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Review

Amyloid beta-protein interactions with membranes and cholesterol: causes or casualties of Alzheimer's disease

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Abstract

Amyloid beta-protein (Aβ) is thought to be one of the primary factors causing neurodegeneration in Alzheimer's disease (AD). This protein is an amphipathic molecule that perturbs membranes, binds lipids and alters cell function. Several studies have reported that Aβ alters membrane fluidity but the direction of this effect has not been consistently observed and explanations for this lack of consistency are proposed. Cholesterol is a key component of membranes and cholesterol interacts with Aβ in a reciprocal manner. Aβ impacts on cholesterol homeostasis and modification of cholesterol levels alters Aβ expression. In addition, certain cholesterol lowering drugs (statins) appear to reduce the risk of AD in human subjects. However, the role of changes in the total amount of brain cholesterol in AD and the mechanisms of action of statins in lowering the risk of AD are unclear. Here we discuss data on membranes, cholesterol, Aβ and AD, and propose that modification of the transbilayer distribution of cholesterol in contrast to a change in the total amount of cholesterol provides a cooperative environment for Aβ synthesis and accumulation in membranes leading to cell dysfunction including disruption in cholesterol homeostasis.

Keywords: Alzheimer's disease; Amyloid beta-protein; Apolipoprotein E; Cholesterol; Fluidity; Lipid raft; Statin

1. Introduction

Neuritic plaques and neurofibrillary tangles in brain are characteristic neuropathological features of Alzheimer's disease (AD). Amyloid beta-protein (Aβ) is a primary component of neuritic plaques. Aβ is 39–43 amino acid residues long and that is derived in part from the transmembrane region of the amyloid precursor protein (APP) [1,2]. Observations that Aβ was neurotoxic in cells provided the first evidence that Aβ might be directly involved in neurodegeneration in individuals with AD [3,4]. Concomitantly, it was proposed that the initial pathophysiology induced by Aβ involved alterations in membrane structure and function [5,6]. Subsequently, there have been an extensive number of studies on effects of Aβ on membranes and cell function (reviewed in Refs. [1,7,8]). There is certainly a physico-chemical interaction between Aβ and membranes, including but not limited to changes in fluidity, binding to membranes and lipids including cholesterol. The majority of studies examining the effects of Aβ on membranes have looked at changes in fluidity but there is no agreement on effects of Aβ on membrane fluidity, and an explanation for this lack of consistency will be discussed in this review.

Cholesterol is an important component of membranes and there has been a mounting body of data on Aβ and cholesterol and linking disturbances in cholesterol homeostasis with AD. Sparks et al. [9] were one of the first groups to suggest a possible link between cholesterol and AD. They found that patients with critical coronary artery disease also showed deposition of Aβ similar to that seen in AD patients. Subsequent work of that group showed that high-cholesterol diets in rabbits induced accumulation of Aβ in brain [10]. Linkage of cholesterol and AD was also suggested by the presence of the apolipoprotein E4 allele and occurrence of AD [11]. ApoE is an apolipoprotein that transports cholesterol and individuals with the apoE4 allele are at a greater risk of developing AD compared to individuals with the apoE2 or -3 alleles. Further strengthening the association between cholesterol and AD is recent epidemiological data

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showing that the prevalence of AD is diminished in patients taking statins [12,13]. Results from experimental studies in vivo and in vitro provide additional and important support for a role of cholesterol in AD.

In spite of the large body of data on cholesterol and AD, there are several major issues that remain unaddressed regarding the role of cholesterol in AD and these issues will be examined in this paper. For example, there is little if any consensus that total or bulk brain cholesterol is altered in AD patients. Changes in cholesterol domains may be important in AD in contrast to changes in bulk cholesterol. The mechanisms of action of statins in brain are not well understood and not all statins are equally effective. There are data showing that cholesterol may actually inhibit action of Aβ. The purpose of this review is to discuss: (1) data on actions of Aβ in membranes; (2) the role that cholesterol plays in Aβ dynamics and AD; and (3) cholesterol domains and Aβ. This review focuses on membranes and cholesterol but it is important to point out that other lipids (gangliosides, phospholipids, fatty acids) besides cholesterol interact with Aβ and those lipids also may be essential in the pathogenesis of AD [14–21].

2. Aβ is associated with membranes

Localization of $A\beta_{1-42}$ on the cell surface plasma membrane of brain from patients with AD was revealed using electron microscopy [22]. The authors concluded that $A\beta_{1-42}$ deposition on the cell surface plasma membrane was an initial event in formation of diffuse plaques that gradually develop into fibrillar amyloid. Similar findings were recently reported in aged dogs, which are an accepted model of AD, since dogs exhibit age-dependent cognitive decline that is correlated with the accumulation of Aβ [23]. Neuronal labeling indicated that $A\beta_{1-42}$ was associated with the neuronal plasma membrane and it was suggested that the peptide may be produced at the dendritic plasma membrane. Two pools of insoluble Aβ were identified in human prefrontal cortices [24]. One pool was located in a cholesterol-enriched low-density membrane domain while the second pool comprised extracellular Aβ deposits. In the same study, it was shown that in PDAPP mice that develop plaques and accumulate Aβ, low-density membrane domains showed accumulation of $A\beta_{1-42}$. Other studies have also reported that APP and Aβ were associated with cholesterol-rich low-density membrane domains [24–27].

