thematic review

Cholesterol: from heart attacks to Alzheimer's disease

Robert L. Raffaï* and Karl H. Weisgraber[1,*,†]

Department of Pathology* and Cardiovascular Research Institute,† Gladstone Institutes of Cardiovascular Disease and Neurological Disease, University of California, San Francisco, CA 94141-9100

Abstract The accumulation and aggregation of the amyloid-β peptide (Aβ) in the brain are important contributing factors to Alzheimer's disease (AD). Consequently, blocking the generation of Aβ is a potentially important treatment strategy. Recent work on the metabolism of Aβ has identified several cellular proteins and proteases that collectively promote or prevent the generation of Aβ. In addition, accumulating in vitro and in vivo evidence suggests a role for cholesterol in modulating the cellular processing of Aβ with the potential to affect AD.—Raffaï, R. L., and K. H. Weisgraber. **Cholesterol: from heart attacks to Alzheimer's disease. *J. Lipid Res.* 2003.** 44: **1423–1430.**

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Cholesterol is a major lipid component of eucaryotic plasma membranes, imparting both flexibility and stability, and it is the precursor for the biosynthesis of bile acids as well as adrenal, pituitary, and sex hormones. In these capacities, cholesterol is essential for life. However, elevated concentrations of plasma cholesterol are a well-established risk factor for cardiovascular disease, and emerging evidence suggests that cholesterol metabolism plays a direct role in the pathogenesis of Alzheimer's disease (AD). This review focuses on the link to AD.

AD

Age is a major risk factor for AD, and as people continue to live longer in the United States and other developed countries, the incidence of the disease is rising. In the United States alone, more than four million people have AD, and the number is projected to double by 2025. This devastating neurodegenerative disorder is characterized by progressive and irreversible loss of short-term memory and cognition. The cost of caring for individuals with AD is estimated to be more than $100 billion annually and will undoubtedly increase significantly in the future. The psychological and emotional costs to families and health care providers are beyond measure. The disease occurs in two forms. Early-onset AD (before age 65) is associated with specific genetic mutations and accounts for less than 2% of AD cases. The more prevalent late-onset form may be of the familial or sporadic variety. Approved drugs are effective only for a short time and do not slow the progression of the disease or act in advanced cases.

The pathological hallmarks of AD, as shown by histological analysis of AD brains at autopsy, are two types of insoluble protein deposits: extracellular amyloid plaques and intracellular neurofibrillary tangles. Tangles are composed primarily of tau, a microtubule-binding protein, that is hyperphosphorylated. How tau phosphorylation and tangle formation contribute to AD is unclear. The major component of amyloid plaques is the amyloid-β peptide (Aβ), a 40- to 42-residue peptide that is derived from the amyloid precursor protein (APP). Similar to the genetic links between plasma cholesterol levels and heart disease, compelling genetic evidence supports a role for Aβ in AD, known as the amyloid hypothesis (1). The familial early-onset form of AD is associated with mutations in three genes, APP and presenilin 1 and 2, that promote the accumulation of Aβ in the brain (1). How to slow or reverse the formation of Aβ is the focus of much AD research (1, 2).

Generation of Aβ

More is known about the origins of Aβ than about its pathogenic role in promoting neurodegeneration and AD (3). Aβ is derived from cellular APP, a type I membrane protein that is cleaved by two distinct proteolytic pathways (**Fig. 1**). In the major pathway, APP is cleaved in a late secretory compartment or at the cell surface, by ADAM10 (a disintegrin and metalloprotease) which cuts the protein at the α-secretase site within Aβ (solid rectangle in Fig. 1) (4, 5). The two products, the neurotrophic amyloid precursor proteins α (APPsα) fragment and a carboxyl-terminal fragment, are not pathological.

