Lipid Free Apolipoprotein E Binds to the Class B Type I Scavenger Receptor I (SR-BI) and Enhances Cholesteryl Ester Uptake from Lipoproteins

The Class B type I scavenger receptor I (SR-BI) is a physiologically relevant high density lipoprotein (HDL) receptor that can mediate selective cholesteryl ester (CE) uptake by cells. Direct interaction of apolipoprotein E (apoE) with this receptor has never been demonstrated, and its implication in CE uptake is still controversial. By using a human adrenal cell line (NCI-H295R), we have addressed the role of apoE in binding to SR-BI and in selective CE uptake from lipoproteins to cells. This cell line does not secrete apoE and SR-BI is its major HDL-binding protein. We can now provide evidence that 1) free apoE is a ligand for SR-BI, 2) apoE associated to lipids or in lipoproteins does not modulate binding or CE-selective uptake by the SR-BI pathway, and 3) the direct interaction of free apoE to SR-BI leads to an increase in CE uptake from lipoproteins of both low and high densities. We propose that this direct interaction could modify SR-BI structure in cell membranes and potentiate CE uptake.

The Class B type I scavenger receptor I, SR-BI, binds HDL and mediates the selective uptake of HDL cholesteryl esters (CE) in cultured cells. Another property of the Class B scavenger receptor, shared with CD 36, is to bind either native (2, 3) or modified lipoproteins (acetylated or oxidized (3–5)). They are also the first defined receptors to be able to specifically bind anionic but not cationic or zwitterionic lipoproteins (6). However CD 36 is less efficient than SR-BI in promoting CE-uptake from native lipoproteins to cells (7).

There are probably different binding sites on SR-BI. HDL compete for the binding of LDL to SR-BI but LDL poorly inhibit the binding of HDL, and there is no reciprocal cross-competition between these two ligands (1, 8). The study of several mutants of SR-BI also supports for the proposal that the interaction of SR-BI with HDL differs from that with LDL (9).

Apolipoproteins (apo) AI, AII, and CIII of HDL either associated with lipids or in lipid free forms can directly mediate their binding to SR-BI (10). Williams et al. (11) demonstrated that SR-BI can interact with multiple sites in apoAI and identified the Class A amphipathic α-helix as a recognition motif. The specific role of apoAI in the delivery of cholesterol to adrenal cells was clearly demonstrated in mice deficient either in apoAI or apoAII (12). We have demonstrated, in a human adrenal cell line, that apoAII, which binds SR-BI with high affinity, can act as an antagonist of apoAI in CE-HDL uptake (13).

ApoE is a constituent of triglyceride-rich lipoproteins and is essential for the receptor-mediated uptake of their remnants (14) and for their catabolism by pathways involving heparan-sulfate proteoglycans (15). ApoE deficiency in mice leads to impaired catabolism of these remnants and increased atherosclerotic lesions (for review, see Ref. 16). Direct interaction of apoE with SR-BI has never been demonstrated, and its implication in CE uptake is still controversial.

It was previously shown that HDL binds murine SR-BI (1), human SR-BI (4), and human CD 36 (2) independently of the removal of apoE by chromatography on heparin-Sepharose (for review, see Ref. 17). These results clearly demonstrated that HDL binding can occur with high affinity in the absence of apoE.

Different results, obtained before the description of SR-BI as an HDL receptor, implicated cellular apoE in enhancing HDL binding to cells and CE uptake from HDL. Selective uptake of CE by HepG2 cells was reduced by antibodies directed against the receptor binding domain of apoE (18). The enhancing effect of apoE on CE uptake was not due to the transfer of apoE to HDL or to the LDL-receptor pathway. Inversely, Ji et al. (19) demonstrated, in cultured hepatocytes, that if apoE (in apoE-enriched HDL) enhanced the uptake of HDL particles, it had little effect on the selective uptake of CE-HDL. Similar results were described by Rinninger et al. (20). More recent studies demonstrated that apoE expression by mouse adrenal cortical cells increased both endocytic and selective uptake of CE-LDL but had little influence on CE uptake from HDL (21). Basal CE-selective uptake could be observed in the absence of secreted apoE but was enhanced by the apoE synthesis.

