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In Vivo Regulation of Scavenger Receptor BI and the Selective Uptake of High Density Lipoprotein Cholesteryl Esters in Rat Liver Parenchymal and Kupffer Cells*

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High density lipoprotein cholesteryl esters (HDL-CE) are selectively taken up by liver parenchymal cells without parallel apolipoprotein uptake. This selective uptake route forms an important step in the so-called reverse cholesterol transport. Scavenger receptor BI (SR-BI) is the only known HDL receptor which can mediate selective uptake of HDL-CE. In the present study we investigated its regulation in liver cells. The down-regulation of SR-BI expression in liver by 17α-ethinyl estradiol (EE) treatment was found by immunoblotting to be the consequence of down-regulation of SR-BI in parenchymal cells, while SR-BI expression in Kupffer cells was up-regulated. The selective uptake of HDL-CE *in vivo* by parenchymal and Kupffer cells was measured by labeling of HDL with [^{3}H]CE and analysis of the cellular uptake at 10 min after injection. After EE treatment, uptake of [^{3}H]CE-labeled HDL by parenchymal cells decreased by 85%, while Kupffer cells showed a 4-fold increase in selective uptake of [^{3}H]CE-labeled HDL. *In vitro* studies with isolated parenchymal cells indicated that after EE treatment, the selective uptake of [^{3}H]CE labeled HDL was 3–4-fold lower, indicating that the *in vivo* observations are also reflected *in vitro*. A 2-week high-cholesterol diet leads to lowering of SR-BI expression in parenchymal cells, while the expression in Kupffer cells is increased. Like EE treatment, the selective uptake of [^{3}H]CE-labeled HDL by the two hepatic cell types *in vivo* correlated with the changes in expression of SR-BI. Our results thus demonstrate that within the liver, the regulation of SR-BI expression by EE treatment or a high-cholesterol diet, correlates with changes in the selective uptake of HDL-CE, supporting a function of SR-BI to mediate the selective uptake of HDL-CE in the liver parenchymal cells. The contrasting regulatory effect on parenchymal cells and Kupffer cells might indicate a different function of SR-BI in the latter cell type.

High-density lipoproteins (HDL)[1] may exert the anti-atherogenic effects by various mechanisms (1, 2). Reverse cholesterol transport as originally proposed by Glomset (3) is a widely accepted mechanism of anti-atherogenic action. In this concept, HDL accepts excessive cholesterol from extrahepatic cells for transport to the liver parenchymal cells (3, 4). The direct uptake of HDL cholesteryl esters (HDL-CE) by liver parenchymal cells is fundamentally different from that of the classical LDL receptor pathway in that HDL-CE are taken up selectively without simultaneous uptake of the holoparticle (5, 6). This so-called selective uptake of HDL-CE in the liver parenchymal cells is efficiently coupled to bile acid formation and secretion (4).

The precise mechanism of selective uptake of HDL-CE is largely unestablished. It is restricted to the adrenals, ovary, testis, and liver (5, 7), while within the liver the parenchymal cells are solely responsible for the selective uptake of HDL-CE (2, 4). Several proteins have been described which can bind specifically HDL (8, 9). However, Acton *et al.* (10) provided recently the first evidence that scavenger receptor class BI (SR-BI), a member of the CD 36 family (11), not only binds HDL but also can mediate selective uptake of HDL-CE. *In vivo*, SR-BI is expressed in the steroidogenic organs and liver of rodents (10, 12, 13), which all display selective uptake of HDL-CE. In the steroidogenic tissues SR-BI expression is coordinately regulated with the steroidogenesis by adrenocorticotropic hormone (ACTH), human chorionic gonadotropin, and estrogen (12, 14). Furthermore, SR-BI expression in adrenals is up-regulated in apoA-I knock-out mice, hepatic lipase knockout mice, and lecithin cholesterol acyltransferase knockout mice (13, 15), indicating that SR-BI is under feedback regulation in response to changes of cellular cholesterol stores. Unlike the steroidogenic tissues, SR-BI expression in the liver is down-regulated by estrogen treatment of rats (12).

