Full-length review

The neurobiology of apolipoproteins and their receptors in the CNS and Alzheimer's disease

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Abstract

The importance of apolipoproteins in the central nervous system became increasingly clear with the association in 1993 of the $\epsilon 4$ allele of apolipoprotein E with familial and sporadic late-onset Alzheimer's disease. Apolipoprotein E is a ligand for several receptors, most of which are found to some extent in the brain. This review summarizes the various apolipoproteins and lipoprotein receptors found in the brain. A growing body of evidence now implicates irregular lipoprotein metabolism in several neurodegenerative disorders. We then focus on research linking apolipoprotein E and Alzheimer's disease, from clinical studies to biochemical models, which may explain some of the complex neurobiology of this disorder. © 1998 Elsevier Science B.V. All rights reserved.

Contents

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1. Introduction

The discovery of apolipoproteins and their receptors was due in large part to their role in plasma lipoprotein metabolism and cholesterol homeostasis. However, new functions for these proteins have been discovered, especially with regards to the nervous system. This review will focus on the role of apolipoproteins and their receptors in the central nervous system (CNS), with emphasis on apolipoprotein E (ApoE) in the neurodegenerative disorder Alzheimer's disease (AD). The pathways for receptor-mediated lipoprotein metabolism, cholesterol homeostasis and apoE structure–function relationships have been extensively studied and reviewed elsewhere. For a review of the low-density-lipoprotein (LDL) receptor pathway, see Brown and Goldstein [30]; for a review of the role of apoE in the redistribution of cholesterol among cells and tissues, see Mahley [147]; for a review of apoE structure–function relationships, see Weisgraber [282].

2. (Apo)lipoproteins in the central nervous system

Cholesterol and other lipids are used for membrane synthesis and for many other anabolic or catabolic activities by cells throughout the body including those of the CNS, a site of high lipid turnover [6,33,229]. Although cells composing the nervous tissue are capable of de novo synthesis of lipid molecules, they can also bind and take-up lipoproteins made available in the local environment for their lipid requirements [202,209]. Since the blood–brain barrier presumably prevents the passage of whole macromolecular complexes, lipoprotein particles are thought to

be assembled locally in the CNS using components imported from the plasma or, alternatively, they would originate from local synthesis and secretion. However, the size and composition, or even the presence of such particles in the CNS interstitium have not been determined so far. On the other hand, lipoprotein particles the size of plasma high-density lipoprotein (HDL) or larger, are known to be present in the cerebrospinal fluid (CSF) [24,123,203,230]. Some of these particles are believed to derive from the brain tissue and to be involved in the process of reverse cholesterol transport of excess cholesterol from the brain to the liver [203]. Lipoproteins in the CSF might represent a potential source of lipids for cells of the nervous parenchyma as well. In that respect, it is of note that receptors for lipoproteins have been identified on ependymal cells, as well as on perivascular and CSF-contacting astrocytic foot processes, and thus have been suggested to play a role in lipid homeostasis and transport into the CNS [121,142,203,292,298].

In addition to lipids, as their name also implies, lipoproteins contain a protein moiety referred to as apolipoproteins (apos), some of which are regulators for extracellular enzymatic reactions involved in lipid metabolism. Other apos are ligands for cell receptors that mediate the influx of lipoprotein particles, and their subsequent intracellular metabolism. Little is known about the presence of the different apos in the brain parenchyma. Messenger RNAs coding for most of the known apos such as apoA-I, apoA-II, apoA-IV, apoB, apoC-II, apoC-III, apoF and apoH could not be detected in total brain extracts from various mammalian species by Northern blot, RNA dot blot or RNase protection analyses, although these results cannot exclude the possibility of low levels of

mRNA expression in specific cell populations [27,46,48,56,64,128,176,203,251,255]. On the other hand, with the notable exception of intact apoB, several apos including apoA-I, apoA-II, apoA-IV, apoC-I, apoC-II, apoC-III, apoD, apoE and apoJ were shown to be present in the CSF of various species, mostly in the form of lipoproteins [24,36,62,123,203,230,238]. These apolipoproteins would arise from local synthesis and/or filtration from plasma. In humans, the CSF concentration for apoA-I is less than 0.5% of its plasma concentration [230]. For most other apos, including apoC-II, apoC-III, apoE, and apoJ, the CSF-to-plasma ratio is 2–5% [36,230]. Using minimal disruptive fractionation procedures, Borghini et al. [24] have shown that CSF apoA-I- and/or apoE-containing lipoproteins are in the same size range of small plasma low-density lipoproteins. A third subpopulation of lipoprotein particles had a larger and more heterogeneous diameter in the range of the intermediate-to-very low-density lipoproteins of plasma [24].

2.1. Peripheral apolipoproteins found in the CNS

The demonstration that apoA-IV, a ~ 44 kDa protein and major component of newly synthesized chylomicrons from human intestine and also of HDL in rats, acts centrally to inhibit gastric acid secretion and regulate food satiety, suggests that this apolipoprotein might enter the brain through the blood–brain barrier or circumventricular organs [62,185]. Moreover, apoA-I, the major protein constituent of plasma HDL and activator for lecithin:cholesterol acyltransferase (LCAT: a key enzyme in reverse cholesterol transport catalyzing cholesterol esterification), was shown to be synthesized and secreted by endothelial cells from capillaries of the mammalian brain [27,166]. Furthermore, apoA-I mRNA was reported to be present, albeit at relatively low levels, in extracts from pig choroid plexus, and it was postulated that the protein might be secreted into neuronal interstitium [281]. In this regard, it is of interest that LCAT mRNA was found in scattered neurons and glial cells of the gray matter, in ependymal cells, as well as in some cells of the cerebellum in brains of primates [255]. It is noteworthy that LCAT activity has been reported to be present in the CSF of human subjects [103]. Moreover, both human and rat brain contain an additional cholesterol-esterifying activity different from LCAT, which is predominantly localized in the white matter [111]. Thus, the necessary enzymatic machinery to esterify cholesterol may be present throughout the CNS.

2.2. Locally synthesized apolipoproteins of the CNS

2.2.1. Apolipoprotein E
The brain is a major site of apoE mRNA expression in humans, marmosets, rats and mice, ranking second only to liver in humans [55]. Transcripts for apoE are distributed throughout all regions of the brain, and have been local-

ized to astrocytes and microglia by in situ hybridization (see Table 1) [51,55,213,266]. Accordingly, apoE was shown to be synthesized and secreted mostly by glial cells, particularly astrocytes, but not by neurons and to serve as a ligand for the low density lipoprotein receptor (LDLR) in primary cultures of rat brain astrocytes [27,174,203,265]. In contrast with the human brain, the mouse brain contains high levels of apoE in the ependyma and choroid plexus [142].

In the adult human and rat brains, immunoreactivity against apoE appeared of equal intensity for protoplasmic astrocytes of gray matter and fibrous astrocytes of white matter, and was observed in all major subdivisions [27,170]. Specialized astrocytic cells such as Bergmann glia, tanycytes and pituicytes of the neurohypophysis as well as basement membranes at either the pial surface or along blood vessels, also showed positive staining for apoE, whereas oligodendrocytes, ependymocytes, the choroidal epithelium and neurons were immunonegative [27,170]. However, primary cultures of hippocampal neurons from rat embryos have the capacity to internalize apoE-containing lipoproteins [11]. Intraneuronal localization of apoE has been observed in several other studies. It has recently been reported that many hippocampal neurons without neurofibrillary changes in two of six nondemented older human individuals, as well as in several cases of Parkinson's and Alzheimer's disease patients, showed some reactivity towards apoE by immunocytochemistry [79]. Similar findings had been previously reported for aged prosimian primate brains [267]. Abnormal neurons containing neurofibrillary tangles in brains of individuals with AD may also contain apoE [79,175,225,245,290]. Using tissue sections of cortex from younger patients with epilepsy, apoE immunoreactivity in neurons was shown by immunoelectron microscopy to be confined to the cytoplasm of cell bodies and proximal dendrites in association with the external membrane surface of some organelles [78]. These results suggest that apoE may affect neuronal metabolism in additional ways not related to cholesterol homeostasis or, alternatively, be involved in the intracellular transport of lipids.

