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Cellular Catabolism of Lipid Poor Apolipoprotein E *via* Cell Surface LDL Receptor–Related Protein[1]

Masaaki Narita,*,[2] David M. Holtzman,†,‡ Anne M. Fagan,† Mary Jo LaDu,‖ Li Yu,‖ Xianlin Han,‖ Richard W. Gross,‖ Guojun Bu,*,‡ and Alan L. Schwartz*,‡

*Departments of *Pediatrics, †Neurology, ‡Molecular Biology and Pharmacology, ‖Medicine, Washington University School of Medicine and St. Louis Children's Hospital, St. Louis, Missouri 63110, USA; and §Department of Medicine, Evanston Northwestern Research Institute, Evanston, Illinois, 60201, USA*

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Apolipoprotein E (apoE), an apoprotein involved in lipid transport in both the plasma and within the brain, mediates the binding of lipoproteins to members of the low density lipoprotein (LDL) receptor family including the LDL receptor and the LDL receptor-related protein (LRP). ApoE/LRP interactions may be particularly important in brain where both are expressed at high levels, and polymorphisms in the apoE and LRP genes have been linked to AD. To date, only apoE-enriched lipoproteins have been shown to be LRP ligands. To investigate further whether other, more lipid-poor forms of apoE interact with LRP, we tested whether lipid-free apoE in the absence of lipoprotein particles interacts with its cell-surface receptors. No detectable lipid was found associated with bacterially expressed and purified apoE either prior to or following incubation with cells when analyzed by electrospray ionization mass spectrometry. We found that the degradation of lipid-poor ^{125}I-apoE was significantly higher in wild type as compared to LRP-deficient cells, and was inhibited by receptor-associated protein (RAP). In contrast, ^{125}I-apoE–enriched β-VLDL was degraded by both LRP and the LDL receptor. When analyzed *via* a single cycle of endocytosis, ^{125}I-apoE was internalized prior to its subsequent intracellular degradation with kinetics typical of receptor-mediated endocytosis. Thus, we conclude that a very lipid-poor form of apoE can be catabolized *via* cell surface LRP, suggesting that the conformation of apoE necessary for recognition by LRP can be imposed by situations other than an apoE-enriched lipoprotein.

Key words: apoE, endocytosis, LRP, neuron, receptor.

Apolipoprotein E (apoE), a 34-kDa glycoprotein, plays an important role in the transport and metabolism of plasma cholesterol and triglycerides through its ability to interact with cell surface low density lipoprotein (LDL) receptor and LDL receptor-related protein (LRP) (*1–3*). ApoE exists in three isoforms (apoE2, E3, and E4) that differ in their cysteine/arginine content at two polymorphic sites (*1*). In addition to its role in plasma lipoprotein metabolism, the regulation of apoE expression and metabolism in the central nervous system may contribute to the pathogenesis of Alzheimer's disease (AD). Supporting this possibility, there is a genetic association between the frequency of the type 4 allele of apoE and late-onset familial and sporadic AD (*4, 5*).

Further, some genetic studies have also found a linkage between a polymorphism in the LRP gene and AD (*6–8*). In addition to genetic data, *in vitro* data suggest that LRP is also involved in the metabolism of Aβ degradation (*9, 10*). While the mechanism by which apoE4 serves as an AD risk factor is not yet known, two possible contributing mechanisms are that apoE can affect amyloid-β (Aβ) deposition as well as neurite outgrowth/neural repair in an isoform specific manner (*11*). Some studies suggest that the effect of apoE on Aβ deposition may be due in part to its ability to influence apoE-Aβ clearance (*12, 13*), and LRP is a major candidate receptor that may be involved in this process. Effects of apoE-containing lipoproteins on neurite outgrowth have also been shown (*11, 14*). These effects of apoE/lipoproteins on neurite outgrowth can be blocked by specific inhibitors of LRP (*15–17*), suggesting that LRP is the major neuronal receptor mediating the cellular effects of apoE/lipoprotein. Furthermore, the effects of apoE on neurite outgrowth are only observed in the presence of an exogenous lipid source such as β-very low density lipopro-

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[2]To whom correspondence should be addressed at the present address: Institute of Basic Medical Sciences, University of Tsukuba, 1-1-1 Tennohdai, Tsukuba, Ibaraki 305-8575. Tel: +81-298-53-6962, Fax: +81-298-64-6510, E-mail: narita_m@md.tsukuba.ac.jp

