The Apolipoprotein E-dependent Low Density Lipoprotein Cholesteryl Ester Selective Uptake Pathway in Murine Adrenocortical Cells Involves Chondroitin Sulfate Proteoglycans and an \( \alpha_2 \)-Macroglobulin Receptor

Cells acquire lipoprotein cholesterol by receptor-mediated endocytosis and selective uptake pathways. In the latter case, lipoprotein cholesteryl ester (CE) is transferred to the plasma membrane without endocytosis and degradation of the lipoprotein particle. Previous studies with Y1/E/tet/2/3 murine adrenocortical cells that were engineered to express apolipoprotein (apo) E demonstrated that apoE expression enhances low density lipoprotein (LDL) CE uptake by both selective and endocytic pathways. The present experiments test the hypothesis that apoE-dependent LDL CE selective uptake is mediated by scavenger receptor, class B, type I (SR-BI). Surprisingly, SR-BI expression was not detected in the Y1/E/tet/2/3 clone of Y1 adrenocortical cells, indicating the presence of a distinct apoE-dependent pathway for LDL CE selective uptake. ApoE-dependent LDL CE selective uptake in Y1/E/tet/2/3 cells was inhibited by receptor-associated protein and by activated \( \alpha_2 \)-macroglobulin (\( \alpha_2 \)M), suggesting the participation of the LDL receptor-related protein/\( \alpha_2 \)M receptor. Reagents that inhibited proteoglycan synthesis or removed cell surface chondroitin sulfate proteoglycans completely blocked apoE-dependent LDL CE selective uptake. None of these reagents inhibited SR-BI-mediated LDL CE selective uptake in the Y1-BS1 clone of Y1 cells in which LDL CE selective uptake is mediated by SR-BI. We conclude that LDL CE selective uptake in adrenocortical cells occurs via SR-BI-independent and SR-BI-dependent pathways. The SR-BI-independent pathway is an apoE-dependent process that involves both chondroitin sulfate proteoglycans and an \( \alpha_2 \)M receptor.

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Snehastika Swarnakar‡, Jeanette Beers§, Dudley K. Strickland§, Salman Azhar¶, and David L. Williams‡

From the ‡Department of Pharmacological Sciences, University Medical Center, State University of New York, Stony Brook, New York 11794, §American Red Cross, Holland Laboratory, Rockville, Maryland 20855, and ¶Geriatric Research, Education, and Clinical Center, Veterans Affairs, Palo Alto Health Care System, Palo Alto, California 94304

LDL\(^1\) is a major carrier of cholesterol in the plasma, and the concentration of plasma LDL cholesterol is regulated mainly by the LDL receptor pathway in the liver (1, 2). On the other hand, LDL receptor-independent pathways mediate about one-third of LDL removal in normal individuals and are responsible for most of the LDL removal in individuals homozygous for familial hypercholesterolemia who lack functional LDL receptors (3). The LDL receptor processes LDL via receptor-mediated endocytosis in which the LDL particle is delivered to lysosomes where the CE is hydrolyzed to free cholesterol for use by the cell. In addition to receptor-mediated endocytosis, LDL CE also can be taken up by cells via a selective uptake pathway in which lipoprotein CE is transferred into the cell membrane without the uptake and degradation of the LDL particle. Although the selective uptake pathway has been studied predominantly with HDL, numerous studies have shown the selective uptake of LDL CE in steroidalogenic cells of rodents (4–9). In cultured human ovarian granulosa cells, \(~33\%\) of total LDL CE uptake occurs via the selective uptake pathway (10). Furthermore, LDL CE selective uptake occurs in human fibroblasts (11), rat and human liver cells (6, 11–13), and a murine macrophage cell (11). Thus, the selective uptake of LDL CE is a widespread process that occurs in a variety of species and cell types.

Little is known about the mechanism of LDL CE selective uptake or the cell surface receptors responsible for this process. In the Y1-BS1 clone of murine adrenocortical cells, SR-BI mediates a major fraction of LDL CE selective uptake as judged by inhibition with antibodies directed to the extracellular domain of SR-BI (14). Expression of SR-BI in transfected COS-7 cells (14) or Chinese hamster ovary cells (15) showed directly that SR-BI mediates LDL CE selective uptake but with a much reduced efficiency compared with HDL CE selective uptake (14). Thus, SR-BI may contribute to LDL CE selective uptake in some cell types.

