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Membrane Biophysics and Mechanics in Alzheimer's Disease

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Abstract Alzheimer's disease is a chronic neurodegenerative disorder characterized by neuronal loss, cerebrovascular inflammation, and accumulation of senile plaques in the brain parenchyma and cerebral blood vessels. Amyloid-β peptide (Aβ), a major component of senile plaques, has been shown to exert multiple toxic effects to neurons, astrocytes, glial cells, and brain endothelium. Oligomeric Aβ can disturb the structure and function of cell membranes and alter membrane mechanical properties, such as membrane fluidity and molecular order. Much of these effects are attributed to their capability to trigger oxidative stress and inflammation. In this review, we discuss the effects of Aβ on neuronal cells, astrocytes, and cerebral endothelial cells with special emphasis on cell membrane properties and cell functions.

Keywords Amyloid-β · Alzheimer's disease · Membrane properties · Membrane order · Membrane fluidity · Membrane potential · Cerebral endothelium

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, which affects higher cognitive functions, memory, and learning. In AD brains, there is an increased deposition of amyloid plaques together with the increased number of activated microglial cells in the parenchyma and monocytes in the vessel wall [1–6]. Amyloid-β peptide (Aβ) derived from the amyloidogenic pathway of amyloid precursor protein (APP) processing [7] is the primary component of amyloid plaques [8]. Aβ monomers aggregate into oligomers, fibrils, and plaques, which have different impacts on cellular functions [9–13]. In fact, it has been reported that oligomeric Aβ is more toxic than fibrillar and monomeric Aβ [11]. Deposition of Aβ in AD brains and cerebral vessels results in neurovascular dysfunction and chronic neurodegeneration [14]. In addition, oligomeric Aβ can induce oxidative stress, apoptosis, abnormal calcium homeostasis, and long-term potentiation and can self-assemble into large, voltage-independent, and nonselective ion channels at cell membranes. Aβ can also perturb the molecular packing of cell membranes, resulting in subsequent alterations of biophysical properties of membranes, such as membrane microviscosity, membrane molecular order, membrane potential, and permeability. Altered membrane properties, in turn, may disrupt membrane functions, activities of membrane-related proteins, and many cellular pathways. Understanding the mechanisms leading to changes of membranes mechanics and biophysics and how they result in changes in cell functions should prove to provide insights into new therapeutic strategies for prevention and treatment of AD.

Aβ–Membrane Interactions in Neurons

The accumulation of Aβ to form senile plaques is one of the hallmarks of AD. Interactions between Aβ peptides and neuronal membranes play a vital role in the neurotoxicity associated with AD [15–23]. By virtue of its structure, Aβ

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is capable of binding to a variety of biomolecules including lipids and proteins, which, in turn, perturbs the organization and functions of membranes, such as membrane fluidity and the formation of ion channel and neurotoxicity [24, 25]. There are two types of Aβ–membrane interactions: Aβ peptide can either be firmly anchored in a membrane upon proteolytic cleavage, thereby being prevented against release and aggregation, or it can have adverse effects when bound to membrane surfaces by undergoing accelerated aggregation and causing neuronal apoptotic cell death [21]. On the other hand, membrane charge and membrane fluidity can influence the conformational structure of Aβ, Aβ binding, and permeabilization [26]. For example, when Aβ is exposed to small amounts of sodium dodecyl sulfate (SDS), which mimic the negatively charged membrane environment, it is converted to β-sheet [23].

Aβ binding to plasma membranes can cause the disturbance of the structure and function of membranes. Williamson et al. [22] showed that exogenously applied Aβ was redistributed on membrane and accumulated in lipid rafts where the protein composition was altered. After Aβ treatment, neuronal membranes are depolarized [27] and exhibit changes in varicosities along neurites and enhance membrane permeability to propidium iodide [28]. In addition, using mass spectrometric assay of lipid damage, Murray et al. [29] showed that oxidative lipid damage caused by Aβ was accelerated in the presences of ascorbate and copper ion.

In addition to plasma membrane damage, Aβ can cause intracellular membrane damage. For example, disturbances of endosomal/lysosomal system are implicated in the process of neurodegeneration [30]. Aβ in the culture medium can be taken up by neurons and accumulate inside the endosomal/lysosomal system. Exposing cultured neurons to soluble Aβ can trigger free radical generation within lysosomes and disruption of lysosomal membrane proton gradient and lead to the loss of lysosomal membrane impermeability preceding to cell death [16].

