The Low Density Lipoprotein Receptor Regulates the Level of Central Nervous System Human and Murine Apolipoprotein E but Does Not Modify Amyloid Plaque Pathology in PDAPP Mice*  

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Apolipoprotein E (apoE), a chaperone for the amyloid β (Aβ) peptide, regulates the deposition and structure of Aβ that deposits in the brain in Alzheimer disease (AD). The primary apoE receptor that regulates levels of apoE in the brain is unknown. We report that the low density lipoprotein receptor (LDLR) regulates the cellular uptake and central nervous system levels of astrocyte-derived apoE. Cells lacking LDLR were unable to appreciably endocytose astrocyte-secreted apoE-containing lipoprotein particles. Moreover, cells overexpressing LDLR showed a dramatic increase in apoE endocytosis and degradation. We also found that LDLR knock-out (Ldlr−/−) mice had a significant, ~50% increase in the level of apoE in the cerebrospinal fluid and extracellular pools of the brain. However, when the PDAPP mouse model of AD was bred onto an Ldlr−/− background, we did not observe a significant change in brain Aβ levels either before or after the onset of Aβ deposition. Interestingly, human APOE3 or APOE4 (but not APOE2) knock-in mice bred on an Ldlr−/− background had a 210% and 380% increase, respectively, in the level of apoE in cerebrospinal fluid. These results demonstrate that central nervous system levels of both human and murine apoE are directly regulated by LDLR. Although the increase in murine apoE caused by LDLR deficiency was not sufficient to affect Aβ levels or deposition by 10 months of age in PDAPP mice, it remains a possibility that the increase in human apoE3 and apoE4 levels caused by LDLR deficiency will affect this process and could hold promise for therapeutic targets in AD.

Alzheimer disease (AD) is a progressive neurodegenerative disease and is the most common cause of dementia. One of the key pathological hallmarks of AD is the deposition of the 39–43-amino acid amyloid β (Aβ) peptide in the form of both diffuse (thioflavine-S/Congo red-negative) and fibrillar (thioflavine-S/Congo red-positive) plaques. An abundance of data suggests that conversion of Aβ from soluble to insoluble forms is an early event in the pathogenesis of AD. The Aβ peptide is generated from cleavage of the larger amyloid precursor protein (APP) with the predominant species being Aβ40 and, to a lesser extent, Aβ42 (1). Although accounting for <1% of all cases, early-onset, autosomal-dominant forms of AD have been identified that share the common feature of an overall increase in Aβ levels or a relative elevation in the more fibrillogenic Aβ42 throughout life, ultimately resulting in early Aβ deposition and plaque formation. Identification of these familial AD cases has led to the generation of several APP transgenic mouse models that recapitulate many aspects of Aβ deposition and associated pathology (2).

In 1993, the e4 allele of apoE was found to be a genetic risk factor for the most common form of AD (late-onset AD) as well as for cerebral amyloid angiopathy, whereas the e2 allele was shown to be protective (3). Abundant data suggest that apoE is linked to AD and cerebral amyloid angiopathy due to its ability to act as an Aβ chaperone (4) and influence Aβ metabolism. By acting as an Aβ-binding molecule, apoE influences the amount of Aβ deposition and the conformation in which Aβ aggregates, as well as Aβ clearance and toxicity in vivo (5–8). Furthermore, the level of apoE in the brain directly influences all of the aforementioned processes (5–8). For example, APP transgenic mice lacking apoE develop less Aβ deposition and virtually no fibrillar Aβ deposits (5, 9, 10). Thus, understanding the cellular events and receptors that regulate apoE levels in brain may give important insights into AD pathogenesis.