Abbreviations: LDL, low density lipoprotein; LRP, low density lipoprotein receptor-related protein; apoE, apolipoprotein E; RAP, receptor-associated protein; AD, Alzheimer's disease; Aβ, amyloid-β; β-VLDL, β-very low density lipoprotein; HDL, high density lipoprotein; CSF, cerebrospinal fluid; TCA, trichloroacetic acid; PBS, phosphate-buffered saline; BSA, bovine serum albumin; MEF, mouse embryonic fibroblasts; DMEM, Dulbecco's minimal essential medium; ESI-MS, electrospray ionization mass spectroscopy; SEM, standard error of the mean; PBS, phosphate buffered saline; HSPG, heparan sulfate proteoglycan.

tein (β-VLDL), high density lipoprotein (HDL), cerebrospinal fluid (CSF) lipoproteins (HDL-like), or when apoE is secreted by cells in lipoprotein particles (*14–17*). Therefore, the actions of apoE on neurite outgrowth may require the delivery of lipids to the cells. Interestingly, a recent study by DeMattos *et al.* (*18, 19*) demonstrated that apoE3 secreted from a stably-transfected cell line in lipid poor particles can also stimulate neurite outgrowth, suggesting that apoE may perhaps function in the presence of minimal amounts of lipid.

Several receptors that belong to the LDL receptor gene family have been shown to bind apoE/lipoproteins. These include the LDL receptor, LRP, gp330/megalin, the VLDL receptor, and apoE receptor 2 (*15, 20–23*). Interactions between apoE and lipoprotein particles has been found to be important for the interaction of apoE with the LDL receptor (*24, 25*). To further investigate the role of LRP and/or the LDL receptor in metabolizing apoE in lipid-rich *vs.* lipid-poor states, we took advantage of four genetically-generated cell lines that differ in their LRP and LDL receptor expression. Using these cell lines we have found that even in the absence of a detectable association with lipid, apoE can be catabolized *via* cell surface LRP. This finding may be relevant to apoE/LRP interactions under specific conditions such as in the AD brain.

EXPERIMENTAL PROCEDURES

Materials—Human recombinant apoE3 and apoE4 expressed in *Escherichia coli*, rabbit β-VLDL and human HDL were prepared as described previously (*15*). Recombinant human apoE produced in this way is lipid-free. For example, the apoE protein is eluted in free protein fractions as opposed to lipoprotein fractions when analyzed by tandem Superose 6 columns on FPLC (data not shown, *26*). Human plasma apoE3 and apoE4 (lipid-free) were generous gifts from Robert Pitas (Gladstone Institute of Cardiovascular Disease, University of California, San Francisco). Receptor-associated protein (RAP) was isolated and purified from a glutathione S-transferase fusion protein expressed in *E. coli* as described previously (*27*). *N*-Succinimidyl 3-(4-hydroxy-5-[^{125}I]iodophenyl)propionate (Bolton and Hunter reagent) was purchased from Amersham (Oakville, Canada). Cytochrome C, trichloroacetic acid (TCA), heparin and phosphate-buffered saline (PBS) were from Sigma Chemical (St. Louis, MO). Bovine serum albumin (BSA) (fraction V) and Pronase were from Calbiochem-Novabiochem (La Jolla, CA). PD-10 Sephadex columns and Sepharose 6 columns were from Pharmacia Biotech (Uppsala, Sweden). Tissue culture medium was from GibcoBRL (Grand Island, NY).

Cell Culture—Wild type mouse embryonic fibroblasts (MEF-1 cells), fibroblasts homozygous for disruption of the LRP gene (MEF-2 cells), fibroblasts homozygous for disruption of the LDL receptor gene (MEF-3 cells), and fibroblasts homozygous for disruption of both the LRP and the LDL receptor genes (MEF-4 cells), were kindly provided by Joachim Herz (University of Texas Southwestern Medical Center at Dallas). MEF cells were cultured at 37°C in a humidified incubator with 5% CO_2 in DMEM supplemented with 10% fetal calf serum.

ApoE3/MEF4 Media—In order to determine the lipid content of apoE following its incubation with MEF4 cells, 1,000 pmol apoE3 was incubated at 50 nM in DMEM for 30 min at 37°C with MEF4 cells (in which no receptor mediated uptake occurs, see below). Thereafter the medium was harvested, and the apoE3 was purified *via* heparin-agarose chromatography (with ~50% recovery) according to Pitas and Mahley (*28*). The purified apoE was subjected to lipid analysis *via* electrospray ionization mass spectrometry.