A minor proteolytic processing pathway involves β- and γ-secretases and generates the neurotoxic Aβ. All three enzyme activities (α, β, and γ cleavages) are membrane as-

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[1]To whom correspondence should be addressed.
e-mail: kweisgraber@gladstone.ucsf.edu

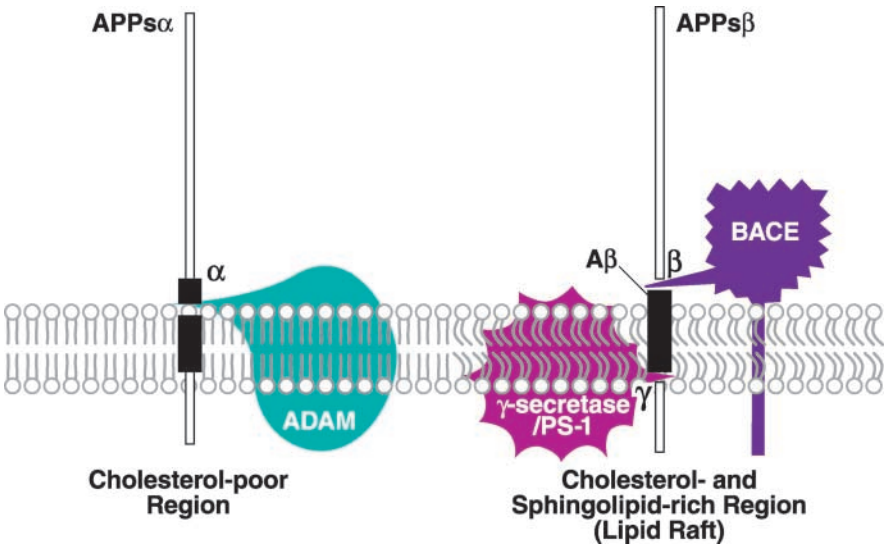

Fig. 1. Influence of membrane lipid composition on amyloid precursor protein (APP) processing. Cholesterol-poor regions favor α-secretase processing, cleaving within the amyloid-β peptide (Aβ) (solid rectangle), and the generation of amyloid precursor proteins α. Cholesterol- and sphingolipid-rich regions, referred to as lipid-rafts, favor both β- and γ-secretase processing and the generation of Aβ (solid rectangle).

sociated. Cleavage appears to be performed at the β-secretase site by β-site amyloid precursor protein cleavage enzyme 1 (6) and at the γ-secretase site by a protease complex containing presenilin-1 (7), primarily within the secretory and recycling compartments. Cleavage at the γ-secretase site in combination with β cleavage leads principally to a 40-amino acid peptide (Aβ-40), while 5% is cleaved into a 42-amino acid peptide (Aβ-42). The Aβ can be either secreted from cells or retained in the endoplasmic reticulum as an insoluble complex (8). Aβ-42 is regarded as the most neurotoxic, as it readily aggregates to form fibrillar structures that ultimately coalesce into amyloid plaques (9). A number of mutations in APP in the vicinity of the cleavage sites enhance the generation of Aβ-42 by γ-secretase and are associated with increased accumulation of amyloid plaques and familial early-onset AD. In vivo studies have revealed that Aβ can be rapidly degraded by several proteases (10). However, as its concentration rises in the brain as a result of enhanced production or inefficient clearance (11), Aβ tends to aggregate into a series of oligomers and eventually into insoluble deposits.

Initially, it was thought that the insoluble amyloid plaques were the pathologic culprits in AD. However, emerging evidence implicates soluble Aβ aggregates as the mediators of neurotoxicity. The Aβ rapidly aggregates by two separate pathways. The first leads to soluble oligomers, referred to as Aβ-derived diffusible ligands (ADDLs), referred to as ADDLs. In a separate pathway, monomers can also form protofibrils that eventually generate fibrillar aggregates that coalesce into the characteristic insoluble amyloid. Several lines of in vivo evidence suggest that ADDLs (12) and protofibrils (13), rather than monomeric Aβ or insoluble amyloid plaques, mediate neurotoxicity. For example, transgenic mice expressing a mutant form of human APP display a loss of synaptic density and behavioral phenotypes before amyloid plaques appear in the brain (14–16). In addition, microinjection of Aβ oligomer preparations into the brains of rats inhibits long-term potentiation, a process involved in memory formation (17).