SR-BI was found to bind a broad spectrum of ligands, including modified lipoproteins, native lipoproteins, and also anionic phospholipids (16). Recently, the HDL binding to SR-BI was shown to be mediated by the major apolipoproteins of HDL, *e.g.* apoA-I, apoA-II, and apoC-III (17). We showed recently that the selective uptake of HDL-CE by isolated rat liver parenchymal cells can be inhibited completely by ligands specific for SR-BI (18), indicating that the expression of SR-BI can be solely responsible for the selective HDL-CE uptake in this cell type. Adenovirus-mediated hepatic overexpression, as recently published by Kozarsky *et al.* (19) resulted in the virtual disappearance of plasma HDL and a substantial increase in biliary cholesterol, demonstrating the importance of hepatic overex-

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[1] The abbreviations used are: HDL, high density lipoprotein; ACTH, adrenocorticotropic hormone; BSA, bovine serum albumin; CE, cholesteryl ester; DMEM, Dulbecco's modified Eagle's medium; EE, 17α-ethinyl estradiol; LDL, low density lipoprotein; SR-BI, scavenger receptor class B, type I.

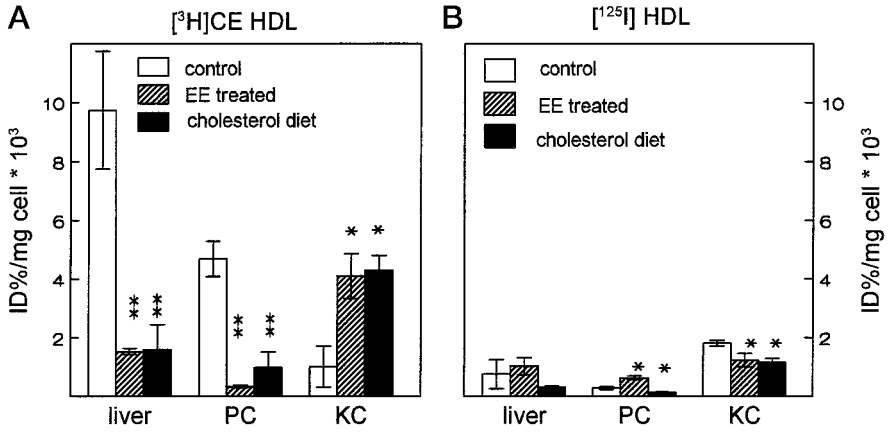

FIG. 1. ***In vivo* distribution of [^{3}H]CE-HDL and ^{125}I-HDL between parenchymal and Kupffer cells, at 10 min after injection in EE-treated rats, rats fed with a high-cholesterol diet, or control rats.** Control rats (*open bars*) or rats treated with EE (5 mg/kg) for 5 days (*hatched bars*) or put on a high-cholesterol diet for 2 weeks (*black bars*). At 10 min after injection of [^{3}H]CE-HDL (*A*) or ^{125}I-HDL (*B*), the liver was perfused and parenchymal cells (*PC*) and Kupffer cells (*KC*) were isolated at 4 °C. Values, expressed as the percentage of the injected dose × 10^3/mg of cell protein, are mean ± S.E. of four experiments. ** indicates very significant difference $p < 0.005$. * indicates significant difference $p < 0.05$ (unpaired Student's *t* test).

pression of SR-BI for HDL catabolism and reverse cholesterol transport. Furthermore, it was shown that in mice with a targeted null mutation in the SR-BI gene, plasma cholesterol concentration increased by 125% due to the formation of large apolipoprotein A-I particles and the adrenal cholesterol content was decreased, indicating that selective cholesterol uptake was inhibited in these animals (20).

SR-BI might also be involved in the efflux of cellular cholesterol to HDL, *i.e.* the first step in reverse cholesterol transport. In SR-BI transfected Chinese hamster ovary cells, cholesterol efflux to HDL was correlated with the expression level of SR-BI (21), while also in cultured macrophages, cholesterol efflux appeared to be correlated with SR-BI expression (21). As the liver contains both tissue macrophages (Kupffer cells) and parenchymal cells, we investigated in the present study the *in vivo* regulation of SR-BI in these cell types, while simultaneously the selective uptake of HDL-CE was studied. Rats were either treated with 17α-ethinyl estradiol (EE) or put on a high-cholesterol diet. It appears that the down-regulation of SR-BI expression in parenchymal cells correlated with changes in the selective HDL-CE uptake, providing further evidence that the regulation of SR-BI expression is responsible for the variation in selective HDL-CE uptake. Surprisingly, SR-BI expression and the selective HDL-CE uptake is up-regulated in Kupffer cells after EE treatment or a high-cholesterol diet, pointing to a different regulatory response in tissue macrophages (Kupffer cells) as compared with parenchymal cells, suggesting a difference in function of SR-BI in these cell types.