According to current views, apoE is the main apolipoprotein produced and secreted within the brain parenchyma, where it is presumably involved in the redistribution of lipids among cells and in the regulation of cholesterol homeostasis, although additional functions unrelated to lipid transport have been proposed [147,284]. However, the brain also represents a major site of synthesis for two other apos found to be associated with specific subclasses of plasma HDL [49,161].

2.2.2. Apolipoprotein J
One of these two is believed to be a multifunctional protein, has a wide tissue distribution, and is referred to as apoJ (also known as clusterin, SP-40,40 or SGP-2; for reviews see Refs. [110,157,165,231]. Transcripts for apoJ

Table 1
Known presence and synthesis of apolipoproteins and their receptors in the central nervous system of various species

	Neurons		Astrocytes		Oligodendrocytes		Microglia		Ependymocytes	
	Protein	mRNA	Protein	mRNA	Protein	mRNA	Protein	mRNA	Protein	mRNA
ApoE	+Human [26] −Rat [78, 164]	−Human [51]	+Human [78,225,170] +Rat [27,202,265]	+Human [51] +Rat [213]	+Rat [26] −Rat [265]		−Rat [27]	+Rat [174,266]	+Human [78] −Rat [27]	+Mouse [142]
ApoJ	+Human [162] +Mouse [180] +Rat [248] −Rat [192]	+Human [158,192] +Rat [65,44,192]	+Human [293] +Rat [301,192]	+Human [192] +Rat [192,301]	−Human [293]		−Rat [192]	−Rat [192]		+Rat [65,44]
ApoD	+Rat [26,187]	+Monkey [255]	+Mouse [193] +Rat [26]							−Monkey [255]
LDLr		+Rabbit [271] +Rat [121]	+Human [225] +Rat [117]		+Rat [117]		+Human [116]	+Human [116]		
VLDLr	+Human [102,37] +Rat [102]	+Rat [94]	+Human [102] −Human [37] +Rat [102]	+Human [94]			+Human [37]		−Human [37] +Rat [102]	
ApoER2		+Rat [121]								+Rat [121]
LRP	+Human [167,292,223,275] +Rat [298]	+Mouse [142] +Rat [32,106]	+Human [167,292,225] +Rat [298]		−Human [167]				−Human [292]	+Mouse [142]
Megalin	−Rat [298]		−Rat [298]						+Rat [298]	
MSR-A	−Human [38]		−Human [38]				+Human [38]			

(+) denotes that protein or messenger RNA has been observed [or not (−)] in the corresponding cell type, on brain sections or cultured cells from the indicated species.
Many or only a few cells of a given type may show positivity.
Signal intensities also vary among positive brain cells within or outside a region.

are distributed throughout the human temporal cortex and hippocampal formation and throughout the rat CNS, although some areas like the ependymal lining of the ventricles and many neuronal populations including motor neurons of the spinal cord, various hypothalamic and brainstem nuclei as well as the habenula, demonstrate a higher prevalence [45,65,192]. Variable levels of apoJ mRNA are found in scattered astrocytes, whereas the messenger appears to be absent from both microglial and endothelial cells [45,192]. It is noteworthy that astrocytes near blood vessels in both the white and gray matter as well as the glia limitans contain relatively high levels of apoJ mRNA, thus supporting the hypothesis that apoJ plays a role in lipid mobilization into and out of the CNS [44]. Furthermore, primary cultures of astrocytes, in contrast to non-stimulated neurons, have been shown to constitutively secrete apoJ, a 70–80 kDa heterodimeric sulfated glycoprotein, into their medium [192,301]. Consistent with this is the observation that astrocytes are, in general, poorly stained in comparison to neurons using immunohistochemistry on tissue sections from rat brain [180,248]. Besides its role in lipid transport, apoJ has been postulated to be involved in complement inhibiting cytolytic activity, intracellular vesicular packaging and cellular adhesion, and is considered as a marker for brain injury and pathology in general [110,165,231]. Indeed, apoJ mRNA dramatically increases locally in rat astrocytes after brain lesioning [44,159,190,191], and high levels of transcripts or protein product have been reported in nervous tissues from patients with AD, Pick's disease, epilepsy, gliomas, retinitis pigmentosa, multiple sclerosis and AIDS [45,53,113,158,293].

2.2.3. Apolipoprotein D

The other apolipoprotein is apoD, a 27–33 kDa glycoprotein. ApoD shares some sequence similarities with members of the lipocalin family, a group of proteins that transport small hydrophobic ligands, and has been suggested to represent a steroid binding/sequestering or multi-ligand protein [218,219]. In the plasma, apoD is found associated with LCAT, and it was postulated that apoD stabilizes the enzyme's activity, unlike two other well-known LCAT activators, apoA-I and apoC-I [264]. Numerous types of cells in various rat tissues contain apoD as shown by immunohistochemistry [26]. Although levels of basal expression can vary considerably, its mRNA has a wide tissue distribution in all species examined, and is expressed mostly in fibroblast-like cells often associated with blood vessels [52,218–220,247,255]. ApoD transcripts transiently increase 40-fold in endoneurial fibroblasts following a crush injury of the rat sciatic nerve [259]. During regeneration of the peripheral nerve, apoD increases in concentration over 500-fold, and accumulates in lipoprotein particles within the nerve's interstitial matrix, where it could be involved in lipid transfer [25]. The rat brain showed strong immunoreactivity against apoD in fibrous astrocytes and pial cells [26]. Positive staining was also seen in protoplasmic astrocytes, oligodendrocytes as well as in some neurons, endothelial and perivascular cells [26]. However, it is unknown whether the latter observations are due to secretion or uptake of apoD by these cells. On the other hand, Northern blot analysis of total RNA extracted from gray and white matters of human brains showed that, in contrast to the gray matter, the white matter is a major site of apoD gene expression, which supports the hypothesis of apoD synthesis in fibrous astrocytes and/or oligodendrocytes [219]. Furthermore, apoD mRNA was mainly localized to the subarachnoid space and to scattered glial cells of the white matter throughout the rabbit CNS by in situ hybridization [219]. Very scattered positive neurons in gray matter areas could not be ruled out in the last study. Scattered glial cells and some neurons were identified as positive in another study on rhesus monkey brain tissue, whereas no hybridization to endothelial nor to ependymal cells was observed [255]. Finally, primary astrocyte cultures from neonatal mouse brain were shown to synthesize and constitutively secrete apoD [193], while oligodendrocytes in the rat spinal cord express apoD mRNA [243]. Thus, different glial cell types and possibly neurons may provide the CNS tissue with apoD.

2.2.4. Apolipoprotein C-I

A messenger RNA coding for yet another apolipoprotein known as apoC-I is relatively abundant (21% relative to liver level) in rat total brain extracts, but less prevalent (5% relative to liver level) in human brain and marmoset cerebral cortices, and seemingly absent from the brain of dogs [132,144,249]. However, the cell type(s) responsible for apoC-I mRNA synthesis in the mammalian brain has not been identified. Human apoC-I is a small polypeptide of 57 amino acids, mainly produced by the liver, that possess high binding capacity for phospholipids, as well as the ability to activate LCAT and to redistribute itself among lipoprotein classes during their metabolic remodeling [3,108,149]. Also, apoC-I can displace apoE from the surface of lipoproteins and thereby affect their binding properties to the low density lipoprotein receptor-related protein (LRP) [270]. Since LRP is expressed in the CNS of mammals, it is thus plausible that apoC-I or the apoC-I/apoE molar ratio play an important role in lipid homeostasis in that compartment, as it does in the periphery [251].

3. Lipoprotein receptors of the central nervous system

In addition to the LRP, several other receptors for lipoproteins were shown to be expressed in the mammalian brain including the LDLR, the very low density lipoprotein receptor (VLDLR), gp330/megalin, and the recently described apolipoprotein E receptor 2 (apoER2) [121]. All of

these receptors are members of a single family of proteins that share structural and functional similarities. They all bind and internalize apoE-containing lipoproteins, although LRP and gp330 possess several other ligands as well, and the LDLR is known as the apoB receptor in peripheral organs [30,288,289]. Interestingly, it was recently demonstrated that apoJ binds with high affinity to gp330 from kidney, and its internalization and degradation in mouse embryocarcinoma F9 cells is also mediated by the gp330 receptor [124]. Antibodies against gp330 failed to stain neurons and astrocytes in rats, but the receptor has been shown to be expressed on the ependyma, mainly on the apical surface, as part of a restricted group of epithelial cells [298]. It remains to be determined if other receptors for apoJ exist within the nervous tissue. No receptors for apoD have been identified to date, whether inside or outside the CNS.