In vivo studies in mice deficient in apoE cannot determine the importance of apoE in CE-selective clearance. Different authors suggest that in mice lacking apoE, selective uptake of CE from remnant lipoproteins is not impaired, but occurs probably via SR-BI and could be facilitated by hepatic lipase (HL).
ApoE Increases SR-BI-dependent CE-selective Uptake

Reconstituted Discoidal HDL (rHDL) Preparation—POPC-AI and POPC-E3 were prepared by the cholate dialysis method (32, 33) at a lipid/apoprotein ratio of 100/1 (mole/mole). DPPC-E complexes (100/1) were prepared according to Jolivalt et al. (34). The functionality of the DPPC-E and DPPC-E4 preparations was verified by their capacity to inhibit $^{125}$I-LDL and DPPC-E uptake at cultured fibroblasts when DPPC-E2 poorly compete with LDL (not shown).

For selective CE uptake experiments, POPC/apoAI/CE, POPC/apoE3/CE, and DPPC/apoE3/CE, containing phospholipids (PL), apolipoprotein, CE in a molar ratio of 100/1/0.3, and trace amounts of $[^{3}H]$/CE, were prepared as described (35).

Lipoprotein Labeling—Native lipoproteins (1 mg) were labeled with $[^{3}H]$/CE as described previously (27). The final specific activities varied between 45 and 400 dpm/ng of cholesterol. Lipoproteins (native or reconstituted) were labeled in protein moiety with $[^{3}H]$iodine using the iodine monochloride method (36). The final specific activities varied between 400 and 1500 dpm/µg of protein.

Binding of $^{125}$I-Labeled Lipoproteins and Competition with Unlabeled Apolipoproteins and Lipoproteins—Cells cultured in 12-well plates were washed and preincubated for 1 h at 37 °C in DMEM-F12 serum-free medium. For direct binding, cells were incubated for 1 h at 37 °C with $^{125}$I-labeled lipoproteins without (total binding) or with a 40-fold excess of unlabeled lipoproteins (nonspecific binding). Cells were then washed and dissolved with 1 mol/liter NaOH, and cell-associated radioactivity was counted. An aliquot was used to quantify cellular proteins (37). Results were expressed as nanograms of bound or degraded proteins per milligrams of cellular proteins. Specific binding was calculated as the difference between total and nonspecific binding.

Competition studies were performed with $^{125}$I-POPC-AI at 2 µg/ml and increasing concentrations of the different unlabeled apolipoproteins or lipoproteins for 1 h at 37 °C. Cells were preincubated, washed, and then counted as in direct binding studies. Results were expressed as a percentage of the binding measured without competitor.

In the experiments indicated, binding studies were performed in the presence of 50 µM chlorpromazine to inhibit endocytosis by coated pit pathway (38).

Selective Uptake of $[^{3}H]$CE-labeled Lipoproteins—Cells cultured in 12-well plates were washed and preincubated for 1 h at 37 °C in DMEM-F12 serum-free medium. They were then washed and incubated for the indicated times with $[^{3}H]$/CE-labeled lipoproteins without (total uptake) or with a 40-fold excess of unlabeled HDL (nonspecific uptake). Cells were then washed and dissolved with 1 mol/liter NaOH, and cell-associated radioactivity was counted. An aliquot was used to quantify cellular proteins (37). Results are expressed as nanograms or milligrams of cellular proteins per milligrams of cellular proteins. In the experiments indicated, CE-selective uptake studies were performed in the presence of 50 µM chlorpromazine to inhibit endocytosis (38).