Studies in vitro, using model membranes and biological membranes, have also shown that Aβ associated with membranes. Electrostatic binding of Aβ to phospholipid polar head groups has been proposed to be a mechanism that may contribute to Aβ neurotoxicity [18,28]. Circular dichroism spectroscopy showed that $A\beta_{1-40}$ interacted with negatively charged unilamellar vesicles but no effect was detected in vesicles containing deuterated phosphatidylcholine mixed with phosphatidylglycerol using deuterium NMR and that $A\beta_{1-40}$ did not penetrate into membranes [28]. $A\beta_{1-40}$ had a more pronounced effect on disrupting and aggregating neg-

Table 1
Aβ perturbation of membranes

Sample	Aβ protein	Technique	Fluidity	Reference
SUV	1–40	Polarization of DPH	Decrease	[6]
Mouse brain membranes	25–35	Anisotropy of DPH	Decrease	[35]
Mouse brain homogenate	25–35	Anisotropy of DPH	Decrease	[36]
Human frontal cortex	25–35; 1–28; 1–40; 1–42; 1–43	Anisotropy of DPH	Decrease	[37]
Human hippocampal tissue, control Human hippocampal tissue, AD	25–35; 1–42	Anisotropy of DPH	Decrease	[38]
Mouse SPM, 3 months of age	25–35; 1–40; 1–42	Anisotropy of DPH	Decrease	[39]
Mouse SPM, 22 months of age	1–42	Anisotropy of DPH	Decrease	
Liposomes	1–39; 1–40	Anisotropy of DPH	Decrease	[40]
Mouse SPM, 3 months of age	25–35; 1–40; 1–42	Pyrene eximer/monomer ratio (bulk fluidity)	No effect	
Mouse SPM, 22 months of age			Increase	
Rat SPM	25–35; 1–40	Pyrene eximer/monomer ratio (bulk fluidity)	Increase	[30,43]
		Pyrene eximer/monomer ratio (annular fluidity)	Increase	
Rat SPM from cortex and hippocampus	1–40	Pyrene eximer/monomer (bulk fluidity)	Increase	[44]
		Pyrene eximer/monomer ratio (annular fluidity)	Increase	
Rat SPM from cerebellum			No effect	
Human brain PM	1–40; 1–42	Anisotropy of DPH	Decrease	[41]
Human brain Golgi			Increase	
Human brain endosomal and lysosomal membranes			Decrease	
Brain lipid extract	1–40	Anisotropy of DPH	Decrease	[42]

atively charged lipid vesicles than zwitterionic vesicles [18]. Conversely, studies of Aβ peptides in both model membranes and biological membranes indicated that soluble or fresh Aβ partitioned into the hydrophobic core of membranes [29,30]. Liposomes consisting of 1-palmitoyl-2-oleoylphosphatidylcholine revealed that the peptide fragment $A\beta_{25-35}$ localized in the membrane hydrocarbon core [29]. Moreover, soluble $A\beta_{1-40}$ and aggregated $A\beta_{1-40}$ were found to differ in their location in rat synaptic plasma membranes (SPM) [30]. Soluble $A\beta_{1-40}$ intercalated into the hydrophobic region of SPM. Aggregated $A\beta_{1-40}$ was positioned at the polar head group region of the membrane. Differences in results of the aforementioned studies may have occurred as a consequence of variations in peptide structure and dissimilarities in structure of liposomes versus biological membranes. Results of studies using the peptide fragment 25–35 have to be viewed cautiously in view of the fact that this fragment is not present in vivo. Nevertheless, in can be seen in Table 1 that Aβ does have a physico-chemical interaction with membranes.

It has been previously proposed that Aβ may interact with lipids both by hydrophobic interactions and electrostatic interactions at the membrane surface particularly with negatively charged phospholipids [18]. The cytofacial leaflet of biological membranes is negatively charged as a result of enrichment of phosphatidylinositol and phosphatidylserine as compared to the zwitterionic exofacial leaflet. Certain cationic drugs have been shown to perturb the negatively charged cytofacial leaflet and anionic drugs perturbed the zwitterionic exofacial leaflet [31,32]. Electrostatic interaction of Aβ with phospholipid polar head groups may be greater in the cytofacial leaflet than the exofacial leaflet and this prediction is consistent with the affinity of Aβ for negatively charged lipids [18,28].