Electrospray Ionization Mass Spectrometry of β-VLDL, HDL, and apoE3/MEF4 Media Extracts—Appropriate amounts of two internal standards, DMPC (1.5 nmol/mg protein) and DMPE (1.5 nmol/mg protein), were added to each protein sample (either 3.6 μg β-VLDL cholesterol, 3.6 μg HDL cholesterol, 250 pmol apoE3/MEF4 media or 250 pmol apoE3 prior to incubation with cells) prior to lipid extraction. The amount of total apoproteins in the β-VLDL and HDL samples was ~250 pmol. Next, lipids were extracted from the lipoprotein into $CHCl_3$ by the method of Bligh and Dyer (*29*). The lipids were dried under a nitrogen stream, redissolved in chloroform and filtered through Millex-HV 0.45 μm filters (Millipore, MA). Finally the lipids were reextracted against H_2O and the $CHCl_3$ layer was dried under a nitrogen stream. The final lipid residue was immediately resuspended in 100 μl of chloroform/methanol 1:2 (v/v) for direct analysis by electrospray ionization mass spectroscopy (ESI-MS).

ESI-MS analyses were performed with a Finnigan TSQ-7000 spectrometer (Finnigan MAT, San Jose, CA) equipped with an ESI source and controlled by Finnigan ICIS software operated through a DEC alpha workstation as described previously (*30, 31*). Typically, a 5-min period of signal averaging in the profile mode was employed for each lipid extract spectrum. All lipid extracts (in 1:2 chloroform/methanol) were supplemented with LiOH (50 nmol/mg protein) dissolved in methanol. The resultant lithiated adduct was directly infused into the ESI source utilizing a Harvard syringe pump at a flow rate of 2 μl/min, and choline glycerophospholipids were quantitated directly as their lithium adducts by comparison with an internal standard (*i.e.*, lithiated DMPC). Similarly ethanolamine glycerophospholipids were quantitated directly in the negative-ion mode after ESI by comparison with the internal standard, DMPE. The ionization selectivity of ESI-MS for distinct phospholipid classes was achieved by exploiting the differential ionization propsensities of each phospholipid class in either the positive or negative ion mode (*30, 32*). Plasmalogen molecular species were distinguished from alkyl-acyl phospholipid molecular species by treating chloroform extracts with acidic vapors prior to mass spectroscopic analyses as described previously (*32*).

Protein Iodination—Iodination of apoE was performed according to Bolton and Hunter (*33*). Specific radioactivities were 5–10 μCi/μg of protein. Briefly, 20 μg of apoE was radiolabelled with 500 μCi of N_2-dried *N*-succinimidyl 3-(4-hydroxy-5-[^{125}I]iodophenyl)propionate and kept at 4°C for 90 min with manual mixing. At the completion of the reaction, the reaction was quenched with 0.4 M glycine buffer and passed through a PD-10 Sephadex column to separate the labelled peptide from unincorporated isotope. In some experiments, ^{125}I-apoE3 (6 μg) was incubated with β-VLDL (3 μg of protein) for 30 min at 37°C before use in cellular assays (*14*).

Cell Binding and Degradation Assays—The assay buffer for ^{125}I-apoE3, ^{125}I-apoE4, or ^{125}I-apoE3/β-VLDL binding

and degradation was DMEM containing 6 mg/ml BSA and 5 mM $CaCl_2$. Cellular degradation assays were performed by incubating cells in assay buffer containing ^{125}I-apoE3 and ^{125}I-apoE4 (5 nM) or ^{125}I-apoE3/β-VLDL (5 nM of apoE3) in the absence or presence of unlabelled RAP (1 μM). This concentration of RAP is sufficient to inhibit ligand-binding to both LRP and the LDL-receptor (*3, 34*). After incubation at 37°C for 4 h, the overlying medium was removed and precipitated with TCA. Ligand degradation was defined as the appearance of TCA-soluble radioactivity in the overlying medium. Non-cellular degradation of ^{125}I-apoE3 or ^{125}I-apoE3/β-VLDL was determined in parallel dishes that did not contain cells and was subtracted from each point. For cellular binding assays, ^{125}I-apoE3 or ^{125}I-apoE4 (5 nM) was incubated with cells in the same buffer as for the degradation assays at 4°C for 1.5 h in the presence or absence of heparin (100 μg/ml). All experiments were repeated at least 3 times. Data are presented as mean ± SEM. Data were analyzed by ANOVA followed by *t*-tests with the Bonferroni correction.