Role of cholesterol in Aβ generation

Emerging from the established genetic dispositions of AD is an association between plasma cholesterol and AD (18, 19). Retrospective analysis of the effect of cholesterol-lowering HMG-CoA reductase inhibitors (statins) on plasma cholesterol levels and coronary heart disease suggests that statins significantly reduce AD development. One study of 57,104 patients over 60 years of age who were taking lovastatin or pravastatin showed a 60–73% lower incidence of AD (20). Another study concluded that individuals 50 years and older who were treated with statins had a substantially lower risk of developing dementia, independent of the presence or absence of hyperlipidemia (21). Whether these apparent benefits are due directly to a reduction in plasma or brain cholesterol or perhaps to a pleiotropic effect of statins is not clear at the present time and will require confirmatory prospective trials (see below).

These suggestive clinical observations correlate with in vivo and in vitro evidence, indicating a role for cholesterol in APP processing and Aβ generation. Rabbits fed a diet enriched with cholesterol had increased levels of Aβ in the brain (22). In transgenic mice expressing a mutant human APP, Aβ deposits increased in the brain along with plasma cholesterol levels (23, 24). Interestingly, the increased Aβ deposits correlated with reduced levels of APPsα (23), suggesting that the hypercholesterolemia

may have altered APP processing, reducing the contribution of the α-secretase pathway (Fig. 1).

A cautionary note must be added to these cholesterol-feeding studies. An increase in plasma cholesterol of several-fold does not commonly occur in humans and raises the possibility of associated vascular damage with these extreme cholesterol concentrations. It is known that the blood-brain barrier is compromised in apoE-knockout mice (25), an animal model characterized by grossly elevated plasma cholesterol levels and accelerated atherosclerosis. Therefore, in the cholesterol-feeding models, it is possible that lipoproteins may "leak" into the brain through a damaged blood-brain barrier, increasing neuronal cholesterol content and thereby affecting Aβ processing.

Consistent with the in vivo observations, plasma membrane cholesterol levels modulate APP processing by the α-secretase pathway in vitro (5). Treatment of neuronal and nonneuronal cell lines with either cholesterol-extracting agents or with statins dramatically increased α-secretase activity and the release of the neurotrophic APPsα fragment, and concomitantly decreased β-secretase activity. Moreover, cellular sites with increased APPsα production were membrane regions with low cholesterol concentrations and high fluidity. Statin-induced reduction of cellular cholesterol levels resulted in reduced generation of Aβ-42 and Aβ-40 both in vitro and in vivo (26). Collectively, these studies support a role for cellular cholesterol in modulating Aβ production.

The mechanism by which cholesterol modulates the proteolytic cleavage of APP is unclear. However, the effect of cholesterol on membrane fluidity is potentially important. As first suggested by in vitro studies, increased plasma membrane fluidity may enhance APP/α-secretase interactions and α-secretase enzymatic activity (5). In contrast, rigid cholesterol-enriched membranes may reduce APP/α-secretase interactions and promote β- and γ-secretase processing (27). In support of this suggestion, γ-secretase activity has been identified in cholesterol- and sphingolipid-rich membrane microdomains known as lipid rafts (27, 28). Lipid rafts appear to promote the accumulation of Aβ and may initiate Aβ aggregation (29). However, the amount of free cholesterol in membranes may not tell the complete story. For example, acetyl-coenzyme A:cholesterol acyltransferase, an enzyme that esterifies cellular cholesterol, appears to play a role in Aβ production by controlling the ratio of esterified to unesterified cholesterol within cells (30).

HOW DOES THE BRAIN MAINTAIN CHOLESTEROL HOMEOSTATIS?

