Regulation by vitamin E of the scavenger receptor BI in rat liver and HepG2 cells

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Abstract The scavenger receptor class B type I (SR-BI) mediates the selective uptake of cholesterol and cholesteryl ester (CE) from high density lipoprotein (HDL) into cells. The high expression in liver and steroidogenic tissues is compatible with a role of SR-BI in reverse cholesterol transport and steroid hormone synthesis. Ways of regulation thus far described include induction by trophic hormones via cAMP-activated protein kinase A (PKA) and the effects of cellular and plasma cholesterol. Here we show that vitamin E (vitE) has a major effect on the expression of SR-BI in rat liver and in a human hepatoma-derived cell line, HepG2. Feeding rats a vitE-depleted diet resulted in an 11-fold increase in the SR-BI protein level in liver tissue. This effect was readily reversed by feeding a vitE-enriched chow. In HepG2 cells, the expression of the human SR-BI homolog was reduced when the vitE content was increased by incubating the cells with vitE-loaded HDL or with phosphatidylcholine/vitE vesicles. The downregulation of human SR-BI (hSR-BI) was accompanied by a reduced level of protein kinase C (PKC) in the particulate cell fraction, and PKC inhibition decreased the expression of hSR-BI and the uptake of vitE and cholesterol from HDL. Our results are consistent with the view that the cellular level of vitE exerts a tight control over the expression of SR-BI. Furthermore, the inhibitory effect of vitE on PKC seems to be involved in the signaling pathway. —Witt, W., I. Kolleck, H. Fechner, P. Sinha, and B. Rüstow. **Regulation by vitamin E of the scavenger receptor BI in rat liver and HepG2 cells. *J. Lipid Res.* 2000.** 41: **2009–2016.**

Supplementary key words high density lipoprotein • protein kinase C • reverse cholesterol transport • α-tocopherol

The almost exclusive mechanism by which mammals eliminate cholesterol from the body is by the formation of bile in the liver. High density lipoprotein (HDL) exerts a major role in the uptake of cholesterol from peripheral organs and the delivery of cholesteryl ester (CE) to the liver, a process called reverse cholesterol transport. On the basis of kinetic data concerning the interaction of HDL with the surface of various cell types, specific receptors were postulated. The scavenger receptor class B type I (SR-BI) in rodents is the first biochemically well-characterized receptor for HDL, but affinity for other typical scavenger receptor ligands was also observed. SR-BI mediates the selective exchange of cholesterol between HDL and plasma membranes, meaning that only the lipid is transported; the HDL particles are not internalized into cells. Evidence is accumulating that the human homolog hSR-BI, also called CLA-1, exerts the same function. The rapidly growing literature dealing with these receptors has been thoroughly summarized (1–3).

SR-BI has been shown to be abundant in steroidogenic glands, in accordance with a central role in supplying cholesterol for steroid hormone synthesis. The level of SR-BI in liver is lower, but because of its mass the liver contains the major part of total body SR-BI. Kinetic analysis revealed that the mechanism by which the liver takes up CE from HDL is fully saturated at physiological concentrations of plasma CE (4). Therefore, changes in SR-BI expression in this organ should have a major impact on cholesterol homeostasis.

Investigations have shown that the expression of SR-BI is influenced by several factors. In steroidogenic tissues, trophic hormones induce the selective uptake mechanism for CE and cholesterol via SR-BI (2, 3), and a feedback inhibition by steroid hormones was observed (5). In parallel, the level of SR-BI in the hormone-producing cells was found to adapt to the supply with cholesterol (6, 7). Some of these studies led to strikingly conflicting results. Wang et al. (6) found an increased expression of SR-BI in the adrenals of apolipoprotein A-I (apoA-I) knockout mice, whereas Spady et al. (4) detected no effects on SR-BI expression in exactly the same mouse model.

In spite of the central role of the liver in reverse cholesterol transport, information about the regulation of SR-BI

Abbreviations: CE, cholesteryl ester; EDTA, ethylenediaminetetraacetic acid; HDL, high density lipoprotein; HEPES, *N*-2-hydroxylethylpiperazine-*N'*-2-ethanesulfonic acid; hSR-BI, human homolog of SR-BI; LDL, low density lipoprotein; PBS, phosphate-buffered saline; PC, phosphatidylcholine; PKA, protein kinase A; PKC, protein kinase C; PMA, 4β-phorbol 12-myristate 13-acetate; PMSF, phenylmethylsulfonyl fluoride; SF-1, steroidogenic factor 1; SR-BI, scavenger receptor class B type I; vitE, vitamin E; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; VLDL, very low density lipoprotein.