Single Cycle Endocytosis—Single cycle endocytosis assays were performed essentially as described previously (*35*). Briefly, initial binding was performed with ^{125}I-apoE3 (5 nM) at 4°C for 1.5 h. The cells were then washed three times with DMEM containing 6 mg/ml BSA and 5 mM $CaCl_2$, followed by incubation at 37°C in the presence of 500 nM RAP. At selected intervals, the buffer overlying each monolayer was removed and subjected to TCA precipitation. The dishes were then quickly chilled on ice and cell monolayers were treated with 0.25% (w/v) Pronase in PBS for 30 min at 4°C to remove residual surface-bound ligand. This treatment also detached cells from the culture wells. The detached cells were then separated from the buffer by centrifugation. Radioactivity associated with the cell pellets represents internalized protease-resistant ligand, whereas radioactivity in the supernatant fraction represents surface protease-sensitive ligand. Degraded ligand was defined as TCA-soluble radioactivity in the overlying buffer. Each determination was performed in triplicate.

RESULTS

Analyses of Individual Phospholipid Molecular Species in β VLDL, HDL, apoE3, and apoE3/MEF4 Media Chloroform Extracts—These analyses were undertaken to determine the extent of lipid present in apoE3 prior to and following incubation with cells. Examination of chloroform extracts from rabbit β VLDL by ESI mass spectroscopy in the positive-ion mode demonstrated a predominance of plasmenylcholine molecular species (*e.g.*, *m/z* 781, lithiated 18:0–18:0 plasmenylcholine and *m/z* 809, 18:0–20:0 plasmenylcholine) and phosphatidylcholine molecular species (*e.g.*, *m/z* 765, lithiated 16:0–18:2 phosphatidylcholine and *m/z* 793, lithiated 18:0–18:2/18:1–18:1 phosphatidylcholines) (Fig. 1A). The positive-ion ESI mass spectrum also demonstrated the presence of sphingomyelin molecular species (*e.g.*, *m/z* 710, lithiated 16:0–18:0 sphingomyelin) (Fig. 1A). Similar choline glycerophospholipid molecular species were also found in the positive-ion ESI mass spectrum of human HDL chloroform extract, but were present in lower abundance (Fig. 1B). Virtually no choline glycerophospholipids were detected in chloroform extracts of apoE3/MEF4 media (Fig. 1C) nor in apoE3 prior to incubation with cells (not shown). The examination of chloroform extracts of β-VLDL, HDL, and apoE/MEF4 media by ESI mass spectroscopy in the negative-ion mode demonstrated no significant peak(s) (spectra not shown) corresponding to a paucity of ethanolamine glycerophospholipids in these samples. These data suggest that there was significantly less lipid associated with apoE after its addition to cells than in physiologically produced lipoprotein particles.

Cellular Degradation of ^{125}I-apoE/β-VLDL or Free ^{125}I-apoE via the LDL Receptor and/or LRP—Several lines of evidence suggest that LRP may serve as an endocytic receptor for apoE-enriched remnant particles (*36, 37*). In order to examine directly the role of LRP and the LDL receptor in the catabolism of apoE-containing β-VLDL, various mouse embryonic fibroblast cell lines including (i) wild type (MEF-1, expressing both LRP and the LDL receptor), (ii) LRP-deficient (MEF-2), (iii) LDL receptor-deficient (MEF-3), or (iv) LRP and LDL receptor–double deficient (MEF-4), were tested for their ability to degrade apoE3-enriched β-VLDL. In addition to these four cell lines, we took advantage of a specific inhibitor of LRP and the LDL receptor, RAP, which, upon application to the cell surface, antagonizes all known ligand interactions with LRP (*35*) and the LDL receptor (*34*). Thus, MEF-1, MEF-2, MEF-3, and MEF-4 cells were incubated with ^{125}I-apoE3–enriched