A detailed discussion of cholesterol homeostasis in the brain was recently published (31). Relevant highlights will be presented here to set the stage for discussing brain cholesterol metabolism in the context of AD. The brain contains about 2% of the total body cholesterol, of which most is unesterified. It is found in the plasma membranes of glial cells, which provide structural and metabolic support to neurons, in neuronal membranes, and in the myelin sheaths that insulate and protect neurons. Under normal conditions, essentially all of the cholesterol in the brain is synthesized locally (31). The blood-brain barrier prevents any real contribution from plasma lipoproteins (**Fig. 2**). Thus, mechanisms that remove cholesterol from the brain are required for cholesterol homeostasis. Outside the brain in the blood, this is accomplished by lipoproteins that transport cholesterol derived from the diet or from peripheral cells to cell-surface lipoprotein receptors in the liver, including members of the LDL receptor family. In the liver, a series of enzymes converts the excess cholesterol into bile acids, which are secreted into the bile and eventually excreted (32). This reverse cholesterol transport process is well understood with respect to lipoprotein carriers, receptors, lipid transfer proteins, cellular cholesterol, bile acid transporters, and regulation by nuclear hormone receptors (31, 32). This is not the case in the brain, where details are just emerging.

To be transported across the blood-brain barrier, most cholesterol is thought to be converted to 24(*S*)-hydroxycholesterol, a soluble oxysterol that can diffuse across the barrier, enter the blood circulation, and be taken up directly by the liver for excretion (Fig. 2) (33, 34). The enzyme suggested to perform this conversion is cholesterol 24-hydroxylase or Cyp46, a new sub-family member of the cytochrome P450 enzymes. Cyp46 is highly expressed in the brain (35) and is expressed in neurons in the cerebral cortex, hippocampus, and dentate gyrus (36) (the same neurons that are preferentially targeted in AD).

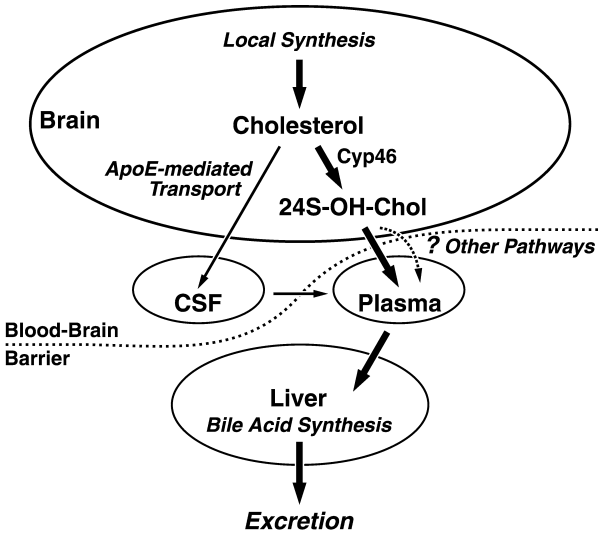

Fig. 2. Brain cholesterol homeostasis. Essentially all brain cholesterol is derived from local synthesis. A major portion of cholesterol exits the brain by conversion to 24*S*-hydroxylcholesterol by Cyp46 and diffuses across the blood-brain barrier. A minor portion exits via an apoE-mediated pathway through the cerebrospinal fluid. Other undefined pathways account for the balance of cholesterol export. Once in plasma, the 24*S*-hydroxylcholesterol or apoE-transported cholesterol is taken up by the liver, converted to bile acids, and excreted.