EXPERIMENTAL PROCEDURES

Materials—[1α,2α(n)-^{3}H]Cholesteryl oleate ([^{3}H]CE) and ^{125}I-carrier free in NaOH were purchased from Amersham (Little Chalfont, Buckinghamshire, United Kingdom). 22-(*N*-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-23,24-bisnor-5-cholen-3β-yl linoleate was purchased from Molecular Probes (Eugene, OR). Egg yolk phosphatidylcholine was purchased from Fluka (Buchs, Switzerland), the PL phospholipids kit, the cholesterol oxidase-peroxidase aminophenazone kit, and the glycerol-phosphate oxidase-peroxidase aminophenazone kit were from Boehringer Mannheim (Mannheim, Germany). Ethylmercurithiosalicylate (thimerosal), bovine serum albumin (BSA, fraction V), and collagenase type I and type IV were obtained from Sigma, while Dulbecco's modified Eagle medium (DMEM) was from Life Technologies, Inc. (Irvine, Scotland). All other chemicals were of analytical grade.

Animals—Throughout the study male Wistar WU rats were used (200–250 g), which had free access to food and water. EE-treated rats were injected subcutaneously for 5 consecutive days with 5 mg/kg body weight of 17α-ethinyl estradiol in propylene glycol. Control rats were injected with an equivalent volume of propylene glycol alone. The weight of the rats was checked (10% weight loss after treatment) as well as the serum cholesterol levels (95% decrease after treatment). For some studies, rats were maintained for 16 days on a cholesterol-rich die (Hope Farms, Woerden, The Netherlands) that included 2% (w/v) cholesterol, 5% (w/v) olive oil, and 0.5% (w/v) cholic acid.

Isolation and Labeling of Lipoproteins—Human HDL and LDL were isolated from the blood of healthy volunteers by differential ultracentrifugation as described by Redgrave *et al.* (22). HDL and LDL were dialyzed against phosphate-buffered saline, 1 mM EDTA. HDL was labeled with [^{3}H]CE by exchange from donor particles as reported previously (2). HDL was iodinated by the ICl method of McFarlane (23) as modified by Bilheimer *et al.* (24). The specific activity of the [^{3}H]CE-labeled HDL varied between 1,000 and 2,000 dpm/μg of HDL protein, and for [^{125}I]HDL varied between 50,000 and 100,000 cpm/μg. The labeled HDL was dialyzed against phosphate-buffered saline, 1 mM EDTA and passed through a heparin-Sepharose affinity column to remove apoE-containing particles (25). Routinely the HDL fraction was checked for the absence of apoE by 10% SDS-polyacrylamide gel electrophoresis, followed by Coomassie Blue staining. After the labeling procedure the radiolabeled HDL was checked for hydrolysis of the cholesteryl ester labels by a Bligh and Dyer extraction (26) followed by thin layer chromatography. Hydrolysis of the cholesteryl ester was always less than 5%. The effect of the labeling procedure on the composition of HDL was analyzed by measurement of phospholipid, cholesterol, cholesteryl ester, and triglyceride content (with the phospholipid kit, cholesterol oxidase-peroxidase aminophenazone kit, and glycerol-phosphate oxidase-peroxidase aminophenazone kit, respectively). The density, electrophoretic α-mobility, and particle size (photon correlation spectroscopy, System 4700 C, Malvern Instruments, Malvern, UK) were also analyzed. Labeled HDL was only used when there was no change observed in the measured composition or physical characteristics as compared with the original unlabeled HDL.