ApoE is expressed at high levels in the liver and the central nervous system (CNS), where it is present in lipoprotein particles. Furthermore, apoE in the CNS is derived from within the CNS and not the plasma (11). However, in the CNS, apoE-containing lipoproteins differ from those found in the periphery in both the amount of sialation and, perhaps more importantly, the type of lipoprotein particle it associates with (12). In the plasma, apoE is in both very low density lipoproteins (VLDLs) and a subset of high density lipoproteins that also contain other
apoE receptors regulate the level of apoE in the brain or mediate apoE-dependent clearance of apoE-binding proteins is unknown. Nearly 20 years ago, Brown and Goldstein (14) elucidated the role of the low density lipoprotein receptor (LDLR) in receptor-mediated endocytosis of lipoproteins in plasma, including apoE. Subsequently, other members of the LDLR family have been identified. Many are expressed in brain, and they have been shown in vitro to bind and endocytose lipid-rich lipoproteins that contain apoE (15). To determine whether LDLR family members influence the level of CNS apoE and Aβ metabolism, an AD mouse model was recently bred to mice lacking the 39-kDa receptor-associated protein (RAP) (16). An increase in Aβ aggregation and deposition was found in these mice. RAP is an endoplasmic reticulum chaperone protein known to influence the folding and level of all LDLR family members, and in this study, levels of both LDLR-related protein (LRP) and LDLR were examined and found to be reduced in the brain of mice lacking RAP (16). Additionally, overexpression of a functional LRP mini-receptor in the PDAPP model led to an increase in the levels of soluble Aβ at advanced stages of deposition, but with no detectable change in Aβ deposition measured histologically (17). Thus, whether other LDLR family members are responsible for brain apoE metabolism and whether they play a role in regulating Aβ levels in vivo remains unclear. Herein, we investigate the role of LDLR in regulation of CNS-derived apoE as well as on brain Aβ levels in a mouse model of AD.

MATERIALS AND METHODS

ApoE Labeling and in Vitro Assays—The immunooaffinity purification of astrocyte-secreted apoE-containing lipoprotein particles has been described previously (18). For apoE degradation studies, astrocyte-secreted apoE3-containing lipoprotein particles were labeled with 125I using an IODO-GEN kit (Pierce). Chinese hamster ovary cells that lack endogenous LRP (LRP-null) were used to stably express LDLR, an LRP mini-receptor (LRP2), and the VLDL receptor (19). Degradation of 125I-labeled apoE was measured as radioactivity soluble in 20% trichloroacetic acid in the cell culture supernatant as previously described (19). 1 μg unlabeled apoE was used to determine specificity, a concentration that inhibits all LDLR family members. For apoE endocytosis assays, apoE lipoprotein particles were labeled with the fluorescent horse radish peroxidase-conjugated anti-apoE antibody EUSA (18). Mice were perfused with PBS, and fixed with 4% paraformaldehyde. These mice were then bred to human PDAPP mice with the 39-kDa receptor-associated protein (Roche Applied Science) were used where indicated. For PBS-soluble Aβ analysis, tissue was first Dounce homogenized in ice-cold PBS with protease inhibitors and immediately spun for 5 min at 20,000 × g. For carbonate-soluble Aβ analysis, the PBS-insoluble pellet was then homogenized in ice-cold 100 mM carbonate, 50 mM NaCl, pH 11.5, with protease inhibitors and immediately spun for 10 min at 20,000 × g. For carbonate-insoluble Aβ analysis, the carbonate-insoluble pellet was lysed for 3 h with rotation in 5 μl guanidine, 50 mM Tris with protease inhibitors. All experimental protocols were approved by the animal studies committee at Washington University.

Biochemical and Histological Analysis—Aβ levels, and, when released by cells, contain apoE as their sole apolipoprotein constituent (12, 13). Despite this information, and, when released by cells, contain apoE as their sole apolipoprotein constituent (12, 13). Despite this information, it remains unclear whether specific apoE receptors regulate the level of apoE in the CNS. Here, we investigate the role of LDLR in regulation of CNS-derived apoE as well as on brain Aβ levels in a mouse model of AD.

RESULTS

To begin to determine whether one of the two major apoE receptors in the brain, LDLR or LRP, could regulate apoE levels in brain, we purified astrocyte-secreted apoE-containing lipoprotein particles as described previously (18) and determined their ability to be endocytosed by mouse embryonic fibroblasts derived from wild-type, Lrp null (Lrp−/−), or double-knock-out Ldlr−/−, Lrp−/− mouse embryos (21). DiI-labeled apoE lipoprotein particles were readily taken up into endocytic vesicles in wild-type and Lrp−/− cells (Fig. 1A). This punctate pattern of staining is typical of receptor-mediated endocytosis and represents an endosomal/lysosomal distribution of endocytosed ligands. However, cells from Ldlr−/− or double-knock-out Ldlr−/−, Lrp−/− cells showed no appreciable endocytosis of astrocyte-secreted, apoE-containing lipoprotein particles (Fig. 1A). To further clarify the role of other LDLR family members in mediating the receptor-mediated endocytosis of astrocyte-
RAP-inhibitable binding of 125I-labeled apoE3 was calculated and is shown for hamster ovary LRP-null cells overexpressing LDLR family members. Lrp particles were readily endocytosed in wild-type and Lrp knockout embryonic fibroblast cells, but not in Ldlr knockout or double-null Ldlr mice (Fig. 1A). We found that apoE levels in cortical tissue homogenized in PBS (as a reflection of extracellular pools of apoE) were significantly increased by 57% in Ldlr−/− mice compared with Ldlr+/+ mice (Fig. 2B). Together, both in vitro and in vivo data suggest that the LDLR plays an important role as an apoE receptor that mediates the uptake, degradation, and level of apoE in the brain.