In a previous study, we found that expression of apoE in murine Y1 adrenocortical cells enhanced the selective uptake of LDL CE by 2–2.5-fold (16). The clone of Y1 cells used in that work, Y1/E/tet/2/3, was engineered to express human apoE4 under control of a tetracycline-regulated promoter, permitting apoE expression to be induced some 20-fold by removal of tet from the culture medium. Experiments in the present study were designed to explore the mechanism by which apoE alters associated protein; Bt-cAMP, dibutyryl cyclic AMP; TBS, Tris-buffered saline; human \(^{125}\)I-labeled \(^{3}H\)HDL\(_2\); \(^{125}\)I-dilactil tyramine-\(^{3}H\)chol-esterol oleyl ether human HDL\(_2\); human \(^{125}\)I-labeled \(^{3}H\)LDL; \(^{125}\)I-dilactil tyramine-\(^{3}H\)cholesterol oleyl ether human LDL.
LDL CE selective uptake and, in particular, to test the hypothesis that the enhancement by apoE occurred via SR-BI-mediated LDL CE selective uptake. Surprisingly, we found that the Y1/E/tet/2/3 adenocortical cell line does not express detectable levels of SR-BI. Furthermore, characterization of the LDL CE selective uptake pathway in Y1/E/tet/2/3 cells showed its properties to be distinct from that mediated by SR-BI and to involve cell surface chondroitin sulfate proteoglycan, apoE, and a member of the LDL receptor family. Thus, these findings identify a new pathway for the selective uptake of LDL CE.

**EXPERIMENTAL PROCEDURES**

*Cell Culture*—Y1-BS1 cells were maintained in a 37 °C humidified 95% air, 5% CO₂ incubator in Ham's F-10 medium supplemented with 12.5% heat-inactivated horse serum, 2.5% calf serum, 2 mM glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin as described (17). Y1/E/tet/2/3 cells were maintained in the same fashion except that the medium also contained 100 µg/ml G418 (Geneticin, Life Technologies, Inc), 200 µg/ml hygromycin (Calbiochem), and 2 µg/ml tetracycline (16). MEFs were maintained in a 37 °C humidified 95% air, 5% CO₂ incubator in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin as described (17).

*Western Blotting*—One day before assay, medium was changed to serum-free medium plus or minus tet containing 2 mM Bt₂cAMP where indicated. Postnuclear supernatants were isolated as previously described (19). Proteins (10–20 µg) were resolved by SDS, 10% polyacrylamide gel electrophoresis (PAGE) or SDS, 4–15% PAGE and transferred to nitrocellulose membranes. The membranes were blocked for 1 h at room temperature in 20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.02% Tween 20 (TBST) containing 7% nonfat milk. The blocked membranes were then incubated with one of the following primary antibodies in TBST-Tween containing 1% nonfat milk: anti-SR-BI C-terminal tail (1:5000) (20), anti-SR-BII C-terminal tail (1:5000) (21), anti-LRP (2 g/ml) (18), anti-apoE (1:5000) (18), and anti-SR-BII C-terminal tail (1:5000) (21), anti-LRP (2 g/ml) (18), and anti-apoE (1:5000) (18). The blotted proteins were visualized by enhanced chemiluminescence (Pierce).

*Proteoglycan Degradation Assays*—Y1/E/tet/2/3 cells were treated with 2 mM Bt₂cAMP in serum-free medium for 40 h at 37 °C in serum-free medium before lipoprotein uptake assays. To inhibit sulfation (27) of glycosaminoglycans, cells were incubated for 20 h at 37 °C in serum-free medium containing 30 mM NaClO₃ before the addition of lipoprotein particles. For selective removal of cell surface heparan sulfate proteoglycans, cells were treated for 3 h at 37 °C with 3 unit/ml heparinase I (EC 4.2.2.7) or heparinase I and III together each at 3 units/ml before the addition of the double-labeled lipoprotein particles. For selective removal of chondroitin sulfate and dermatan sulfate proteoglycans, cells were treated with chondroitinase ABC (EC 4.2.2.4) for 3 h at 37 °C with 1 unit/ml before the addition of the double-labeled lipoprotein particles. Each enzyme (Sigma) was dissolved in F-10 medium and used immediately.