Hepatic Cellular Distribution—The hepatic cellular distribution of HDL was studied by using a low temperature cell isolation technique as described (27). Rats were anesthetized and injected with radiolabeled HDL. Ten minutes after injection, the vena porta was cannulated and the liver was perfused with oxygenated Hanks' buffer, containing Hepes (1.6 g/liter), pH 7.4, at 4 °C. Total liver uptake was determined by taking of a liver lobule 8 min after the start of the perfusion. The perfusion was continued for 15 min with Hanks'/Hepes buffer containing 0.05% (w/v) collagenase (type I, Sigma) and 1 mM $CaCl_2$. Parenchymal cells were isolated after mincing the liver in Hanks' buffer containing 0.3% BSA, filtering through nylon gauze, and centrifugation for three times 30 s at 50 × *g*. The pellets consisted of pure parenchymal cells as judged by light microscopy. The supernatants were centrifuged for 10 min at 400 × *g* to harvest the non-parenchymal cells. The remainder on the nylon gauze was incubated with Hanks'/Hepes/BSA buffer containing 0.25% Pronase for 15 min at 4 °C. This cell suspension was centrifuged for 10 min at 400 × *g* and all non-parenchymal cell pellets were combined. By means of centrifugal elutriation the Kupffer cells were separated from the non-parenchymal cells (28). The purity of each cell fraction was checked by light microscopy after staining for peroxidase activity. Cellular cholesterol concentrations were measured with a commercial kit as mentioned above after a Bligh and Dyer extraction (26) of the cellular lipids.

In Vitro Studies with Freshly Isolated Rat Hepatocytes—Parenchymal liver cells were isolated by perfusion of the livers of male Wistar WU rats (200–250 g) with collagenase at 37 °C as described (28). The viability (>95%) of the obtained parenchymal cells was checked by trypan blue exclusion. The cells from the last centrifugation step were resuspended in oxygenated DMEM supplemented with 2% BSA, pH 7.4. For competition studies 1–2 mg of parenchymal cell protein was incubated with the indicated amount of radiolabeled HDL for 180 min in 1 ml of DMEM containing 2% BSA at 37 °C. Cell incubations were per-

formed in a circulating lab shaker (Adolf Kühner AG, Basel, Switzerland) at 150 rpm. Every hour the incubations were briefly oxygenated. The viability of the parenchymal cells remained higher than 88% during these long-term incubations (28). After incubation the cells were centrifuged for 2 min at 600 rpm in an Eppendorf centrifuge and washed 2 times in 50 mM Tris-HCl, 0.15 M NaCl, 0.2% BSA, pH 7.4, at 4 °C. Subsequently, the cell pellet was washed in a similar medium without BSA. The cells were lysed in 0.1 N NaOH and the protein content and radioactivity were determined.

Western Blotting and Immunolabeling—After isolation of the different liver cell types as described (29), membranes were prepared and solubilized according to the method described by de Rijke and Van Berkel (30). Solubilized membrane proteins were subjected to electrophoresis in 7.5% polyacrylamide gels containing 0.1% SDS. Electrophoresis was performed according to the method of Laemmli (31) under nonreducing conditions. The proteins were transferred to a nitrocellulose membrane by using a Bio-Rad transblot unit (1 h, 130 V, 4 °C). After transfer the blots were blocked by 5% skim milk and 1% BSA in 50 mM Tris-HCl, pH 7.5, 90 mM NaCl, 2 mM $CaCl_2$, and 0.25% (v/v) Tween 20. Subsequently the blots were incubated with anti-SR-BI rabbit antiserum (1:1000). The rabbit antiserum was raised against a region of the extracellular domain of murine SR-BI (amino acids 230–380). After repeated washing the blots were incubated with donkey anti-rabbit immunoglobulin horseradish peroxidase-linked antibody (1:15000) (Amersham, Little Chalfont, Buckinghamshire, United Kingdom). The proteins were visualized by enhanced chemiluminescence (ECL) detection (Amersham, Little Chalfont, Buckinghamshire, United Kingdom). Quantification of the intensity of the protein bands were performed with Image-Quant software (Molecular Dynamics, Sunnyvale, CA).

Corticosterone Determination—Plasma corticosterone was measured by an radioimmunoassay using an antiserum raised against corticosterone-21-hemisuccinate bovine serum albumin as described previously (32).

Protein Determination—Protein was determined according to Lowry *et al.* (33) with BSA as standard.

