Selective Uptake of Low Density Lipoprotein-Cholesteryl Ester Is Enhanced by Inducible Apolipoprotein E Expression in Cultured Mouse Adrenocortical Cells*

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Apolipoprotein (apo) E is expressed at high levels by steroidogenic cells of the adrenal gland, ovary, and testis. The cell surface location of apoE in adrenocortical cells suggests that apoE may facilitate the uptake of lipoprotein cholesterol by either the endocytic or the selective uptake pathways, or both. To examine these possibilities, the human apoE gene was expressed in murine Y1 adrenocortical cells under control of an inducible tetracycline-regulated promoter. The results show that induction of apoE yielded a 2–2.5-fold increase in the uptake of low density lipoprotein-cholesteryl ester (LDL-CE) but had little effect on high density lipoprotein-CE uptake. Analysis of lipoprotein uptake pathways showed that apoE increased LDL-CE uptake by both endocytic and selective uptake pathways. In terms of cholesterol delivery to the adrenal cell, the apoE-mediated enhancement of LDL-CE selective uptake was quantitatively more important. Furthermore, the predominant effect of apoE expression was on the low affinity component of LDL-CE selective uptake. LDL particles incubated with apoE-expressing cells contained 0.92 ± 0.11 apoE molecules/apoB after gel filtration chromatography, indicating stable complex formation between apoE and LDL. ApoE expression by Y1 cells was necessary for enhanced LDL-CE selective uptake. This result may indicate an interaction between apoE-containing LDL and cell surface apoE. These data suggest that apoE produced locally by steroidogenic cells facilitates cholesterol acquisition by the LDL selective uptake pathway.

Apolipoprotein E (apoE)1 is a prominent component of plasma lipoproteins and serves to mediate endocytic uptake of remnant lipoproteins by members of the LDL receptor family (1–6). In contrast to other apolipoproteins, apoE is expressed in many peripheral tissues, including adrenal gland, ovary, testis, brain, adipose, skin, and lung (7–15). Studies with humans, nonhuman primates, and rats show that the apoE synthesis rate and mRNA concentration in the adrenal gland are similar to those in liver (7, 8, 10, 16), indicating that apoE is an abundant protein product of adrenal cells. ApoE mRNA is expressed in adrenocortical zona fasciculata and zona reticularis cells, the sites of steroid production and cholesteryl ester storage in rat adrenal gland (17).

The high expression of apoE in adrenocortical cells and its pattern of regulation suggest that locally derived apoE may facilitate the acquisition of lipoprotein cholesterol, alter cellular cholesteryl ester (CE) storage, or modulate the availability of cholesterol for steroidogenesis (7, 16). Adrenal gland apoE expression is regulated in direct proportion to CE stores and inversely to the level of steroid production (16, 17). A potential role for locally produced apoE in adrenocortical cholesterol metabolism is supported by results showing that constitutive expression of human apoE in murine Y1 adrenocortical cells leads to enhanced accumulation of CE (18). Immunolocalization studies in rat adrenocortical cells show apoE intracellularly within multivesicular bodies of the endocytic pathway and on cell surface microvillar channels (19). Microvillar channels retain LDL and HDL particles and have been proposed to be the site at which the selective uptake of lipoprotein-CE occurs (20, 21). In contrast to lipoprotein uptake by endocytosis, the selective uptake pathway brings lipoprotein-CE into the cell without the uptake and lysosomal degradation of the lipoprotein particle (22–27). LDL-CE selective uptake was first noted in perfused rat ovaries (28) and was later studied in human fibroblasts (29), in the Y1-BS1 subclone of murine Y1 adrenocortical cells (29), and in human hepatoma cells (30). In ovarian tissue (28) and in Y1-BS1 cells (29), most of the LDL-CE delivered to the cells occurs via the selective as opposed to the endocytic pathway.

The presence of apoE within multivesicular bodies of adrenocortical cells and in microvillar channels may indicate that locally synthesized apoE acts to facilitate the uptake of HDL and/or LDL-CE by either the endocytic or selective uptake pathways, or both (19). In the present study, we examined these possibilities by expressing human apoE in murine Y1 adrenocortical cells under control of an inducible tetracycline (tet)-regulated promoter. The results show that apoE expression yielded a 2–2.5-fold increase in the uptake of LDL-CE but had little influence on HDL-CE uptake. Analysis of lipoprotein uptake pathways showed that apoE increased LDL-CE uptake by both endocytic and selective uptake pathways. These data suggest that apoE produced locally by steroidogenic cells facil-
states cholesterol acquisition from LDL particles by two distinct pathways.