Definitive proof that Cyp46 is actually responsible for the hydroxylation and subsequent transport was recently obtained in Cyp46-knockout mice (37), which have significantly reduced levels of 24-hydroxycholesterol in the brain. Sterol balance studies in these mice demonstrated that hepatic cholesterol and bile acid metabolism were unchanged. Interestingly, brain cholesterol synthesis was reduced by 40%, while steady-state concentrations of brain cholesterol were virtually unchanged. These results demonstrate that Cyp46 mediates the turnover of a major portion of brain cholesterol and that the synthesis and secretion of brain cholesterol are coupled. An important question raised by these studies is whether Cyp46 activity changes the distribution of cholesterol in the various brain compartments, thereby affecting APP processing. These studies also indicate that brain cholesterol is removed by mechanisms unrelated to Cyp46 (Fig. 2). For example, a small fraction of brain cholesterol is transported from the cerebrospinal fluid (CSF) to plasma via a pathway mediated by apolipoprotein E (apoE) (38); however, additional pathways likely exist.

RELATIONSHIP OF CYP46 TO AD

Most of the 24-hydroxycholesterol in circulation originates from the brain (36). Since neurodegeneration involves degradation of neuronal cell membranes and release of cholesterol, the relationship of plasma concentrations of this oxysterol to brain cholesterol metabolism was examined. In a study comparing AD subjects with healthy age-matched controls, depressed subjects, and subjects with vascular dementia not related to AD, the plasma levels of 24-hydroxycholesterol were significantly elevated only in subjects with AD or vascular dementia (39). Another study showed increased 24-hydroxycholesterol levels in the CSF of AD subjects (40). These results suggest that neuronal death is coupled with increased flux of cholesterol from the brain. In addition, 24-hydroxycholesterol is neurotoxic and may directly contribute to neurodegeneration (41). However, 24-hydroxycholesterol concentrations are decreased in cases of advanced AD (42). In a recent study, three statins (lovastatin, simvastatin, and pravastatin) and niacin reduced plasma concentrations of 24-hydroxycholesterol in AD subjects (43). It is not clear how much of the reduction was due to decreases in LDL, which transports 24-hydroxycholesterol released from the brain, versus a direct effect on brain cholesterol metabolism. In normal brains, Cyp46 is primarily expressed in neurons, but in AD brains, neuronal expression is decreased and glial expression is markedly increased (44). The significance of this shift in expression and its role in neurodegeneration are not known.

In addition to its role in cholesterol efflux, 24-hydroxycholesterol has a second potential role in the brain as a ligand for the nuclear hormone receptors, liver X receptors (LXRs) (45, 46), which are potent activators of several genes involved in lipid metabolism. Of particular interest, LXRβ is highly expressed in the brain, although its function in brain cholesterol metabolism is unknown. The distribution of brain expression of LXRβ overlaps with that of Cyp46.

In a study of two independent European populations, an intronic polymorphism in *CYP46* was associated with increased Aβ amyloid deposits, increased tau phosphorylation, and increased risk of AD (47). In patients with apoE4, a synergy was noted in these end points. However, it was not determined if the *CYP46* polymorphism actually affected Cyp46 activity. In a study of two different ethnic American groups, this association did not hold up (48). Additional studies will be required to resolve this issue.

One challenge for the future will be to determine the role of Cyp46 activity in brain cholesterol metabolism and AD. Cyp46-knockout mice should prove informative in this regard with the interesting coupling of Cyp46 activity to cholesterol synthesis. The shift from neuronal to glial expression of Cyp46 in AD is also likely to be of importance.

ROLE OF APOE IN CHOLESTEROL TRANSPORT AND AD

The major lipid transport proteins in the central nervous system are apoE and apoA-I, which are present on spherical and discoidal particles of the size of HDLs (38, 49). Therefore, it seems likely that they would be involved in any cholesterol effect on AD through their lipid transport functions. The role of apoA-I in the brain is not clear. Originally, apoA-I in the CSF was thought to result from infiltration from blood. However, recent in situ hybridization evidence suggests that spinal cord neurons express apoA-I (50). ApoA-I is a potent mediator of cholesterol efflux, and this may be its role in brain cholesterol metabolism.