Because Ldlr−/− mice have elevated extracellular levels of apoE in the brain and because apoE acts as an Aβ chaperone for both soluble and insoluble Aβ, we wanted to determine the effect of LDLR on the deposition of Aβ in vivo. To determine whether LDLR has a direct effect on Aβ aggregation and deposition in vivo, we used the PDAPP mouse model of AD to generate PDAPP−/−, Ldr−/− and PDAPP−/−, Ldlr−/− mice (littermates). PDAPP mice express the human APP transgene containing a familial AD mutation at amino acid 717 and overproduce Aβ (particularly Aβ42). In the hippocampus of these mice, the amount of soluble and insoluble Aβ increases in an age-dependent manner beginning between 6 and 9 months of age, when plaques begin to deposit (25, 32). We first examined the levels of both Aβ40 and Aβ42 in young PDAPP−/−, Ldr−/− and PDAPP−/−, Ldlr−/− mice at 3 months of age, well before the deposition of Aβ begins. PBS-soluble levels of Aβ40 and Aβ42 in the hippocampus were not significantly different between the two genotypes (Fig. 3A). Carbonate-soluble levels of Aβ40 and Aβ42 in the hippocampus were also not significantly different between the two genotypes (Fig. 3B). We next examined PDAPP−/−, Ldr−/− and PDAPP−/−, Ldlr−/− mice at 10 months of age to determine the effect of the LDLR on Aβ deposition. The area of the hippocampus covered by Aβ immunoreactive deposits in tissue sections was 31% higher in 10-month-old PDAPP, Ldr−/− mice compared with PDAPP, Ldr−/− mice (Fig. 4A), but this increase was not statistically significant. Similar results were found for thioflavine-S-positive fibrillar deposits, and comparable results were found in the cortex (data not shown). A biochemical analysis of...
carbonate-soluble Aβ levels in the hippocampus revealed slight but nonsignificant increases in both Aβ40 (17%) and Aβ42 (53%) of 10-month-old PDAPP, Ldlr−/− mice as compared with PDAPP, Ldlr+/+ mice (Fig. 4B). Carbonate-insoluble Aβ levels in the hippocampus also revealed slight but nonsignificant increases in both Aβ40 (51%) and Aβ42 (10%) of 10-month-old PDAPP, Ldlr−/− mice as compared with PDAPP, Ldlr+/+ mice (Fig. 4C). Whereas Ldlr−/− mice have an increase in plasma cholesterol of −79% compared with wild-type (Ldlr+/+) mice (Fig. 5A), there were no differences in either brain or CSF cholesterol (Fig. 5, B and C). There was also no evidence that the processing of APP (Fig. 5D) or the levels of total APP protein (data not shown) were different between the genotypes. Additionally, hippocampal LRP levels were not altered in the absence of LDLR (data not shown). Thus, although the level of murine apoE is elevated by about 50% in the extracellular CNS pools in Ldlr−/− mice, these data suggest that this degree of change in the level of apoE is not sufficiently increased to significantly affect Aβ levels at young ages or the early stages of Aβ deposition in PDAPP transgenic mice.

To explore the possibility that the LDLR may differentially regulate the level of human apoE isoforms in the CNS compared with murine apoE, we bred human APOE2, APOE3, and APOE4 targeted replacement mice (knock-in) onto an Ldlr−/− mouse background. To confirm previous findings noted from these mice (33), we assessed plasma cholesterol and apoE and found that both were significantly elevated in the absence of LDLR in all genotypes (data not shown). To determine the extent to which LDLR regulates human apoE levels in the extracellular space of the CNS, we measured apoE levels in the CSF. We found that levels of both human apoE3 and human apoE4 in the CSF were significantly higher by 210% and 380%, respectively, in the absence of the LDLR (Fig. 6A). The level of human apoE2 in the CSF was not significantly altered by the presence or absence of the LDLR (Fig. 6A), as was expected, because the human apoE2 isoform exhibits <2%
binding to the LDLR (34). However, it is interesting to note that the level of human apoE2 is substantially higher than that of apoE3 or apoE4 in the CSF (Fig. 6A). Despite the increase in human apoE levels in the absence of LDLR, there was no significant increase in the level of brain cholesterol in these mice (Fig. 6B).