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expression is scarce. Hepatic SR-BI in rats is susceptible to downregulation by high doses of estrogens (2, 8, 9). Only a few reports deal with the regulatory effects of cholesterol and other plasma lipids and lipoproteins on SR-BI in this organ, and the results are contradictory. Feeding a high cholesterol diet to rats led to a pronounced downregulation of SR-BI in liver parenchyma cells (9), whereas Spady et al. (4) found no change in SR-BI expression in apoA-I knockout mice compared with the wild type, even though these mice lacked normal HDL. Furthermore, a diet rich in polyunsaturated fatty acids increased the level of cholesterol in the liver but not in plasma and induced a moderate (50%) upregulation of hepatic SR-BI expression (10).

Several other factors with a significant effect on the expression of SR-BI were described, including the expression of hepatic lipase (6, 11) and of apoE as well as the proinflammatory stimuli bacterial lipopolysaccharide, interferon γ, and tumor necrosis factor α (12). At least in steroidogenic tissues, the trophic hormone-dependent release of cAMP as second messenger and the activation of protein kinase A (PKA) were identified as components of the signaling pathway for the regulation of SR-BI expression (2, 3). Steroidogenic factor 1 (SF-1) has a binding sequence in the SR-BI promoter, and convincing evidence was presented that SF-1 regulates the transcription in vivo (13). Several lines of evidence, however, have demonstrated that this scheme does not adequately describe the regulatory mechanism in all tissues. The transcription factor SF-1 was not found in liver (10), and several reports clearly showed that posttranscriptional events take part in the regulation of SR-BI expression (8, 12, 14). Taken together, the regulatory mechanism at least in nonsteroidogenic tissues is far from clear. The expression is obviously affected by a multitude of factors and, because interactions among these factors cannot be excluded, some of the described effects may be secondary.

This article aims at determining the effects of vitamin E (vitE) on the expression of SR-BI in the liver and the resulting changes in cholesterol metabolism. A striking upregulation of SR-BI was observed on vitE depletion of liver tissue in a rat dietary model, indicating that the supply of organs with vitE is a major, and thus far not recognized, factor in the control of SR-BI expression. The vitE effect was also observed with hSR-BI in the human liver-derived tumor cell line HepG2. In addition, evidence is presented that PKC is involved in the signaling pathway.

EXPERIMENTAL PROCEDURES

Materials

The preparation of affinity-purified rabbit anti-SR-BI polyclonal antibodies (immunization with amino acids 495–509) is described elsewhere (15). Poyclonal antibodies to SR-BI with similar properties were purchased from Novus Biologicals (Littleton, CO), and donkey peroxidase-conjugated anti-rabbit IgG was from Pierce (Rockford, IL). Polyclonal anti-PKC antibodies were obtained from Calbiochem (Bad Soden, Germany). Other chemicals were obtained from the following sources, or as indicated in text: protease inhibitors, PKC inhibitors and activators, buffer substances, detergents, and lipids, Sigma (Dreieich, Germany); [1α,2α(*n*)-^{3}H]cholesterol (47.0 Ci/mmol), Amersham Pharmacia Biotech (Freiburg, Germany); DL-α-tocopherol (Serva, Heidelberg, Germany); cholesterol test kit (Merck, Darmstadt, Germany); cell culture media and fetal calf serum (GIBCO, Karlsruhe, Germany); peroxidase/chemiluminescence detection kit and protease inhibitor tablets (Complete Mini; Boehringer, Mannheim, Germany). Standard pelleted rat diet and vitE-depleted and vitE-supplemented pellets were obtained from Altromin (Lage, Germany), and HepG2 cells (ATCC HB 8065) were obtained from the German Collection of Microorganisms and Cell Culture (Braunschweig, Germany).

Rat feeding regimen for vitE depletion

Male Wistar rats (80–90 g) from a local animal facility received the standard pelleted rat diet ad libitum for 38–40 days (control group). In parallel, a second group was fed the same diet but depleted of vitE (α-tocopherol content not detectable). Some of these rats were subsequently fastened for 24 h followed by feeding a vitE-supplemented diet (400 mg of α-tocopherol per kg) ad libitum for 48 h.

Cell culture and vitE enrichment

HepG2 cells were grown in culture flasks in RPMI medium with 25 mM glucose supplemented with 10% fetal calf serum, streptomycin (50 μg/ml), and 2 mM glutamine, in 5% CO_2 at 37°C. The medium was changed every 2–3 days. For modifying the vitE content, about 0.5×10^6 cells were transferred to 10-cm petri dishes. The cells were grown for 1 day in RPMI medium and for 2 days in the lipoprotein-depleted medium Seromed-BMS (Biochrom KG, Berlin, Germany), supplemented with trypsin inhibitor (1 mg/ml) from soy bean, to reach about 80% confluence. The cells were washed three times in BMS medium before human HDL, to a final concentration of 34.7 μg of protein (18 μg total HDL cholesterol) per ml of BMS medium, was added, either in native form (α-tocoperol content, 6.2 μg/mg protein) or enriched with α-tocopherol to a concentration of 19.3 μg/mg protein. The cells were then incubated for another 16 h. HDL enriched in vitE was prepared as described (15). Alternatively, the cells were incubated under the same conditions with mixed egg yolk phosphatidylcholine (PC)/α-tocopherol vesicles in two concentrations, 50 μg of α-tocopherol plus 100 μg of PC per ml of medium and 250 μg of α-tocopherol plus 500 μg of PC per ml of medium. The lipids were emulsified by sonication (5 strokes, 3 s each, at 200 W; Labsonic, Braun, Melsungen, Germany) with cooling on ice. Cells were finally washed and scraped from the dishes in ice-cold phosphate-buffered saline (PBS).