3. Aβ and membrane fluidity

Aβ disrupts membrane fluidity. Studies have examined the effects of Aβ peptides on membrane fluidity in model membranes and biological membranes of mice, rats and humans (Table 1). We use the term fluidity in the broadest sense to describe an average lateral motion in the membrane lipid environment without making a distinction between dynamic and static states of fluorescent probe motion. This issue is discussed later in this section. It is well recognized that changes in the physico-chemical state of the membrane can markedly alter activity of various membrane proteins [32,33]. Effects of Aβ on membrane fluidity have been proposed as contributing to disruption in different cell functions (e.g., calcium signaling; activity of various enzymes, lipid transport) [5,34,35].

$A\beta_{1-40}$ was reported to increase polarization of diphenylhexatrine (DPH) in small unilamellar vesicles (SUV) consisting of phosphatidylethanolamine (PE), phosphatidylcholine (PC) and cardiolipin [6]. An increase in polarization of DPH is indicative of a more ordered membrane state. In one of the first studies on Aβ and fluidity using biological tissue, the peptide fragment $A\beta_{25-35}$ increased anisotropy (i.e., reduced fluidity) of DPH in mouse and rat brain membranes [35,36]. Several subsequent studies including brain tissue from humans with and without AD have shown that different Aβ peptides reduce membrane fluidity [37–42]. Aβ would appear to have a rigidifying effect on membranes. However, it has been reported that Aβ disorders or increases fluidity of membranes [30,40,41,43,44]. $A\beta_{1-40}$ increased both annular fluidity and bulk fluidity in SPM using energy transfer from protein tryptophan residues and excitation of pyrene (annular fluidity) and pyrene excitation alone (bulk fluidity) [30,40,43,44]. Anisotropy of DPH was reduced in human brain Golgi membranes in the presence of $A\beta_{1-40,42}$ but just the opposite effect was noted in endosomal, lysosomal and mitochondrial membranes of the same human brain samples [41]. FTIR-PATR spectroscopy demonstrated that $A\beta_{1-40}$ slightly disordered SUV containing gangliosides [17].

There is no agreement on effects of Aβ proteins on membrane fluidity. Certainly, differences in effects of Aβ on fluidity could result from the usual suspects such as tissue source and preparation, whether Aβ is soluble or aggregated, and age of the organism. We propose, however, that the differences in effects of Aβ on fluidity are largely the result of differences in the location of the fluorescence probes in the membrane environment and the lifetime of the fluorescence probes. The majority of studies examining Aβ and fluidity have used steady-state fluorescence of DPH and pyrene fluorescence. It can be seen in Table 1 that, generally, studies reporting a reduction in fluidity induced by Aβ have measured polarization or anisotropy of DPH. In contrast, studies finding that Aβ increases membrane fluidity have measured energy transfer and excitation of pyrene. Structurally, DPH and pyrene differ and this difference can influence their behavior in membranes [45,46]. DPH is a rodlike structure whose axis is parallel to the acyl groups of membranes and pyrene is spherical in structure and is positioned at the terminal end of the acyl groups. Lifetime of a fluorescent probe establishes the duration of time for which the probe interacts in its environment such as membranes [47]. The lifetimes of DPH and pyrene differ: average lifetimes approximately 10 vs. 400 ns, respectively [47]. The longer lifetime of pyrene may increase interaction with different areas of the membrane. We conclude that the most parsimonious explanation for the reported differences in Aβ effects using fluorescence of DPH and pyrene is that the fluorescent probes are reporting on behavior of Aβ in different membrane environments as a function of probe location and probe lifetime.

Most of the studies on Aβ and membranes have examined fluidity. However, there are additional membrane structural properties that could be altered by Aβ. Membrane fluidity is a general term used to describe the movement of lipids in membranes. Actually, fluidity consists of different components including, for example, rate of probe motion, a dynamic component, and extent of probe motion, a static component

[48–50]. Time-resolved fluorescence of DPH can be used to differentiate between limiting anisotropy (order) and rotational relaxation time (rate). Aβ is an amphipathic molecule and another amphipathic molecule, ethanol, has been shown to reduce limiting anisotropy but had no effect on rotational relaxation time [51]. Effects of Aβ on limiting anisotropy and rotational relaxation time have not been reported. Lifetimes of fluorescence probes were discussed in the preceding paragraph and it should be mentioned that a fluorescent probe can have multiple lifetimes and would be indicative of different membrane environments [47]. Lifetime distribution may be altered by Aβ and effects dependent on Aβ protein structure.

Another structural property of membranes that may play an important role in protein function is curvature of the bilayer [52,53]. Both positive and negative curvature strain may facilitate function of proteins depending on the lipid composition of the membrane and the specific protein [52]. The action of certain peptides is thought to involve changes in curvature strain [53]. It has been suggested that Aβ may induce negative curvature strain in membranes [17] and this conclusion was based on results showing Aβ promotion of dehydration of lipid interfacial groups and some reduction in ordering of acyl groups.