To measure the effect of direct interaction of apoE3 with cells, cells were preincubated for 1 h at 37 °C in DMEM-F12 serum-free medium with 10 µg/ml (or the indicated concentrations) of apoE3. In some experiments apoE3 (100 µg) was pretreated with thrombin (2 µg) in 100 mM bicarbonate buffer for 24 h as described previously by Bradley et al. (39, 40). The efficiency of the cleavage of recombinant apoE3 (40 kDa) was verified by the presence of two major fragments (10-kDa C-terminal fragment and 26-kDa N-terminal fragment) after SDS-PAGE in reducing conditions.

To inhibit proteoglycan synthesis, cells were preincubated for 20 h with 1 mM p-nitrophenyl-β-D-glucopyranoside (β-glucoside), a general inhibitor of proteoglycan synthesis (41), and during the lipoprotein uptake assay. The effect of β-glucoside on glycosaminoglycan synthesis was measured by simultaneous incubation of cells with Na2$^{35}$SO4 (20 µCi/ml) (42, 43). After incubation cells were washed six times with phosphate-buffered saline and cells were then solubilized with 1% NaOH or were incubated with 12.5 µg/ml trypsin in phosphate-buffered saline with 1 mM EDTA for 15 min at 37 °C to release cell surface proteoglycans. Total cells and trypsin-released materials were counted for $^{35}$S incorporation. The decrease by 24 ± 5% of Na2$^{35}$SO4 incorporation in cells and of 49 ± 11% in cell surface trypsin-released materials demonstrates that their proteoglycan content was effectively decreased during xyloside incubation.

RESULTS

Effect of Free ApoE on Ligand Binding to SR-BI—We previously demonstrated that reconstituted HDL, POPC-AI, bind to NCI-H295R adrenal cells by direct interaction with SR-BI, which was the major if not the unique receptor for these ligands (13). Among the tested ligands, POPC-AI had the highest affi-
ApoE Increases SR-BI-dependent CE-selective Uptake

finite for SR-BI. We wanted to know if apoE was a ligand for SR-BI. We first analyzed the competition of apoE with POPC-AI to bind to SR-BI. In Fig. 1A we can see that free apoE competes with 125I-POPC-AI. There is no significant difference between the recombinant apoE2, apoE3, and apoE4 isomers, but their affinity for SR-BI was slightly lower than free apoAI. In the conditions used for competition experiments, apoE did not displace 125I-apoAI from 125I-POPC-AI, since more than 95% of 125I-apoAI can be reisolated in POPC-AI by ultracentrifugation at a density of 1.21 g/ml (not shown).

We then studied the competition of free apoE3 with other lipoproteins, which are also ligands of SR-BI, and we showed that free apoE failed to compete efficiently with native 125I-lipoproteins (HDL2, LDL, or VLDL) (Fig. 1B).

After incubation with cells in the presence of apoE, 125I-POPC-AI and 125I-lipoproteins were reisolated by ultracentrifugation at a density of 1.21 g/ml and the apoE content of the different fractions was measured by ELISA (25). For 125I-lipoproteins, the main part of apoE was found in the bottom (>90%) but during the competition with 125I-POPC-AI, for higher apoE concentrations, a part of apoE was found in the top, indicating a partial apoE association with lipids.

Effect of Phospholipids on ApoE Binding to SR-BI—We then reconstituted lipoproteins, DPPC-E and POPC-E complexes, to evaluate the role of lipids in the interaction of apoE with SR-BI, and we studied the competition between these complexes and POPC-AI. As shown in Fig. 2, DPPC-E poorly competes with 125I-POPC-AI to bind to SR-BI. There is no difference between the different apoE isoforms. POPC-E3 also fails to compete with 125I-POPC-AI. In a control experiment, competition for the binding of 125I-POPC-AI by DPPC-AI was lower than POPC-AI but much greater than DPPC-E.