MATERIALS AND METHODS

Preparation of Stably Transfected Cell Lines—Murine Y1 adrenal cells (American Type Culture Collection) 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% heat-inactivated fetal calf serum, 2 mg glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin complete medium. Y1 cells transfected with pUHD15-1, pUHD10-3, and pUHD13-3 kindly provided by H. Bujard. The expression vector pUHDapoE was made as follows. The 4.2-kilobase pair BamHI/EcoRI fragment from the vector pEF2 (32), which encodes the human apoE cDNA genomic sequence from base pair 8 to base pair 4200, was ligated to a BamHI/EcoRI adapter sequence. This fragment was then cloned into BamHI-linearized pUHD10-3, which encodes the tTA-responsive promoter. Cell lines were constructed in two steps. First, Y1 cells were co-transfected with pUHD15-1, which encodes the tet transactivator (tTA) protein, and pSV2neo, which encodes resistance to G418 sulfate, at a ratio of 9:1, by calcium phosphate-mediated gene transfer as described (32). Cell clones were selected in complete media containing 200 μg/ml G418 sulfate (Geneticin, Life Technologies, Inc.) and screened for expression of the tTA protein by transient transfection of pUHD13-3, which encodes a luciferase reporter gene expressed from a tTA-responsive promoter. One clone, Y1UHD7, which expressed high levels of the tTA protein was secondarily transfected with pUHDapoE, together with pCMV hygromycin (Calbiochem) at a ratio of 9:1. To make control cell lines, Y1UHD7 cells were transfected with the empty pUHD10-3 vector together with pCMV hygromycin. Hygromycin-resistant clones (Y1/E/tet or Y1/con/tet cells) were selected in complete medium containing 200 μg/ml hygromycin B (Calbiochem) and 200 μg/ml G418 sulfate. Tetracycline (2 μg/ml) was included during selection to suppress expression of apoE. Following selection, cell lines were maintained in complete medium containing 100 μg/ml hygromycin, and 2 μg/ml tet. Inducible Y1/E/tet cell lines were identified by Western blotting of medium following removal of tet. Two cell lines (Y1/E/tet/2/3 and Y1/E/tet/2/5) showed strong induction of apoE and two control cell lines (Y1/con/tet/1/2 and Y1/con/tet/1/6) were used for subsequent experiments.

Western Blotting and ELISA—Proteins were separated by 8% SDS-PAGE, electrophoretically transferred to a nitrocellulose membrane, and blocked for 6 h at room temperature in 20 mM-Tris-HCl pH 7.4, 150 mM NaCl (TBS) containing 7% nonfat milk and 0.05% Tween 20. The blocked membrane was incubated with polyclonal goat anti-human apoE antibody (Calbiochem) (1/10000 dilution) overnight at room temperature in TBS containing 1% nonfat milk and 0.2% Tween 20. The membrane was washed three times with TBS containing 0.05% Tween 20 and once with 0.1% Tween 20 in TBS. A horseradish peroxidase-conjugated donkey anti-goat IgG (Sigma) (1/100,000 dilution) for 1 h at room temperature in TBS containing 1% nonfat milk and 0.05% Tween 20. Bands were visualized by enhanced chemiluminescence (Amersham). ApoE concentration in conditioned medium was determined by ELISA using an affinity-purified goat anti-human apoE antibody (Biodesign) as described (33). Samples were assayed in triplicate using human apoE (PanVera) as standard.

Preparation of [125I]Diactiloyl Tyramine-[3H]Cholesteryl Oleoyl Ether hHDLs and [125I]Diactiloyl Tyramine-[3H]Cholesteryl Oleoyl Ether hLDLs—Human (h) HDLs (1.125 g/ml; ρ < 1.121 g/ml) and human LDL (1.019 g/ml; ρ < 1.063 g/ml) were doubly labeled with [125I]diactiloyl tyramine and [3H]cholesteryl oleoyl ether as described (34). The specific activity of the [125I]H1HhHDL ranged from 46 to 70 dpm/ng protein for △125I and from 6 to 28 dpm/ng protein for △3H. The specific activity of the [125I]H1HhLDL ranged from 25 to 75 dpm/ng protein for △125I and from 30 to 30 dpm/ng protein for △3H.