3.1. Low-density lipoprotein receptor

Astrocytes in vitro have been shown initially to express the prototype LDLR in the CNS and to be downregulated following internalization of lipoprotein cholesterol [202]. Astrocytic localization was subsequently confirmed by immunohistochemistry performed on brains from rats, marmosets and humans, where expression was found to be higher in areas of white matter [203,225]. Moreover, pial cells gave strong immunoreactivity, whereas little reactivity was observed with ependymal cells [203]. Other in vitro or ex vivo studies in various species, including human, demonstrated the presence of the LDLR on oligodendrocytes, microglia and brain capillary endothelial cells, and suggest that many cell types in the CNS can express the LDLR [50,116,117,163]. Although only occasional neurons were immunoreactive for LDLR in human brain, transcripts for LDLR were observed in various neuronal populations of the rabbit nervous system, and particularly in sensory and motor nuclei [203,271]. Levels of LDLR have been shown not to vary with age, indicating that LDL receptors are required even in the adult brain when new myelin synthesis is minimal [91].

3.2. Very low density lipoprotein receptor

As far as the VLDLR is concerned, immunofluorescence studies using an anti-VLDLR antibody on human fetal brain cultures gave intense labeling on neurons, whereas GFAP-positive astrocytes were weakly labeled [102]. VLDLR immunoreactivity was found in neurons and microglia from autopsy brain tissue in control and AD brains [37]. Neuronal staining was seen at the level of dendritic processes of cortical and hippocampal pyramidal cells as well as granule cells in the dentate gyrus [37]. Moreover, VLDLR transcripts were detectable in the rat cerebellar cortex and hippocampus [121]. However, the cell types responsible for their synthesis were not identified in this last study.

3.3. Low density lipoprotein receptor-related protein

The LRP antigen was localized by immunohistochemistry in the normal human brain to neuronal cell bodies and proximal processes, whereas other cell types were immunonegative, except perhaps for some lightly stained perivascular astrocytic foot processes [225,292]. However, it should be mentioned that one group reported the expression of LRP in some fibrillary and protoplasmic astrocytes of normal human brain [167], while others claim it to be expressed on astrocytes only when reactive [225]. Immunoreactivity in humans and in situ hybridization in rats and mice were substantial on granule and pyramidal neurons of the hippocampal formation [32,142,223]. LRP mRNA were expressed throughout the brain and, in addition to the hippocampus, they were also abundant in the cerebellum, the cerebral cortex and the brainstem [32,142].

3.4. Apolipoprotein E receptor 2 / LR8B

Three new members have recently been added to the LDLR family. The cDNA coding for the human apoER2 [121], the chicken and mouse LR8B [179] receptors, and the human sorLA-1/rabbit LR11 [109,295] have been isolated from their respective species brain tissues. These receptors are predicted to have an important future with regards to the CNS, since they all are predominantly expressed in the brain. ApoER2 has 50% amino acid sequence similarity in its ligand binding domain to both the human LDL and VLDL receptors, and has been shown to bind and internalize apoE-rich β-VLDL [121]. In rabbit tissue, mRNA for apoER2 was detected most intensely in the brain and testis, but was undetectable in almost all other tissues. Using in situ hybridization, apoER2 transcripts were detected at highest levels in the cerebellar cortex, choroid plexus, ependyma, hippocampus, olfactory bulb and, to a much lesser extent, in the cerebral cortex in adult rat brain [121]. The cDNAs coding for the chicken and mouse LR8B were cloned using RT-PCR and were shown to be 73% identical to apoER2 [179]. LR8B and apoER2 have subsequently been shown to be structural, and perhaps functionally distinct variants of a counterspecies protein and to arise from differential splicing of corresponding genes [29]. Although the receptor shows strong homology to the LDL and VLDL receptors, its physiological ligand remains to be determined.

3.5. SorLA-1 receptor

The sorLA-1 receptor is a receptor-associated protein (RAP)-binding receptor, a chaperone known to bind to

members of the LDLR family [31]. It is a hybrid-type receptor that contains a sortilin domain and fibronectin type III repeats in addition to LDLR class A and YWTD repeats [109]. The rabbit homologue LR11 is found predominantly in the cerebrum but is also present in the cerebellum and brainstem [295]. Immunoreactivity was shown in the hippocampus, dentate gyrus, cerebral cortex and at lower levels in the thalamus on rabbit brain sections [295]. The abundant expression in the CNS of the latter three receptors suggests that they have important functions in the metabolism of lipid particles in the brain.

3.6. Macrophage scavenger receptor class A

Finally, the macrophage scavenger receptor class A (MSR-A) was shown to be expressed on brain macrophages and microglia, but not on neurons or astrocytes using immunohistochemistry [13,38]. Increased immunoreactivity on activated microglia and recruited macrophages was observed in Alzheimer's disease, and in response to different forms of injury to the CNS in rodents [38,13,68]. Although the MSR-A is not structurally related to the members of the LDLR family, like the LRP and megalin, it is a multifunctional receptor capable of binding and internalizing both non-lipoprotein and lipoprotein ligands [126]. However, unlike the LDLR-related receptors, the macrophage scavenger receptor mediates the endocytosis of chemically modified LDL, such as oxidized LDL, rather than native LDL. This property of the MSR-A suggests that it may play a major role in protecting the nervous tissue against oxidative damage by clearing oxidatively modified macromolecules from degenerating cells and other potential source of free radicals. Some evidence suggests the existence of more than one receptor for modified LDL [126,268], and new receptors involved in CNS lipid homeostasis are likely to be discovered. Mice deficient in type I and type II MSR-A have been generated in order to gain insights into the role of scavenger receptors in atherosclerosis, cell proliferation, apoptosis, and immune functions [268,236,273,60]. Such animal models may prove to be valuable in the dissection of the respective role of these multifunctional proteins and other differentially regulated scavenger receptors in response to neurodegeneration [75]. The reason for the existence of so many different receptors capable of lipoprotein uptake into cells is presently unclear, but emphasizes the importance of lipid transport and metabolism, particularly in organs with a high lipid turnover such as the brain.

Thus, the mammalian CNS has the capability of synthesizing many of the components found in the periphery, including lipoprotein lipase [19,142], that are necessary for lipid transport and utilization. Local synthesis of these components may ensure rapid responses to high demands in lipids for cellular activity, maintenance, and plasticity particularly after brain injury.

4. Apolipoprotein E in neuronal plasticity

4.1. Neuronal plasticity in the peripheral nervous system

Initial work demonstrating a neuronal function for apoE dates back to 1986. At that time, models were characterized, in which a role for apoE was proposed for the coordinated storage and redistribution of cholesterol among cells of injured and regenerating peripheral nerves [100,256]. Following sciatic nerve crush in the rat, macrophage-secreted apoE levels increased 100- to 200-fold compared to controls [100]. ApoE synthesis peaked after about one week and slowly returned to baseline levels by eight weeks, when peripheral nerve regeneration is mostly complete [28]. From these data, it was proposed that apoE produced within the lesion vicinity scavenges cholesterol from the cellular and myelin debris, and delivers the lipids for storage in macrophages. The stored apoE may then be reutilized for axonal regeneration. To this end, the regenerating nerve sends out numerous growth cones or neurites, which express high levels of LDL receptors on their growing tips, presumably to recycle the lipids delivered by apoE for the purpose of new membrane synthesis [101]. This peripheral nerve model of axonal regeneration and remyelination involving apoE and LDL receptors is now well established, and serves as the basis for models characterizing the function of apoE in the CNS.

4.2. Neuronal plasticity in the central nervous system

A major difference between peripheral nerves and central nerves is that neurons in the CNS are unable to regenerate. However, specific areas such as the hippocampus demonstrate limited synaptic plasticity or reinnervation following injury. The entorhinal cortex (EC) is the main gateway of neural input to the hippocampus proper, and receives widespread neocortical and subcortical afferents. This large supply of multimodal information is sent into the body of the hippocampus via the perforant path [240]. Denervation of the dentate gyrus, due to loss of the perforant pathway projection from the EC, is proposed to contribute to the pathophysiology of AD [95,96]. Entorhinal cortex-lesioned (ECL) rats have been used extensively as a model to examine the molecular mechanisms associated with deafferentation and reinnervation in the CNS.