RESULTS

Intrahepatic Cellular Uptake of HDL-CE: Effect 17α-Ethinyl Estradiol Treatment or a High-cholesterol Diet—Treatment of rats with EE for 5 consecutive days was reported to lower expression of SR-BI in the liver (12). To test whether this change correlates with a change in the selective uptake of HDL cholesteryl esters, the liver uptake of [^{3}H]CE-labeled HDL was determined, as well as the association of iodinated HDL to analyze total particle association. To identify the changes in the cellular uptake sites for [^{3}H]CE HDL, parenchymal cells and the liver tissue macrophages (Kupffer cells) were isolated (Fig. 1). For total liver, the association of [^{3}H]CE-HDL was 12-fold higher as compared with ^{125}I-HDL in the control situation, in accordance with earlier data (2), clearly demonstrating selective uptake of HDL-CE. Treatment of rats with EE for 5 days resulted in a 85% decrease in [^{3}H]CE-HDL uptake by the liver, while uptake of ^{125}I-labeled HDL was not significantly changed. Thus the selective uptake of HDL-CE was greatly inhibited by treatment of rats with EE, in accordance with the supposed role of SR-BI as the mediator of selective HDL-CE uptake. This decrease in selective uptake of HDL-CE by the liver can be explained by a 93% decrease in [^{3}H]CE-HDL uptake by the parenchymal cells, while no decrease in ^{125}I-HDL association was observed after EE treatment, and actually an increase was observed. In contrast, the Kupffer cells showed a significant 4-fold increase ($p < 0.05$) in uptake of [^{3}H]CE-HDL, while cell association of ^{125}I-HDL was slightly lowered.

Rats were also fed a high-cholesterol containing diet for 2 weeks. The diet increased the plasma cholesterol levels 20-fold as compared with the control animals, while total cholesterol concentration in the liver increased more than 10-fold. The total cellular cholesterol concentration in parenchymal cells increased from the control value of 11 ± 0.9 μg/mg of cell protein up to 136 ± 18 μg/mg of cell protein ($n = 3$, ± S.E.),