*RESULTS*—Detection of SR-B1 Expression in Y1/E/tet/2/3 and Y1-BS1 Cells—Y1/E/tet/2/3 cells were prepared from the Y1 parent cell line by two rounds of cloning to place apoE expression under control of the tet-off system described by Gossen and Bujard (28). Y1/E/tet/2/3 cells express apoE at 2–2.5 µg/ml in the medium in the absence of tet and suppress apoE expression to 0.1 µg/ml in the presence of tet (16). To examine the effect of Bt₂cAMP on SR-B1 expression in Y1/E/tet/2/3 cells and to compare with the level of SR-B1 expression in Y1-BS1 cells, post-nuclear supernatant was used for Western blot analysis using antibody raised against the C-terminal tail of murine SR-BI (20). Fig. 1 shows that Y1/E/tet/2/3 cells plus or minus tet in the presence or absence of Bt₂cAMP did not show detectable SR-B1 expression. As expected, SR-B1 expression was readily detected in Y1-BS1 cells and showed the expected increase in the presence of Bt₂cAMP (19). These results indicate that Y1/E/tet/2/3 cells do not make detectable SR-B1 in the presence or absence of apoE expression or upon activation of the protein kinase A pathway by Bt₂cAMP. A Western blot using an antibody to the C-terminal tail of SR-B1 also failed to detect this splice variant of the class B scavenger receptors in the presence or absence of apoE expression (Fig. 2). Note that SR-B1 expression was detected in the parent Y1 cell line, from which the Y1/E/tet/2/3 clone was generated, at ~14% of the expression level in the Y1-BS1 line as determined by quantitative Western blotting (14). The basis for the loss of SR-B1 expression in the Y1/E/tet/2/3 cell line is unknown, but a wide range of SR-B1 expression levels has been observed in unselected subclones of the parent Y1 cell line isolated by limited dilution cloning (14). Effect of RAP on LDL CE Uptake—Since neither SR-B1 nor SR-BII seemed likely to be the basis for the apoE-mediated enhancement of LDL CE selective uptake in Y1/E/tet/2/3 cells, we surveyed the cells for other apoE receptors that are mem...
bers of the LDL receptor superfamily. As shown in Fig. 2, the LRP/\(\alpha_2\)M receptor and the VLDL receptor were both detected in Y1/E/tet/2/3 cells with no reproducible differences in expression in the presence or absence of tet were analyzed by SDS, 4–15% PAGE under reducing conditions and electroblotted to nitrocellulose membranes. Membrane strips were probed with the indicated antibody for 3 h at room temperature followed by incubation with secondary antibody and development via chemiluminescence. Positive controls (cont) were provided by \(\alpha\)-SR-BII (mouse testis extract), \(\alpha\)-LR8B (extract of COS-7 cells transfected with expression vector for LR8B), \(\alpha\)-LRP (extract of mouse embryo fibroblast cells), \(\alpha\)-VLDLR (extract of Y1-BS1 cells).

Although this antibody against chicken LR8B/aPOER2 readily detected this receptor in extracts of mouse SR-BI, the mobility of molecular weight markers are shown on the right side of the gel.

To determine whether these members of the LDL receptor family might play a role in apoE-dependent LDL CE selective uptake, Y1/E/tet/2/3 cells were incubated with RAP, which inhibits the binding of apoE as well as other ligands to many members of this receptor family (30, 31). As shown in Fig. 3A, increasing concentrations of RAP blocked the apoE-dependent component of LDL CE selective uptake in Y1/E/tet/2/3 cells but had no effect on LDL CE selective uptake in the absence of apoE expression. In a separate experiment with Y1/E/tet/2/3 cells induced to express apoE, RAP inhibited LDL CE selective uptake but had no effect on HDL CE selective uptake (Fig. 3B). Fifty percent of the apoE-dependent LDL CE selective uptake component was inhibited at a RAP concentration of 50 nM.