4.3. Entorhinal cortex lesioned rats as a model of CNS plasticity

Under defined conditions, entorhinal cortex lesions disrupt the perforant path, thereby removing cortical connections to the hippocampus, and causing a loss of nearly 60% of the synaptic input to the granule cell layer. However, this loss of synapses is transient. Beginning a few days

after denervation, new synapses are formed, compensating for the lost inputs within 2 months [156]. These new synapses originate from cholinergic septal neurons [146], glutamatergic commissural–associational pyramidal cells of the CA3/hilus areas [145,244] and, to a lesser extent, from neurons of the contralateral entorhinal cortex [263]. Ultrastructural studies of the hippocampal molecular layer of the dentate gyrus following ECL showed that throughout the 2–11 days postlesion, astrocytes progressively engulf both presynaptic terminals and preterminal axons [134]. Once metabolized, these neuron-derived particles generate a large astroglial store of lipids, providing a convenient and readily retrievable pool for membrane synthesis of precursors used in the formation of neuronal sprouts, and in the reorganization of the dendritic field of granule cell neurons (Fig. 1). Cholesterol, phospholipids and apoE are then combined to form an uncharacterized lipoprotein complex that may be secreted into the circulation and/or directed to specific target sites in the CNS. This sequence of compensatory changes associated with ECL has been shown to coincide with the increased ex-

pression of apoE in the deafferented zone of the molecular layer. The induction of apoE gene expression was shown to coincide with the early phase of reactive synaptogenesis and terminal proliferation. The increased expression of apoE in the deafferented zone appears to be restricted to the local astrocyte population [213].

The recycling of cholesterol may explain the reduced levels of cholesterol synthesis and increased apoE expression during active synaptogenesis [207]. Autoradiographic analysis of the LDL receptor binding sites in the deafferented zone of the hippocampus following ECL revealed an increased expression of the LDL receptor in granule cell neurons (undergoing dendritic remodeling and synaptogenesis) during the acute phase of the reinnervation process [209]. This occurred with a concomitant decrease in the activity of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (the rate-limiting enzyme in cholesterol synthesis and cell–cycle progression [70]), a finding that had also previously been reported in peripheral nerve tissue [72]. Hence, accumulation of cholesterol in astrocytes responsible for terminal degradation in the
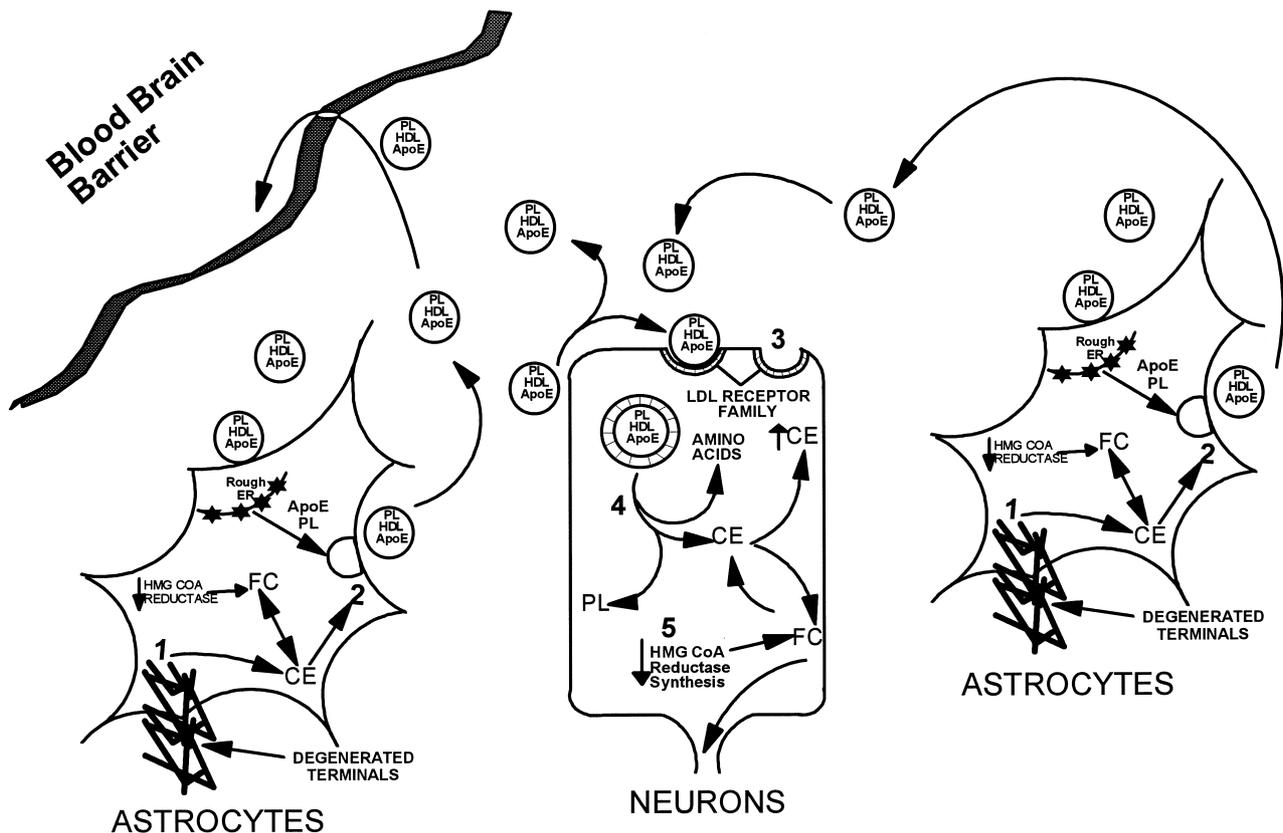
Fig. 1. Representative model of hypothesized cholesterol/phospholipid recycling mechanism in the injured hippocampus following entorhinal cortex lesions in the rat. CE: esterified cholesterol, ER: endoplasmic reticulum, FC: free cholesterol, HDL: high-density lipoprotein particle, PL: phospholipid. Lesions of the entorhinal cortex cause nerve terminals in the hippocampus to degenerate. These nerve terminals are initially internalized and degraded by astrocytes. The cholesterol derived from the membranes of nerve terminals (1) is used as FC and is then assembled into an apoE/FC/lipoprotein complex (2), or converted to CE for further storage. The complexes are then directed (a) into the circulation presumably through ependymal cells surrounding the ventricles and/or (b) to specific brain cells requiring lipids. ApoE complexes are then thought to be internalized (3) into neurons through an apoE receptor (LDL, LRP, etc.) and the cholesterol released (4) for the purpose of dendritic proliferation and/or synaptic remodeling. The increased levels of free cholesterol in the cells leads a reduction in the synthesis of HMG–CoA reductase (5).

molecular layer of the dentate gyrus could result in a cholesterol-mediated suppression of the HMG–CoA reductase transcription in astrocytes. Together, these data explain the downregulation of hippocampal HMG–CoA reductase activity, and suggest that apoE-containing lipoprotein complexes are being used in the CNS to recycle cholesterol/cholesterol esters derived from degraded terminals to sprouting neurons and neurons undergoing dendritic reorganization.

5. Polymorphic nature of human ApoE

An important biochemical characteristic of human apoE stems from a genetic polymorphism, first established by Utermann et al. [277] using isoelectric focusing. Polymorphisms within the human apoE gene (located on chromosome 19) account for the three major apoE isoforms, designated apoE2, apoE3 and apoE4, arising from respective alleles $\epsilon2$, $\epsilon3$ and $\epsilon4$. The result of this polymorphism is three homozygous genotypes ($\epsilon2/\epsilon2$, $\epsilon3/\epsilon3$, and $\epsilon4/\epsilon4$) and three heterozygous genotypes ($\epsilon2/\epsilon3$, $\epsilon2/\epsilon4$, and $\epsilon3/\epsilon4$). The most common isoform apoE3, differs from apoE2 and apoE4 by amino acid substitutions at residues 112 and 158 (apoE is 299 amino acids long). The apoE2 isoform has cysteine residues at sites 112 and 158, apoE3 has cysteine at site 112 and arginine at site 158, while apoE4 has arginine at both sites [221,222,283]. These single amino acid substitutions lead to a charge difference detectable by isoelectric focusing. The distribution of the alleles in the general population is approximately 8% for $\epsilon2$, 75% for $\epsilon3$ and 15% for $\epsilon4$ [277]. Functionally, apoE3 and apoE4 have a much higher affinity for the LDL receptor and LRP than does apoE2 [125,283]. This leads to lower serum apoE levels in $\epsilon3$ and $\epsilon4$ homozygotes than in $\epsilon2$ individuals [47]. The functional defect in binding of apoE2 to LDL receptors has been associated with familial type III hyperlipoproteinemia, a genetic disorder characterized by elevated plasma cholesterol levels and accelerated coronary artery disease [148,149]. In the nervous system, the importance of the polymorphic nature of apoE has recently been revealed, with regards to function in neuronal plasticity and with respect to other pathologies such as Alzheimer's disease (discussed in detail below).