Aβ has several different effects on membrane structure. Aβ-induced changes in neuronal functions (e.g., ion flux, calcium homeostasis, enzyme activity and signal transduction) may be initially precipitated by the cumulative effects of disruptions in different membrane structural properties.

4. Cholesterol and AD

There is growing interest in the potential contribution of cholesterol in the pathogenesis of AD. Actually, this contribution can be considered from the perspective of two major questions. The first question is whether changes in cholesterol homeostasis are a causative factor in AD. The second question asks if cholesterol homeostasis is a target of AD particularly with respect to Aβ. Ostensibly, these questions may appear to be an exercise in ‘‘circular reasoning’’. However, it is our view that the answer to both questions is indeed yes.

Data of clinical studies on cholesterol and AD indicate that patients on statins have a lower risk of developing AD as compared with individuals not taking statins [12,13]. Lovastatin and pravastatin were associated with a reduced risk of AD, but treatment with simvastatin was not associated with a lower risk. Several interesting conclusions and questions can be drawn from these data. Obviously, statins as a drug class do not equally act on expression of AD. Statin-induced inhibition of HMG-CoA reductase and reduction of cholesterol do not explain the lower AD risk reported. All three drugs inhibit HMG-CoA reductase and reduce plasma cholesterol. Effects of pravastatin and lovastatin imply both a peripheral and potential CNS effect of the two drugs, respectively. Pravastatin is hydrophilic, does not readily partition into cell membranes, does not cross the blood–brain barrier and is thought to be taken up into cells by an active transport mechanism [54,55]. Both lovastatin and simvastatin are hydrophobic and can intercalate into membranes and probably do cross the blood–brain barrier. Lovastatin has been detected in the CSF of normal human subjects whereas pravastatin was not detected [55].

Another important distinction among the three statins is the active form of the drug that inhibits HMG-CoA reductase. Lovastatin and simvastatin are administered as lactones and then metabolized to lovastatin acid and simvastatin acid, each having an open hydroxyl moiety, and it is this acid metabolite that acts on HMG-CoA reductase whereas pravastatin acts directly on the enzyme [54]. There is evidence suggesting that lovastatin and simvastatin in the lactone form cross the blood–brain barrier by simple diffusion and the acid form of the two statins crosses the blood–brain barrier by means of a transport mechanism for monocarboxylic acid [56]. An overriding conclusion is that there is very little information on the pharmacokinetics and pharmacodynamics of statins in brain. This is a topic that is sorely in need of attention. It is not known if it is the lactone or acid form of lovastatin that may reduce the risk of AD. Moreover, what is the mechanism whereby a statin that does not cross the blood–brain barrier appears to be associated with reducing the risk of AD? It could be argued that plasma cholesterol levels and brain cholesterol levels are in equilibrium and lowering plasma cholesterol also lowers brain cholesterol. However, plasma cholesterol and brain cholesterol are not in equilibrium. A very pointed example of differences in plasma cholesterol and brain cholesterol is comparisons between wild-type mice and apoE-deficient mice. Plasma cholesterol was approximately sevenfold higher in apoE-deficient mice as compared with wild-type mice [57]. There were no differences in brain cholesterol amounts between the two groups. Similar results have been reported for SPM of wild-type mice and apoE-deficient mice [58]. Effects of statins on AD expression may be independent of their action on cholesterol levels. It has been shown for example that statins induce upregulation of COX-2 and stimulation of apoptosis, and reduce expression of endothelin-1 [59–61]. Recently, it was reported that lovastatin and compactin inhibited vasoconstriction and inflammation induced by soluble $A\beta_{1-40}$ in rat aortae [62]. Lovastatin also reduced $A\beta_{1-40}$ induction of prostaglandin E_2 and $F_{2\alpha}$ in rat aortae. There is considerable interest in the role of inflammation in AD and that topic has been reviewed in detail elsewhere [2,63]. Risk of AD may be lowered by statins as a result of a reduction in proinflammatory products occurring both peripherally and in brain.

If bulk brain cholesterol amount was a factor in AD, then it is reasonable to predict that cholesterol content in brain of AD patients would differ as compared to brain cholesterol of non-AD individuals. However, data on cholesterol content of brain tissue of Alzheimer’s patients have been equivocal. There was a small but significant increase in frontal cortex gray matter of AD patients (2.65 ± 0.14 mg/g wet tissue