The effect of RAP on both LDL CE selective uptake and HDL CE selective uptake was also tested in Y1-BS1 adrenocortical cells in which the majority of LDL CE and HDL CE selective uptake is due to SR-BI (14, 32). At neither of two lipoprotein concentrations (10 \(\mu\)g/ml or 100 \(\mu\)g/ml) did RAP at 1 \(\mu\)M have an appreciable effect (<10% inhibition) on LDL CE selective uptake or HDL CE selective uptake in Y1-BS1 cells (data not shown). Thus, SR-BI-mediated LDL CE and HDL CE selective uptake is not sensitive to RAP inhibition in Y1-BS1 cells, whereas apoE-dependent LDL CE selective uptake in Y1/E/tet/2/3 cells that do not express SR-BI is completely sensitive to RAP.

**Role of LRP/\(\alpha_2\)M Receptor in LDL CE Uptake**—Based on the RAP inhibition data, we asked whether the LRP/\(\alpha_2\)M receptor might be responsible for apoE-dependent LDL CE selective uptake. Functional activity of the LRP/\(\alpha_2\)M receptor in Y1/E/tet/2/3 cells in the presence of apoE was first tested by monitoring the degradation of \(^{125}\text{T}-\alpha_2\)M*. As shown in Fig. 4A, Y1/E/tet/2/3 cells degraded \(^{125}\text{T}-\alpha_2\)M* at a rate similar to that of

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Fig. 1. Immunoblotting of SR-BI in Y1/E/tet/2/3 and Y1-BS1 cells Ten \(\mu\)g of post-nuclear supernatant of each cell extract was electrophoresed on a 10% SDS-polyacrylamide gel under reducing conditions and electroblotted onto nitrocellulose. The blot was probed with antibody raised against the C-terminal tail of murine SR-BI. The mobilities of molecular weight markers are shown on the right side of the gel.

Fig. 2. Immunoblotting of SR-BII and LDL receptor family members Post-nuclear supernatant (20 \(\mu\)g of protein) of 2 mM Bt2cAMP-treated Y1/E/tet/2/3 cells grown in the presence or absence of tet were analyzed by SDS, 4–15% PAGE under reducing conditions and electroblotted to nitrocellulose membranes. Membrane strips were probed with the indicated antibody for 3 h at room temperature followed by incubation with secondary antibody and development via chemiluminescence. Positive controls (cont) were provided by \(\alpha\)-SR-BII (mouse testis extract), \(\alpha\)-LR8B (extract of COS-7 cells transfected with expression vector for LR8B), \(\alpha\)-LRP (extract of mouse embryo fibroblast cells), \(\alpha\)-VLDLR (extract of Y1-BS1 cells).
mouse embryo fibroblast cells, which are known to express the LRP/α2M receptor. Both RAP and α2M* blocked the degradation of 125I-α2M* in Y1/E/tet/2/3 cells. In addition, RAP inhibited 50% of α2M* degradation by Y1/E/tet/2/3 cells at a concentration of 50–100 nM (Fig. 4B), which is similar to the concentration dependence for inhibition of apoE-dependent LDL CE selective uptake (Fig. 3B).

Inhibition by RAP is consistent with the participation of the LRP/α2M receptor but is not diagnostic for this receptor alone, since RAP interacts with other members of the LDL receptor family (30, 31). In contrast, α2M* is specific for the LRP/α2M receptor. The experiment in Fig. 5A shows that α2M* inhibited ~30% of total LDL CE selective uptake activity in apoE-expressing Y1/E/tet/2/3 cells. The enhancement of LDL CE selective uptake by apoE expression in this experiment was ~2-fold (Fig. 5B); hence, the 30% inhibition of total LDL CE selective uptake by α2M* reflects a 60% inhibition of apoE-dependent LDL CE selective uptake. In three such experiments, α2M* inhibited an average of 68% of apoE-dependent LDL CE selective uptake but inhibited less than 10% of LDL CE selective uptake in Y1/E/tet/2/3 cells not induced to express apoE or in Y1-BS1 cells that do not express apoE (data not shown).