Determination of HDL and LDL Cell Association, Selective CE Uptake, and Apolipoprotein Degradation—For all experiments, six-well plates were seeded with Y1/E/tet or Y1/con/tet cells at 0.8 × 10^5 cells/well in complete medium in the presence or absence of tet. At day 1 and day 3, medium was changed and, at day 4, medium was changed to the above medium lacking serum, plus or minus tet, containing 2 mM dibutyryl cAMP. After 24 h, half of the medium was removed and double-labeled [125I]H1HhHDL at 50 μg/ml (protein) or [125I]H1HhLDL at 50 μg/ml (protein) (except where indicated) was added, and the incubation was continued for 4 h. Cells were washed three times with 0.1% bovine serum albumin in phosphate-buffered saline, pH 7.4; one time with phosphate-buffered saline, pH 7.4; and lysed with 1.5 ml of 0.1 N NaOH. The lysate was processed to determine trichloroacetic acid-soluble and -insoluble △125I radioactivity and organic solvent-extractable △3H radioactivity as described (34, 35). Values are expressed as micrograms of cholesterol/mg of cell protein. The LDL concentration dependence for each of these parameters was modeled by a simple binding isotherm composed of a high affinity saturable process and a low affinity nonsaturable process. P_total = [P_max] [LDL]/(K HA [LDL] + [LDL]), where P_total is the measured parameter, [P_max] is the high affinity parameter at saturating levels of LDL, K HA is the apparent high affinity constant, and C is the slope of the low affinity nonsaturable process. For each parameter, P_total was resolved into high affinity and low affinity components by determining C and subtracting [(C/LDL)] from P_total to generate the curve for high affinity LDL concentration dependence (37).

Size Fractionation of hHDL—hHDL was chromatographed on Bio-Gel A-15 m (90 × 1.6 cm) at 6 ml/h in 5 mM Na-PO4, 150 mM NaCl, 0.25 mM EDTA, pH 7.4. Pooled fractions were concentrated in a Centriprep-30 (Amicon) concentrator and resolved by nondenaturing 12%–25% gradient PAGE in Tris-borate buffer, pH 8.3, for 2000 V-h at 4 °C as described (38). The gel was stained with Coomassie Blue. In some experiments, medium from cells incubated with or without LDL was resolved by chromatography on Bio-Gel A-15 m as above, and the fractions were assayed for apoE by ELISA and for LDL by monitoring △125I radioactivity.

RESULTS

Characterization of Y1/E/tet and Y1/con/tet Cell Lines—In order to examine the effects of apoE on lipoprotein uptake, we prepared Y1 cell lines in which apoE expression is inducible. This approach permits the influence of apoE to be tested within a clonal cell line and eliminates the variables inherent in selecting and comparing lipoprotein uptake in different clones that do or do not express apoE. With the tet-regulated system of Gossen and Bujard (31), apoE expression is suppressed in cells that do not express apoE and for LDL by monitoring △125I radioactivity.

### Table I

| Cell line       | tet   | apoE (μg/ml) | 24 h |
|-----------------|-------|-------------|------|
| Y1/con/tet/1/2  | +     | ND          |      |
| Y1/con/tet/1/6  | +     | ND          |      |
| Y1/con/tet/1/8  | +     | ND          |      |
| Y1/E/tet/2/5    |       | 0.16 ± 0.02 |      |
| Y1/E/tet/2/5    |       | 2.5 ± 0.07  |      |
| Y1/E/tet/2/3    |       | 0.11 ± 0.02 |      |
| Y1/E/tet/2/3    |       | 2.1 ± 0.08  |      |

* ND, not detected.