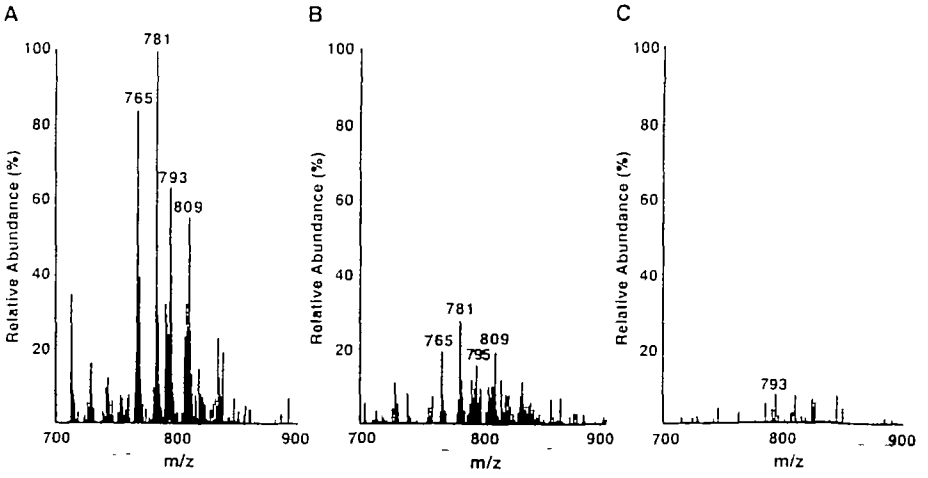

Fig. 1. **Positive-ion electrospray ionization mass spectra of choline glycerophospholipids in lipid extracts of rabbit β-VLDL, human HDL, and apoE/MEF4 media.** Phospholipids from β-VLDL (A), HDL (B), and apoE3/MEF4 media (C) were infused directly into the ESI chamber utilizing a syringe pump at a flow rate of 2 μl/min as described in "EXPERIMENTAL PROCEDURES." Ion peaks *m/z* 765, 781, and 809 correspond to lithiated 16:0–18:2 phosphatidylcholine, lithiated 18:0–18:0 plasmenylcholine, lithiated 18:0–18:2/18:1–18:1 phosphatidylcholines and lithiated 18:0–20:0 plasmenylcholine, respectively. Ion peak intensities in all three panels were normalized to the ion peak intensity of the internal standard.

β-VLDL (5 nM ^{125}I-apoE) in the absence or presence of 1 μM RAP at 37°C for 4 h. Cellular degradation of ^{125}I-apoE3–enriched β-VLDL was assessed by TCA precipitation of the overlying medium after incubation with cells. We found that MEF-1 cells degrade ^{125}I-apoE3/β-VLDL and that RAP inhibits this degradation by 56% ($p < 0.05$) (Fig. 2). When analyzed in LRP-negative MEF-2 cells, degradation of ^{125}I-apoE3/β-VLDL was reduced by 40% as compared to that seen in MEF-1 cells ($p < 0.05$), and was further reduced by 35% by the addition of RAP (although this was not statistically significant). When analyzed in LDL receptor–negative MEF-3 cells, the degradation of ^{125}I-apoE3/β-VLDL was observed, 60% of which was inhibited by the presence of RAP ($p < 0.05$). There was little degradation of ^{125}I-apoE3/β-VLDL in LRP- and LDL receptor–negative MEF-4 cells, with no observable effect of RAP. These results indicate that apoE-containing β-VLDL is catabolized by fibroblasts *via* both the LDL receptor and LRP.

In order to determine whether apoE in the absence of exogenously added lipoprotein can also be catabolized through the LDL receptor and/or LRP, lipid free recombinant ^{125}I-apoE3 alone was incubated with MEF-1, 2, 3, and 4 cells, and the degradation products were evaluated. Figure 3 shows that MEF-1 cells degrade ^{125}I-apoE3 and that RAP significantly inhibits this degradation ($p < 0.05$). When analyzed in LRP-negative MEF-2 cells, the degradation of ^{125}I-apoE was 43% of that seen in MEF-1 cells ($p < 0.05$) and was not further reduced by the presence of RAP. The same results were obtained when plasma purified apoE3 and apoE4 was used instead of bacterially produced recombinant apoE3 and apoE4, with no consistent differences observed between the isoforms (data not shown). When analyzed in LDL receptor–negative MEF-3 cells, a significant degradation of ^{125}I-apoE3 was still observed, 62% of which was inhibited by the presence of RAP ($p < 0.05$). The amount of ^{125}I-apoE3 degradation in the absence of RAP in MEF-1 cells *versus* MEF-3 cells was not significantly different. Furthermore, the amount of ^{125}I-apoE3 degradation in the presence and absence of RAP in MEF-4 cells was very small. These results suggest that ^{125}I-apoE3 can be degraded *via* an LRP-mediated pathway, even when in a very lipid-poor state.

To determine whether there is a dose-dependent degradation of ^{125}I-apoE3 that is LRP-dependent, increasing concentrations of ^{125}I-apoE3 were incubated with MEF-1 and MEF-2 cells, and the amount of degradation was assessed.

