Scavenger receptor class B type I is solely responsible for the selective uptake of cholesteryl esters from HDL by the liver and the adrenals in mice

Ruud Out,[1] Menno Hoekstra,[1] John A. A. Spijkers, Johan K. Kruijt, Miranda van Eck, Ingrid S. T. Bos, Jaap Twisk, and Theo J. C. Van Berkel[2]

Leiden/Amsterdam Center for Drug Research, Division of Biopharmaceutics, Gorlaeus Laboratories, Leiden University, 2300 RA Leiden, The Netherlands

Abstract **Scavenger receptor class B type I (SR-BI) has been identified as a functional HDL binding protein that can mediate the selective uptake of cholesteryl ester (CE) from HDL. To quantify the in vivo role of SR-BI in the process of selective uptake, HDL was labeled with cholesteryl ether ([^{3}H]CEt-HDL) and ^{125}I-tyramine cellobiose ([^{125}I]TC-HDL) and injected into SR-BI knockout (KO) and wild-type (WT) mice. In SR-BI KO mice, the clearance of HDL-CE from the blood circulation was greatly diminished (0.043 ± 0.004 pools/h for SR-BI KO mice vs. 0.106 ± 0.004 pools/h for WT mice), while liver and adrenal uptake were greatly reduced. Utilization of double-labeled HDL ([^{3}H]CEt and [^{125}I]TC) indicated the total absence in vivo of the selective decay and liver uptake of CE from HDL in SR-BI KO mice. Parenchymal cells isolated from SR-BI KO mice showed similar association values for [^{3}H]CEt and [^{125}I]TC in contrast to WT cells, indicating that in parenchymal liver cells SR-BI is the only molecule exerting selective CE uptake from HDL. Thus, in vivo and in vitro, SR-BI is the sole molecule mediating the selective uptake of CE from HDL by the liver and the adrenals, making it the unique target to modulate reverse cholesterol transport.**—Out, R., M. Hoekstra, J. A. A. Spijkers, J. K. Kruijt, M. van Eck, I. S. T. Bos, J. Twisk, and T. J. C. Van Berkel. **Scavenger receptor class B type I is solely responsible for the selective uptake of cholesteryl esters from HDL by the liver and the adrenals in mice.** ***J. Lipid Res.*** **2004.** 45: **2088–2095.**

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In both mice (1, 2) and humans (3) there is a strong inverse relation between the blood levels of HDL and the development of atherosclerosis. The atheroprotective effect of HDL is ascribed to its role in reverse cholesterol transport (RCT), as first proposed by Glomset (4), in which HDL accepts cholesterol from peripheral cells, including those in the arterial wall, and delivers it to the liver for biliary secretion (reviewed in Refs. 4–10). In addition, HDL can deliver its cholesteryl ester (CE) to the adrenals and testis or ovary for steroid hormone synthesis (11, 12). At both the liver and steroidogenic tissues, cholesterol delivery occurs via selective cellular uptake of HDL-CE without stoichiometric degradation of HDL protein (13, 14). Acton and coworkers (15) provided the first evidence that scavenger receptor class B type I (SR-BI) can mediate the selective uptake of HDL-CE in Chinese hamster ovary cells stably transfected with mouse SR-BI. Furthermore, treatment of the adrenocortical cell line Y1-BS1 with antibodies directed against mouse SR-BI resulted in a dramatic decrease in the selective uptake of HDL-CE (16).

In vivo, the expression levels of rat and mouse SR-BI mRNA and protein are highest in liver and steroidogenic tissues (adrenal gland, testis, and ovary) (17, 18), all tissues that display selective uptake of HDL-CE. We showed earlier (19) that changes in SR-BI expression in rat liver, induced by estradiol treatment or a high-cholesterol diet, correlated with changes in the selective uptake of HDL-CE in vivo, supporting a function of SR-BI in mediating the selective uptake of HDL-CE. Adenovirus-mediated hepatic overexpression of SR-BI in mice on both sinusoidal and canalicular surfaces of hepatocytes resulted in the virtual disappearance of plasma HDL and a substantial increase in biliary cholesterol (20). A similar decrease in plasma HDL cholesterol levels was found in transgenic mice overexpressing SR-BI under the control of the apolipoprotein A-I promoter (21). These studies indicated that SR-BI expression in the liver can regulate blood HDL me-

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Abbreviations: CE, cholesteryl ester; CEt, cholesteryl ether; FCR, fractional catabolic rate; ID, injected dose; KO, knockout; oxLDL, oxidized LDL; RCT, reverse cholesterol transport; SR-BI, scavenger receptor class B type I; TC, tyramine-cellobiose; WT, wild-type.

[1] R. Out and M. Hoekstra contributed equally to this study.

[2] To whom correspondence should be addressed.
e-mail: t.berkel@lacdr.leidenuniv.nl

tabolism and influence cholesterol secretion into bile. Studies with transgenic mice with liver-specific overexpression of SR-BI showed increased total and selective uptake of HDL-CE by the liver compared with nontransgenic controls (22, 23). In addition, in SR-BI attenuate (att) mice with an SR-BI promoter mutation resulting in decreased expression of the receptor, the hepatic uptake of HDL-CE was decreased accordingly (24). Further evidence for the role of SR-BI in RCT was provided by studies in SR-BI knockout (KO) mice. These animals displayed impaired biliary cholesterol secretion (25, 26) and increased plasma cholesterol concentration in large apolipoprotein A-I-containing particles, together with low adrenal gland cholesterol content (27, 28).