ApoE in the brain is derived from local synthesis, primarily by glial cells (51), with little contribution from plasma (**Fig. 3A**) (52). Evidence also suggests that, at least under certain conditions, neurons can express apoE (53, 54). The lipoproteins that are synthesized and secreted by the glial cells provide lipids to neurons for membrane synthesis during synaptogenesis and repair (Fig. 3A). Recently, it was suggested that neurons might depend entirely on cholesterol from extra-neuronal sources as a way of conserving the cost of sterol synthesis, allowing the neuron to focus its energy resources on its specialized function of generating electrical activity (55). Supporting a role for apoE in neuronal plasticity and repair is the demonstration that glia-derived cholesterol, delivered by apoE to neurons, promotes synaptogenesis (56).

As a major lipid transporter in the brain, apoE takes on added significance. ApoE-4, one of the three common human isoforms, is a major risk factor for AD, accounting for 40–60% of the genetic variation in the disease (57–59). ApoE-4 is also a significant risk factor in other forms of neuronal damage, including poor recovery from head injury (60) and other central nervous system stresses (61). It was hypothesized that apoE plays a key role in the normal

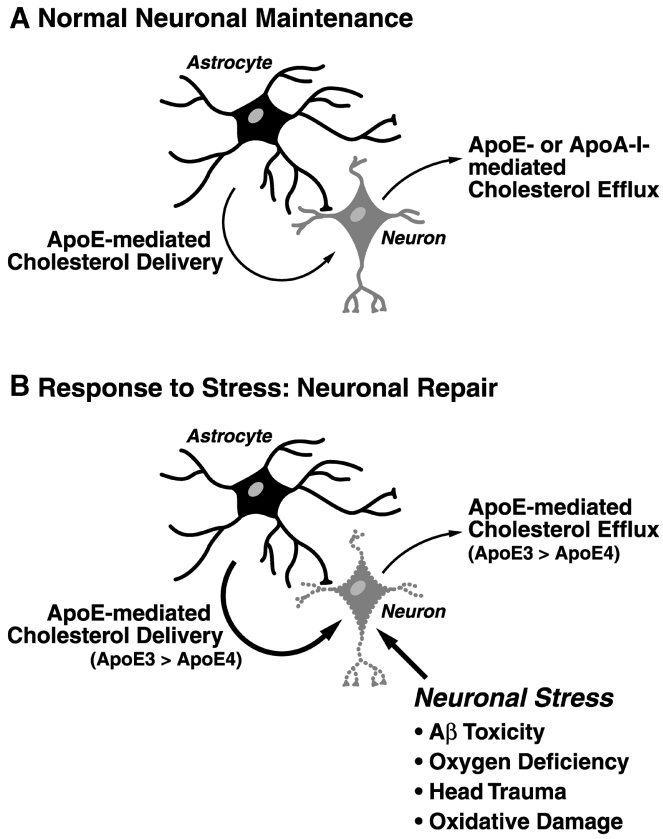

Fig. 3. Neuronal maintenance, plasticity, and repair. A: In normal neuronal maintenance and plasticity, cholesterol required for membrane synthesis is supplied by astrocytes by apoE-mediated transport, and excess cholesterol is effluxed to apoE- or apoA-I-containing lipoproteins. B: In response to stresses that require major neuronal repair, cholesterol from astrocytes is delivered more effectively by apoE-3 than apoE-4. ApoE-3 may also be more effective than apoE-4 in mediating cholesterol efflux and maintaining neuronal cholesterol homeostasis.

maintenance and remodeling (plasticity) of neurons, as well as repair in response to injury, and that apoE-4 is much less effective in these processes than apoE-3 or apoE-2 (62). Several studies in apoE knockout mice support this role for apoE. For example, it was demonstrated that apoE knockout mice had significant reductions in the levels of brain cholinergic and noradrenergic nerve terminals and these deficits were reversed in apoE transgenic mice on the apoE knockout background (63). Also, more severe neurological and cognitive deficits were observed following closed head injury in apoE knockout mice than controls (64).