6. ApoE isoforms on neurite outgrowth

Peripheral nerves normally contain low levels of apoE, however dramatic increases in the levels of apoE occur following denervating crush injury (see above) [25,28,100,133,256]. In vitro experiments using PC12 cells (a pheochromocytoma cell line) further demonstrated that apoE-containing lipoproteins obtained from regenerating nerves are internalized by a receptor mediated mechanism

[101]. In mixed cultures from fetal dorsal root ganglion cultures, incubation with β-very low density lipoprotein (β-VLDL) particles, which are rich in both apoE and cholesterol, increased neurite outgrowth and branching [80]. These results have been the basis for proposing that apoE-containing lipoproteins are involved in the mobilization and redistribution of lipid in the repair and maintenance of myelin and axonal membranes following peripheral nerve injury.

More recently, due to the increased significance of apoE isoforms and the particular role of apoE4 in AD, investigators reexamined these models for apoE isoform specific changes. Using dorsal root ganglion cells, it was found that apoE3-β-VLDL increased neurite extension and decreased the amount of branching, while apoE4-β-VLDL decreased both extension and branching [177]. Interestingly, neither apoE3 nor apoE4 lacking lipid particles had any effect on neurite branching or extension in this model. Similarly, in a central nervous system-derived neuronal cell line, apoE3 but not apoE4 was shown to increase neurite extension [92]. The effect of apoE3 was blocked at low nanomolar concentrations by a purified 39-kDa protein receptor associated protein (RAP). RAP regulates ligand binding to the low density lipoprotein receptor-related protein (LRP), as well as other receptors including the LDLR, VLDLR and gp330. Anti-LRP antibody also completely abolished the neurite-promoting effect of apoE3 [92]. These results all imply that a receptor-mediated event is likely responsible for the uptake of apoE-containing lipoproteins. However, although maximal effects on neurite extension were obtained with β-VLDL and VLDL-type lipoproteins, the presence of these particles in the CNS is still unclear. However, recent experiments using HDL-like lipid particles obtained from bovine plasma and CSF did reveal parallel results to those obtained using β-VLDL [14,57]. Taken together, these data suggest that apoE4, which has been associated with late onset familial and sporadic AD, may be potentially detrimental to neurons by inhibiting regeneration and thereby contribute to the pathogenesis of the disease. More importantly, Arendt et al. recently confirmed this hypothesis with data demonstrating that Alzheimer's disease patients carrying one or two apoE $\epsilon4$ alleles had more severe neuronal degeneration as well as significantly less neuronal plasticity in several brain areas than in patients lacking the $\epsilon4$ allele [8].

7. ApoE-deficient mice

Due to the key role of apoE in lipid transport and the pathology of atherosclerosis, apoE-deficient or knockout mice were created [206]. These mice have recently been used for investigations into the potential importance of apoE in the nervous system. Popko et al. [216] and Goodrum et al. [73] demonstrated that peripheral nerve regeneration following sciatic nerve crush occurred equally

as well in apoE deficient mice compared to control animals. These results indicated that nerve repair and reutilization of cholesterol were not totally dependent on apoE, perhaps indicative of the redundancy of apolipoproteins in peripheral nerve regeneration. However, Masliah et al. [154] found an age-related 15–40% loss of synaptophysin-positive nerve terminals, and microtubule-associated protein 2-immunoreactive dendrites in the neocortex and hippocampus in apoE deficient mice when compared to controls. These results imply that in the CNS, apoE indeed plays an important role in plasticity and integrity of synapses and nerve terminals during aging, perhaps due to the absence of other functionally similar apolipoproteins that are more readily available in the PNS. Masliah et al. [153] further analyzed the patterns of denervation and reinnervation in the dentate gyrus after perforant pathway transection of apoE-deficient mice. Their results indicate that the absence of functional apoE can lead to abnormal synaptic regeneration. Moreover, a significant loss of synapses, disruption of the cytoskeleton and a poor reparative ability after lesion were observed in homozygous apoE-deficient mice. Behaviorally, apoE deficient mice display impairments in cognitive performance using the Morris water maze [74,127,212]. When injected with either recombinant apoE3 or apoE4 isoforms into the lateral ventricles, apoE-deficient mice demonstrated significant improvements in their learning capacity in the Morris water maze compared to saline-infused mice [155]. Furthermore, this infusion of apoE was able to reverse the synaptodendritic pathology in these animals. Currently however, it is still unclear precisely how the apoE is transported to locations where it would be most needed in order to function. Similar experiments in LDL receptor-deficient, double apoE/LDL receptor-deficient and human apoE2, E3 and E4 transgenic mice may reveal more clues about the importance of this pathway in the plasticity of the CNS [104,105,294].

8. ApoE and Alzheimer's disease

8.1. ApoE as a risk factor

Using nonparametric linkage analysis methods, genetic markers from chromosome 19 (where both the genes for apoE and the LDL receptor are located), suggested linkage to late-onset familial AD [197]. It was then demonstrated that the frequency of the $\epsilon4$ allele of apoE was increased in late-onset familial AD when compared to age-matched controls [267]. This result was quickly confirmed in sporadic AD patients, which account for approximately 95% of all late-onset AD cases [210]. This result has since been confirmed in numerous other studies in both early-onset and late-onset familial and sporadic cases of AD [1,10,40,43,61,143,160,184,194,241,242,267]. In both sporadic and familial late-onset AD, the prevalence of the $\epsilon4$

allele is increased from approximately 15 to 40% [210,242]. About 80% of familial and 64% of sporadic late-onset AD cases carry at least one copy of the $\epsilon4$ allele compared to 31% of controls [43]. More importantly, population-based studies have now confirmed these initial findings in both early and late-onset forms of AD [129,279]. To date, apoE genotype represents the most important genetic risk factor for AD. Estimates indicate that as much as 50% of the risk associated with AD is due to apoE genotype [43]. However, not everyone possessing an $\epsilon4$ allele will develop AD, and many who lack the allele also develop AD. It should be emphasized that several other genetic loci besides apoE have been identified as contributing to AD, including the amyloid precursor protein [69], the presenilin 1 gene [250], the presenilin 2 gene [140], and several as yet unidentified genes on chromosomes 4, 6, 12 and 20 [196].

8.2. ApoE and age of onset of Alzheimer's disease

ApoE genotype also has a clear impact on the age of onset of clinical symptoms in AD. Each additional $\epsilon4$ allele shifts the age of onset to a younger age; on average, the age of onset decreased from 84 to 68 years with increasing number of apoE $\epsilon4$ alleles in a study of familial late-onset AD [43]. If the apoE $\epsilon4$ allele is a strong risk factor for AD, then the prevalence of this allele would be very small in very old populations. Indeed, approximately 75% of the AD patients with an age of onset at age 55–60 possess at least one copy of allele $\epsilon4$, this percentage drops to only 30% for AD patients with an age of onset of 90. Subjects with the $\epsilon4/\epsilon4$ genotype may account for 40% of all AD patients at age 55, but less than 5% after age 91 [224]. Further to the increased $\epsilon4$ allele frequency in AD, several studies have also observed a decrease in the frequency of the $\epsilon2$ allele, suggesting a possible protective effect [35,41,272,285]. The presence of the $\epsilon2$ allele delays the onset of clinical symptoms of AD, and also decreases the risk of AD. Interestingly, apoE genotype also influences the age of onset in families with mutations in the amyloid precursor protein (APP), but not in families with presenilin-1 (PS1) mutations [86,261,278]. For more detailed information and summaries on the genetic association between apoE and AD, readers are directed to several thorough reviews [83,93,118,232].