weight) with the apoE4 genotype compared with apoE4 control subjects (2.04 ± 0.18) [64]. Conversely, it was reported that cholesterol content was lower in the temporal gyrus of autopsied brains of AD patients in contrast to control subjects [65]. The cholesterol-to-phospholipid ratio of the temporal gyrus was reduced by 30% in the AD brains and no differences were observed in the cholesterol-to-phospholipid ratio in cerebellum of the two groups. The reduction in the cholesterol-to-phospholipid ratio in the temporal gyrus was attributed to cholesterol because the phospholipid-to-protein ratio was similar in brains of both groups. Cholesterol content did not differ in hippocampal tissue of AD patients as compared with control subjects [38]. HMG-CoA reductase mRNA levels in brain were indistinguishable between AD samples and control samples and it was suggested that cholesterol synthesis may be unaffected by AD [66]. While levels of HMG-CoA reductase mRNA were similar between the two groups, there could be posttranslational changes in the enzyme that could alter its activity and in turn modify cholesterol synthesis. Activity of HMG-CoA reductase has been shown to be regulated by processes such as phosphorylation and cAMP that could be altered in AD patients. In addition, a metabolite of brain cholesterol, 24S-hydroxycholesterol, was found to be elevated in CSF of AD patients compared with control subjects and it was concluded that there was an increased turnover of cholesterol in AD patients [67]. This oxysterol is thought to be important in regulation of brain cholesterol homeostasis [68]. Changes in levels of 24S-hydroxycholesterol would imply changes in cholesterol turnover but it is not clear how such changes relate to the amount of cholesterol in brain. A humbling fact is that the biosynthesis of cholesterol is most complicated, requiring over 30 different enzymes and several cofactors [69]. An understanding of cholesterol biosynthesis in brain and its role in AD is only beginning.

Whether the total amount of cholesterol in brains of AD patients is either increased or decreased has not been established. Numerous differences (e.g., other pathology, tissue preparation and brain region) in the studies could account for the lack of consistent effects. More importantly, cholesterol amounts may differ depending on brain region and some brain regions more affected by AD than other brain regions. To that end, the amount of SPM cholesterol has a distribution with the hippocampus>cerebral cortex>cerebellum [44]. Effects of $A\beta_{1-40}$ on SPM fluidity were positively correlated with cholesterol amount. Cholesterol domains in contrast to changes in the total amount of cholesterol may be targets of AD [70,71] and this issue will be discussed later in this review.

5. Cholesterol and expression of Aβ and APP

Changes in the amount of cholesterol modify expression of APP and Aβ. Rabbits administered with dietary cholesterol showed an accumulation of Aβ in brain [10]. Administration of diets high in cholesterol increased Aβ accumulation in brain tissue of double-mutant transgenic mice (PSAPP) overexpressing APP (Tg2567) and presenilin 1 (PS1) as compared with transgenic controls [72]. There was a small but significant increase (16.68 mg/g) in cholesterol in brain tissue of mice fed the high-cholesterol diets compared with controls (14.76 mg/g). In contrast to the studies reporting that cholesterol diets increased Aβ expression, it was found that a high-cholesterol diet reduced Aβ expression in brain of APP-gene-targeted mice [73]. Total brain cholesterol was unaffected by the cholesterol diet and a small but significant increase in cholesterol was observed in the frontal cortex of mice on the cholesterol diet. The most striking finding was the large increase in apoE in serum and frontal cortex of the mice on cholesterol diets. Differences in the genetic background of the mouse animal models employed may account for the contrasting results as previously suggested [72]. Levels of $A\beta_{1-42}$ and $A\beta_{1-40}$ were reduced by simvastatin in brain and CSF of guinea pigs [74]. Interestingly, brain cholesterol content was unaffected by simvastatin treatment in guinea pigs in contrast to an 83% reduction in plasma cholesterol. There was a significant reduction in brain lathosterol, which is one of the last two precursors to cholesterol [75], and it was concluded that simvastatin treatment reduced de novo brain cholesterol synthesis. If brain cholesterol synthesis was reduced, any effects would be negligible at best because total brain cholesterol was unaffected and the half life of cholesterol in rat brain has been calculated to be approximately 6 months [76]. The absence of effects of simvastatin on brain cholesterol amount differs from a study showing that lovastatin significantly reduced brain cholesterol in C57BL/6J mice [57]. Furthermore, as discussed earlier, human studies indicated that lovastatin reduced AD risk and simvastatin had no effect [12,13].

Regardless of the effects of alterations in cholesterol on Aβ expression, changes in brain cholesterol are modest. Administration of cholesterol in diets may have a substantial effect on cholesterol domains as compared with the total amount of cholesterol. It also is possible that redistribution of cholesterol occurs within the cell involving different intracellular organelles or perhaps transport between cell types such as astrocytes and neurons. Another issue is the extent to which cholesterol administered in the diet can cross the blood–brain barrier. A low-density lipoprotein receptor has been identified in endothelium of brain capillaries [77] and it has been suggested that cholesterol and other lipids could be delivered to the brain by the transcytosis of LDL across the blood–brain barrier [78]. However, data on LDLR-deficient mice would argue against such a mechanism. It was reported that the total amount of SPM cholesterol was similar for LDLR-deficient mice as compared with control mice [58]. If the LDLR is involved in transport of cholesterol across the blood–brain barrier, then it would be expected that brain cholesterol content would be lower in LDLR-deficient mice. On the other hand, cholesterol content in other membrane types, cell types or brain regions may be differentially