Cholesterol Modulates Aβ–Membrane Interactions

Cholesterol plays an important role in the pathogenesis of AD [31–36]. It has been shown that cholesterol can modulate the interactions between Aβ and membrane [37] and that membrane insertion ability of Aβ is critically controlled by the ratio of cholesterol to phospholipids. In membranes with low concentrations of cholesterol, Aβ prefers to stay within the membrane surface region and is mainly in the β-sheet structure. In contrast, as the ratio of cholesterol to phospholipids rises, Aβ can insert spontaneously into the lipid bilayer due to its hydrophobic C-terminus [38].

In addition, membrane cholesterol can modulate the cleavage of amyloid precursor protein (APP) and alter the production of Aβ [33, 36, 39]. In fact, it has been reported that membrane cholesterol depletion decreases the content of APP in cholesterol and sphingolipid-enriched membrane microdomains and subsequently inhibits the amyloidogenic pathway to produce Aβ. Moreover, depletion of cellular cholesterol levels reduces the ability of Aβ to act as a seed for further fibril formation [34, 35]. There is evidence that plasma membrane cholesterol controls the toxicity of Aβ [40] and protects cells from apoptosis induced by soluble oligomers but not fibrils of Aβ [41]. There are some contradictory results from different studies [42–44], possibly due to using different models and experimental conditions. Consequently, more systematic studies are needed to address the discrepancy.

Aβ Induces Membrane Damage and Dysregulation of Calcium Concentration

Dysregulation of ion homeostasis has been implicated in the pathogenesis of AD. Dysregulated inositol triphosphate (IP3) signaling in cortical neurons of knock-in mice expressing an Alzheimer's-linked mutation in presenilin1 results in exaggerated Ca^{2+} signals and altered membrane excitability [45]. Protein kinase C inhibits the transplasma membrane influx of Ca^{2+} triggered by 4-aminopyridine in Jurkat T lymphocytes [46]. Apolipoprotein E epsilon 4 allele (apoE4) has a clear association with AD. ApoE4 molecules can rapidly suppress the activities of delayed rectifier potassium (IK) channels in hippocampal neurons when they are applied on the inner side of the neuronal membrane [47]. Overproduction of apoE4 in neurons may suppress normal IK channel activities and thus be responsible for the neuronal damages related to the pathogenesis of AD.

Aβ has been shown to form Ca^{2+} channels in lipid vesicles and alter the concentration of intracellular Ca^{2+} in neuronal cells and astrocytes [48–55]. Aβ decreases plasma membrane Ca^{2+}-ATPase (PMCA) activity purified from normal brain, which plays a crucial role in controlling cytosolic Ca^{2+} [56]. Aβ also invoked the release of calcium from the endoplasmic reticulum (ER) and subsequently triggered apoptotic pathway [11, 57, 58]. In turn, calcium signaling increased the aggregation of early protofibrillar structures and markedly increased conversion of protofibrils to mature amyloid fibrils, which play a role in the pathogenesis of AD [59].

Phospholiases A_2 and Cell Membrane Properties in AD

Aβ deposits are associated with the activation of phospholipase A_2 (PLA_2s) [60–62]. These enzymes are ubiquitous in mammalian cells for catalyzing the cleavage of fatty acids from *sn*-2 position of phospholipids. PLA_2s are

classified into three major families: calcium-dependent cytosolic PLA_2 ($cPLA_2$), secretory PLA_2 ($sPLA_2$), and calcium-independent PLA_2 ($iPLA_2$). These enzymes are responsible for maintenance of phospholipid homeostasis in cell membranes. They are also important in the production of lipid mediators, such as arachidonic acid (AA), a precursor for synthesis of eicosanoids [63, 64]. Activation of PLA_2s occurs in a number of pathologic conditions including AD [63–72].

It has been reported that immunoreactivity of $cPLA_2$ (group IVA) increased in reactive astrocytes in severe AD patient brains [60, 61]. In addition, increases in immunoreactivity of $sPLA_2$-IIA in astrocytes were found in postmortem inferior temporal gyrus and hippocampal dentate gyrus and CA3 field of AD brains [62]. Up-regulation of $sPLA_2$-IIA mRNA was reported in the hippocampus (confined mainly to dentate gyrus and CA3 field) of AD patients [62]. $cPLA_2$ mRNA was also up-regulated in the hippocampal CA1 field of AD patients [73]. Furthermore, Aβ has been shown to activate $cPLA_2$ in primary rat or mouse cortical neurons and in PC12 cells [74–77].