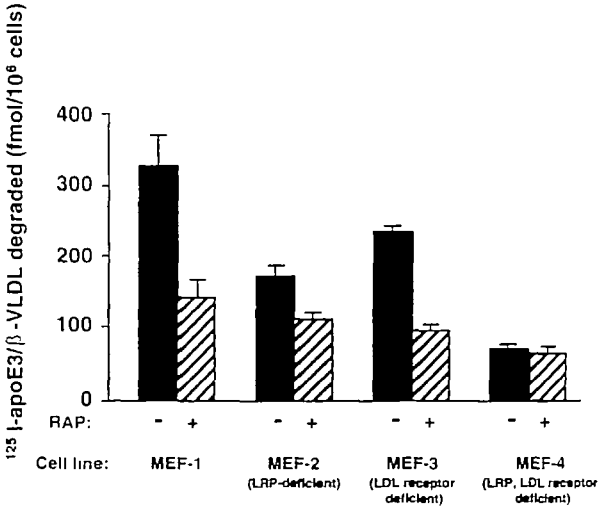

Fig. 2. **Degradation of ^{125}I-apoE3/β-VLDL in MEF-1, MEF-2, MEF-3, and MEF-4 cells.** MEF-1 (wild type), MEF-2 (LRP deficient), MEF-3 (LDL receptor deficient), and MEF-4 (LRP and LDL receptor double deficient) cells were incubated with ^{125}I-apoE3/β-VLDL in the absence or presence of 1 μM RAP for 4 h at 37°C. Thereafter, the degraded ligands were assessed by precipitating the overlying medium with 20% TCA. Symbols represent the average results of 5 determinations ± SEM from one of three independent experiments. Non-specific degradation of ^{125}I-apoE/β-VLDL in parallel dishes without cells was subtracted from each assay.

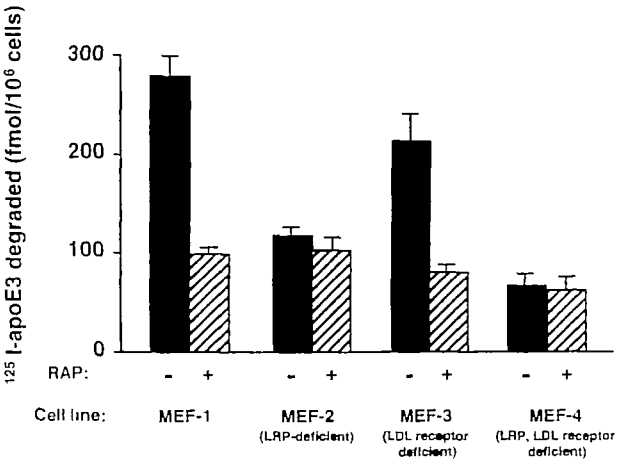

Fig. 3. **Degradation of lipid-free ^{125}I-apoE3 in MEF-1, MEF-2, MEF-3, and MEF-4 cells.** MEF-1 (wild type), MEF-2 (LRP deficient), MEF-3 (LDL receptor deficient), and MEF-4 (LRP and LDL receptor double deficient) cells were incubated with ^{125}I-apoE3 alone in the absence or presence of 1 μM RAP for 4 h at 37°C. The degradation of ^{125}I-apoE3 under each condition was analyzed *via* TCA-precipitation of the overlying medium. Symbols represent the average results of 5 determinations ± SEM from one of three independent experiments. Non-specific degradation of ^{125}I-apoE in parallel dishes without cells was subtracted from each assay.

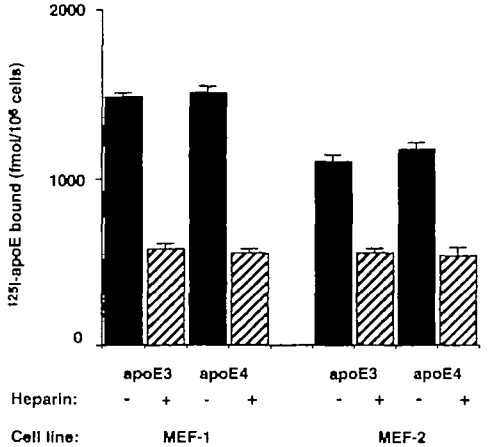

Fig. 4. **Cellular binding of lipid-free ^{125}I-apoE3 and ^{125}I-apoE4 in MEF-1 and MEF-2 cells in the presence and absence of heparin.** MEF-1 and MEF-2 cells were incubated with ^{125}I-apoE3 and ^{125}I-apoE4 (5 nM) in the absence or presence of heparin (100 μg/ml) for 1.5 h at 4°C. Symbols represent the average results of three determinations ± SEM from one of three independent experiments.