Determination of vitE and cholesterol uptake

Human HDL was enriched in vitE (α-tocopherol) or labeled with [^{3}H]cholesterol as outlined in previous articles (15, 16). HepG2 cells were grown in 10-cm culture dishes to 80% confluence before they were incubated at 37°C for 1 or 2 h with 10 ml of HDL solution (15 μg of protein per ml) in RPMI medium. The vitE content of enriched HDL was 13.9 μg/mg protein, and the cholesterol-labeled HDL comprised 427 μg of cholesterol per mg protein (4.5 μCi/mg cholesterol). To stop the uptake, the cells were washed twice with PBS. The cells were then scraped from the plates in 2 ml of *N*-2-hydroxylethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES)/sucrose buffer (see below) and homogenized by sonication (40 strokes, 1 s each, 70 W). Aliquots were removed for liquid scintillation counting in 4 ml of scintillation cocktail (Wallac Optiphase HiSafe; Fisher Chemicals, Loughborough, UK) and for the determination of vitE.

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TABLE 1. Plasma lipid constituents and the distribution of total cholesterol among lipoproteins as affected by vitE depletion over 38 days and subsequent refeeding for 2 days

Treatment of Rats	vitE	Cholesterol	Bile Acids	HDL	LDL	LDL
	μg/ml	*μg/ml*	*mM*	*% of total lipoprotein-cholesterol*		
Control	8.0 ± 1.1	460 ± 50	ND	73 ± 4	20 ± 4	7 ± 1
vitE depletion	1.6 ± 0.3[a]	610 ± 150	9.3 ± 2.0	73 ± 3	18 ± 7	9 ± 7
vitE refeeding	19.0 ± 5.0[b]	490 ± 30	3.8 ± 0.8	81 ± 6	12 ± 6	5 ± 3

Data represent means ± SD, n = 3. ND, Not detectable.
[a] Significantly different from controls, $P < 0.005$.
[b] Significantly different from controls, $P < 0.05$.

Preparation of rat liver membrane protein extracts

Rats were anesthetized with pentobarbital (30 mg/100 g) and killed by bleeding before the livers were excised. The tissue was sliced and homogenized in ice-cold buffer [0.25 M sucrose, 10 mM HEPES/NaOH (pH 7.5), 2 mM phenylmethylsulfonyl fluoride (PMSF), leupeptin (2.5 μg/ml), aprotinin (5 μg/ml)] by 15 strokes in a glass/Teflon Potter device at 1,400 rpm. The complete membrane fraction was prepared according to Fisher et al. (17). The membrane pellet was extracted for proteins in Dulbecco's PBS without Ca^{2+} and Mg^{2+}, comprising 1% (w/v) octyl-β-D-glucopyranoside, 2.5 mM ethylenediaminetetraacetic acid (EDTA), and the protease inhibitors as described above. These extracts were routinely subjected to electrophoresis and quantification of SR-BI. Basically the same results were obtained with the crude liver homogenate, but the quantitative evaluation was difficult because of overloading of gels.

Determination of membrane-bound PKC

Freshly harvested HepG2 cells (about 5×10^6) were collected by centrifugation at 150 *g*. The cell pellet was resuspended in homogenization buffer [20 mM Tris-HCl (pH 7.5), 1 mM EDTA, 1 mM ethyleneglycol-bis(β-aminoethyl ether)-*N,N,N,N*-tetraacetic acid, 250 mM saccharose, 1 mM dithiothreitol, 1 mM PMSF, aprotinin (10 μg/ml), leupeptin (10 μg/ml), and a Boehringer protease inhibitor tablet/10 ml] according to Gobran and Rooney (18), and the cells were lysed by sonication (Sonoplus HD60, 2 × 20 s; Bandelin Electronics, Berlin, Germany). The particulate fraction was collected by centrifugation at 100,000 *g* for 1 h. Proteins in the pellets were solubilized in homogenization buffer comprising 1% (w/v) Triton X-100 for 2 h on ice with occasional vortexing. Nondissolved material was removed by centrifugation at 10,000 *g*. All steps were carried out at 0–4°C. The supernatants were subjected to electrophoresis for analysis of PKC and SR-BI by immunoblotting.