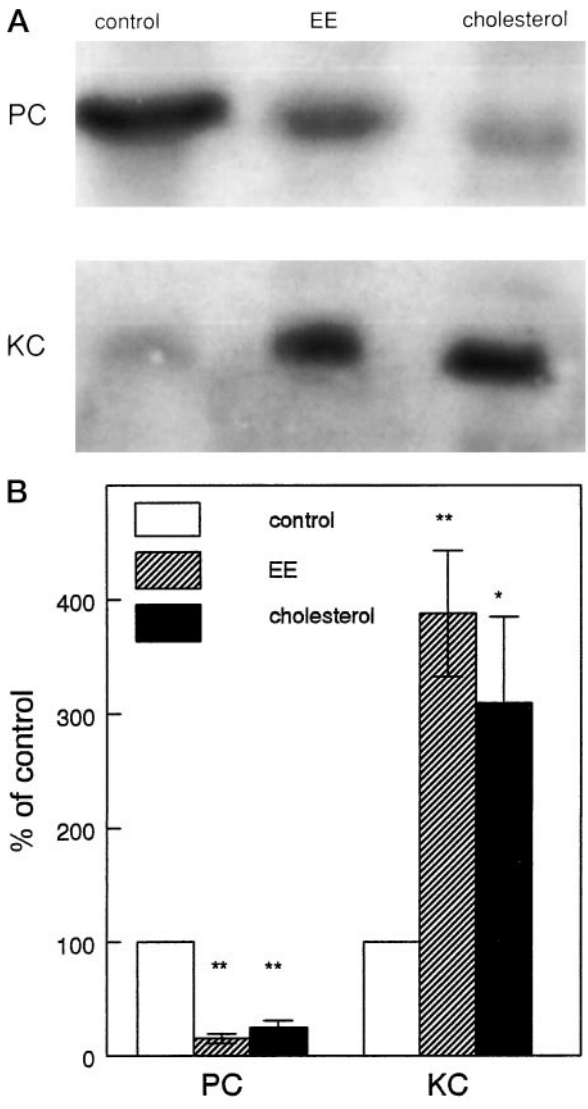

FIG. 2. **Western blot analysis of hepatic SR-BI expression after EE treatment or a high-cholesterol diet.** Cell membranes from parenchymal and Kupffer cells were isolated from control rats, rats treated with EE (5 mg/kg) for 5 days, or from rats that had been fed a high-cholesterol diet for 2 weeks. Solubilized membrane proteins were subjected to SDS-polyacrylamide gel electrophoresis and blotted onto nitrocellulose membranes. SR-BI was visualized by immunolabeling followed by enhanced chemiluminescence (ECL) detection (*A*). *Top*, parenchymal cell membranes (20 μg/lane) from control rats, EE-treated rats, and rats fed with 2% cholesterol chow for 2 weeks. *Bottom*, Kupffer cell membranes (20 μg/lane) from control rats, EE-treated rats, and rats fed with 2% cholesterol chow for 2 weeks. The SR-BI bands on blot (three separate experiments) after EE treatment or cholesterol diet were quantitated and normalized to the intensity of the control (*B*). Values are mean ± S.E. of three experiments. ** indicates very significant difference $p < 0.005$. * indicates significant difference $p = 0.05$ (unpaired Student's t test).

while the cholesterol content in the Kupffer cells increased from 6.8 ± 0.2 μg/mg of cell protein in the control animals up to 155 ± 66 μg/mg of cell protein after the 2-week diet ($n = 3$, ± S.E.). This diet resulted in a decrease in selective uptake of [^{3}H]CE-HDL by the liver similarly as was observed after the EE treatment (Fig. 1). The 2-week high-cholesterol diet inhibited only the parenchymal cell uptake of [^{3}H]CE-HDL (80%), while Kupffer cell uptake of [^{3}H]was 4-fold increased, like after the EE treatment. ^{125}I-HDL uptake by parenchymal cells was decreased for 50%, while Kupffer cells also showed a significant 35% decrease in ^{125}I-HDL uptake ($p < 0.05$).

Western Blot Analysis of Hepatic SR-BI Expression: Effect of

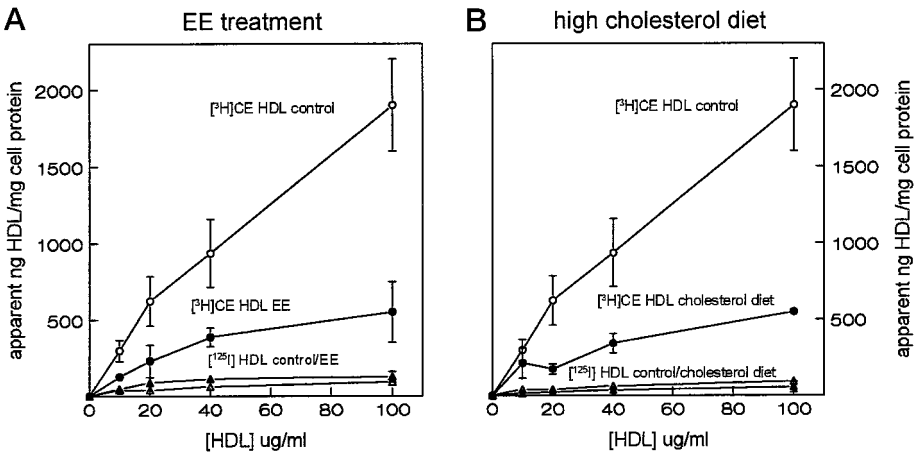

FIG. 3. **Concentration dependence of [^{3}H]CE-HDL and ^{125}I-HDL association to rat liver parenchymal cells isolated from control rats, EE-treated rats, or cholesterol fed rats.** Rat liver parenchymal cells were isolated from control rats, rats treated with EE (5 mg/kg) for 5 days (*A*), or rats on a high-cholesterol diet for 2 weeks (*B*). Rat liver parenchymal cells were incubated for 3 h at 37 °C with the indicated amount of labeled HDL in DMEM with 2% BSA (w/v). Data are expressed in terms of apparent particle uptake (2, 5). The values are corrected for nonspecific cell association in the presence of a 20-fold excess of HDL. The results are given as mean ± S.E. (n = three separate cell isolations).

EE Treatment or a High-cholesterol Diet—Cell membranes from parenchymal and Kupffer cells were isolated from control rats, rats treated with EE, or from rats that had been fed a high-cholesterol diet for 2 weeks. These membranes were used for Western blotting. SR-BI was detected by immunolabeling, using rabbit antiserum directed against SR-BI. The changes in intensity of the SR-BI bands after EE treatment or the cholesterol diet were quantitated and compared with the intensity of the control. A very significant 80% decrease ($p < 0.005$) in SR-BI expression was observed in parenchymal cell membranes from EE-treated rats and rats fed with a high-cholesterol diet as compared with control rats (Fig. 2). In contrast, SR-BI expression was increased 3–4-fold in Kupffer cells after a high-cholesterol diet or EE treatment, respectively (Fig. 2).