Effect of ApoE, Free or Associated to Lipids, on the Selective Uptake of Cholesteryl Esters from Lipoproteins Devoid of ApoE—We then wanted to evaluate the effect of apoE, free or associated to lipids, on the selective uptake of cholesteryl esters from HDL. To inhibit the LDL-receptor pathway, the selective uptake experiments were performed in the presence of chlorpromazine, an inhibitor of endocytosis (38). We incubated cells with free apoE3, then measured the selective CE uptake from [3H]CE-lipoproteins to cells. Chlorpromazine does not modify selective uptake from apoE3 free lipoproteins (not shown). Surprisingly, we can see in Fig. 3 that the presence of free apoE3 in the culture medium increases 2–3-fold the selective CE uptake mediated by SR-BI from human [3H]CE-HDL3 (Fig. 3A) or from [3H]CE-VLDL prepared from apoE-deficient mice (Fig. 3B). We verified that human plasma apoE3 has the same stimulating effect that the recombinant peptide (not shown). This effect was not obtained with free apoAI (Fig. 3, A and B), which, at higher concentrations, plays a competitive role in the selective uptake process (13). The DPPC-E preparation (Fig. 3A) fails to inhibit or enhance CE-selective uptake from HDL instead of POPC-AI, which strongly inhibits selective uptake from HDL as found previously (13).

Since apoE interaction with lipids in lipoproteins is not required to stimulate selective uptake from HDL, it was interesting to determine whether the N-terminal fragment of apoE was sufficient to elicit effect on SR-BI-mediated selective CE uptake. We incubated apoE3 with thrombin for 24 h as indi-
concentrations expressed in /H9262 presence of 50 /H9262 spond to 7.5 100% corresponds, respectively, to 0.25 Procedures. was measured after NaOH digestion as indicated under After incubation, cells were washed, and cell-associated radioactivity was measured after NaOH digestion as indicated under “Experimental Procedures.” 100% corresponds to 0.28 ± 0.02 µg of CE internalized per mg of cellular proteins in the absence of free apoE. Results are mean ± S.D. of two independent experiments performed in duplicate. B, SDS-PAGE (10%) in reducing conditions of recombinant apoE3 (lane 2) and thrombin-digested recombinant apoE3 (lane 3, CT, 10-kDa C-terminal fragment and NT, 26-kDa N-terminal fragment). Standard molecular weights are shown in lane 1.

FIG. 3. Effect of free apoE or in DPPC-E complexes on cholesteryl ester uptake by cells from lipoproteins devoid of apoE. NCI-H295R cells were incubated for 1 h at 37 °C in the presence of 30 µg/ml 3H]CE-HDL, from human plasma (A) or 2 µg/ml 3H]CE-VLDL from apoE-deficient mice (B) with increasing concentrations of free apoE3 (○), apoAI (□), DPPC-E3 (▲), or POPC-AI (■). Lipoprotein concentrations expressed in µg/ml apolipoprotein were chosen to correspond to 7.5 µg/ml total cholesterol. Experiments were performed in the presence of 50 µM chlorpromazine to inhibit the endocytic pathway. After incubation, cells were washed, and cell-associated radioactivity was measured after NaOH digestion as indicated under “Experimental Procedures.” 100% corresponds to 0.28 ± 0.02 µg of CE internalized per mg of cellular proteins in the absence of free apoE. Results are mean ± S.D. of two independent experiments performed in duplicate.

Effects of ApoE Present in Lipoproteins or in rHDL on CE-selective Uptake from These Lipoproteins—Despite former results, which suggest that only free apoE can have a competitive role in rHDL binding and an enhancing role in CE-lipoprotein uptake, we investigated whether the presence of apoE in lipoproteins had an influence on CE-selective uptake. For this purpose, we prepared lipoproteins naturally containing apoE and compared them with lipoproteins devoid of apoE. We compared 3H]CE-selective uptake from total human HDL2 (1.063 < d < 1.12 g/liter) and from the fraction of human HDL2 not retained on the heparin-Sepharose column (free apoE HDL2). Fig. 5 does not show any difference between these two HDL subfractions either for competition with 125I-POPC-AI (Fig. 5A) or for direct CE-selective uptake from these lipoproteins (Fig. 5B).