8.3. ApoE and Alzheimer's disease progression and duration

Analysis of the incidence of the $\epsilon4$ allele in pre-symptomatic subjects with age-related memory decline showed that a strong association exists between high Alzheimer disease assessment scale-cognitive component (ADAS-Cog) scores (i.e., poor performance) and the incidence of $\epsilon4$ in these subjects [22]. Patients with a diagnosis of probable AD also show segregation of symptoms on the basis of their genotype. Early-stage AD patients with two

ϵ4 allele showed a lower score on immediate and delayed tests assessing verbal memory [137]. Among 20 fraternal twin pairs discordant for the presence of ϵ4, analysis in subclinical changes in cognition, indicate that the twins with the ϵ4 allele demonstrated poorer mean performance than their co-twins without the ϵ4 allele [226]. These results suggest that not only is the age of onset different for ϵ4 AD carriers, but that the rate of progression prior to and possibly after onset is ϵ4-dependent. Several studies have attempted to monitor the rate of progression of the key symptoms in heterogenous population of AD subjects with mixed results. Frisoni et al. [61] showed that both mini mental state examination (MMSE) scores and clinical dementia rating (CDR) were linked to apoE allele dose in 62 AD patients with age of onset of more than 70. The progression of patients with fast progression decreased, and with slow progression increased with increasing the ϵ4 gene dose. Stern et al. [262] reported a similar profile of progression in a large cohort of AD subjects living in New York city. In contrast, Basun et al. [10] and Growdon et al. [76] did not observe any change in the rate of clinical progression in ϵ4 subjects when compared to non-ϵ4 patients. In a more recent study, we took advantage of the fact that AD patients enrolled in clinical drug trials represent a very homogeneous population of subjects, i.e., they usually exhibit a narrow range of: age of onset, severity (mild to moderate) and duration. Furthermore, the mean age of enrollment is usually around 75 years of age, and genders are carefully balanced. Analysis of the placebo arm of two different drug trials (Tacrine: Parke-Davis, Xanomeline: Eli Lilly) revealed a clear difference in the rate of progression as monitored by variation in the ADAS-Cog over a period of 6 months [208]. The non-ϵ4 subgroup showed a significantly faster rate of progression when compared to the ϵ4 subgroup. It is clear that the use of these homogenous populations of subjects allows us to better monitor disease progression by reducing intrinsic variability due to the nonlinear decline of function in AD patients.

8.4. ApoE and ethnic variation in Alzheimer's disease

A meta-analysis of apoE allele frequencies from 40 research teams enabled the calculation of odds ratios (OR) for AD stratified by ethnic background, including Caucasian, African–American, Hispanic and Japanese populations [59]. This study found that among Caucasians, the risk for AD increased with increasing ϵ4 allele number, yielding ORs of 2.6, 3.2 and 14.9 for apoE genotypes ϵ2/ϵ4, ϵ3/ϵ4, and ϵ4/ϵ4, respectively relative to ϵ3/ϵ3 [59]. The apoE ϵ4 genotype effect was weaker in African–Americans and Hispanics; however, there was a significant heterogeneity in ORs in the African–American studies examined [59]. In Japanese populations, the ϵ4 AD association was found to be even stronger than in Caucasians. The ϵ2/ϵ3 genotype was found to be protective

(OR = 0.6 relative to ϵ3/ϵ3) across all ethnic groups. Together, these data confirm that the ϵ4 allele of apoE is a major risk factor for AD in all ethnic groups.

8.5. ApoE and gender in Alzheimer's disease

The association of the ϵ4 allele of apoE and AD also holds true for both sexes. However, within the AD group, Poirier et al. [210] originally reported that the frequency of the ϵ4 allele is higher in women compared to men. In a study of 52 late-onset AD families, Payami et al. [194] observed an increased risk for women with one ϵ4 allele compared to men. According to Corder et al. [42], nearly 100% of women by 85 years with one ϵ4 allele were affected with AD as compared to 50% of men with one ϵ4 allele, although the overall difference was not statistically significant due to small sample size. A direct comparison of ϵ4 heterozygous men and women revealed a significant twofold increased risk for AD in women [195]. To further address this point, a meta-analysis of 5930 AD patients revealed that at most ages and across all apoE genotypes, women were more likely to develop AD than men [59]. This indicates that women may have a higher susceptibility to AD regardless of apoE genotype, perhaps due to independent factors such as estrogen. Interestingly, estrogen has recently been shown to regulate apoE expression in astrocytes and microglia, and may thereby provide a protective role by increasing neuronal sprouting as seen in hippocampal slice cultures [234,266,274].

9. ApoE ϵ4 gene dose effect on Alzheimer's disease pathophysiology

9.1. Senile plaques

One of the characteristic neuropathological features of AD is the presence of amyloid-containing senile plaques (SP). SPs comprise aggregates of beta-amyloid (Aβ) protein, which is derived from APP, the amyloid precursor protein. The role of Aβ in the pathogenesis AD is strongly supported by findings that associate specific point mutations in APP with families having autosomal dominant early-onset familial AD [34,69]. The first report to link apoE to amyloid plaques was by Namba et al. [175] in 1991. In their study, they demonstrated that apoE immunoreactivity was associated with amyloid deposits in both SPs and neurofibrillary tangles (NT) of confirmed AD brain tissue. With the later knowledge that the E4 isoform of apoE is a risk factor for AD, investigators naturally wanted to know if the phenotype of AD pathology in patients with the ϵ4 allele is different in patients without an ϵ4 allele. Indeed, when senile plaque density was correlated to apoE genotype, several groups found that ϵ4/4 patients demonstrated significantly increased

plaque numbers compared to $\epsilon 3/3$ patients [12,67,71,97,172,186,188,215,225,245,299]. These studies demonstrate a clear gene dose effect, where plaque density numbers correlate to apoE genotype: $\epsilon 4/4 \gg \epsilon 3/4 \gg \epsilon 3/3$. These data support the hypothesis that inheritance of the $\epsilon 4$ allele of apoE is a strong susceptibility factor for AD. On the other hand, a few groups have also recently provided data demonstrating no correlation between apoE $\epsilon 4$ to senile plaques [107,131,168]. Differences in ethnic populations, varying stages of plaque evolution, and technical considerations, may be responsible for the discrepancies in these studies. To this end, Gearing et al. [66] have demonstrated that the increased plaque frequency observed in apoE $\epsilon 4$ individuals may be due to an increase in Aβ_{1-40}-positive plaques, while finding that Aβ_{1-42}-positive plaques were similar for all three apoE genotypes.

9.2. Neurofibrillary tangles

Another characteristic feature of AD neuropathology is the presence of intraneuronal neurofibrillary tangles (NTs). In this case, the data appear to be even more controversial. Initial reports indicated that the average number of NTs was greater in $\epsilon 4/4$ than in $\epsilon 3/3$ patients; however, the NT count also correlated positively with apparent duration of AD [245]. Therefore, the increase in NT counts in $\epsilon 4/4$ individuals was attributed to an increase in duration of illness associated with apoE4. To further investigate this result, Ohm et al. [181] used a histological staging system that considers the gradual development of AD-related histological changes over time, and correlated them to cognitive decline ante mortem. Their study revealed that the mean stage for NTs is significantly increased in $\epsilon 4$ carriers, representing an earlier onset of the histopathological process of about 10 years. Several studies have since found a positive relationship between NT and the $\epsilon 4$ allele of apoE [12,172,181,215]; however, there is also considerable data to the contrary. Numerous studies to date have not found a correlation between the $\epsilon 4$ allele and NT [17,71,107,131,168,186,188,299].