PLA_2 plays key roles in modulation of membrane properties under pathologic and physiologic conditions. For instance, the treatment of immortalized astrocytes (DITNC) with Aβ promotes the reactive oxygen species (ROS) production from nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and activation of $cPLA_2$, which, in turn, increased membrane molecular order [78]. Membrane microdomains, which are enriched in cholesterol, sphingolipids, and saturated phospholipids, are highly ordered and tightly packed [79, 80]. There is less water content in these membrane microdomains. The emission spectrum of LAURDAN, an environment sensitive probe, is sensitive to membrane phase properties (e.g., molecular order). The generalized polarization (GP) of LAURDAN has been defined to characterize the change of the emission spectrum due to changes in membrane phase properties [81–86]. Methylarachidonyl fluorophosphonate, the inhibitor of $cPLA_2$ and $iPLA_2$, suppressed the increase in membrane order, but bromoenol lactone (BEL), the specific inhibitor of $iPLA_2$, did not. These results suggest that $cPLA_2$ but not $iPLA_2$ mediated the Aβ-induced membrane molecular order increase [78]. In primary rat cortical astrocytes, ROS induced by menadione, a redox active agent, also alters astrocyte membrane molecular order through activation of $cPLA_2$ [87].

Membrane fluidity is another important parameter for characterizing the physiologic state of the cells. In AD brains, there is evidence for reduced membrane fluidity together with the decreased PLA_2 activity [88–90]. Other studies also show that inhibition of PLA_2 reduces membrane fluidity [91, 92]. Injection of PLA_2 inhibitor into the CA1 area of rat hippocampus resulted in reduced membrane fluidity as compared with control [91, 92]. In addition, inhibition of PLA_2 activity also impaired the formation of short- and long-term memory [91, 92]. This finding is interesting and may have important implications in potential therapy for AD.

Mitochondrial Dysfunction in AD

Several lines of evidence suggest that mitochondrial dysfunction plays an important role in AD pathogenesis. Studies of postmortem brains indicated that neurons from AD brains were deficient in pyruvate dehydrogenase and cytochrome oxidase activity [93]. Mitochondrial encoded gene expressions were aberrant in AD postmortem brains and transgenic mice overexpressing human mutant APP [94, 95]. Several other studies showed decreased ATP production and increased production of free radicals, lipid peroxidation, oxidative DNAs, and protein damages in AD brains [96–99]. Several groups found that Aβ can accumulate in mitochondrial membrane and subsequently induce mitochondrial dysfunction and ROS production [100–103]. The result from rat mitochondria showed that Aβ is transported into mitochondria via the translocase of the outer membrane (TOM) machinery, and after the import, Aβ is associated with the inner membrane fraction [102]. Aβ progressively accumulates in mitochondria and is associated with the decreased enzymatic activity of respiratory chain complexes (III and IV) and the reduction in the rate of oxygen consumption and increased ROS production [100, 103]. Importantly, mitochondria-associated Aβ was detected as early as 4 months, before extensive extracellular Aβ deposits in APP transgenic mice [100]. These studies delineate a new means, through which Aβ potentially impairs neuronal energetics, contributing to cellular dysfunction in AD [100, 103]. Although the mechanism is still not fully understood, Aβ-induced mitochondrial dysfunction is also associated with abnormal mitochondrial dynamics [104].

Aβ-induced activation of PLA_2 may play a role in mitochondrial dysfunction. In fact, our studies demonstrated that the Aβ-induced activation of PLA_2 led to loss of mitochondrial membrane potential ($\Delta\Psi_m$) and mitochondrial ROS production in primary rat cortical astrocytes [105]. In this study, oligomeric Aβ was shown to activate $cPLA_2$ through the NADPH oxidase and mitogen-activated protein kinase pathway as well as methylarachidonyl fluorophosphonate, inhibitor of both $cPLA_2$ and $iPLA_2$, completely suppressed loss of Aβ-induced $\Delta\Psi_{m,}$ indicating that activation of PLA_2 is required for the $\Delta\Psi_m$ loss in astroctyes [105]. On the other hand, BEL, a specific inhibitor of $iPLA_2$, can only suppress Aβ-induced $\Delta\Psi_m$ loss during the first 12 to 15 minutes, suggesting that $iPLA_2$