Electrophoresis and immunoblotting

The procedures for separating proteins by standard sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 8% gels, for electroblotting of the bands to nitrocellulose, and for visualization of the PKC and SR-BI bands using rabbit anti-PKC and anti-SR-BI antibodies and peroxidase-conjugated second antibodies have been outlined in previous articles (15, 19). The PKC antibodies were used at a dilution of 1:250. The peroxidase/chemiluminescence-produced bands on X-Omat films (Eastman Kodak, Rochester, NY) were quantified by scanning, using densitometers with automatic calibration [Image Master DTS (Pharmacia, Uppsala, Sweden) and GS-710 Imaging Densitometer (Bio-Rad, Hercules, CA)].

Lipid analytical procedures

The separation of rat plasma lipoprotein fractions for analytical purposes was carried out on agarose gels, using an electrophoresis analyzer (Rapid EP; Helena Laboratories, Sunderland, UK). Plasma (2 μl) was applied to the gels and separated at pH 7.0 according to the recommendations of the manufacturer. Cholesterol in the lipoprotein fractions was determined by scanning after staining by an enzymatic procedure with nitrotetrazolium blue as substrate and cholesterol esterase, cholesterol dehydrogenase, and diaphorase as auxiliary enzymes. The procedures to determine cholesterol and vitE in plasma, liver tissue, HepG2 cells, and rat diets, were carried out as previously described (15, 19). Bile acids were measured with a commercial test kit (Merck, Darmstadt, Germany).

Miscellaneous procedures

Northern blot analysis was carried out with an SR-BI single-stranded DNA probe as previously reported (15). Radioactive signals were quantified by means of a GS-250 Molecular Imager (Bio-Rad) with a β-actin probe as internal standard. Protein was determined by the bicinchoninic acid procedure, using a test kit (Pierce, Rockford, IL), with bovine serum albumin as standard. Human HDL was prepared from the plasma of healthy, normolipidemic volunteers by KBr density gradient centrifugation and subsequent dialysis against Tris-buffered saline as described elsewhere (15).

RESULTS

In vivo effects of dietary vitE depletion

The in vivo effect of vitE on the expression of SR-BI in rat liver was investigated by feeding a vitE-depleted diet over 38–40 days. A control group of rats received the standard rat chow while a third group was treated in the same way as the first group, but the rats were fed a α-tocopherol-enriched chow for 2 days before the animals were killed. The treatment with the depleted food reduced the level of vitE in plasma to 20% (**Table 1**) and in the liver to 29% (**Table 2**) of the value in controls, while feeding the enriched diet over 2 days led to a massive accumulation of vitE in the liver (Table 2). The alimentary vitE status did

TABLE 2. Effect of dietary vitE on lipid constituents in rat liver tissue

Treatment of Rats	Vitamin E	Cholesterol	Bile Acids
	μg/mg protein		*μmol/mg protein*
Control	0.101 ± 0.011	6.4 ± 0.5	0.8 ± 0.1
vitE depletion	0.029 ± 0.006[a]	6.2 ± 0.3	0.9 ± 0.2
vitE refeeding	2.163 ± 0.507[a]	7.6 ± 0.5	0.6 ± 0.2

Each value represents the means ± SD for data obtained from three animals.
[a] Significantly different versus controls, $P < 0.005$.

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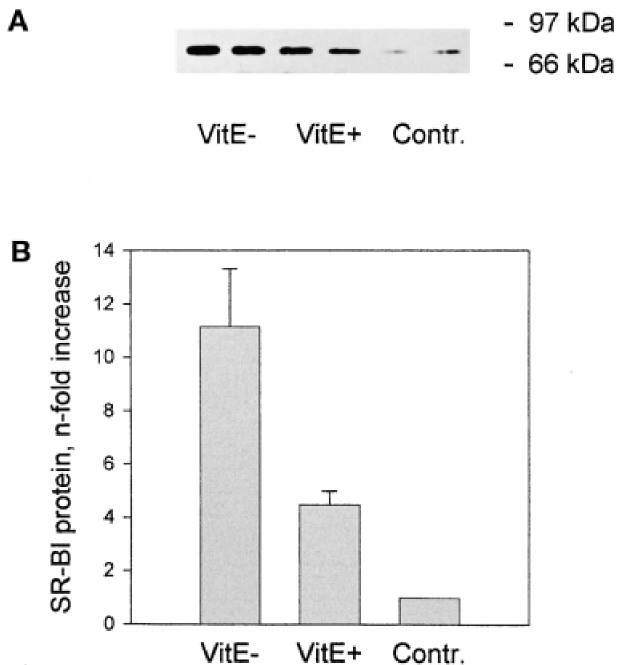

Fig. 1. Induction of SR-BI in rat liver by vitamin E depletion. (A) Immunoblotting of liver protein extracts from vitE-depleted (VitE−), vitE-refed (VitE+), and control (Contr.) animals. (B) Quantification of SR-BI in liver membrane protein extracts by scanning of immunoblots. The data of both vitE-treated groups are significantly different from controls (VitE−, $P < 0.025$; VitE+, $P < 0.01$). Data represent means ± SD (n = 3).

not significantly affect the cholesterol level in plasma and liver (Tables 1 and 2) and the cholesterol distribution among the lipoprotein classes (Table 1).