DISCUSSION

In the present study, we found that the LDLR, but not other LDLR family members, was able to efficiently bind, endocytose, and degrade astrocyte-secreted apoE-containing lipoprotein particles and that Ldlr−/− mice have −50% higher levels of apoE in CSF and extracellular pools of brain tissue. However, when the PDAPP mouse model of AD was bred onto an Ldlr−/− background, there was not a significant increase in the levels of Aβ40 and Aβ42 at young ages before deposition of Aβ begins. Additionally, there was not a significant increase in Aβ deposition defined by immunohistochemical and biochemical measures between 10-month-old PDAPP, Ldlr−/− and PDAPP, Ldlr+/− mice. Interestingly, using human APOE knock-in mice, we found that the increase in the level of apoE3 and apoE4 (but not apoE2) on an Ldlr−/− background was much greater than that for murine apoE on this same Ldlr−/− background.

Our in vitro data indicate that LDLR family members including LRP, VLDL receptor, and apoER2 are not able to appreciably internalize and degrade astrocyte-secreted apoE-containing lipoproteins. Previous work has clearly demonstrated that these LDLR family members are able to efficiently bind and endocytose apoE reconstituted in large, cholesterol-rich lipoprotein particles termed β-VLDL (35). However, these β-VLDL particles differ from astrocyte-secreted apoE in lipid composition, the presence of other apoproteins, and the amount of sialation (12). The apolipoprotein lipidation state does alter receptor binding characteristics. For example, our previous studies have shown that recombinant apoE in the absence of lipid prefer binding to LRP over the LDLR (22). This is not a form of apoE that has been shown to be present under physiological conditions. Together, these results indicate that the lipid content and form of apoE lipoprotein particles can alter their receptor binding specificity. They also demonstrate that the LDLR is an important apoE receptor that regulates human and murine apoE endocytosis and levels in the brain. Although

![Figure 5](http://www.jbc.org/)

**FIG. 5.** LDLR influences levels of plasma cholesterol but has no effect on hippocampal and CSF cholesterol or APP processing. A, the level of total plasma cholesterol was 68.2 ± 4.63 mg/dl in Ldlr+/− mice (n = 6) as compared with 124 ± 8.82 mg/dl in Ldlr−/− mice (n = 6) (***, p < 0.001, unpaired two-tailed t test). Level of total cholesterol in the hippocampus (B), CSF (C), and APP processing as assessed by semiquantitative Western blotting of α-C-terminal fragment (α-CTF; D) was not significantly different between the genotypes (n = 5 in each group).

![Figure 6](http://www.jbc.org/)