Role of Cell Surface Proteoglycan in ApoE-dependent LDL CE Selective Uptake—Cell surface proteoglycans are known to be important for the endocytic uptake of apoE-containing β-VLDL particles by the LRP/α2M receptor (33, 34). In addition, direct internalization of lipoproteins bound to the cell surface via heparan sulfate proteoglycans, particularly synde-
can and perlecan, may occur without the participation of an LDL receptor family member (35, 36). To test the role of proteoglycan in apoE-dependent LDL CE selective uptake in Y1/E/tet/2/3 cells, we used two approaches to inhibit proteoglycan formation. In the first, the sulfation of glycosaminoglycan side chains was inhibited by treatment of cells with 30 mM sodium chlorate for 20 h before the addition of 125I-labeled [3H]LDL. In three experiments, treatment of Y1/E/tet/2/3 cells with sodium chlorate caused about 75% inhibition of the apoE-dependent LDL CE selective uptake (Fig. 6A) but had no effect on SR-BI-mediated LDL CE selective uptake in Y1-BS1 cells that do not make apoE (Fig. 6B). Cells were processed to determine LDL CE selective uptake. The percent inhibition by chlorate of the apoE-dependent component of LDL CE selective uptake was determined by subtracting the −apoE value from the +apoE value in both treatment groups and then expressing the difference between the resultant values as a percent of the apoE-dependent value for the control group. Panel B, Y1-BS1 cells were cultured in serum-free medium plus 30 mM sodium chlorate for 20 h at 37 °C. Double-labeled LDL (50 μg/ml) was then added and incubated for 4 h. Cells were processed to determine LDL CE selective uptake. Values represent the mean ± S.E. (n = 3).

Effect of Heparinase and Chondroitinase on ApoE-dependent LDL CE Uptake—To test the specific effect of heparan sulfate proteoglycan and chondroitin sulfate proteoglycan in apoE-dependent LDL CE selective uptake, Y1/E/tet/2/3 cells were subjected to enzymatic digestion of glycosaminoglycan side chains by proteoglycan lyases. When cells were treated with 3 units/ml heparinase I or heparinase I and III together for 3 h at 37 °C there was at most a 20% reduction of apoE-dependent LDL CE selective uptake (Fig. 8). In addition, both heparinase II and III had an inhibitory effect of less than 20% on apoE-dependent LDL CE selective uptake (data not shown). However, upon treatment of Y1/E/tet/2/3 cells with 1 unit/ml chondroitinase ABC at 37 °C for 3 h, apoE-dependent LDL CE selective uptake was inhibited by greater than 90%, suggesting the participa-
tion of chondroitin sulfate and/or dermatan sulfate proteoglycans. Neither chondroitinase ABC nor the heparinases had any effect on the apoE-independent component of LDL CE selective uptake in Y1/E/tet/2/3 cells (Fig. 8). Note in the above studies with proteoglycan synthesis inhibitors and proteoglycan lyases the reductions in apoE-dependent LDL CE selective uptake suggested that a member of the LDL receptor family might be involved. Western blots showed that Y1/E/tet/2/3 cells expressed three members of the LDL receptor family, two of which (LRP/αM receptor and VLDL receptor) are inhibited by RAP concentrations similar to those that inhibit apoE degradation. Furthermore, approximately two-thirds of the apoE-dependent LDL CE selective uptake was inhibited by apoE. Since the LRP/αM receptor is the only known receptor for apoE (30, 31), these data provide strong evidence that the LRP/αM receptor participates in the apoE-dependent selective uptake of LDL CE. These results, however, do not eliminate the potential participation of other LDL receptor family members, some of which have only recently been identified (49).

The mechanism through which the LRP/αM receptor participates in LDL CE selective uptake is unclear. Results of studies with inhibitors of proteoglycan biosynthesis clearly show that proteoglycans are necessary for apoE-dependent LDL CE selective uptake. This result is further supported by the finding that treatment of cells with chondroitinase ABC completely abolished apoE-dependent LDL CE selective uptake. Thus, the participation of the LRP/αM receptor in the LDL CE selective uptake process requires cell surface proteoglycans. In addition it appears likely that a portion of the apoE-dependent LDL CE selective uptake occurs as a result of proteoglycan interactions independently of the LRP/αM receptor since apoE degradation. Furthermore, approximately two-thirds of the apoE-dependent LDL CE selective uptake was inhibited by apoE. Since the LRP/αM receptor is the only known receptor for apoE (30, 31), these data provide strong evidence that the LRP/αM receptor participates in the apoE-dependent selective uptake of LDL CE. These results, however, do not eliminate the potential participation of other LDL receptor family members, some of which have only recently been identified (49).