Cells were cultured in complete medium plus or minus tet for 4 days with changes of media on days 1 and 3. On day 4, medium was changed to serum-free medium. Medium was removed after 24 h for estimation of apoE by ELISA. Values represent mean ± S.E. (n = 6).
Western blot analysis showed that, following a medium change at 4 days after tet removal, secreted apoE accumulated to a steady state by 24 h (Fig. 1, panel A (–tet) and B). When tet was not removed, a faint apoE band was detected by Western blotting by 36–48 h after the medium change (Fig. 1, panel A (+tet)), confirming the conclusion from the ELISA (Table I) of low level apoE expression in the presence of tet. The time necessary to induce maximal apoE expression upon tet removal was determined by Western blotting of medium samples collected in 24-h intervals over a 15-day period. As shown in Fig. 1 (panel C), apoE accumulation per 24 h was maximal by day 4 and remained stable for up to day 15. In subsequent lipoprotein uptake studies, Y1 cells were withdrawn from tet for 4 days to induce maximal apoE expression, switched to serum-free medium at day 4 to permit secreted apoE to accumulate, and experiments were initiated by addition of labeled lipoprotein to the medium on day 5.

**Selective Uptake of CE from LDL**—The results in Table II illustrate the effects of tet withdrawal and apoE expression on LDL-CE uptake. With Y1/conv/tet/1/6 control cells not expressing apoE, tet withdrawal had little or no effect on cell association, selective uptake, or endocytic uptake of LDL-CE. In contrast, with Y1/E/tet/2/3 cells that do express apoE, tet withdrawal led to a 2–3-fold increase in LDL-CE selective and endocytic uptake and a 1.4-fold increase in cell association of LDL particles. These results indicate that the enhanced LDL-CE uptake and cell association reflect the expression of apoE and not the influence of tet. Similar results were obtained with Y1/conv/tet/1/2 and Y1/E/tet/2/5 cell lines (data not shown).

To determine whether the apoE-mediated enhancement of LDL-CE uptake was specific for LDL, the uptake of LDL-CE and HDL-CE were compared. The results in Table III show that in contrast to the marked apoE-mediated enhancement of LDL-CE cell association and uptake, apoE expression had only a modest effect on HDL-CE uptake. When data from seven experiments were analyzed, HDL-CE selective uptake was 30% greater in apoE-expressing cells, but this difference was not statistically significant (p > 0.5, data not shown). This result indicates that the effect of apoE expression is primarily on LDL-CE uptake.

The concentration dependence for LDL-CE uptake in the presence and absence of apoE expression is shown in Fig. 2. These data indicate that, in the presence or absence of apoE, most LDL-CE uptake at all LDL concentrations tested occurred via selective uptake as opposed to endocytic uptake. apoE expression enhanced LDL-CE uptake by both endocytic and selective pathways, with the -fold enhancement of endocytic uptake being somewhat greater (2.35 ± 0.13-fold, n = 29) as compared with selective uptake (2.04 ± 0.10-fold, n = 29) when data in numerous experiments were averaged. However, in terms of total LDL-CE delivery to the cell, the major effect of apoE was on the selective uptake pathway. For example, at 50 μg/ml LDL, apoE expression increased LDL-CE uptake by the selective uptake pathway by about 2000 ng of CE/mg of cell protein, whereas the enhancement by the endocytic pathway was about 225 ng of CE/mg of cell protein (Fig. 2).

The LDL concentration dependence for selective CE uptake and for endocytic CE uptake was indicative of both high and low affinity components. This point is illustrated in Fig. 3, which shows the LDL concentration dependence for selective (panel A) and endocytic (panel B) uptake for apoE-expressing Y1/E/tet/2/3 cells resolved into high and low affinity compo-
nents. These data show that, at LDL concentrations greater than 50 μg/ml, most of the LDL-CE selective uptake (panel A) was due to the low affinity component; this component increased further at higher LDL concentrations, whereas the high affinity component was saturated above 20 μg/ml LDL. A similar result was seen with endocytic uptake of LDL-CE (panel B), except that the contribution of the low affinity component was less at lower LDL concentrations; in this case, the low affinity and high affinity components were equivalent at about 150 μg/ml LDL. The cell association of LDL-CE, most of which is believed to reflect cell surface bound LDL particles, showed a similar LDL concentration dependence and a similar enhancement by apoE expression throughout the LDL concentration range as was seen for LDL-CE selective and endocytic uptake (Tables II and III and data not shown).