9.3. Hippocampal volume

One region of the brain in which the neuropathological markers (SP and NT) diagnostic of AD appear earliest is the hippocampal formation [95,96]. The hippocampus is also thought to be critical for the establishment of memories, the loss of which are established clinical hallmarks of AD. Magnetic resonance imaging (MRI) studies allow for the measurement of damage to the hippocampus due to decreases in synapse density in elderly individuals afflicted with AD. Finnish researchers have recently correlated the volumes of hippocampus in AD individuals with apoE genotype using MRI. They found that AD patients with the $\epsilon 4/4$ genotype had smaller volumes of the hippocampus than those not carrying an $\epsilon 4$ allele [136]. These data

suggest that AD individuals with $\epsilon 4/4$ genotype suffer greater damage to the hippocampus very early in the disease process, and differ significantly from non-$\epsilon 4$ carriers. Similar results were also found in a larger study by the same group [138]. Another study investigating nondemented elderly subjects also found a correlation between the $\epsilon 4$ allele of apoE and reduced hippocampal volume [258]. Together, these data suggest that carrying an $\epsilon 4$ allele increases the risk for synaptic loss and hippocampal lesions characteristic of AD. To further these data, positron-emission tomography (PET) has now been used to establish preclinically that the presence of the $\epsilon 4$ allele is a risk factor for AD. Small et al. [253] used PET to measure cerebral glucose metabolism in members of AD families and found that the $\epsilon 4$ allele of apoE was associated with reduced cerebral parietal metabolism and increased asymmetry in non-demented relatives at risk for probable AD. Reiman et al. [227] extended these data by showing that cognitively normal $\epsilon 4$ homozygotes had significantly reduced rates of glucose metabolism in several brain regions, the same regions affected in patients with probable AD.

9.4. ApoE levels in Alzheimer's disease

In AD, apoE mRNA levels may be unchanged or decreased in the hippocampus and cortex of AD subjects [188,214]. Blennow et al. [21] reported a reduced CSF apoE concentration in affected AD patients compared to controls. A similar but less significant reduction was found in patients with frontal lobe dementia [21]. These results have been confirmed and extended by studies reporting reduced apoE levels in the CSF of AD patients vs. controls; however, none of the studies reported a difference between the subjects carrying the $\epsilon 4$ allele and those without the $\epsilon 4$ allele of apoE [130,135,204,252]. Studies of apoE protein levels from AD and control brain tissue have demonstrated decreased apoE levels in hippocampus, but not cortex of AD subjects [18,87]. Pirttila et al. [205] also reported lower apoE levels from frontal and temporal cortex of AD patients compared to controls. Furthermore, Bertrand et al. [18] demonstrated an $\epsilon 4$ allele dose-dependent reduction of apoE and concomitant augmentation of apoJ levels in both the hippocampus and cortex of AD subjects. These results implied that apoJ may be attempting to compensate for the reduced levels of apoE found in AD subjects carrying one or two $\epsilon 4$ alleles of apoE.

10. ApoE, cholinergic dysfunction and treatment in Alzheimer's disease

The role of apoE in the CNS is particularly important in relation to the function of the cholinergic system, which relies heavily on lipid availability to synthesize acetylcholine (ACh) in neurons (Fig. 2). Brain membrane phos-
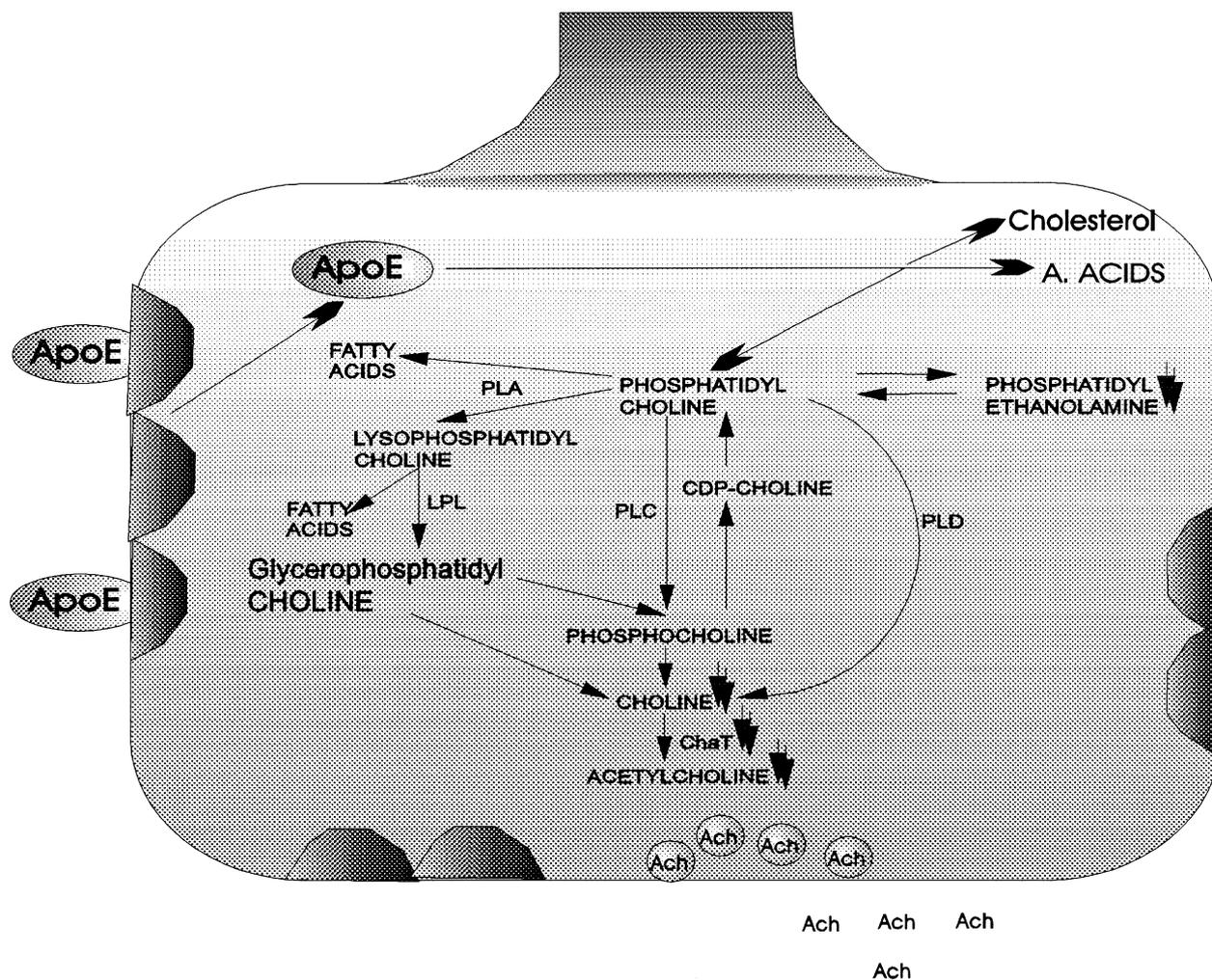
Fig. 2. Schematic showing the potential interaction of apolipoprotein E in cholinergic metabolism. A. Acids: amino acids, ACh: acetylcholine, ChaT: choline acetyltransferase, LPL: lipoprotein lipase, PLA: phospholipase A, PLC: phospholipase C, PLD: phospholipase D. Double arrows indicate reduced levels of indicated protein. ApoE could play a crucial role in this cascade, as it is one of the key transporters of phospholipids and cholesterol in the central nervous system. The presence of the apoE4 isoform in the brain may impair phospholipid homeostasis in cholinergic neurons and indirectly compromise acetylcholine synthesis.

pholipids, particularly phosphatidylcholine (PC) and phosphatidylethanolamine (PE), have been shown to serve as donor intermediates for choline, a rate-limiting precursor of ACh [23]. The release from PC of free choline precursor for ACh synthesis is accomplished in a one-step process through a phospholipase-D type enzyme in cholinergic neurons. Brain levels of choline are decreased by up to 40–50% in frontal and parietal cortices [178] of AD patients (with unknown apoE genotype), whereas cholesterol, which is required for the proper functioning of nicotinic receptor subtype [112], was shown to be reduced in AD vs. control subjects [269].