affected in comparison with SPM. What is needed is a detailed study of subcellular membrane fractions of different brain regions of animals administered with dietary cholesterol. In addition, such an approach would be very useful with respect to animal models of AD. A question that has not been fully answered is whether there are differences in either total cholesterol or specific cholesterol domains in animal models of AD. If cholesterol actually plays a role in expression of APP and Aβ, then a reasonable prediction is that brain cholesterol content or cholesterol domains would differ between animal models of AD and wild-type controls without dietary manipulation. However, a recent report showed that there were no differences in cholesterol levels, cholesterol precursors and metabolites in brain homogenates of different age groups (3, 6, 9, 12 and 18 months of age) of wild-type and APP23 transgenic mice[79]. A caveat regarding those data is that a brain homogenate preparation is heterogeneous in brain region and cell type. Moreover, differences between wild-type and APP mice in cholesterol distribution and cholesterol domains could occur in the absence of differences in the total amount of cholesterol.

Studies in cell culture have shown that modification of cholesterol amounts altered APP and Aβ expression. Cholesterol added to APP 751 stably transfected human embryonic kidney cells reduced the production of soluble APP, which is a nonamyloidogenic derivative, but increased production of APP holoprotein [80]. Cholesterol reduction in human embryonic kidney cells and astroglioma cells stimulated production of soluble APP [81]. Depletion of cholesterol by lovastatin and methyl-β-cyclodextrin inhibited the production of Aβ formation in hippocampal neurons that had been infected with Semliki Forest virus encoding human APP695 [82]. In the same study it was observed that a portion of APP was located in fraction that has been described as lipid rafts. Other studies have found that APP, as well as Aβ, and presenilin-1 were located in cholesterol-enriched membrane domains [25,27,83,84]. Caveolin-3 that is associated with a type of membrane domain identified as caveolae was found to be upregulated in astrocytes nearby senile plaques in brain tissue of AD patients and brain tissue of mice that overexpress the human APP with the Swedish mutation [85]. In addition to the importance of cholesterol domains in Aβ synthesis, inhibition of intracellular transport of cholesterol by U18666A reduced cleavage of Aβ from APP in neuronal cells [86].

A question that has not been addressed in the cell culture studies of APP, Aβ and cholesterol reduction is what other effects does reduction of large amounts of cholesterol have on normal cell structure and function. It is certainly well established that cholesterol in mammalian cells is required for membrane structure and activity of various proteins [87–89]. For example, a relatively small reduction (5% to 10%) of cholesterol in SPM resulted in a 40–50% decrease in $Ca^{2+}+Mg^{2+}$-ATPase activity [90]. Membrane interdigitation was significantly reduced and membrane fluidity was significantly increased in that study. Cholesterol reduction in synaptosomes and SPM produced a significant impairment in sodium-dependent GABA uptake, a reduction of GABA-binding sites [91]. On the other hand, cholesterol enrichment of neurons altered GABA receptor function, particularly in the presence of neurosteroids [92–94].

Reducing cholesterol levels appear to decrease the promotion of APP and Aβ. However, there are data indicating that cholesterol may act to attenuate the effects of Aβ. Cholesterol protected PC12 cells from Aβ toxicity in vitro and inhibited effects of Aβ on cellular calcium signaling [35,95]. This observation was supported by recent findings of enhanced cholesterol levels and reduced disordering effects of Aβ peptides in SPM of aged mice [39].

6. Aβ modifies cholesterol dynamics

Cholesterol modifies APP and Aβ expression. On the other hand, Aβ impacts on cholesterol homeostasis. $Aβ_{1-40}$ and $Aβ_{1-28}$ inhibited cholesterol esterification in plasma [96]. Both free and esterified cholesterol synthesis were reduced by $Aβ_{1-40}$ in HepG2 cells [97]. Cholesterol esterification was inhibited by $Aβ_{1-40}$ in rat neurons of primary cell culture [98]. Cholesterol transport into and out of cells was altered by Aβ. Aβ stimulated the uptake of apoE–cholesterol complexes into rat astrocytes [99]. $Aβ_{1-40}$ induced the removal of cholesterol from rat hippocampal neurons to 2-hydroxypropyl-β-cyclodextran [98]. A recent study reported that oligomeric $Aβ_{1-40}$ stimulated the release of cholesterol, PC and GM1-ganglioside from rat cultured neurons and astrocytes [100]. Fresh Aβ and fibrillar Aβ had little if any effect on lipid release. Effects of Aβ on lipid efflux may result from direct interaction of Aβ with lipids and modification of Golgi function. $Aβ_{1-40}$ preincubated for different periods of time up to 24 h binds lipids with binding of cholesterol>stearic acid>PC [101]. Lipid binding occurred when Aβ polymers were present as compared with monomers and dimmers. The Golgi complex is involved in regulation of cholesterol efflux [102,103] and Aβ modifies cholesterol content in the Golgi complex of astrocytes and neurons from primary cell culture [104]. Soluble $Aβ_{1-42}$ significantly increased cholesterol in the Golgi complex in astrocytes and neurons. Aggregated $Aβ_{1-42}$ had quite the opposite effect with a significant reduction in cholesterol content occurring in the Golgi complex of both cell types. Reduction of cholesterol in the Golgi complex by aggregated Aβ may be in response to the removal of cholesterol from the cell plasma membrane induced by Aβ.