Since SR-BI is known to be regulated by stress hormones (12) we measured the effect of both the EE treatment and the cholesterol diet on corticosterone plasma levels. However, either treatment did not change the plasma corticosterone levels significantly. The control sham injected animals had a corticosterone level of 12.4 ± 3.6 μg/dl (n = 3, ± S.E.), while the EE-treated animals and animals on a cholesterol diet had corticosterone levels of 10.2 ± 2.8 μg/dl (n = 3, ± S.E.) and 13.4 ± 3.3 μg/dl (n = 3, ± S.E.), respectively. Therefore, the observed changes in SR-BI expression in the parenchymal and Kupffer cells cannot be explained by induction of stress by the EE treatment or the high-cholesterol diet.

Selective Uptake of HDL-CE in Vitro by Isolated Liver Parenchymal Cells and Kupffer Cells: Effect of EE Treatment or a High-cholesterol Diet—The effects of EE treatment or a high-cholesterol diet on the selective uptake of HDL-CE was also studied *in vitro*. Hepatic parenchymal cells were isolated from both EE-treated rats and rats that had been fed a high-cholesterol diet for 2 weeks. The concentration dependence of the cell association of [^{3}H]CE-HDL or ^{125}I-HDL was studied (Fig. 3). Data are expressed in terms of apparent particle uptake as originally devised by Pittman (5). Both parenchymal cells isolated from EE-treated rats or from rats fed on a high-cholesterol diet showed a 3-fold decrease in [^{3}H]CE-HDL association *in vitro* (Fig. 3). The marked decrease of [^{3}H]CE-HDL association was not accompanied by an effect on the ^{125}I-HDL association (Fig. 3). These *in vitro* data thus illustrate that the changes in *in vivo* uptake are also reflected *in vitro* with the isolated parenchymal cells.

The observed *in vivo* increase in [^{3}H]CE-HDL association by Kupffer cells after the high-cholesterol diet could also be demonstrated *in vitro*. Kupffer cells isolated from rats on a cholesterol diet showed a significant almost 2-fold increase ($p < 0.05$, two-way anova) in the uptake of [^{3}H]CE-HDL (Fig. 4).

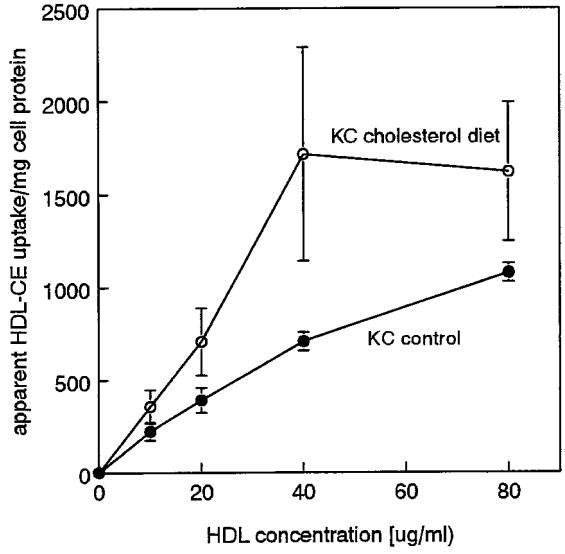

FIG. 4. **Concentration dependence of [^{3}H]CE-HDL association to rat liver Kupffer cells isolated from control rats or cholesterol fed rats.** Rat liver Kupffer cells were isolated from control rats and rats on a high-cholesterol diet for 2 weeks. The cells were incubated for 3 h at 37 °C with the indicated amount of labeled HDL in DMEM with 2% BSA (w/v). Data are expressed in terms of apparent particle uptake (2, 5). The results are given as mean ± S.E. (n = 4 separate cell isolations).