Although the aforementioned studies are consistent with a regulatory role of SR-BI in HDL metabolism, there is no report that addresses the quantitative contribution of SR-BI in vivo to the selective uptake of HDL-CE by the liver and the adrenals. By using SR-BI KO mice in the present study, we made a quantitative comparison of the metabolism of HDL-CE in SR-BI KO and wild-type (WT) mice to assess the contribution of SR-BI to the serum decay and hepatic and adrenal uptake of HDL-CE. The data obtained are compared with data for holo-particle uptake, determined by analyzing the fate of ^{125}I-tyramine-cellobiose-labeled HDL ([^{125}I]TC-HDL) in the animals, to assess whether additional HDL binding proteins perform selective CE uptake from HDL. In addition, studies with isolated parenchymal liver cells from WT and SR-BI KO mice demonstrate the quantitative importance of SR-BI for the high level of selective uptake of HDL-CE by these cells.

MATERIALS AND METHODS

Egg yolk phosphatidylcholine was from Fluka (Buchs, Switzerland). Cholesteryl [1α,2α(n)-^{3}H]oleoyl ether ([^{3}H]CEt), cholesteryl[1α,2α(n)-^{3}H] ester ([^{3}H]CE), and ^{125}I (carrier free in NaOH) were obtained from Amersham (Piscataway, NJ). The PL phospholipids kit, the CHOD-PAP (cholesterol oxidase-peroxidase aminophenazone) kit, and the GPO-PAP (glycerolphosphate oxidase-peroxidase aminophenazone) kit were from Roche Diagnostics (Mannheim, Germany). Hypnorm and Thalamonal were from Janssen Pharmaceutica (Titusville, NJ), and ketamine was from Bela-Pharm (Vechta, Germany). Ethylmercurithiosalicylate (thimerosal), BSA (fraction V), and collagenase type IV were from Sigma-Aldrich (St. Louis, MO). DMEM was from BioWhittaker (Walkersville, MD). All other chemicals were of analytical grade.

Animals

Heterozygous *srbI*$^{+/-}$ mice on a 129(agouti)/C57BL/6 background were kindly provided by Dr. Monty Krieger (Massachusetts Institute of Technology). The offspring of these mice was analyzed by polymerase chain reaction as described (27) for the presence of the targeted or WT SR-BI alleles. Experiments were carried out with homozygous mutant (*srbI*$^{-/-}$) progeny. The WT (*srbI*$^{+/+}$) littermates were used as controls. All animals used were between 8 and 10 week old males. Animals were maintained on a 12 h light/dark cycle and had unlimited access to regular chow diet (SRM-A; Hope Farms, Woerden, The Netherlands) and water. Animal welfare was in accordance with institutional guidelines.

Phospholipid liposome preparation

Unilamellar liposomes were prepared from egg yolk phosphatidylcholine and labeled with [^{3}H]CEt or [^{3}H]CE as described (29).

Isolation and labeling of lipoproteins

Human HDL and LDL were isolated from blood of healthy subjects by differential ultracentrifugation as described by Redgrave, Roberts, and West (30) and dialyzed against PBS with 1 mM EDTA. HDL ($1.063 < d < 1.21$) was labeled with [^{3}H]CEt or [^{3}H]CE via exchange from donor particles as reported previously (31). Donor particles were formed by sonication of egg yolk phosphatidylcholine supplemented with 50 μCi of either [^{3}H]CE of [^{3}H]CEt. Sonication was carried out with a MSE soniprep 150 for 40 min (amplitude, 12 μm) at 52°C under a constant stream of argon in a 0.1 M KCl, 10 mM Tris, 1 mM EDTA. 0.025% NaN_3 buffer, pH 8.0. Donor particles with a density of 1.03 g/ml were isolated by density gradient centrifugation. HDL was labeled by incubating HDL with donor particles (mass ratio of HDL protein/particle phospholipid = 8:1) in the presence of human lipoprotein-deficient serum as the CE transfer protein source (1:1, v/v) for 8 h at 37°C in a shaking-water bath under argon. Ethylmercurithiosalicylate (thimerosal; 20 mM) was added to stimulate CE transfer and to inhibit phospholipid transfer and lecithin:cholesterol acyltransferase activity. Radiolabeled HDL was then isolated by density gradient ultracentrifugation.