7. Transbilayer distribution of cholesterol and AD: a hypothesis

Cholesterol is associated with AD but evidence suggesting that changes in the bulk amount of brain cholesterol are a contributing factor in the pathogenesis of AD has not been

forthcoming. We propose instead (Fig. 1) that alterations in the transbilayer distribution of cholesterol in SPM in contrast to changes in the total amount of cholesterol acts to promote synthesis of Aβ and could also restrict efflux of the peptide from membranes. The two greatest risk factors for late-onset AD are increasing age and inheritance of the apolipoprotein E4 allele. SPM cholesterol asymmetry is altered in aged mice and SPM of human apoE4 knockin mice [105,106]. The SPM

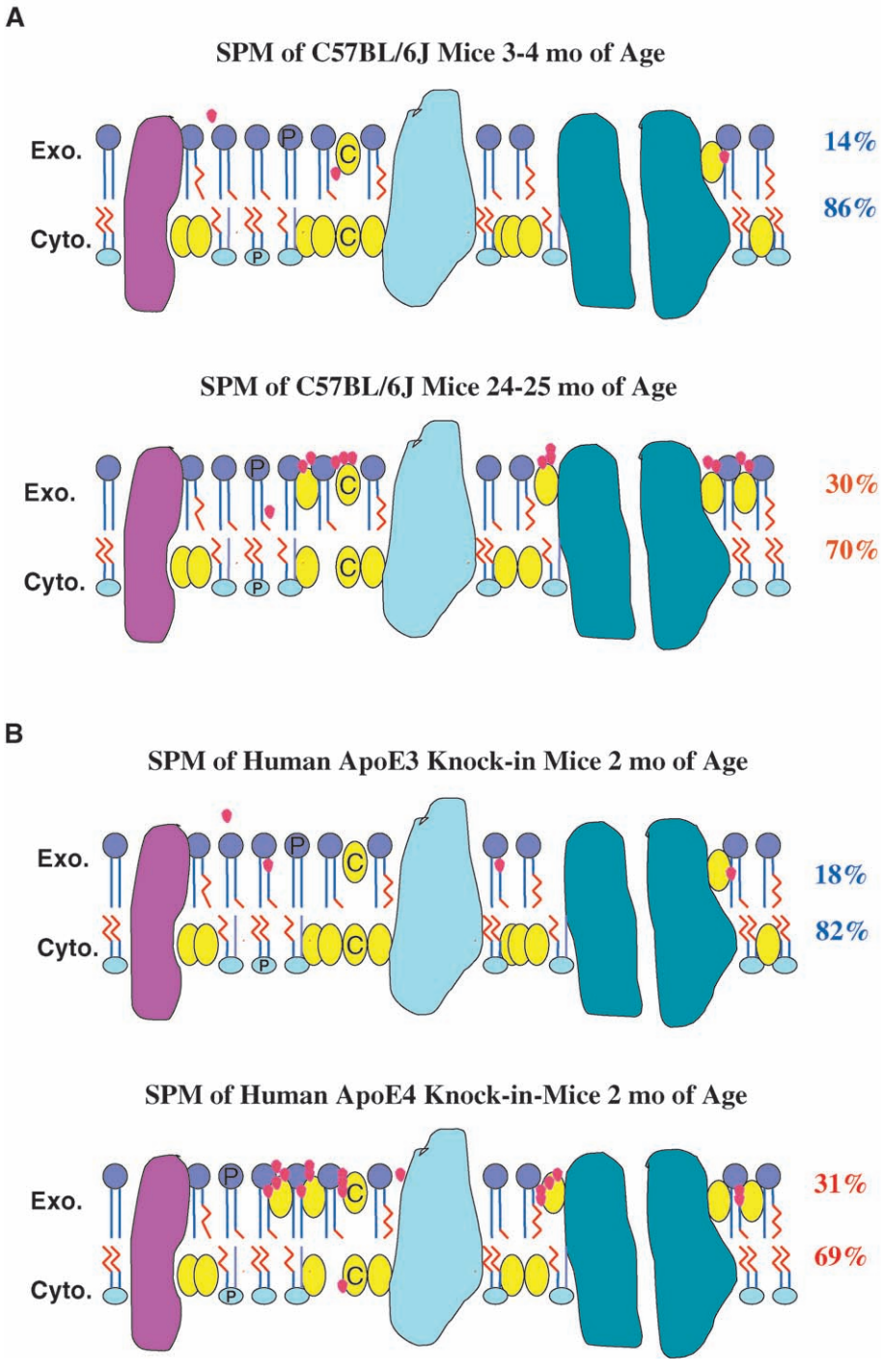

Fig. 1. Model of transbilayer distribution of cholesterol in the SPM exofacial (Exo.) and cytofacial (Cyto.) leaflets of C57BL/6J mice (3–4 and 24–25 months of age) and human ApoE3 and ApoE4 knock-in mice (2 months of age). Panel A shows the transbilayer cholesterol distribution in SPM of mice 3–4 and 24–25 months of age. Panel B shows transbilayer cholesterol distribution in SPM of human ApoE3 and ApoE4 knock-in mice. Percent distribution of cholesterol is shown for each leaflet. Aβ synthesis and movement into and out of membranes may be affected by an increase in cholesterol of the exofacial leaflet. Both accumulation of cholesterol and Aβ could promote peptide polymerization, alter lipid rafts, and disrupt integral membrane proteins. C, cholesterol; P, phospholipids; red structures, amyloid beta-protein. Modified from Ref. [71].

exofacial leaflet contains approximately 15% of total SPM cholesterol and the cytofacial leaflet 85% [58,105–107]. There was a doubling of cholesterol in the SPM exofacial leaflet of aged mice as compared with younger mice [105]. Total SPM cholesterol did not differ with age. Recently, it was reported that mice expressing human apoE4 had substantially more cholesterol in the exofacial leaflet in contrast to the exofacial leaflet of mice expressing human apoE3 and wild-type mice [106]. Total SPM cholesterol did not differ among the three groups. As discussed earlier in this paper, an increase in cholesterol amount enhances expression of APP and Aβ. In addition, APP, Aβ and presenilin-1 have been identified in cholesterol enriched membrane domains [24,25,27,83,84]. It has been suggested that these cholesterol domains may reside in the membrane exofacial leaflet [108]. Cholesterol catalyzed the fibrillogenesis of soluble Aβ [109,110]. Taken together, the structure and lipid composition of the exofacial leaflet may play an important role in Aβ dynamics. Certainly there is much more work that needs to be done in understanding the potential role of cholesterol asymmetry