In contrast, the vitE depletion caused a strong (11-fold) increase in the expression of the SR-BI protein. The effect was partly reversed by refeeding the vitamin for 2 days, showing that the expression of SR-BI rapidly responded to the vitE content of liver tissue (**Fig. 1**). The constant level of the SR-BI messenger RNA (**Table 3**) indicates that the effect of vitE is mediated on the posttranscriptional level.

In our dietary rat model, a significant change in cholesterol in liver tissue and plasma was not observed (Tables 1 and 2), but the level of bile acids in the plasma was markedly increased in vitE-depleted rats (Table 1). Obviously, the induction of SR-BI favors cholesterol uptake in the liver, but the capacity of the degradation pathway to bile acids is sufficient to prevent the accumulation in liver tissue.

The expression of SR-BI may also be influenced by steroid hormones, for example, by high doses of estrogens in the liver (8, 9). To exclude hormone effects, the level of cortisol, testosterone, β-estradiol, and progesterone in plasma was determined. No significant differences were found among vitE-deficient and refed rats as well as controls (results not shown). Furthermore, the low and constant level of the indicator enzymes aspartate and alanine aminotransferase, indicates that the liver tissue was not damaged by vitE depletion (results not shown).

TABLE 3. Effect of dietary vitE regimens on SR-B1 mRNA level in rat liver tissue

Treatment of Rats	SR-B1 mRNA
	arbitrary units
Control diet	0.82 ± 0.11
vitE depletion	0.70 ± 0.06
vitE refeeding	0.77 ± 0.33

Values are expressed as means ± SD (n = 4).

hSR-BI in HepG2 cells as affected by vitE

The change in vitE level in vivo requires lengthy feeding regimens that may lead to largely unknown adaptation processes. In addition, the various cell types in an organ sample may respond in different ways to regulatory factors (9). To avoid these ambiguities, a cell line derived from human liver tumor cells, HepG2, was used to verify the vitE effects in vitro.

The extremely low vitE content in HepG2 cells (below the detection limit of 5 ng/mg protein) was increased by incubating the cells with native HDL to 37 ng/mg protein (**Table 4**), a level well below the normal rat liver vitE content of about 100 ng/mg protein (Table 2), whereas a significantly higher value than in liver tissue was reached by incubating HepG2 cells with vitE-enriched HDL (Table 4), although the vitE level in the medium (0.67 μg/ml) constituted only about 10% of the physiological concentration of vitE in rat plasma (Table 1). The treatment of HepG2 cells with mixed PC/vitE vesicles resulted in up to 6.4-fold higher vitE levels compared with rat liver, but pronounced variation among the concentration values was observed (Table 4). We must assume that only a minor part of the vitE was internalized by the cells, while a large and highly variable amount of the vesicles was merely adsorbed to the cell surface. The content of total cholesterol in HepG2 cells did not vary under any of these treatments (Table 4).

The SR-BI band at 82 kDa was significantly decreased when the vitE level was increased by loading the HepG2 cells with HDL (**Fig. 2**). A less pronounced decrease was induced by incubating the cells with PC/vitE vesicles, although the apparent vitE content was much higher compared with HDL-treated cells (Table 4), a result support-

TABLE 4. Levels of vitE and cholesterol in HepG2 cells after incubation for 16 h with mixed PC/vitE vesicles and human HDL enriched in α-tocopherol

Experimental Condition	Content in HepG2 Cells	
	vitE	Cholesterol
	ng/mg protein	*μg/mg protein*
Control	ND	102 ± 40
PC/vitE (50 μg of vitE/ml)	441 ± 275	113 ± 46
PC/vitE (250 μg of vitE/ml)	643 ± 123[a]	105 ± 37
Native HDL	37 ± 8	93 ± 23
vitE-enriched HDL	177 ± 14[a]	99 ± 11

Each value represents the means ± SD of data from three experiments. ND, Not detectable.
[a] Significantly different versus native HDL, $P < 0.005$.

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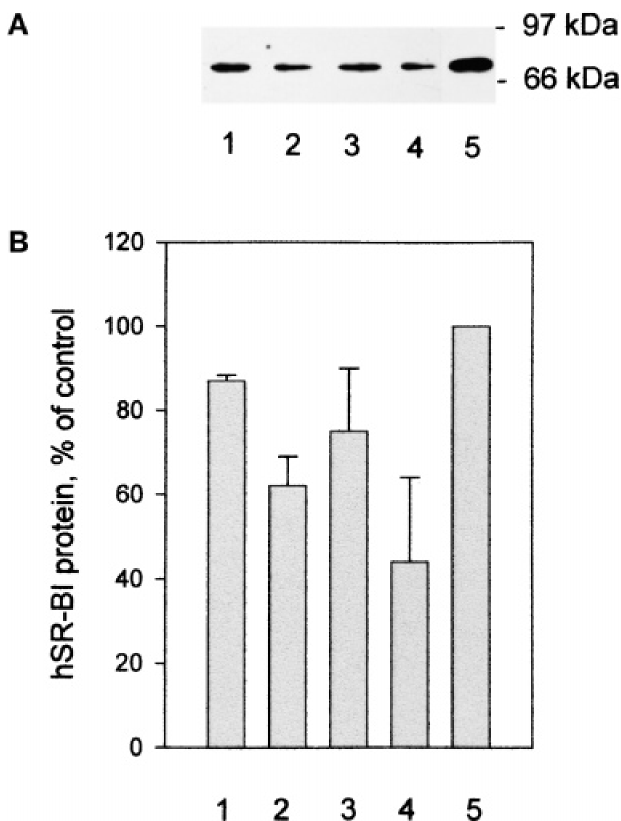