The specific activity varied between 5 and 8 dpm/ng protein. For some experiments, HDL was doubly labeled with [^{125}I]TC. Synthesis and subsequent radioiodination of TC were performed as described earlier (32). Coupling of [^{125}I]TC to HDL was done as described by Bijsterbosch, Ziere, and van Berkel (33). To 50 μl of 0.3 mM [^{125}I]TC were successively added 20 μl of 0.75 mM cyanuric chloride in acetone and 10 μl of 3.0 mM NaOH. After 20 s, 20 μl of 2.25 mM acetic acid was added. The resulting activated ligand was added to 1–2 mg of HDL in 1 ml of 20 mM sodium tetraborate buffer, pH 9.0, containing 0.12 M NaCl and 1 mM EDTA. After 30 min at room temperature, the reaction was quenched by the addition of an equal volume of 0.2 M NH_4HCO_3. Unbound label was removed by exhaustive dialysis against phosphate-buffered saline (10 mM sodium phosphate buffer, pH 7.4, containing 150 mM NaCl and 1 mM EDTA). Less than 1% of the labeled material was trichloroacetic acid soluble. The specific activity was 20–30 cpm/ng protein. Before use, the radiolabeled HDL was checked for hydrolysis of the CE label and for its composition and physical properties (29). Hydrolysis of the CE was <5%. Labeled HDL was used only when there were no differences in physical behavior and composition compared with the unlabeled HDL.

LDL was dialyzed against PBS with 10 μM EDTA and, where indicated, oxidized by exposure to $CuSO_4$ as described (34).

Plasma decay, organ association, and liver cell distribution

A dose of 200 μg (apolipoprotein) ± 1.2×10^6 dpm (plasma decay and organ association) or 500 μg (apolipoprotein) ± 3×10^6 dpm (liver cell distribution) of [^{3}H]CEt-HDL (total volume of 100 or 250 μl) was injected into the tail vein of WT and SR-BI KO mice. At 3 min after injection, a blood sample was drawn to verify the injected dose. At the indicated time points, blood samples were drawn to measure serum decay. For analysis of organ association, the liver, adrenals, and various other organs (stomach, intestines, thymus, pancreas, spleen, kidneys, thyroid, heart, aorta, veins, lungs, lymph nodes, muscles, skin, adipose tissue, bones, and reproductive organs) were isolated for radioassay at 1, 2, 4, and 24 h after tracer injection. The organs were weighed, solubilized, and counted for ^{3}H radioactivity in a Packard liquid scintillation unit and for ^{125}I in a γ counter (Packard). Correction was made for the radioactivity in the blood present in the organs at the time of sampling as determined by injection of screened

[^{125}I]BSA. The values are in μl/g organ (means ± SEM; n = 4): liver, 84.7 ± 8.9; heart, 68.1 ± 2.9; lung, 125.9 ± 13.2; stomach, 22.9 ± 3.2; kidney, 135.2 ± 7.2; adrenal, 110.2 ± 9.5; spleen, 64.6 ± 9.1; small intestine, 46.3 ± 9.0; large intestine, 35.2 ± 3.0; skin, 13.1 ± 3.4; muscle, 13.7 ± 2.5; and bone, 30.1 ± 5.2.

When double-labeled HDL was used, the [^{3}H]CEt radioactivity was measured in a Packard β counter with a double label program. Within the used ^{3}H/^{125}I activity ratios the percentage recovery for ^{3}H is 100 ± 2%. Computer analysis using an interactive curve peeling program was applied to fit a least-squares multiexponential curve to each set of serum decay data and to calculate fractional catabolic rates (FCRs) according to the model of Matthews (35). The uptake by liver parenchymal, endothelial, and Kupffer cells was determined at 2 h after injection. After a liver lobule was tied off (total liver sample), the parenchymal liver cells were isolated as indicated under In Vitro Studies with Freshly Isolated Hepatocytes. The endothelial and Kupffer cells were collected from the parenchymal cell supernatant by centrifugation for 10 min at 500 *g* and separated further via centrifugal elutriation as described (36). The purity of each fraction was examined via a staining reaction for peroxidase activity followed by light microscopy. The purity was 80–90% and >95% for Kupffer cells and endothelial cells, respectively. The total liver sample and the cell isolations were solubilized and analyzed for protein content and radioactivity.

Protein determination

Protein was determined according to Lowry et al. (37) with BSA as the standard.

In vitro studies with freshly isolated hepatocytes

Between 10 and 11 AM, WT (*srbI*$^{+/+}$) and SR-BI KO (*srbI*$^{-/-}$) mice were anesthetized by subcutaneous injection with a mixture of 1.5% (w/v) ketamine, 8.5% (v/v) Thalamonal, and 1.5% (v/v) Hypnorm in PBS at a dose of 5–7.5 ml/kg. Collagenase perfusion [0.06% (w/v) collagenase] of the liver was then performed for 12 min as described (38). Liver cells were collected by mincing the liver in ice-cold Hank's buffer containing 0.3% (w/v) BSA followed by filtration through nylon gauze to remove large debris. Three subsequent washing steps (10 min, 50 *g*, 4°C) with ice-cold Hank's buffer were performed to separate the parenchymal cells (pellet) from the endothelial and Kupffer cells (supernatant). The pellet consisted of pure parenchymal cells as judged by light microscopy. Cell viability was examined by trypan blue exclusion and was 80–90% in every case. After resuspension in oxygenated DMEM supplemented with 2% (w/v) BSA (pH 7.4), 1 mg of parenchymal cell protein was incubated with the indicated amounts of ligand at 37°C for 3 h in a final volume of 0.5 ml. During the incubation, the cells were shaken at 150 rpm and briefly oxygenated every hour. The viability of the cells remained greater than 80% during these incubations. Subsequently, the cells were washed twice with wash buffer (0.15 M NaCl, 2.5 mM $CaCl_2$, and 50 mM Tris-HCl, pH 7.4) containing 0.2% (w/v) BSA and once with the same buffer without BSA, after which the cells were lysed in 0.1 N NaOH and protein content and radioactivity were measured.