of the exofacial and cytofacial leaflets and Aβ dynamics. It would be worthwhile to determine if differences in the transbilayer distribution of cholesterol occur in brain tissue from humans with the apoE3 and apoE4 alleles. In addition, mechanisms regulating cholesterol asymmetry are not understood. Several potential mechanisms have been proposed (sterol carrier protein-2, fatty acid binding proteins, apoE, LDLR) whose dysfunction may precede the development of AD [70,71,88].

8. Conclusions

Aβ is thought to be a primary factor in the pathogenesis of AD. This protein has a physico-chemical relationship with membranes and cholesterol. Aβ-induced changes in membrane structure can certainly contribute to alterations in cell function. The effects of Aβ on membrane structure are heterogeneous, acting dissimilarly in different membrane locations. Cholesterol is a major component of plasma membranes and cholesterol may be both a promoter and a target of Aβ. Certain statins that lower cholesterol levels would appear to reduce the risk of AD. However, whether changes in the total amount of cholesterol, peripherally or in brain, are responsible for the reduced risk of AD is not known. Pharmacokinetics and pharmacodynamics of statins in brain is an area of research that is sorely in need of attention. Administration of statins in cell culture is problematic for two important reasons. Both lovastatin and simvastatin have to be metabolized before these drugs can inhibit HMG-CoA reductase. In the absence of brain levels of these drugs, it is not known what form of the drug or how much of the drug is in the brain. Another concern is that typically cholesterol is reduced by over 50% in cell culture studies. While APP and Aβ expressions are reduced many other proteins as well as the membrane environment will be altered. Finally, a hypothesis is proposed that does not rely on changes in the total amount of cholesterol but instead predicts that redistribution of cholesterol from the cytofacial leaflet to the exofacial leaflet acts to promote APP and Aβ expressions and disrupts membrane structure, including fluidity and lipid rafts and various cell functions such as ion flux and cholesterol transport. Increasing age and inheritance of the apoE4 allele are the two greatest risk factors for late-onset AD and both conditions result in the doubling of cholesterol in the exofacial leaflet, which in combination could be additive. Obviously there are many more questions than there are answers concerning the role of cholesterol in the pathogenesis of AD. An important benefit of the widening interest in cholesterol and AD is that it has substantially increased an understanding of the biochemistry of brain cholesterol.

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