Analysis of hLDL Size Heterogeneity and hLDL-ApoE Interaction—Size heterogeneity within the LDL particle population potentially could bias the selective uptake measurements if the LDL contained a significant fraction of large CE-rich particles that were taken up in preference to the bulk of the LDL. To address this point, LDL was fractionated by chromatography on Bio-Gel A-15m. The profile contained no particles larger than LDL (data not shown), and, within the LDL region of the chromatogram, the particles eluted in a near normal distribution (Fig. 4, panel A). The LDL profile was divided into three fractions (A, B, and C) corresponding to the leading edge, the peak, and the trailing edge, respectively, which were analyzed by nondenaturing gradient gel electrophoresis. As shown in panel B, the LDL contained two major species with the larger and smaller species recovered preferentially in fractions A and C, respectively, and similar amounts of both species recovered in fraction B. Equal amounts of LDL from each fraction (20 μg/ml protein) were compared for LDL-CE uptake with Y1/E/tet/2/3 cells with and without apoE induction. The results in Fig. 5 show that the fractions differed little in selective (panel A) or endocytic (panel B) uptake, with the peak fraction of the LDL profile, fraction B, being 25–50% more active than either the leading or trailing fractions of LDL. The apoE-mediated enhancement of LDL-CE selective or endocytic uptake was similar among the LDL fractions. Similar results were seen
with two independent preparations of LDL that were analyzed. These data indicate that LDL-CE selective uptake by murine Y1 adrenocortical cells and the enhancement by apoE are properties of the bulk LDL population and not due to a small fraction of large CE-enriched particles.

To address the question of whether there is a direct interaction between LDL and apoE, LDL was re-isolated by gel filtration chromatography after incubation with cells expressing apoE. The elution profile in Fig. 6 (panel A) shows that apoE eluted with the LDL peak as well as in a second lower molecular weight peak. The profile in panel B shows that the association of apoE with LDL was dependent upon apoE expression by the Y1 cells and not due to apoE contamination in the purified LDL. The profile in panel C shows that the presence of apoE in the LDL fraction required the addition of LDL. These data indicate that LDL particles acquired apoE when incubated with apoE-expressing Y1 cells. The stoichiometry of this association was estimated by comparing the quantity of apoE recovered in the LDL fraction as determined by ELISA with the apoB content as determined from the apoB radioactivity. These measurements gave a value of 0.92 ± 0.11 (n = 3) apoE molecules/apoB, suggesting that each LDL particle acquired one apoE molecule that was stable to gel filtration.

Role of Cell-associated and LDL-associated ApoE in Mediating LDL-CE Selective Uptake—ApoE accumulates in the medium and associates with LDL particles, but is also present on the surface of apoE-expressing Y1 cells (data not shown) and adrenocortical cells in vivo (19). Thus, both LDL-associated or cell surface-associated apoE, or both, could account for the increased LDL-CE selective uptake. To test this point, conditioned medium (from cells expressing or not expressing apoE) was added to cells (expressing or not expressing apoE) immediately before LDL particles were added for the 4 h uptake assay. As shown in Fig. 7, with the controls, for which the medium was not changed, apoE expression gave a 3-fold increase in LDL-CE selective uptake (compare samples 1 and 2). Adding fresh unconditioned medium prior to LDL reduced but did not eliminate the apoE-enhancement of LDL-CE selective uptake in the apoE-expressing cells (sample 3 versus 1) and gave a slight increase in cells not expressing apoE (sample 4 versus 2) that was not statistically significant (p > 0.08). Partial retention of the apoE-mediated increase upon addition of
fresh medium in sample 3 could be due to cell surface apoE or to secreted apoE that would accumulate to some extent during the 4-h uptake assay (about 20% of the steady state level; see Fig. 1, panel B). When medium from apoE-expressing cells was added back to apoE-expressing cells, full restoration of LDL-CE selective uptake was seen (sample 5 versus 1). However, the addition of medium from apoE-expressing cells to cells not expressing apoE (sample 6) did not increase LDL-CE selective uptake (sample 6 versus 4). The level of LDL-CE selective uptake in sample 6 was the same as seen when cells not expressing apoE received either fresh medium (compare sample 6 versus 4) or medium from cells not expressing apoE (compare sample 6 versus 7). These data indicate that apoE expression by the cells is necessary for the apoE-mediated enhancement of LDL-CE selective uptake. The partial loss in the apoE-mediated enhancement upon addition of fresh medium (sample 1 versus 3), and the restoration of this loss by addition of apoE-containing medium (sample 3 versus 5, p = 0.001) suggest that LDL-associated apoE also contributes to the enhancement of LDL-CE selective uptake. However, the enhancement of selective uptake by LDL-associated apoE only occurred when cells also were expressing apoE.