RESULTS

Serum decay and liver and adrenal association

Upon injection of [^{3}H]CEt-HDL into WT mice, 51.7 ± 3.1% of the injected label was removed from the blood during the first 4 h. In SR-BI KO mice, a severely delayed clearance was noted (**Fig. 1**). In WT mice, the liver uptake of CEt from HDL increased gradually up to 4 h after injection. At this time point, 31.3 ± 4.9% of the injected dose (ID) was recovered in the liver (Fig. 1). This indicates that, in agreement with data obtained in rats (30), also in mice the liver is responsible for the majority of the removal of CEt from HDL. In SR-BI KO mice, the liver uptake of CEt from HDL was greatly diminished: at 4 h after injection, only 3.3 ± 0.8% of the ID accumulated in the liver, indicating an almost 90% inhibition of liver uptake in the absence of SR-BI ($P < 0.05$). Serum decay kinetics and the rate at which the liver accumulated CEt tracer were determined in both WT and SR-BI KO mice. The activity of the liver was expressed as organ FCR per gram of tissue. This represents the fraction of the plasma pool of the traced HDL component cleared per hour per gram of tissue. The serum decay rate and the liver FCR of the [^{3}H]CEt-HDL were higher in WT mice compared with SR-BI KO mice. In WT mice, the serum FCR was 0.106 ±

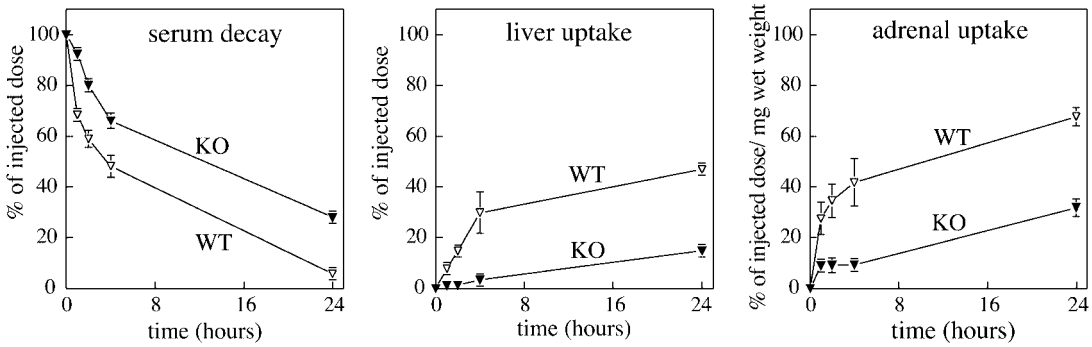

Fig. 1. Serum decay, liver association, and adrenal association of HDL labeled with cholesteryl ether ([^{3}H]CEt-HDL) in wild-type (WT) and scavenger receptor class B type I (SR-BI) knockout (KO) mice. A dose of 200 μg (apolipoprotein; 1.2 × 10^{6} dpm) of [^{3}H]CEt-HDL was injected into the tail vein of WT (open symbols) and SR-BI KO (closed symbols) mice. At the indicated time points, the animals were killed, serum was collected, and liver and adrenal biopsies were taken. The tissue samples were weighed, combusted in a Hewlett-Packard 306 sample oxidizer, and counted for radioactivity. Serum decay and liver and adrenal association were calculated. Correction was made for the radioactivity in the blood present in the organs at the time of sampling (see Materials and Methods). For serum and liver, data are expressed as percentage of injected dose (ID), whereas for the adrenal, association values are expressed as percentage of ID/mg wet weight × 10^{3}. Values are means ± SEM of four animals for each time point.

0.004 pools/h (n = 4); serum FCR was 0.043 ± 0.004 pools/h in SR-BI KO animals (n = 4; $P = 0.002$). The liver FCR was 38.7 ± 6.6 × 10^{-3} serum pools/h/g in WT mice vs. 4.8 ± 0.80 × 10^{-3} serum pools/h/g in KO mice ($P = 0.036$). These values indicate a severely delayed capacity for CE uptake in the SR-BI KO mice.