The major finding in this study is the identification of an LDL CE selective uptake pathway in murine adrenocortical cells that requires apoE but is independent of SR-BI. The apoE-dependent LDL CE selective uptake pathway involves both an αM receptor and cell surface proteoglycans. As judged by quantitative comparisons of apoE-dependent LDL CE selective uptake in Y1/E/tet/2/3 cells with SR-BI-dependent LDL CE selective uptake in Y1/BS1 cells (Figs. 6 and 7, for example), both pathways have the capacity to take up similar amounts of LDL CE (3–6 μg of CE/4 h/mg of cell protein at 50 μg/ml LDL). Interestingly, with both the apoE-dependent pathway (16) and the SR-BI-dependent pathway (14) the majority of LDL CE is taken up by cultured adrenal cells by the selective pathway as opposed to endocytic processing of LDL particles. LDL CE selective uptake has been observed in steroidogenic cells and the liver in vivo (4, 6) as well as in a variety of cultured cells such as human fibroblasts (11), rat and human liver cells (6, 11–13), and a murine macrophage cell (11). The contributions of the SR-BI-dependent versus apoE-dependent LDL CE selective uptake pathways in each of these cell types in culture and in vivo is currently unknown. However, many of these cell types including macrophage, hepatocytes, and steroidogenic cells synthesize substantial quantities of apoE (37–48), raising the possibility that apoE-dependent LDL CE selective uptake is a widespread process in LDL metabolism.

The finding that RAP inhibited the apoE-dependent component of LDL CE selective uptake suggested that a member of the LDL receptor family might be involved. Western blots showed that Y1/E/tet/2/3 cells expressed three members of the LDL receptor family, two of which (LRP/αM receptor and VLDL receptor) are inhibited by RAP concentrations similar to those that inhibit apoE degradation. Furthermore, approximately two-thirds of the apoE-dependent LDL CE selective uptake was inhibited by apoE. Since the LRP/αM receptor is the only known receptor for apoE (30, 31), these data provide strong evidence that the LRP/αM receptor participates in the apoE-dependent selective uptake of LDL CE. These results, however, do not eliminate the potential participation of other LDL receptor family members, some of which have only recently been identified (49).

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thesis completely blocked apoE-dependent CE uptake. RAP binds with high affinity to most LDL receptor family members but also binds with lower affinity to cell surface proteoglycan with a $K_d$ of greater than 200 nM (50, 51). The more gradual inhibition of LDL CE selective uptake at higher RAP concentrations (100–2000 nM, Fig. 2) may reflect the inhibition of LDL CE selective uptake due to direct interaction of apoE-enriched LDL with proteoglycan.

Previous studies showed that an LDL particle acquires approximately one molecule of apoE upon incubation with Y1/E/tet/2/3 cells (16). In addition, the enhancement of LDL CE selective uptake by apoE also requires apoE expression by Y1/E/tet/2/3 cells (16), suggesting that both LDL-bound apoE and cell surface-bound apoE are necessary. A likely scenario is that apoE serves a bridging function to localize LDL particles to the cell surface via interactions with proteoglycans via the LRP/αM receptor or to a ternary complex of apoE-enriched LDL, the LRP/αM receptor, and proteoglycans. The observation that inhibition of proteoglycan synthesis or treatment of cells with chondroitinase ABC blocks apoE-dependent LDL cell association as well as LDL CE selective uptake suggests that the primary role of apoE is at the step of LDL binding to the cell surface. The findings reported here on apoE-dependent LDL CE selective uptake have striking parallels to previous studies (16). The LRP/αM receptor or proteoglycans in chylomicron remnant uptake into liver cells or response to apoE enrichment of β-VLDL particles (33, 34, 51–54). Very similar proposals have been made to explain the roles of the LRP/αM receptor and proteoglycans in chylomicron remnant uptake by hepatocytes. In that case, however, the uptake of β-VLDL CE is believed to occur primarily via endocytic uptake of intact β-VLDL particles (33).