The mechanisms by which apoE-4 exerts its effects in neurodegeneration and neuronal repair are largely unknown. Many possibilities have been suggested that are not related to lipid or cholesterol and that include direct interactions with Aβ, tau, or the cytoskeleton, [reviewed in refs. (62, 65–67)]. Here the focus will be limited to potential apoE effects related to cholesterol transport and metabolism.

Since apoE binds to lipoproteins in an isoform-specific manner (68), it is likely that lipoproteins containing apoE-3 differ in composition from those containing apoE-4. There is experimental evidence to support this suggestion. When lipoproteins from primary cultures of astrocytes from human apoE-3 and apoE-4 transgenic mice on a mouse apoE-knockout background were analyzed, lipoproteins from the apoE-4-expressing cells were slightly larger than those from the apoE-3-expressing cells (49). In astrocytes from mice in which the human apoE gene was "knocked" into the mouse *Apoe* locus, apoE-3-containing lipoproteins contained more cholesterol per particle than apoE-4-containing lipoproteins, suggesting that apoE-3 may be more effective in delivering cholesterol to neurons for normal maintenance, synaptogenesis, or repair (69). Consistent with this finding, in cocultures of astrocytes and neurons from human apoE transgenic mice, apoE-3-containing lipoproteins supported neurite outgrowth more effectively than apoE-4-containing lipoproteins (70). ApoE also appears to have isoform-specific effects on cholesterol efflux from neurons, with exogenously added apoE-3 being more effective than apoE-4 (71). A polymorphism in the ATP-binding cassette transporter AI (ABCAI), which mediates cholesterol efflux from cells, lowers CSF cholesterol levels and is associated with a delay of 1.7 years in AD onset in three different populations (72). In vitro studies on the effect of ABCAI on Aβ production are inconclusive (70, 73, 74).

These observations suggest that cholesterol efflux from neurons is an important aspect of neuronal maintenance. Perhaps there is a parallel between atherosclerosis and AD in which, if the input of cholesterol exceeds output, the balance is tipped toward a pathological state. Evidence to date indicates that apoE is critical in the transport of cholesterol and other lipids in the brain for normal neuronal maintenance or repair after an injury. Neuronal injury could result from Aβ-induced injury, deprivation of oxygen, acute head trauma, oxidative stress, or any other insult that requires a repair response (Fig. 3B). Since AD manifests symptoms after decades, the relative inability of apoE-4 to respond effectively to chronic insults provides, in addition to its nonlipid-related effects, a potential explanation for the strong association of apoE-4 with AD.

CONCLUSIONS

Evidence from epidemiological, in vitro, and in vivo studies suggests that brain cholesterol metabolism may play role in AD. While the exact nature and magnitude of this role is unknown, a number of possibilities have emerged, including modulation of APP cleavage pathways and Aβ production and clearance, apoE-mediated cholesterol transport, and cholesterol efflux from the brain. At this point, the evidence is circumstantial and key questions remain. For example, does the plasma cholesterol concentration, or a particular class of lipoproteins, directly influence brain cholesterol metabolism or Aβ production in the presence of an intact blood-brain barrier?

The suggested link between cholesterol metabolism and AD has opened a new area for AD research with the potential to identify new therapeutic strategies for treating this devastating disorder. In this regard, the preliminary evidence with statins suggesting their beneficial effects are of potential importance. Based on these results and the suggested link between cholesterol and AD, the National Institute of Aging is organizing a nationwide clinical trial to determine the safety and efficacy of simvastatin in slowing the progression of AD. This 18-month trial (Cholesterol-Lowering Agent to Slow the Progression of Alzheimer's Disease Study, or CLASP) will recruit 400 participants with mild to moderate AD. The results from this study should help clarify the benefits of the long-term use of statins in delaying the onset of AD. Hopefully, this study will also provide insight to distinguish between the importance of plasma or brain cholesterol-lowering effects and the potential pleiotropic effects mediated by statins.

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