We observed earlier that SR-BI deficiency induced a 1.8-fold increase in total serum cholesterol levels (28). Interestingly, this increase in total cholesterol levels was mainly the result of a 3.2-fold increase in free cholesterol, and CEs were only increased by 1.3-fold. Rigotti et al. (27) reported that apolipoprotein A-I levels were not different between WT and SR-BI KO mice. Correction for the HDL-CE pool size indicates that the HDL-CE mass influx into the liver was reduced from 22.0 ± 3.8 μg HDL-CE/h in WT mice to 4.1 ± 0.7 μg HDL-CE/h in SR-BI KO animals. To verify the possible presence of a selective CE decay and uptake in the absence of SR-BI, we injected double-labeled HDL, whereby the protein moiety was additionally labeled with [^{125}I]TC (**Fig. 2**, **Table 1**). In WT mice, the decay rate of [^{3}H]CEt was higher than that of [^{125}I]TC, indicative of selective CE removal from serum. In contrast, the decay rates of [^{3}H]CEt-HDL and [^{125}I]TC-HDL in SR-BI KO mice were identical, reflecting the absence of a selective CE removal pathway in these animals. The liver uptake values of [^{3}H]CEt-HDL were similar to those for single CE-labeled HDL (30.3 ± 1.2% for WT mice and 4.4 ± 0.9% for KO mice), whereas the uptake values for [^{125}I]TC (4.8 ± 0.6% for WT mice and 5.4 ± 0.4% for KO mice) indicated that no residual selective uptake of CE from HDL was observed in SR-BI KO mice. Because the observed liver uptake did not account fully for the serum decay, the distribution of radioactivity over extrahepatic tissues was also analyzed at various time points after injection; the data for 24 h are given in **Table 2**. It was found that the majority of the extrahepatic label was present in skin (15–18%), bones (6–8%), muscle (3%), and intestine (4%), whereas the other tissues contained <1% for each tissue. The total recovery of label was between 80% and 90% for both WT and KO mice in every case. There was no significant difference in extrahepatic distribution between WT and SR-BI KO animals except for the adrenals. The major differences in adrenal uptake occurred during the first 4 h after injection (Fig. 1), during which the uptake in WT mice increased to 45.3 ± 5.0% of the ID/mg wet weight × 10^3, whereas uptake was only 6.3 ± 1.1% of the ID/mg wet weight × 10^3 in the SR-BI KO mice (Table 1).

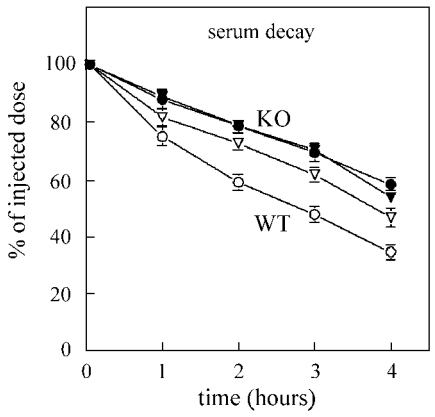

Fig. 2. Serum decay of double-labeled [^{3}H]CEt-HDL and HDL labeled with tyramine-cellobiose ([^{125}I]TC-HDL) in WT and SR-BI KO mice. A dose of 200 μg (apolipoprotein) was injected into the tail vain of WT [open circles ([^{3}H]CEt-HDL) and open triangles ([^{125}I]TC-HDL)] and SR-BI KO [closed circles ([^{3}H]CEt-HDL) and closed triangles ([^{125}I]TC-HDL)] mice. At the indicated time points, blood was taken and radioactivity was determined as described in Materials and Methods. The data are expressed as percentage of ID ± SEM of four animals at each time point.

TABLE 1. Liver and adrenal association of [^{3}H]CEt-HDL and [^{125}I]TC-HDL at 4 h after injection into SR-BI KO mice and WT mice

	[^{3}H]CEt-HDL		[^{125}I]TC-HDL	
Tissue	WT Mice	SR-BI KO Mice	WT Mice	SR-BI KO Mice
Liver (% ID)	30.3 ± 1.8	4.4 ± 0.9[a]	4.8 ± 0.6	5.4 ± 0.4
Adrenals (% ID/mg wet weight × 10^3)	45.3 ± 5.0	6.3 ± 1.7[a]	6.1 ± 1.6	3.1 ± 0.2

HDL labeled with tyramine-cellobiose ([^{125}I]TC-HDL) and cholesteryl ether ([^{3}H]CEt-HDL) was injected into the tail vein of wild-type (WT) and scavenger receptor class B type I (SR-BI) knockout (KO) mice. At 4 h after injection, the animals were killed, serum was collected, and liver and adrenals were taken. Tissue samples were weighed, solubilized, and together with the serum samples assayed for ^{3}H or ^{125}I counts. Correction was made for the contribution of serum to the measured organ-associated radioactivity (see Materials and Methods). Data are expressed as percentage of injected dose (ID) for liver and as percentage of ID/mg wet weight × 10^3 for the adrenals. The values are means ± SEM of five to eight animals.

[a] Significant difference for [^{3}H]CEt-HDL ($P < 0.05$; unpaired Student's *t*-test) between WT and KO mice. The WT [^{125}I]TC-HDL values did not differ significantly from the KO [^{3}H]CEt-HDL values or the KO [^{125}I]TC-HDL values (unpaired Student's *t*-test).