The degradation of ^{125}I-apoE3 in MEF-1 cells increased in a dose-dependent manner and a significant amount was RAP-inhibitable, whereas the degradation of ^{125}I-apoE3 by MEF-2 cells was not inhibited by RAP (data not shown). We also compared the binding of ^{125}I-apoE3 and ^{125}I-apoE4 to MEF-1 and MEF-2 cells. There was no significant difference in the binding of apoE3 and apoE4 to MEF-1 or MEF-2 cells, although less binding was seen in MEF-2 cells compared with MEF-1 cells (Fig. 4). In addition, binding in both cell lines was significantly inhibited by heparin (Fig. 4). These data are consistent with a portion of apoE binding requiring HSPG and the HSPG-LRP pathway.

Single Cycle Endocytosis of ^{125}I-apoE by MEF-1 Cells—To confirm that ^{125}I-apoE is catabolized by LRP *via* a receptor-mediated endocytic pathway, we performed single cycle endocytosis analyses. Figure 5 shows the fate of ^{125}I-apoE3 during a single cycle of endocytosis in MEF-1 cells. ^{125}I-apoE (5 nM) was incubated with MEF-1 cells for 1.5 h at 4°C to allow surface binding, and then incubated at 37°C for selected intervals to allow ligand uptake. After each interval, aliquots of medium were removed and subjected to TCA precipitation, whereas cell monolayers were quickly cooled to prevent further ligand internalization. The cells were then treated with Pronase at 4°C to remove residual surface ligand. The partitioning of ligand after each interval was assessed as described in "EXPERIMENTAL PROCEDURES." As shown in Fig. 4, there was a loss of 50% of surface bound ligand within 10 min. Concomitantly, ligand was internalized rapidly and reached a peak at 15–20 min after which the level declined. Ligand appeared in the medium simultaneously with the disappearance of cell-surface ligand. TCA-precipitable radioactivity (representing degraded ligand) was detected only after a delay of 15–20 min. The presence of degraded ligand coincided with the disappearance of internalized ligand. Thus, the kinetic distribution pattern of ^{125}I-apoE during a single cycle of endocytosis is typical of that of other ligands degraded in a receptor-mediated fashion (*38*), suggesting that lipid-free ^{125}I-apoE follows this cellular pathway.

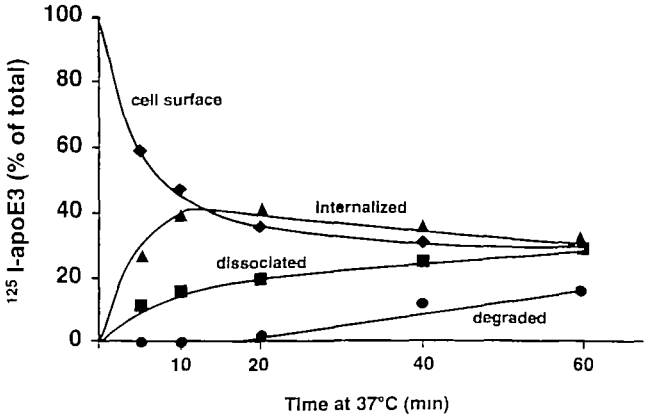

Fig. 5. **Distribution of ^{125}I-apoE3 during a single cycle endocytosis in MEF-1 cells.** Binding of ^{125}I-apoE3 (5 nM) to MEF-1 cells was performed at 4°C for 1.5 h. After washing, cells were incubated at 37°C for selected intervals in the presence of 500 nM RAP. The overlying buffer was then removed and subjected to TCA-precipitation, whereas cell monolayers were chilled and treated with Pronase. Cell-associated ligand (Pronase-sensitive, ♦) and internalized ligand (Pronase-resistant, ▲) were quantified for cells following Pronase treatment. Dissociated ligand (TCA-precipitable, ■), as well as degraded ligand (TCA-soluble, ●) associated with the medium were also determined. Symbols represent means of triplicate determinations. The absolute amount of ligand at the cell surface at 0 min was 63 fmol/10^6 cells; the amount degraded at 60 min was 12 fmol/10^6 cells.

