid SR-BII C-terminal cytoplasmic tails is completely different. This marked difference in the C-terminal tail does not alter the intracellular distribution of the receptor since SR-BII, like SR-BI, is targeted to caveolae. Our evidence indicates that SR-BII is modified by thioester-linked palmitate, a feature shared with many other caveolar proteins, including SR-BI (21). Based on the deduced amino acid sequence, the likely site of SR-BII palmitoylation is Cys462 which is predicted to lie at the junction of a transmembrane domain and the C-terminal cytoplasmic tail. SR-BI shares Cys462 with SR-BII and contains an additional potential site for palmitoylation at Cys470. Further studies are required to determine whether SR-BI is palmitoylated at both of these potential sites and whether differential fatty acylation underlies the observed difference in selective lipid transfer. Johnson and co-workers (37) recently reported that the three C-terminal amino acid residues of SR-BI comprise a peroxisomal targeting sequence. The significance of this observation is not known, but the fact that SR-BII does not contain this motif presents another distinction between these isoforms. The data presented here may provide important insights for future structure-function studies of SR-BI and SR-BII.

In conclusion, our results demonstrated the expression of SR-BII receptor in various mouse tissues and the presence of significant levels of SR-BII mRNA in human cells. The SR-BII isoform, while having a markedly different cytoplasmic tail to that of SR-BI, mediates both the selective uptake of lipid from HDL and cellular cholesterol efflux, although with lower efficiency. It remains possible that other functional differences not yet detected exist between the two isoforms. Clearly, the relative expression and functional activities of these isoforms represent a potential mechanism for regulating selective lipid transfer between HDL and cells.

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