We then prepared VLDL from rabbits fed a cholesterol-rich diet. These VLDL were apoE-rich, but the apoE content depends on the duration of the cholesterol-rich diet (40). We obtained three VLDL preparations with increasing apoE content. After labeling, 3H]CE-selective uptake from these different VLDL preparations was measured, and no difference in CE-selective uptake was observed (Fig. 6). However, lipoproteins containing apoE and devoid of apoE are often found to be also different as regards to their lipid composition, and this could modify per se the CE-selective uptake (44, 45). We then artificially prepared apoE-enriched VLDL and HDL3 by the method described previously (31). VLDL from KO E mice and human HDL3 were incubated at 37 °C with free apoE3. In the conditions described under “Experimental Procedures” (31), nearly all the apoE3 was incorporated in the lipoproteins, and this was verified by ELISA after reisolation of lipoproteins. Before incorporating apoE into the lipoproteins, they were doubly labeled with 125I and 3H]CE. In the presence of chlorpromazine, no significant effect of apoE3 enrichment of lipoprotein, either on the binding measured by cell association of 125I-labeled proteins (Fig. 7A), or on 3H]CE-selective uptake, could be observed for HDL3 and VLDL (Fig. 7B). In the conditions used here, there was no cellular degradation of the protein moiety of lipoproteins (<1% of equivalent CE internalized for HDL and <5% for VLDL). These results confirm that the association of apoE to lipoproteins does not modify their interaction with SR-BI or their capacity to transfer CE to cells.

To measure direct selective uptake from discoidal rHDL (100/10/0.3, PL/ApoAI/CE or PL/ApoE3/CE), dual labeled rHDL (125I-apolipoproteins and 3H]CE) at 5 µg/ml were incubated...
with cells for 2 h at 37 °C in the presence of chlorpromazine. The data summarized in Table I show that apoE3 or apoAI-containing rHDL can bind to cells but that the binding of POPC/apoE3/CE or POPC/apoE3/CE cannot be effectively displaced by a 40-fold HDL excess but partly by LDL. It is therefore

**DISCUSSION**

**In vitro** and **in vivo** experiments have demonstrated that SR-BI is a physiologically relevant HDL receptor that can mediate selective CE uptake in the liver and in steroidogenic tissues (for review, see Refs. 47 and 48). Apolipoprotein E is a ligand of lipoprotein receptors from the LDL-receptor family (15, 49), but was not shown as a specific ligand of scavenger receptors and especially of SR-BI. However many papers have reported a role of apoE either in facilitating CE-selective uptake by cells or selective clearance of CE-HDL **in vivo** by the liver (50).

In this paper we have demonstrated in a human adrenal cell line NCI-H295R, which does not secrete apoE, that 1) free apoE competes with POPC-AI in binding to SR-BI, 2) apoE associated with lipids or in lipoproteins does not modify CE-selective uptake, but 3) strikingly, free apoE3 can interact with SR-BI and that this interaction leads to an increase in CE-selective uptake from VLDL and HDL.