In vivo liver cell distribution

At 2 h after injection of [^{3}H]CEt-HDL, parenchymal, endothelial, and Kupffer cells were isolated from the livers

TABLE 2. Percent radioactivity in tissues determined 24 h after injection of [^{3}H]CEt-HDL in WT and SR-BI KO mice

Organ	WT Mice	SR-BI KO Mice
Liver	46.9 ± 2.4	14.8 ± 1.2
Heart	0.4 ± 0.1	0.7 ± 0.2
Lung	0.7 ± 0.1	0.7 ± 0.2
Stomach	0.6 ± 0.2	0.6 ± 0.2
Kidney	0.6 ± 0.1	0.4 ± 0.2
Adrenal	0.5 ± 0.1	0.2 ± 0.1
Spleen	0.7 ± 0.2	1.0 ± 0.2
Small intestine	4.0 ± 0.3	3.3 ± 0.2
Large intestine	2.5 ± 0.3	2.0 ± 0.2
Skin	14.7 ± 2.4	18.1 ± 4.8
Muscle	4.1 ± 0.3	3.7 ± 0.4
Bone	8.2 ± 0.6	9.3 ± 1.9
Serum	4.9 ± 0.7	32.0 ± 1.0
Recovery	88.8%	86.8%

[^{3}H]CEt-HDL was injected into the tail vein of WT and SR-BI KO mice. At 24 h after injection, the animals were killed, serum was collected, and tissues were removed. Tissues were assayed for ^{3}H counts, and correction was made for the contribution of serum to the measured organ-associated radioactivity (see Materials and Methods). The values are means ± SEM of four animals in each group.

of WT and SR-BI KO mice to examine the potential roles of SR-BI in the process of CEt uptake by these various cell types. For total liver, there was an 81.6% lower association of CEt in SR-BI KO animals compared with WT mice (13.1 ± 1.3% and 71.3 ± 3.6% of the ID/mg protein × 10^{-3} for KO and WT mice, respectively) (**Fig. 3**). It can be calculated that in the WT liver, parenchymal cells were responsible for 88% of the [^{3}H]CEt uptake from HDL, when taking into account the contribution of these cells to total liver mass. The absence of SR-BI from the liver parenchymal cells resulted in an 87% decrease of CEt uptake (62.4 ± 1.1% uptake of the ID/g protein × 10^{-3} in WT parenchymal cells vs. 8.0 ± 0.6% uptake of the ID/mg protein × 10^{-3} in SR-BI KO parenchymal cells). For liver endothelial cells, no significant difference was observed. In the Kupffer cell fraction from SR-BI KO mice, the uptake of CEt from HDL was 51.5% lower compared with WT mice (69.6 ± 13.9% and 143.4 ± 7.5% of the ID/mg protein × 10^{-3}, respectively). Because parenchymal liver cells are the predominant cell type in the liver (92.5%), the diminished uptake of CEt by the SR-BI KO liver is mainly the consequence of the absence of SR-BI in the parenchymal liver cells and is only to a minor extent caused by the SR-BI deficiency in the Kupffer cells.

In vitro parenchymal liver cell association

Parenchymal liver cells from WT and SR-BI KO mice were isolated and incubated for 3 h at 37°C with different concentrations of HDL doubly labeled with [^{3}H]CE and [^{125}I]TC. As described by Pittman et al. (39), values for CE association are expressed in terms of apparent particle uptake (i.e., from the amount of ^{3}H-labeled tracer associated with the cells, the amount of HDL protein apparently taken up is calculated). In WT parenchymal liver cells, the CE association increased with increasing ligand concentration and exceeded the association to SR-BI KO cells by ~3- to 5-fold (**Fig. 4**). In SR-BI KO parenchymal liver cells, at every ligand concentration used, the association of HDL-derived [^{3}H]CE was similar to the [^{125}I]TC association, indicating that SR-BI KO parenchymal liver cells performed no residual selective CE uptake from HDL (Fig. 4). With [^{3}H]CEt-HDL, similar data were obtained as with the [^{3}H]CE label.

Effect of LDL and oxidized LDL on selective uptake of [^{3}H]CE from HDL

Previously, we had shown that oxidized LDL (oxLDL) is a very potent inhibitor of HDL-CE association with rat parenchymal liver cells. Based on these inhibition characteristics, we suggested that SR-BI mediates the efficient uptake of HDL-CE by these cells (28). In the present study, the availability of SR-BI KO mice enabled us to determine if this assumption is true. Incubation of freshly isolated WT and SR-BI KO mouse parenchymal liver cells with 10 μg/ml [^{3}H]CE-HDL for 3 h at 37°C resulted in an association of 476.3 ± 37.8 ng/mg cell protein for WT cells and a 5-fold lower association of 89.5 ± 18.9 ng/mg cell protein for SR-BI KO cells (**Fig. 5**). The efficiency of modified LDL to compete for this [^{3}H]CE-HDL association was examined by including the indicated amounts of unlabeled native or oxidized LDL in the incubation (Fig. 5). Addition of native LDL only marginally decreased the cell association (18% in WT cells and 20% in SR-BI KO cells at the highest