Fig. 2. The expression of the scavenger receptor hSR-BI in HepG2 cells as affected by loading with vitE via PC/vitE vesicles and HDL. Cells were incubated for 16 h with vesicles containing α-tocopherol at a concentration of 50 μg/ml (lane 1) or 250 μg/ml (lane 2), with native HDL (lane 3), or with HDL enriched in α-tocopherol (lane 4); lane 5, control. (A) The SR-BI bands in cell membrane protein extracts as visualized by immunoblotting; (B) densitometric evaluation of the immunoblots. Treatment data are significantly different from control data (lane 1, $P < 0.0005$; lane 2, $P < 0.005$; lane 3, $P < 0.05$; lane 4, $P < 0.01$). Data represent means ± SD (n = 3).

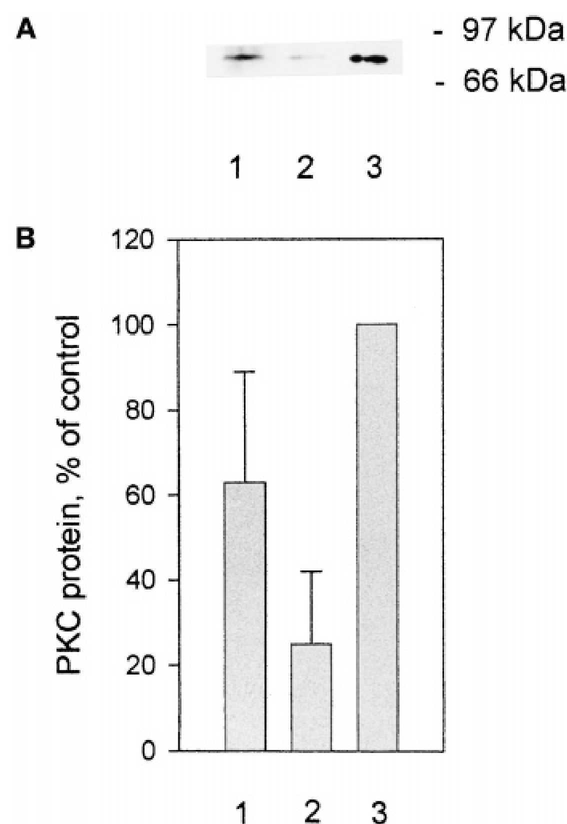

Fig. 3. Particulate PKC protein in HepG2 cells as influenced by vitE loading. The vitE content of HepG2 cells was increased by incubating the cells with native HDL (lane 1) or with α-tocopherol-enriched HDL (lane 2); lane 3, control. The conventional isoforms of membrane-bound PKC were detected by immunoblotting (A), and the protein content was quantified by densitometric scanning (B). $P < 0.005$, lane 2 versus controls. Data represent means ± SD (n = 3).

ing the view that only a limited part of the cell-associated vitE was internalized.

Downregulation of SR-BI expression by PKC inhibition

Several investigations of different physiological effects of vitE have shown that a common mechanism is involved, the inhibition of PKC stimulation (20). To test the hypothesis that the vitE-induced downregulation of SR-BI is also mediated by PKC, the activity of the kinase in HepG2 cells was modified. Long-term exposure of cells to the PKC activator 4β-phorbol 12-myristate 13-acetate (PMA) is known to downregulate the enzyme (20). The PMA treatment (50 μM, 16 h) resulted in a significant ($P < 0.001$) reduction in SR-BI expression to 5.5 ± 12.3% of controls (mean ± SD, n = 5). These results support our assumption that PKC is involved in the signaling of SR-BI regulation.

Particulate PKC protein as affected by vitE status

The association of the PKC protein with membranes is generally considered a prerequisite for the transfer of the enzyme into the active state (21). To obtain additional information about the participation of PKC in the regulation of SR-BI in HepG2 cells, variations in the level of particle-bound PKC protein were measured by immunoblotting. The loading of HepG2 cells with vitE via HDL resulted in the reduction to 63% (native HDL) and to 25% (vitE-enriched HDL) of controls (**Fig. 3**), whereas chronic treatment with PMA reduced the particulate PKC level to 44% of controls (results not shown). It should be noted that the PKC antibodies used in this study preferentially detected the conventional forms of PKC, isoforms α, βI, βII, and γ. Only the conventional isoforms α and βII were found in HepG2 cells, both migrating at about 80 kDa in SDS-polyacrylamide gels (22, 23), whereas the novel and atypical isoforms of PKC in HepG2 cells, δ, ε, and ζ, show clearly different electrophoretic mobility (22, 23). Therefore, it can be assumed that the PKC band in Fig. 3 at 80 kDa represents the isoforms α and βII. The complete analysis of all isoforms was beyond the scope of the present investigation.