We have shown that lipid free apoE (independently of the isoform E2, E3, or E4) competes with POPC-AI in binding to NCI-H295R cells. As SR-BI is the major cellular receptor for POPC-AI in these cells (13), this is a demonstration of the direct interaction of lipid free apoE with SR-BI in cells. In the competition with POPC-AI, high apoE concentrations induced apoE association with lipids, but we further demonstrated that the association of apoE to phospholipids highly decreased the competition between apoE and POPC-AI. We obtained direct binding of POPC/apoE3/CE and DPPC/apoE3/CE to NCI-H295R cells, but this binding was not displaced by POPC-AI or by a large HDL excess but partly by LDL. It is therefore
possible that the binding of POPC/apoE3/CE reflects interactions with other receptors or with binding sites on SR-BI independent of apoAI or HDL. Competition curves with POPC-AI are the best indicators of the differential interaction of lipid free apoE and DPPC-E or POPC-E with SR-BI but on a particular site (the POPC-AI binding site). The competition of DPPC-E or POPC-E is low but negligible and is coherent with the difference in binding affinity to SR-BI published by different authors (35, 51) between POPC-AI (about 4 μg/ml) and POPC-E (about 35 μg/ml). Thuanhain et al. (35) showed a direct interaction between reconstituted lipoproteins (POPC/CE/apoE or DPPC/CE/apoE, 100/0/3) and SR-BI-transfected cells, with these lipoproteins transferring their CE to cells. We also observed transfers of CE to NCI-H295R cells from reconstituted POPC/apoE3/CE, but the selective uptake from these reconstituted HDL is very much lower than from native lipoproteins, certainly because of the low availability of CE in these lipoproteins. ApoAI in these rHDL is more efficient in promoting CE uptake than apoE. In the same way we demonstrated that the presence of apoE in lipoproteins does not modify their interaction with cells and does not impair the selective uptake of CE from these lipoproteins to cells in presence of chlorpromazine, which inhibits the LDL receptor pathway. These results were obtained either with native lipoproteins (HDL, VLDL) or with lipoproteins devoid of apoE and further in vitro enriched with apoE (VLDL from mice lacking apoE gene or human HDL). This was previously described for HDL by different authors (1, 4), but we extended these results to VLDL. The low affinity for SR-BI of apoE associated with lipids does not modify the affinity for this receptor of apoE-containing lipoproteins, which can interact through other apo-lipoproteins present at their surface and/or by their lipid moiety.

The most interesting result is the capacity of free apoE to increase the selective CE uptake from different lipoproteins without any effect on their binding capacity. In our experiments free apoE3, at low concentrations (from 2 to 10 μg/ml), is allowed to interact with cells for 1 h before adding lipoproteins. Then apoE is maintained in the cell medium during CE-selective uptake experiments. However, simultaneous addition of lipoproteins and apoE3 led to nearly the same stimulation of CE-selective uptake (not shown). ApoE incorporation in lipoproteins cannot occur in cell medium when free apoE is added to labeled lipoproteins because of the very low concentration of the lipoproteins (<0.1 mg/ml). Our leading hypothesis of different binding sites for lipoproteins and free apolipoproteins is coherent with the described “nonreciprocal cross-competition” between LDL and HDL for SR-BI binding (1) or the dissociation of LDL and HDL binding activity of murine SR-BI by retrovirus library-based activity dissection (9), with these two papers showing that the multiple ligands of SR-BI can bind to different sites on the extracellular loop of the protein. Direct interaction of apoE with SR-BI, without playing a role in the binding of CE-rich lipoproteins, could therefore modify the structure of the complexes and enhance CE-selective uptake mechanism. An important feature is that a constant apoE3 concentration (10 μg/ml) has the same stimulating effect on CE uptake from lipoproteins independently of their own concentration (up to 100 μg/ml). This strengthens the idea that the apoE effect does not modify any interaction of apoE with lipoproteins but rather a direct interaction with SR-BI. This direct interaction implies the whole apoE peptide, since