**DISCUSSION**

The major finding in this study is that apoE expression markedly increased LDL-CE uptake into adrenocortical cells via both endocytic and selective uptake pathways. The enhancement of LDL-CE uptake by apoE expression may play a quantitatively important role in LDL-CE uptake into adrenal cells in vivo since apoE expression occurs in adrenal cells of all mammalian species examined (7, 8, 10, 13, 16). ApoE also is expressed by steroidogenic cells of the ovary and the testis (14, 39–41), suggesting that apoE may play a general role in LDL-CE uptake in steroidogenic cells.

Expression of apoE increased LDL-CE selective uptake by 2–2.5-fold over a broad range of LDL concentrations. ApoE markedly enhanced LDL-CE selective uptake, but this process does not, in itself, appear to require apoE since it also occurred in control Y1 cells that do not express apoE (Table II) (29). The LDL concentration dependence for CE selective uptake was similar in the presence and absence of apoE expression, with apoE increasing LDL-CE selective uptake throughout the concentration range examined. The concentration dependence showed both low and high affinity components for the LDL-CE selective uptake process. At low LDL concentrations (<50 µg/ml), most of the LDL-CE selective uptake occurred via the high affinity component, but at higher LDL concentrations (>50 µg/ml), the low affinity component predominated and increased linearly as a function of LDL concentration (Fig. 3). This result suggests that the low affinity component may provide greater cholesterol delivery than the high affinity component in vivo in species with high LDL levels. In the mouse, which has very low LDL levels (42, 43) and relies on HDL-CE selective uptake to provide cholesterol to steroidogenic cells (22, 23, 26), LDL-CE selective uptake would not be expected to play a major role in steady state cholesterol delivery to adrenal cells. However, in

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**FIG. 4.** [125I,3H]hLDL fractionation by gel filtration chromatography. Panel A, doubly labeled [125I,3H]hLDL (2 mg) was fractionated on Bio-Gel A-15m as described under “Materials and Methods.” Regions of the profile indicated by A, B, and C were collected and concentrated in a Centriprep-30. Panel B, samples of each fraction and the starting LDL (L) were analyzed by electrophoresis on a nondenaturing 2–16% polyacrylamide gel as described under “Materials and Methods.” The Coomassie Blue stain of the gel is shown.

**FIG. 5.** Selective and endocytic uptake of cholesteryl ester from subfractions of [125I,3H]hLDL. Y1/E/tet2/3 cells were cultured in complete medium in the presence or absence of tet for 4 days. At day 4, medium was changed to serum-free medium containing 2 mM Bt2-cAMP plus or minus tet. After 24 h, [125I,3H]hLDL was added to 20 µg/ml (protein). After 4 h, cells were processed to determine LDL-CE selective uptake (panel A) and endocytic uptake (panel B) as described under “Materials and Methods.” Results are the mean ± S.E. (n = 3) from a representative experiment.
humans and other species with high LDL levels, LDL-CE selective uptake and the enhancement by apoE expression may be of greater quantitative importance.

The enhancement of LDL-CE selective uptake by apoE appears to involve both secreted apoE and apoE expression by Y1 cells. Secreted apoE associated with LDL particles with approximately one molecule of apoE recovered per LDL particle after gel filtration chromatography. This is a minimal estimate because more weakly associated apoE molecules may not have been stable to chromatography. Interestingly, addition of apoE-containing medium to Y1 cells did not enhance LDL-CE selective uptake unless the cells were expressing apoE. This suggests that cell surface apoE or the continuous production of apoE was required to enhance LDL-CE selective uptake.