Effect of hSR-BI decrease on lipid uptake from HDL

The results of the present study support the view that the expression of SR-BI in the liver and in HepG2 cells is

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TABLE 5. Lipid uptake into HepG2 cells as affected by the inhibition of PKC by chronic exposure to PMA

Treatment of Cells	Uptake of vitE		Uptake of Cholesterol
	1-h Uptake	2-h Uptake	
	ng/mg protein		*ng/mg protein × 2 h*
Control	45.4/52.0	84.0/82.0	550 ± 40[a]
PMA	12.6/18.6	38.0/36.2	340 ± 35[a]

Data represent two independent experiments and the means ± SD of three experiments.

[a] Significantly different versus control, $P < 0.01$.

regulated by the vitE status in a PKC-dependent way. To investigate the physiological consequences of this effect, the uptake of vitE and cholesterol from HDL into HepG2 cells was measured. **Table 5** shows that the decrease in SR-BI after long-term incubation with PMA (16 h, 50 μM) was accompanied by a reduction in vitE uptake from HDL to 32% of controls (1-h uptake period) and to 45% (2-h uptake period). A clearly lesser effect, reduction to 62% of controls, was observed when the uptake of cholesterol was measured (Table 5).

DISCUSSION

The high expression of SR-BI in the liver is consistent with a role of this receptor in reverse cholesterol transport. The regulation of SR-BI expression should therefore substantially affect cholesterol homeostasis. Investigations from many laboratories have shown that a multitude of factors can influence SR-BI, and in some cases conflicting results were presented, especially concerning the effects of plasma cholesterol and lipoproteins as well as cholesterol loading of cells (2, 3). In the present article we showed that vitE represents another compound with a striking effect on SR-BI expression. It is shown by dietary variation of the plasma and tissue levels of vitE that an inverse correlation between these parameters and the expression of SR-BI exists. The human homolog hSR-BI responded in the same way in an in vitro model. These observations may shed some light on the aforementioned investigations of the expression of SR-BI. Vitamin E is transported in the plasma in a lipoprotein-bound form, and it is taken up by some cell types preferentially from HDL (15). Any change in the HDL content in the plasma or in the composition of HDL particles in the diverse subfractions of HDL may lead to impaired transport of the vitamin into tissues and, consequently, altered expression of SR-BI. Furthermore, attempts to modify plasma lipids by special diets should be considered with caution. In one review, pronounced effects on the physiology of laboratory animals by variations of the vitE content of commercial diet formulations have been described (24). To our knowledge, these issues have never been taken into account in investigations of SR-BI expression.

The results of Table 3 show that the level of SR-BI mRNA in the liver did not vary in response to vitE. Obviously, the regulation occurs at a posttranscriptional level. Similar observations were reported by other authors, who investigated vitE effects on the expression of cell adhesion molecules (25) and interleukin 1β (26), but the underlying mechanisms were not characterized. Arai et al. (14) reported that the SR-BI upregulation in apoE knockout mice is also due to posttranscriptional events. In addition, the downregulation of hSR-BI by bacterial lipopolysaccharides in human macrophages was attributed to a modification and subsequent destabilization of the mRNA (12), and indirect evidence of the posttranscriptional regulation of SR-BI in several organs of the rat was presented (8). Because posttranscriptional events also dominate the downregulation of SR-BI by vitE, transcription factors such as SF-1 or the SREBP family are not involved in this process, and the effects of minor regulatory pools of cholesterol on the release of SREBP (27) can also be excluded.

The dietary rat model used in this study showed consistently the inverse correlation of vitE supply and SR-BI expression in liver tissue, but it must be taken into account that SR-BI expression is regulated by multiple factors, some of which are probably not yet known, and the lengthy feeding regimen for achieving vitE depletion may have led to adaptation processes that may have influenced SR-BI expression in a secondary way. Furthermore, different, even opposing, effects in the various cell types, as demonstrated by Fluiter, van der Westhuijzen, and van Berkel (9) in Kupffer cells and liver parenchyma cells, may have obscured the results. We therefore decided to verify the in vivo results by means of a cell culture model, the human liver tumor cell line HepG2. This cell line also gives the opportunity to study the human SR-BI homolog. Furthermore, HepG2 cells were used to get information on the signaling mechanism.