Irrespective of the details of how the apoE-enriched LDL particle is bound to the cell surface, it seems likely that the apoE-dependent selective uptake of LDL CE differs in important ways from the process mediated by SR-BI. For example, SR-BI is believed to be localized to cholesterol/sphingolipid-enriched membrane microdomains or caveolae that appear to be the site of CE uptake from HDL particles (9, 55, 56). In contrast, the LRP/αM receptor and other members of the LDL receptor family are targeted for endocytosis via coated pits (57). As recently discussed (33), cell surface binding of remnant-like particles in the livers of LDL receptor-deficient mice is very rapid, but endocytosis via an apoE-dependent pathway (presumably the LRP/αM receptor) is very slow compared with that mediated by the LDL receptor (58). This suggests that the LRP/αM receptor is relatively inefficient at endocytosis and that the residence time on the cell surface for an apoE-enriched LDL bound to the LRP/αM receptor may be very long. It is also of note from other studies that LDL bound to cell surface proteoglycan occurs in two kinetic pools, one of which appears to be a sequestered pool that is internalized slowly, if at all (59). Although speculative, we suggest that a prolonged residence time of apoE-enriched LDL on the cell surface, whether bound to the LRP/αM receptor or proteoglycan, will lead to selective CE transfer to the cell membrane. Whether this occurs because some of the bound LDL is directed to caveolae or this process is independent of these membrane domains is unknown. Interestingly, the recently reported enhancement of LDL CE selective uptake by lipoprotein lipase (60) also requires cell surface proteoglycan as well as interaction of lipoprotein lipase with both LDL and the proteoglycan. These data as well as previous results (16) and the results of the present study suggest the hypothesis that bridging proteins such as apoE or lipoprotein lipase or hepatic lipase facilitate the selective uptake of lipoprotein CE by holding the lipoprotein particle on the cell surface for prolonged periods of time.

The present results on apoE-dependent LDL CE selective uptake show an interesting difference in comparison to the secretion/capture hypothesis for apoE-mediated chylomicron remnant uptake into hepatocytes due to the LRP/αM receptor and/or proteoglycans (33). In the case of β-VLDL uptake into hepatocytes, heparan sulfate proteoglycans had only modest effects, whereas chondroitinase completely abolished apoE-dependent LDL CE selective uptake. This result suggests that apoE-enriched LDL interacts with chondroitin sulfate or dermatan sulfate proteoglycans on adrenocortical cells. This difference with the results of β-VLDL uptake in hepatoma cells (33) could reflect cell-type differences in proteoglycan composition or differences in how apoE-enriched LDL versus apoE-enriched β-VLDL interact with different proteoglycans. Interestingly, Burgess et al. (61) noted that a major pool of HepG2 cell surface apoE is associated with chondroitin sulfate proteoglycans. Similarly, there is ample documentation of binding of chondroitin/dermatan sulfate proteoglycan to LDL both in vivo and in vitro (62–64). In this regard, it is of interest that a basal level of LDL CE selective uptake occurs in the Y1/E/tet/2/3 cells in the absence of apoE expression (Figs. 3A, 5B, 6A, 7A, and 8). Although the nature of this pathway is not understood, we have observed that heparin blocks this apoE-independent process (data not shown). This result is consistent with the possibility that the apoE-independent LDL CE selective uptake in the Y1/E/tet/2/3 cells is due to an apoB100/proteoglycan interaction that serves to localize LDL particles to the cell surface.

In summary, we report here that delivery of LDL CE by the selective uptake process occurs via multiple pathways in mouse adrenocortical cells. One pathway is apoE-dependent and involves the LRP/αM receptor and proteoglycans. A second pathway is SR-BI-dependent, occurs in the absence of apoE, and does not require cell surface proteoglycan. Additional studies will be required to assess the contributions of these pathways to LDL CE selective uptake in various cell types both in culture and in vivo.

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The Apolipoprotein E-dependent Low Density Lipoprotein Cholesteryl Ester Selective Uptake Pathway in Murine Adrenocortical Cells Involves Chondroitin Sulfate Proteoglycans and an α2-Macroglobulin Receptor

Snehasikta Swarnakar, Jeanette Beers, Dudley K. Strickland, Salman Azhar and David L. Williams

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