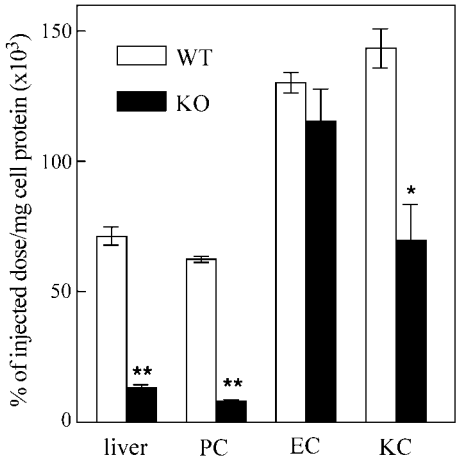

Fig. 3. In vivo distribution of [^{3}H]CEt-HDL in parenchymal, liver endothelial, and Kupffer cells in WT and SR-BI KO mice at 2 h after injection. A dose of 500 μg (apolipoprotein; 3 × 10^6 dpm) of [^{3}H]CEt-HDL was injected into the tail vein of WT (open bars) and SR-BI KO (closed bars) mice. At 2 h after injection, a liver lobule was tied off and the liver was perfused at 37°C. Parenchymal cells (PC), endothelial cells (EC), and Kupffer cells (KC) were further isolated at 4°C. The total liver sample and the cell isolations were solubilized and counted for radioactivity. Values are means ± SEM of four isolations in both groups. ** Very significant difference ($P < 0.005$) between WT and KO mice; * significant difference ($P < 0.05$) between WT and KO mice (unpaired Student's *t*-test).

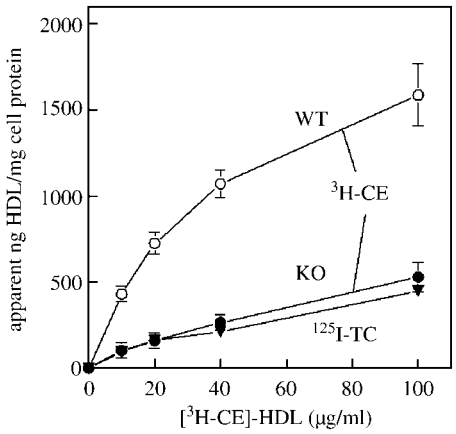

Fig. 4. Concentration dependence of HDL labeled with cholesteryl ester ([^{3}H]CE-HDL) and [^{125}I]TC-HDL association to isolated parenchymal liver cells from WT and SR-BI KO mice. Parenchymal liver cells from WT (open symbols) and SR-BI KO (closed symbols) mice were incubated for 3 h at 37°C with the indicated concentrations of HDL doubly labeled with [^{3}H]CE (circles) and [^{125}I]TC (triangles) in DMEM with 2% BSA. The association of [^{3}H]CE is expressed as apparent uptake [i.e., the amount of HDL protein that would deliver the measured amounts of [^{3}H]CE (39)], whereas for [^{125}I]TC the association is expressed in ng HDL/mg cell protein. Values are means ± SEM of four cell isolations from both groups. The [^{125}I]TC values for WT and SR-BI KO mice are graphically similar in the plots and do not differ significantly from the [^{3}H]CE-HDL values in the SR-BI KO mice (Student's *t*-test).

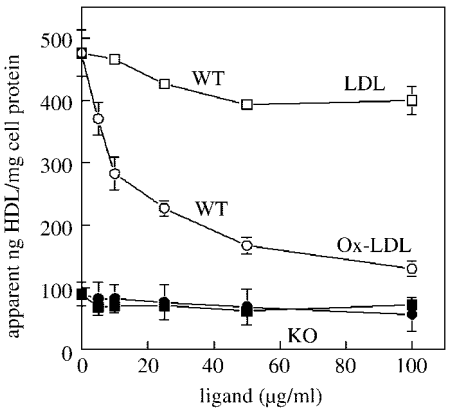

Fig. 5. Effect of increasing concentrations of LDL or oxidized LDL (oxLDL) on the association of [^{3}H]CE-HDL to WT and SR-BI KO mouse parenchymal liver cells. Parenchymal liver cells from WT (open symbols) and SR-BI KO (closed symbols) mice were incubated for 3 h at 37°C with 10 μg/ml [^{3}H]CE-HDL and the indicated concentrations of LDL (squares) or oxLDL (circles) in DMEM with 2% BSA. The association is expressed as apparent uptake [i.e., the amount of HDL protein that would deliver the measured amounts of [^{3}H]CE (39)]. Values are means ± SEM of four cell isolations. Where error bars are not visible, they fall within the symbols.

concentration of 100 μg/ml). Competition with oxLDL at a concentration of 100 μg/ml resulted in a 73% reduction of [^{3}H]CE-HDL cell association to WT cells. With an oxLDL concentration as low as 25 μg/ml, a 52% decrease was achieved (Fig. 5). In contrast, in the SR-BI KO parenchymal liver cells, oxLDL did not efficiently decrease the association of [^{3}H]CE-HDL. This indicates that apparently oxLDL is a very efficient inhibitor of the SR-BI-mediated selective uptake of CE from HDL.