DISCUSSION

The role of SR-BI as a functional HDL receptor is supported by three phenomena. First, SR-BI can bind HDL, and this binding is mediated through interaction with apoA-I, apoA-II, and apoC-III (16). Second, SR-BI is most highly expressed in tissues that have previously been shown to be the principle sites of selective HDL-CE uptake *in vivo* in rodents (12, 13). Third, SR-BI was shown to mediate selective uptake of HDL-CE (10). Recently, it was demonstrated that the human CD36 and LIMPII analogous-1 (CLA-1) receptor, which is the human form of SR-BI, can also mediate selective uptake of HDL-CE *in vitro* and is strongly expressed in human liver and adrenal glands (34). In the present study we focused on the role of SR-BI in the liver and studied its potential role in the selective uptake of HDL-CE.

In control rats it was observed that SR-BI is expressed mainly in parenchymal cells, the cell type which is also responsible for the selective uptake of HDL-CE within the liver (1, 2). However, a low level of SR-BI expression can also be found in Kupffer cells, which in the control situation do not show selective uptake of HDL-CE *in vivo* (4). It might be that SR-BI in

Kupffer cells is acting as part of the innate immune system, as CD36, SR-BI, and also CLA-1 have been reported to bind apoptotic cells (34, 35). Recently, it was reported that SR-BI in macrophages can mediate the efflux of cholesterol to HDL (21). For reasons that Kupffer cells do readily internalize oxidized LDL, it might be that the presence of SR-BI is related to cholesterol efflux rather than uptake.

SR-BI is regulated differently in steroidogenic tissues as compared with the liver. In steroidogenic tissues SR-BI expression is up-regulated in response to treatment of rats with EE, while in liver SR-BI is down-regulated (12). We now show that this previously reported down-regulation of SR-BI expression (12) does only occur in the parenchymal cells. Furthermore, we found that this is accompanied by an almost complete inhibition of selective uptake of HDL-CE by parenchymal cells *in vivo*. In contrast, the expression of SR-BI is increased in Kupffer cells. The increase in SR-BI expression in Kupffer cells is coupled to a 4-fold increase in the uptake of HDL-CE. However, since the parenchymal cells make up for more than 90% of the total amount of liver protein, total liver uptake of HDL-CE is still decreased by 85%. The down-regulation in selective CE uptake by parenchymal cells is accompanied by an increase in ^{125}I-HDL association. However, it must be realized that EE treatment leads to a 17-fold increase in the expression of the LDL receptor, specifically in parenchymal cells (36). Although our HDL preparation was made free from human apoE, it cannot be excluded that circulating apoE associates to a limited extent with the HDL preparation and may mediate LDL receptor mediated uptake (37).

A high-cholesterol diet for 2 weeks induced a similar change in expression pattern of SR-BI in the liver cell types as compared with a 5-day treatment with EE, with a decreased expression in parenchymal cells and a 4-fold increased expression in Kupffer cells. Parenchymal cells showed *in vivo* a 93% inhibition of HDL-CE selective uptake, while Kupffer cells showed an increased uptake of HDL-CE. It thus appears that the changes in selective uptake of HDL-CE by parenchymal and Kupffer cells do correlate with the expression of SR-BI. It has been suggested that SR-BI expression is under feedback regulation in response to changes of cellular cholesterol stores (13). Parenchymal cells indeed lower the expression of SR-BI as a result of cholesterol loading. The capacity to relieve the parenchymal cells of excess cholesterol by bile acid secretion may be rate-limiting as might also be the case after EE treatment (4). The down-regulation of SR-BI may thus prevent overloading of the cells with HDL-CE under these conditions. Anyway, the concomitant regulation of SR-BI expression and selective uptake of HDL-CE by estradiol treatment and cholesterol diet does support the unique role of SR-BI in HDL-CE uptake in parenchymal liver cells. Furthermore, it appears that SR-BI and the selective uptake of HDL-CE is differentially regulated in parenchymal and Kupffer cells upon EE treatment or a high-cholesterol diet, suggesting that SR-BI might have a different function in the tissue macrophages (Kupffer cells). Recently, it was suggested that SR-BI may promote HDL-mediated cellular cholesterol efflux in different cell types, including macrophages. In 6 different cell types cholesterol efflux rates correlated well with the expression levels of SR-BI (21). Upon a high-cholesterol diet Kupffer cells accumulate a high amount of cholesteryl esters and the increased expression of SR-BI in these tissue macrophages may be related to the suggested function in HDL-mediated cholesterol efflux.

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