**FIG. 6.** LDLR influences the levels of human apoE3 and apoE4 but not apoE2 in the cerebrospinal fluid. A, the level of apoE in the cerebrospinal fluid was measured in human APOE2+/+, APOE3 itus+, +/+, and APOE4+/+ targeted replacement mice (knock-in) in the presence (Ldlr+/+) or absence (Ldlr−/−) of the endogenous LDLR. The level of human apoE was significantly higher in APOE2+/+ mice (5.54 ± 0.78 µg/ml) compared with APOE2−/− (1.94 ± 0.25 µg/ml) and APOE4+/+ (1.28 ± 0.26 µg/ml) mice (a, p < 0.001 by analysis of variance with post-hoc Tukey t test). As expected from previous studies, the level of human apoE was not significantly different between APOE2+/+, Ldlr+/+ and APOE2−/−, Ldlr−/− mice. The level of human apoE was significantly higher in APOE3+/+ mice (4.04 ± 0.51 µg/ml) compared with APOE3−/−, Ldlr−/− mice (1.94 ± 0.25 µg/ml) (**, p < 0.05 by analysis of variance with post-hoc Tukey t test). The level of human apoE was also significantly higher in APOE4+/+, Ldlr+/+ mice (4.87 ± 0.47 µg/ml) compared with APOE3−/−, Ldlr−/− mice (1.28 ± 0.26 µg/ml) (***, p < 0.001 by analysis of variance with post-hoc Tukey t test). B, the level of total cholesterol in the cortex was not increased in human APOE2+/+, APOE3+/+, and APOE4+/+ knock-in mice in the absence of the LDLR. Interestingly, human APOE2+/+ had a slight but significant decrease in the level of cholesterol in the cortex in the absence of the LDLR (†, p < 0.05).
recent findings show that increasing or decreasing cellular cholesterol can influence cellular Aβ secretion (36). Although LDLr−/− mice have elevated plasma cholesterol levels, we found that the level of brain cholesterol is unaffected in these mice as has been previously reported (37). The exact mechanism by which apoE affects AD pathogenesis is unclear. However, a large body of evidence suggests that one mechanism by which it influences the age of onset of AD is by acting as a chaperone for both soluble and insoluble Aβ, thereby influencing Aβ clearance and fibrillogenesis (3, 5, 7, 32). Our current results suggest that although the LDLR regulates extracellular levels of apoE in the brain, a 50% increase in the level of murine apoE over endogenous levels is not sufficient to alter Aβ levels at young ages or the early stages of Aβ deposition in PDAPP mice at 10 months of age. This may represent a ceiling effect of the level of murine apoE or indicate that a greater extent than murine apoE and therefore influence Aβ as a chaperone for both soluble and insoluble Aβ, thereby influencing Aβ clearance and fibrillogenesis (3, 5, 7, 32). Our current results suggest that although the LDLR regulates extracellular levels of apoE in the brain, a 50% increase in the level of murine apoE over endogenous levels is not sufficient to alter Aβ levels at young ages or the early stages of Aβ deposition in PDAPP mice at 10 months of age. This may represent a ceiling effect of the level of murine apoE or indicate that a greater extent than murine apoE and therefore influence Aβ as a chaperone for both soluble and insoluble Aβ, thereby influencing Aβ clearance and fibrillogenesis (3, 5, 7, 32). Our current results suggest that although the LDLR regulates extracellular levels of apoE in the brain, a 50% increase in the level of murine apoE over endogenous levels is not sufficient to alter Aβ levels at young ages or the early stages of Aβ deposition in PDAPP mice at 10 months of age. This may represent a ceiling effect of the level of murine apoE or indicate that a greater extent than murine apoE and therefore influence Aβ as a chaperone for both soluble and insoluble Aβ, thereby influencing Aβ clearance and fibrillogenesis (3, 5, 7, 32). Our current results suggest that although the LDLR regulates extracellular levels of apoE in the brain, a 50% increase in the level of murine apoE over endogenous levels is not sufficient to alter Aβ levels at young ages or the early stages of Aβ deposition in PDAPP mice at 10 months of age. This may represent a ceiling effect of the level of murine apoE or indicate that a greater extent than murine apoE and therefore influence Aβ as a chaperone for both soluble and insoluble Aβ, thereby influencing Aβ clearance and fibrillogenesis (3, 5, 7, 32). Our current results suggest that although the LDLR regulates extracellular levels of apoE in the brain, a 50% increase in the level of murine apoE over endogenous levels is not sufficient to alter Aβ levels at young ages or the early stages of Aβ deposition in PDAPP mice at 10 months of age. This may represent a ceiling effect of the level of murine apoE or indicate that a greater extent than murine apoE and therefore influence Aβ as a chaperone for both soluble and insoluble Aβ, thereby influencing Aβ clearance and fibrillogenesis (3, 5, 7, 32). Our current results suggest that although the LDLR regulates extracellular levels of apoE in the brain, a 50% increase in the level of murine apoE over endogenous levels is not sufficient to alter Aβ levels at young ages or the early stages of Aβ deposition in PDAPP mice at 10 months of age. This may represent a ceiling effect of the level of murine apoE or indicate that a greater extent than murine apoE and therefore influence Aβ as a chaperone for both soluble and insoluble Aβ, thereby influencing Aβ clearance and fibrillogenesis (3, 5, 7, 32). Our current results suggest that although the LDLR regulates extracellular levels of apoE in the brain, a 50% increase in the level of murine apoE over endogenous levels is not sufficient to alter Aβ levels at young ages or the early stages of Aβ deposition in PDAPP mice at 10 months of age. This may represent a ceiling effect of the level of murine apoE or indicate that a greater extent than murine apoE and therefore influence Aβ as a chaperone for both soluble and insoluble Aβ, thereby influencing Aβ clearance and fibrillogenesis (3, 5, 7, 32).
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J. Biol. Chem. 2005, 280:25754-25759.
doi: 10.1074/jbc.M502143200 originally published online May 11, 2005

Access the most updated version of this article at doi: 10.1074/jbc.M502143200

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