It was recently proposed that the low levels of apoE reported in the brain and CSF of $\epsilon 4$ AD subjects may compromise cholesterol and phospholipid delivery in the CNS, and selectively restrict cholinergic neurotransmission [207]. As losses of cholinergic neurons and/or choline

acetyltransferase (ChAT: the enzyme responsible for ACh synthesis) activity are well known neurochemical hallmarks of AD [198,287], investigation of their relationship to apoE genotype is very relevant. Three independent studies have demonstrated that ChAT activity is significantly reduced in the hippocampus and cortex of $\epsilon 4$-AD subjects when compared to control subjects [8,207,257]. Furthermore, the total number of cholinergic neurons determined using ChAT and NGF-receptor immunoreactivity were significantly reduced in the nucleus basalis of Meynert (the primary cholinergic input into the cortex and hippocampus) in $\epsilon 4$ allele carriers suffering from AD compared to non-$\epsilon 4$ allele carriers [8]. Similarly, cholinergic neuronal cell density as determined by acetylcholinesterase (AChE) staining reveals fewer cells in AD patients carrying an $\epsilon 4$ allele compared to $\epsilon 4$-negative subjects [211]. Nicotinic receptor sites, which have a presy-

naptic location in the hippocampus, were shown to be significantly reduced in ϵ4 allele carriers when compared to non-ϵ4 AD cases or control subjects [211]. These results are consistent with the unique preference of cholinergic neurons to use lipids, particularly choline for the purpose of synthesizing both ACh and PC, which may contribute to their selective vulnerability in AD. When physiologically active, cholinergic neurons may use free choline from the 'reservoir' of membrane PC to synthesize ACh, and may thereby indirectly alter membrane and synaptic integrity. Alternatively, neurons facing an important shortage of lipid due to an impaired delivery, as predicted in ϵ4 carriers, may elect to shut down cholinergic neurotransmission in order to maintain membrane integrity and plasticity.

The integrity of the cholinergic system has also been studied in apoE deficient mice. Interestingly, although apoE-deficient mice displayed behavioral impairments in the Morris water maze, measurements of several cholinergic markers revealed no dramatic changes when compared to control animals [127]. Indeed, binding of the cholinergic ligands cytisine (nicotinic receptors), pirenzepine (M1 muscarinic), AFDX-384 (M2 muscarinic) [127], as well as AChE staining [5,127], revealed no significant changes in young and mature apoE-deficient mice compared to controls. Data concerning the synthesis of ACh reveal that ChAT activity is either unchanged [5,127,189] or decreased in the hippocampus and neocortex [74] in apoE-deficient mice. However, the lack of cholinergic deficits in apoE deficient animals does not preclude the possibility of cholinergic deficits in AD, since ϵ4 carriers still possess a functional form of apoE. Future studies comparing mice with different human apoE isoforms on a mouse apoE-deficient background may reveal more clues to the cholinergic changes seen in AD [294].

The effect of the ϵ4 allele of apoE on the intrinsic cholinergic activity in the brain of AD cases raises another fundamental question regarding existing cholinergic drugs designed to target cognitive symptoms associated with AD. Is the efficacy of cholinomimetic agents used for the treatment of AD dependent upon the apoE genotype of the patients? Preliminary results obtained in small and large-scale drug trials suggest that apoE genotype has a significant impact on drug responsiveness in AD subjects treated with the AChE inhibitor tacrine. Patients lacking the ϵ4 exhibit a stronger response on the ADAS-Cog [208,211] and on clinical interview-based impression of change (CIBIC) [208] scale. A strong gender and/or estrogen effect was also observed in the non-ϵ4 group [58,246]. These results should not be interpreted as absolute evidence of the presence or absence of drug responsiveness in tacrine-treated patients but instead, it should be seen as a genotype-dependent difference in the quality (and size) of the response. Consistent with this observation is the fact that tacrine's ability to increase cortical alpha wave activity in AD subjects was dependent on apoE genotype [228].

In summary, both pathological and clinical data clearly suggest that the ϵ4 genotype of apoE influences the function and integrity of the cholinergic system in the brain. This observation should have significant impact on the design of future cholinomimetic-based trials in AD in addition to focusing efforts on mechanisms involving apoE4 in cholinergic deficits in AD.

11. ApoE ϵ4 gene dose as a risk factor for other central nervous system pathologies

Since the many neuropathological features of AD are not exclusive to this disease, many studies have been performed examining the extent of apoE allele distribution in other CNS pathologies. Dementia associated with cortical Lewy bodies is the second most common form of amyloid-forming degenerative dementia in the elderly after AD [81]. A genetic association of the ϵ4 allele of apoE has now been reported with most Lewy body-related disorders, including senile dementia of the Lewy body type [15,16,20,85,89,168,260], the Lewy body variant of AD [63,82,119], diffuse Lewy body disease [120,152], and Lewy body disease with or without pathological aging [141,199]. Unfortunately, as is evidenced by the numerous terminology used with this disease, no definitive diagnostic criteria for Lewy body disease exists, and much confusion can be found in the classification and characterization of this disease, making comparisons between studies difficult. All of these data are, however, consistent with the hypothesis that the apoE genotype does affect the neuropathology associated with Lewy body disease.

Other CNS pathologies with which the apoE ϵ4 allele frequency did not differ from control subjects include alcoholic dementia [171], amyotrophic lateral sclerosis [169], chromosome 14 encoded AD [278], Creutzfeldt–Jakob disease [173,200,239,241,296,297], Down's syndrome [9,84,150,168,199,233,241,280,291], familial amyloidotic polyneuropathy [241], Huntington's chorea [89], ischemic cerebrovascular disease (or stroke) [77], lobar atrophy [201], multiple sclerosis [235], Parkinson's disease [15,54,85,89,99,122,151,152,168,235,286], progressive supranuclear palsy [7], schizophrenia with progressive dementia [152], sporadic inclusion body myositis [88], and vascular dementia [20,237]. Several exceptions are of note and include work by Amouyel et al. who found that the ϵ4 allele of apoE was a risk factor for Creutzfeldt–Jacob disease (CJD); however, the discrepancy with the aforementioned studies may lie in selection bias, i.e., the inclusion of familial CJD in this study [4]. It should be noted that in the case of Down's syndrome, the ϵ2 allele of apoE was found to be associated with longevity, and the absence of clinical evidence of dementia [84,233], while the ϵ4 allele was associated with an increased risk for developing higher levels of amyloid accumulation [98]. An association

of the apoE ϵ4 allele was recently reported with bulbar-onset motor neuron disease [2], but has already been contradicted by another study [254]. Similarly, Harrington et al. [90] reported an increased apoE ϵ4 allele frequency in schizophrenia patients, but this report has also been countered with data showing equal representation of the ϵ4 allele in schizophrenics and control subjects [114,115].

12. Other potential risk factors related to lipoprotein metabolism in Alzheimer's disease

Although apoE4 has been postulated to be involved in neurofibrillary tangles and plaque formation, its exact role in AD remains to be established. The fact that approximately one-third to one-half of late-onset AD patients do not carry the ϵ4 allele indicates that other risk factors must be involved in the pathogenic process. One hypothesis involves defects in uptake of cellular lipoproteins leading to lipid metabolism dysfunction in these subjects. Poor cholesterol and phosphatidylcholine delivery, for example, would presumably give rise to impaired synaptic and cellular plasticity, as well as a decrease in neurotransmitter synthesis in cholinergic neurons. Factors potentially responsible for such an impairment could include downregulation of or mutations in the genes coding for apolipoproteins, lipoprotein receptors, or other proteins involved in lipid metabolism and synthesis such as HMG-CoA-reductase.

So far, evidence linking downregulation or functional mutations of candidate lipoprotein-related genes to AD is lacking. Single-strand conformation polymorphism (SSCP) analysis of the apoJ gene did not reveal any association between five different polymorphism in exons 2 and 7 and susceptibility to AD in several hundred Caucasians, Hispanics and African–Americans [276]. Other polymorphic sites in the apoJ gene and their relationship to AD have yet to be determined.

On the other hand, two groups of investigators have recently reported an association between a polymorphic site at either the VLDLR or the LRP loci and AD. The frequency of a 5-time repeated polymorphic triplet in the 5′-untranslated region of the VLDLR gene was significantly higher in a Japanese population of sporadic AD patients compared to Japanese controls [182]. However, this association could not be replicated in several other studies involving Caucasian populations [183,39,217,139]. The significant differences in the allele frequencies in Japanese and white populations were suspected to be responsible for this discrepancy, and warrants further studies in other populations.

Moreover, a genetic association between the 87 bp allele of the LRP gene with AD is believed to represent a minor risk factor that may modify the susceptibility to the disease in Caucasians [139]. Alleles for the LRP gene differ by one tetranucleotide repeat located in the 5′ region

of the gene, and are designated on the basis of the size of a PCR product [300]. The precise location of this polymorphism, and whether it has any consequence on LRP receptor protein levels or function are presently unknown. It will be interesting to see whether the polymorphism is in linkage disequilibrium with genetic variation elsewhere in the LRP gene that could affect receptor function, and also if it can be confirmed in other studies.

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