DISCUSSION

Selective delivery of CE from HDL to the adrenals and the liver is important for the supply of substrate for steroid hormone synthesis and the maintenance of lipid homeostasis, respectively. Based on the following evidence, SR-BI is suggested to be a functional receptor for HDL: *i*) SR-BI is highly expressed in the steroidogenic organs and the liver in rats (17) and mice (18); *ii*) SR-BI can bind HDL via interaction with its apolipoproteins (40); and *iii*) SR-BI can mediate the selective uptake of HDL-CE (15). In the present work, we used SR-BI KO mice to assess the quantitative role of SR-BI in the selective uptake of CE from HDL in vivo by comparing the uptake in these animals with that in WT mice. Our data show that, in the intact animal, the initial rate at which CE is taken up from HDL by the liver and the adrenals is greatly diminished in SR-BI KO animals compared with WT animals. Calculation of the liver FCR for WT and KO animals indicated that CE uptake from HDL by the liver occurred eight times faster in WT animals. This shows the quantitative importance of SR-BI as a rate-limiting factor in the process of selective uptake of CE from HDL by the liver. It is consistent with the finding that in SR-BI KO mice biliary cholesterol secretion is impaired (25, 26) and that plasma HDL cholesterol concentrations are increased by 125% (27). In agreement with the rate-determining role of SR-BI in the selective CE uptake from HDL, it has been reported that overexpression of SR-BI in vivo resulted in a dramatic decrease in plasma HDL cholesterol levels (20–22) and increased total and selective uptake of HDL-CE by the liver (22) compared with nontransgenic controls. Furthermore, our data are consistent with the decreased CE uptake from HDL by the liver observed in SR-BI att mice, which have an attenuated expression of the SR-BI receptor (24). Analysis of the organ distribution of [^{3}H]CE at various times after injection indicated that in addition to the liver, only the uptake by the adrenals is impaired in the absence of SR-BI. This is in accordance with the previously reported low adrenal gland cholesterol content in homozygous SR-BI KO mice (27). Comparison of the uptake values obtained for [^{3}H]CE and [^{125}I]TC, a nondegradable and accumulating label located in the protein moiety of HDL, indicates that in SR-BI KO mice the residual CE uptake can be ascribed to HDL holo-particle association and/or uptake. This implies not only that SR-BI is the determining factor for the selective uptake of CE from HDL but also that no additional routes for the selective uptake of CE from HDL are active in vivo. Our data obtained with SR-BI KO animals compared with WT mice thus form the final proof that SR-BI is solely responsible for the selective uptake of CE from HDL in the blood circulation by the liver and the adrenals.

Recently, in a preliminary report, Rinninger's group (41) came to the same conclusion by studying the kinetics of autologous HDL in SR-BI KO mice.

The liver does contain, in addition to the parenchymal cells, endothelial and Kupffer cells. We have shown previously (19) that, in rats, SR-BI protein is expressed in both the parenchymal and Kupffer cells. Treatment with estradiol or a high-cholesterol diet led to downregulation of SR-BI protein expression in the parenchymal cells and resulted in an upregulation of SR-BI protein expression in the Kupffer cells. These changes in SR-BI protein expression correlated with changes in the ability of these cells to perform selective uptake of CE from HDL (19). In the present study, isolation of parenchymal, endothelial, and Kupffer cells at 2 h after in vivo administration of [^{3}H]CEt-HDL revealed that in the SR-BI KO mice CEt-HDL uptake is greatly impaired in parenchymal cells but in liver endothelial cells no difference in uptake of CEt from HDL was observed. Kupffer cells from SR-BI KO mice showed a 2-fold decrease in CE uptake from HDL compared with WT mice. These data obtained in mice are consistent with our earlier data for the rat. Because the parenchymal liver cells account for 92.5% of total liver protein, we conclude that the SR-BI-mediated selective uptake of CE from HDL by the liver is mainly exerted by the parenchymal cells.

In view of the major role of the parenchymal liver cells in the removal of CE from HDL, additional in vitro studies were performed with these cells from WT and SR-BI KO mice. Pioneering studies by Glass and coworkers (11, 14)

have shown that the uptake route of HDL-CE by liver parenchymal cells is characterized by the selective uptake of the CE without simultaneous uptake of the holo-particle. We have shown previously that the selective uptake of HDL-CE by rat parenchymal liver cells can be blocked efficiently by oxLDL (29) and also that changes in SR-BI protein expression, induced by a high-cholesterol diet or estradiol treatment, correlated with changes in the selective uptake of HDL-CE in vitro (19). Our present data with isolated parenchymal liver cells from SR-BI KO mice indicate that the uptake of HDL-CE is greatly diminished compared with WT parenchymal liver cells and also that inhibition by oxLDL of the selective uptake process no longer occurs. This indicates that, similar to the in vivo observations, SR-BI expression is essential for the efficient uptake of CE from HDL. In addition, the present data support our earlier suggestion (29) that the inhibition of CE uptake from HDL by oxLDL indicates that SR-BI is mainly responsible for the selective uptake process. By simultaneous measurement of [^{3}H]CE uptake and [^{125}I]TC uptake (reflecting holo-particle uptake) from HDL in parenchymal cells from SR-BI KO mice, we conclude that these cells, in contrast to WT parenchymal liver cells, no longer exert selective CE uptake from HDL. These data are consistent with the in vivo uptake values and indicate that for this major liver cell type SR-BI is essential for the selective uptake of CE from HDL. Furthermore, the data indicate that the parenchymal liver cells do not contain additional receptor systems that can facilitate this unique uptake route.

In conclusion, both our in vivo and in vitro data prove that SR-BI is mainly responsible for the efficient and selective uptake of HDL-CE by the liver and the adrenals, making it an important target for pharmaceutical interference with the RCT process.

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