The present results showing an apoE-mediated enhance-ment of the LDL-CE selective uptake pathway show many parallels to the effects of apoE on the endocytic uptake of \( \beta \)-VLDL in hepatoma cells. ApoE is localized to the cell surface of adrenocortical cells (19) and hepatocytes (44) and is known to associate with cell surface proteoglycan when secreted from hepatoma cells in culture (45, 46). In hepatoma cells, apoE expression enhances cell surface binding and endocytic uptake of \( \beta \)-VLDL, an effect that involves heparan sulfate proteoglycan and the LDL receptor-related protein (46). Conditioned medium from apoE-expressing cells gave the full apoE enhancement of \( \beta \)-VLDL binding when added to nontransfected hepatoma cells, suggesting that the enhanced binding required secreted apoE that associated with \( \beta \)-VLDL particles (46). However, nontransfected hepatoma cells also expressed endogenous rat apoE, which may have been present on the cell surface and contributed to the enhanced uptake. Consistent with this possibility, \( \beta \)-VLDL binding to apoE-expressing hepatoma cells at 4 °C was increased without the addition of exogenous apoE, again suggesting a role for cell surface apoE (46). This is similar to the present results with adrenocortical cells in which enhancement of LDL-CE selective uptake by apoE required apoE expression by the cells. The current data in adrenal cells and the results with hepatoma cells (46) may indicate that apoE-enriched lipoprotein particles interact with cell surface apoE to facilitate CE uptake by both endocytic and selective uptake pathways. The extent to which apoE expression enhances the endocytic versus the selective uptake pathway may depend on the cell type, the type of lipoprotein particle, and the spectrum of lipoprotein receptors expressed by the cells.

The biochemical mechanism of LDL-CE selective uptake and the manner in which apoE enhances uptake are poorly understood. The LDL concentration dependence showed that apoE enhanced selective uptake throughout the concentration range tested (Fig. 2). Thus, both the high and low affinity uptake processes were increased. This was also true for the uptake of LDL-CE via the endocytic pathway (Fig. 2). Similarly, the cell association of LDL particles (data not shown), most of which is believed to reflect cell surface LDL binding, was also increased.
by apoE throughout the LDL concentration range. Although we cannot rule out that apoE expression enhances each of these parameters by a different mechanism, we consider that possibility unlikely. We speculate that the primary effect of apoE is to enhance cell surface LDL binding, thereby increasing the local surface concentration of LDL particles available to both the low and high affinity components of the endocytic and selective uptake pathways.

The cell surface receptors responsible for the selective uptake of lipoprotein CE are not well understood. In the case of HDL, scavenger receptor BI can mediate HDL-CE selective uptake in transfected cells (47) and is the major route for high affinity HDL-CE uptake and delivery to the steroidogenic pathway in cultured adrenal cells (37). Scavenger receptor BI binds native LDL (48), but it is not known whether scavenger receptor BI mediates high or low affinity selective uptake from LDL particles. Recent studies show that cell surface proteoglycans can mediate the endocytosis of LDL particles via a bridging molecule of lipoprotein lipase (49). A major component of this LDL endocytosis appears to occur via direct endocytosis of a syndecan proteoglycan without the participation of other cell surface receptors (50). Interestingly, proteoglycan-bound LDL occur in two kinetic pools, one which is internalized rapidly and the other which appears to be a sequestered cell surface LDL pool with a prolonged residence time (49). It is not known whether this sequestered pool of LDL, by virtue of its prolonged cell surface residence time, might make LDL particles available to the selective uptake pathway, but this is a prime candidate to test in future studies. We speculate that a “blanket” of apoE on the cell surface (19) and LDL-associated apoE act as a bridging mechanism to localize LDL particles to the cell surface proteoglycans.

In summary, the results of this study showed that inducible apoE expression in Y1 adenocortical cells enhanced the selective uptake of LDL-CE by 2–2.5-fold over a broad range of LDL concentrations. Endocytic uptake of LDL was also increased by apoE expression, but this was quantitatively less important in low concentrations. Endocytic uptake of LDL was also increased by 2–2.5-fold over a broad range of LDL concentrations. Endocytic uptake of LDL was also increased by apoE expression, but this was quantitatively less important in low concentrations.