DISCUSSION

The present observations demonstrate that (i) a very lipid-poor form of ^{125}I-apoE3 and ^{125}I-apoE4 can be taken up and degraded by fibroblasts *via* LRP, (ii) the uptake and degradation of ^{125}I-apoE3–containing β-VLDL is mediated by both LRP and the LDL receptor, and (iii) the kinetics and distribution of lipid-poor ^{125}I-apoE3 during a single cycle of endocytosis are typical of a receptor-mediated pathway. Taken together, these results suggest that apoE can assume a conformation required to interact with cell surface LRP even when present in a very lipid-poor form.

At least two different pathways exist for the cellular uptake of apoE-containing lipoproteins. First, apoE/lipoproteins can interact directly with the cell surface LDL receptor and be taken up by cells (*39*). Second, a two-step process has been proposed in which both LRP and cell-surface heparan sulfate proteoglycans (HSPG) are involved (*40*). It appears that the initial binding requires the interaction of apoE/lipoproteins with HSPG. ApoE/lipoprotein may subsequently be transferred to LRP for internalization, or apoE/lipoprotein/HSPG/LRP may form tertiary complexes and be taken up by cells. This latter pathway of apoE/lipoprotein metabolism is referred to as the HSPG/LRP pathway (*41*). For each of these pathways, it is generally believed that only when apoE is present on lipoprotein particles is it able to serve as a ligand for the LDL receptor and LRP (*1, 3, 11, 36*). However, our present results clearly demonstrate that ^{125}I-apoE3 in the absence of exogenous lipoproteins can be metabolized by cells *via* LRP. It is interesting to note that previous studies that demonstrated that apoE can interact with LRP did so by showing that the addition of apoE to β-VLDL facilitates the binding and uptake of labeled β-VLDL to cells (*36*). Those studies did not assess whether lipid-free apoE added to cells can bind LRP. Consistent with our results, DeMattos *et al.* (*18*) recently showed that cell-secreted apoE3 associated with minimal lipid is able to stimulate neurite outgrowth in the absence of exogenously applied lipoprotein. Of note, lipid-free apoE bound to a plastic surface exhibits high affinity binding to an LDL receptor fragment containing the ligand-binding domain (*42*). In addition, Dyer and Curtiss (*43*) demonstrated that in the absence of exogenous lipid a dimeric synthetic apoE peptide possesses LDL receptor-binding activity. Furthermore, when this peptide was aminoterminally acetylated, it interacted with LDL and was able to mediate cellular LDL uptake (*44*). Perhaps, the same epitope on lipid-free apoE can interact with the LDL receptor and LRP. The precise conformation of lipid-free apoE in physiological solutions is not known; however, our preliminary results utilizing nondenaturing gradient gel electrophoresis suggest it is in a multimeric form (unpublished observations). Perhaps such a form is required to expose appropriate epitopes for receptor binding. Lipid-poor apoE appears to prefer LRP to the LDL receptor. These observations taken together support the possibility that apoE in a lipid-poor form can directly interact with members of the LDL receptor gene family.

Although the physiological function of lipid-poor apoE metabolism mediated *via* LRP is not clear at present, such a form may exist *in vivo*, particularly in disease states. For example, specific interactions of lipid-free and cell-secreted purified apoE with amyloid-β protein (Aβ) have been observed (*45, 46*). These kinds of interactions may be important in the AD brain where apoE associates with Aβ in plaques (*47, 48*). While apoE-containing lipoproteins may initially interact with Aβ, it is possible that a lipid-poor form of apoE is present in plaques. *In vitro* data suggest that lipidated apoE can influence Aβ metabolism and that this probably occurs through cellular apoE receptors (*49*). ApoE/Aβ interactions may be particularly relevant *in vivo* as recent data suggest a role for human apoE in Aβ clearance in both human AD brain (*12*) as well as in a transgenic model of AD (*13*). While apoE/lipoproteins are more likely to play a role in the clearance of the "soluble" Aβ that is found in CSF lipoproteins (*50*), plaque-associated apoE may be in a lipid-poor form. Thus, it is conceivable that lipid-poor apoE may participate in Aβ catabolism in an LRP-dependent manner and that this finding may have important implications for understanding the relationship between apoE and AD.

While we have shown that essentially lipid-free apoE can exist in the medium in the presence of cells, our studies do not prove that it cannot, once associated with the cell surface, interact with membrane phospholipids prior to receptor mediated uptake. Our studies do, however, show that a very lipid-poor form of apoE can be metabolized *via* LRP independent of exogenous lipoprotein particles. Further studies to examine and compare cellular interactions of the lipid-poor form as well as brain cell (*e.g.*, astrocyte)–derived apoE/lipoproteins should provide further insights into relevant cellular pathways of apoE metabolism in normal and disease states.

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