In accordance with the in vivo results, the loading of HepG2 cells with vitE resulted in the downregulation of the SR-BI protein (Fig. 2) with a concomitant reduction of the PKC protein in the cell particle fraction (Fig. 3). Although this inactivation of PKC by vitE is a well-known effect, the mechanism is still a matter of debate (20). Direct inhibition of the kinase activity as well as prevention of stimulation by interference with membrane binding were suggested as potential inhibitory mechanisms. Our results are more compatible with the latter view, because the particle-bound fraction of PKC was affected by vitE loading of the HepG2 cells. Together with the downregulation of SR-BI by inactivating PKC after chronic exposure to the activator PMA, these observations clearly demonstrate a functional relationship between SR-BI regulation and PKC activity. This conclusion is compatible with the results of Pedreño, Vila, and Masna (28), who described the downregulation of the binding sites for HDL_3 on human platelets by a PKC-dependent mechanism, although these binding sites were not identified.

Investigations using specific antibodies have shown that five of the numerous PKC isoforms are detectable in HepG2 cells (22, 23). In the present study, only the conventional isoforms α and βII could be visualized by immunoblotting, because of the limited specificity of the antiserum (Fig. 3). Downregulation of PKC by PMA had a pro-

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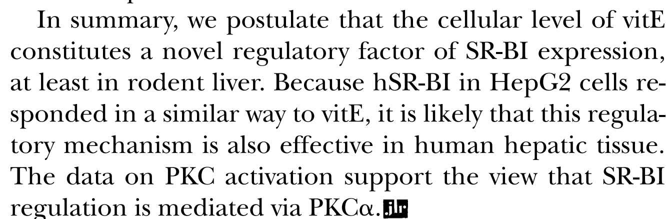
nounced effect on the expression of SR-BI, and detailed investigations of the different isoforms showed that predominantly PKCα is susceptible to PMA inactivation in HepG2 cells (23, 29). We therefore conclude that mainly the α isoform is involved in the regulation of SR-BI.

Numerous investigations have demonstrated that PKC activation in many cell types is affected merely by the binding of HDL or HDL apolipoproteins to the cell surface (28, 30–33). Because HDL was used as carrier for vitE in the present study, it was necessary to present additional evidence to show that SR-BI regulation was not influenced by HDL constituents other than vitE. By using mixed PC/vitE vesicles for loading HepG2 cells it was demonstrated that the effect was not dependent on the presence of HDL (Fig. 2). This result therefore also supports our assumption that vitE itself affects the level of SR-BI protein.

The pronounced change in SR-BI expression by vitE depletion or by modification of PKC activity is supposed to affect the uptake of lipids from HDL, but the reduction of the SR-BI level in HepG2 cells to 5.5% of controls by PMA resulted only in a reduction of vitamin E uptake to 32%, and the transport of cholesterol was reduced only to 62% of controls (Table 5). The reasons for this discrepancy are not known, but it may be speculated that the receptor protein is not in a fully functional state or is localized to functionally inadequate cell sites. Alternatively, the lipid exchange may also proceed via other mechanisms that were not regulated by PKC and vitE. Furthermore, the intracellular transport after uptake, especially of cholesterol, may be rate limiting.

The scavenger receptor SR-BI, the splicing variant SR-BII, and the human homolog hSR-BI are so far the only known receptors to facilitate the selective uptake of cholesterol (1–3). The term "selective" uptake is defined as transfer of a certain lipid component, usually cholesterol and CE, from HDL to the surface of an acceptor cell without internalization of the lipoprotein particle or apolipoproteins. In one study, convincing evidence was presented that the uptake of vitE into HepG2 cells follows the same mechanism (34). In addition, the uptake of vitE preferentially from HDL compared with low density lipoprotein (LDL) was observed with several cell types, including alveolar type II epithelial cells (15), HepG2 cells (34), and skeletal muscle myoblasts (35). The induction of SR-BI by vitE depletion, though not as pronounced as in liver, was also found in lung type II cells (15). Together with the vitE uptake experiments in the present study, these observations are compatible with the view that SR-BI constitutes an important transport mechanism for vitE. A continuously high expression of SR-BI under conditions of vitE depletion may serve to maintain a sufficient intracellular level to prevent oxidative damage.

The human homolog hSR-BI is structurally and functionally closely related to rodent SR-BI (36, 37). hSR-BI was therefore considered as a potential therapeutic target with the aim to reduce plasma cholesterol (38). In this respect, the interaction via SR-BI of vitE and cholesterol metabolism described here should be taken into account. A high level of vitE would exert a beneficial effect on redox-related injuries such as atherosclerosis (39, 40) whereas the concomitant downregulation of hSR-BI in peripheral organs and the liver would interfere with reverse cholesterol transport.



In summary, we postulate that the cellular level of vitE constitutes a novel regulatory factor of SR-BI expression, at least in rodent liver. Because hSR-BI in HepG2 cells responded in a similar way to vitE, it is likely that this regulatory mechanism is also effective in human hepatic tissue. The data on PKC activation support the view that SR-BI regulation is mediated via PKCα.

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