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**Fig. 7. Binding and cholesteryl ester transfer to cells from lipoproteins devoid of apoE after enrichment with free apoE.** Doubly labeled [125I]-[3H]CE-lipoproteins devoid of apoE, VLDL from apoE-deficient mice, and human HDL3 at 0.5 mg/ml were enriched with apoE by incubation with 0.3 mg/ml recombinant apoE3 for 1 h at 37°C. These lipoproteins devoid (white columns) or enriched with apoE (black columns) were incubated for 1 h at 37°C with NCI-H295R cells. Lipoprotein concentrations expressed in μg/ml apolipoprotein were 30 μg/ml [125I]-[3H]CE-HDL3 or 1 μg/ml [125I]-[3H]CE-VLDL and chosen to correspond to 7.5 μg/ml of total cholesterol. After incubation, cells were washed, and cell-associated radioactivity [125I] (A) or [3H] (B) was measured after NaOH digestion as indicated under “Experimental Procedures.” Binding of apoE-enriched [125I]-[3H]-labeled lipoproteins (A) and selective uptake from [3H]CE-apoE-enriched lipoproteins (B) were expressed as a percentage of binding or CE-selective uptake from the corresponding lipoproteins free of apoE. Results are mean ± S.D. of two independent experiments performed in triplicate.

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**Table 1**

| rHDL          | rHDL binding | CE-selective uptake from rHDL |
|---------------|--------------|-------------------------------|
|               | Total        | With HDL excess               | Total        | With POPC-AI excess | With HDL excess | With LDL excess |
| POPC/apoAI/CE | 113 ± 5      | 22 ± 0.5                      | 2.51 ± 0.28  | 0.85 ± 0.16         | 1.01 ± 0.13    | 3.25 ± 0.15    |
| POPC/apoE3/CE | 305 ± 25     | 279 ± 7                       | 0.955 ± 0.08 | 1.22 ± 0.23         | 0.75 ± 0.08    | 0.20 ± 0.05    |
| DPPC/apoE3/CE | 210 ± 3      | 170 ± 6                       | 0.85 ± 0.17  | 0.96 ± 0.30         | 0.75 ± 0.17    | 0.76 ± 0.16    |
In cells cultured in the presence of D-xyloside, apoE main-
ly resides in the extracellular matrix (41, 54). In cells cultured in
the presence of proteoglycans was important in mediating the
apoE role in selective uptake, and we cultured cells with
the presence of proteoglycans—D-xyloside, apoE main-
bound lipases could be good candidates to bind apoE for this
effect as co-receptor. They play a major role in apoE
interaction with LDL-receptor-related protein and in lipoprotein
metabolism (53), and their implication was suggested in HDL
interactions with cells (18–20). We wanted to know whether the
presence of proteoglycans is important in mediating the
apoE role in selective uptake, and we cultured cells with
β-xyloside, a general inhibitor of proteoglycan synthesis (41, 54).
In cells cultured in the presence of β-xyloside, apoE main-
tains a high activating effect on CE-selective uptake, indicating that
proteoglycans, and indirectly lipases, do not play a major
role in that apoE effect. However our results do not exclude the
participation of another cellular protein in a complex interac-
tion with SR-BI.

What is the in vitro relevance of the stimulating effect of apoE
on CE-selective uptake? Arai et al. (24) showed that apoE-
deficient mice had a decreased clearance of CE-HDL, which
was not further impaired by attenuation of the SR-BI gene.
Free apoE does not exist in plasma in substantial amount, but
the potential physiologic relevance is probably in the capacity of
most tissues to secrete apoE and especially liver and adrenal
cells. ApoE is not secreted by NCI-H295R cells in the culture
medium, but in vitro a cell surface “blanket” of apoE was de-
tected on rat adrenocortical cells (55). This apoE could play a
major role in speeding up CE-selective uptake by SR-BI in adrenals.
In vitro expression of apoE was induced in a murine
adrenocortical cell line (21), although its effect on enhancing
CE-selective uptake was only shown on LDL and not on HDL.
The discrepancy with our in vitro results can arise from differ-
ences in species or from SR-BI differential interaction with exogenous or endogenous apoE, and the same authors in fur-
ther studies demonstrated the implication of proteoglycans in
the effect of apoE on CE-LDL selective uptake by these cells
(54). In the liver, another tissue with a high level of CE-
selective uptake, it has been demonstrated that hepatocytes

ApoE Increases SR-BI-dependent CE-selective Uptake

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