is involved in the initial $\Delta\Psi_m$ loss [105]. Since BEL is specific for $iPLA_2$ inhibition, these results suggest that a lag time of about 12 to 15 minutes was required for $cPLA_2$ to induce $\Delta\Psi_m$ loss. Consistently, confocal fluorescence microscopy analysis demonstrated increase in colocalization between p-$cPLA_2$ and mitochondria 20 minutes after Aβ treatment [105]. Taken together, these data suggest a mechanism for Aβ-induced initial ROS production through NADPH oxidase, which leads to activation of PLA_2, and activated $cPLA_2$ and $iPLA_2$ in turn target mitochondria and subsequently cause the $\Delta\Psi_m$ loss and mitochondrial ROS production.

Since PLA_2 is responsible for hydrolysis of membrane phospholipids and for the release of free fatty acids and lysophospholipids, enhanced PLA_2 activity may have a number of physiologic consequences. Free fatty acids are classical uncouplers of mitochondrial respiratory chain [106, 107], and lysophospholipids possess detergent properties. AA release by PLA_2 has been shown to trigger a Ca^{2+}-dependent apoptotic pathway by opening mitochondrial permeability transition pores (mPTP) [108]. However, the mechanisms linking Aβ, PLA_2, and mitochondrial dysfunctional are still poorly understood and require additional studies.

Membrane Biophysics and APP Processing

Aβ is derived from cleavage of amyloid precursor protein (APP) by β- and γ-secretases [7]. Alternatively, APP can be cleaved by α-secretase and produce neurotrophic and neuroprotective soluble APP ($sAPP_\alpha$) in a nonamyloidogenic pathway [109]. Since APP and α-, β-, and γ-secretases are membrane proteins, APP processing can be affected by the local membrane environment. The cleavage of APP by β-secretase (BACE), the primary step to produce Aβ [110, 111], occurs mainly in lipid rafts, which are highly ordered membrane microdomains enriched with cholesterol, sphingolipids, and saturated phospholipids [112–117]. On the other hand, the activity of α-secretases is favorable in nonraft domains [31]. Therefore, APP processing can be altered by manipulating membrane lipid composition, such as removal of cholesterol and sphingolipids [36, 118–120]. Since PLA_2 can alter membrane properties, it is reasonable that these enzymes can also affect APP processing and increase $sAPP_\alpha$ production [121]. In our recent study, we demonstrated the capability of $sPLA_2$-III and AA to increase $sAPP_\alpha$ secretion and alter membrane fluidity in neuronal cells [122]. In another study, $sPLA_2$-III was shown to increase membrane fluidity in hippocampal neurons in vivo [123]. Besides AA, docosahexenoic acid (DHA) can also increase membrane fluidity and $sAPP_\alpha$ secretion in human embryonic kidney 293 cells (HEK) cells and in neuronal SH-SY5Y overexpressing APP cells [124]. Other studies demonstrated effects of benzyl alcohol (C_6H_5OH) to increase membrane fluidity and $sAPP_\alpha$ secretion, whereas Pluronic F68 (PF68) decreased membrane fluidity and $sAPP_\alpha$ secretion [125]. In turn, Aβ itself accelerates the amyloidogenic processing of APP by reducing membrane fluidity [125]. These results suggest that compounds capable of altering membrane fluidity can modulate $sAPP_\alpha$ production. Study by Kojro et al. [36] showed that treatment with methyl-β-cyclodextrin (MβCD) to reduce cellular cholesterol increased membrane fluidity, APP accumulation at the cell surface, and $sAPP_\alpha$ secretion. Our study also showed that $sPLA_2$-III and AA treatment increased the accumulation of APP at cell surface [122]. These results are consistent with the notion that Aβ production mainly occurs in endosomes [120, 126–130].

Taken together, increase in membrane fluidity seems to result in APP accumulation at the cell surface and increase in $sAPP_\alpha$ secretion. Since $sAPP_\alpha$ is neuroprotective and neurotrophic, and α-secretase cleavage of APP may compete with the BACE cleavage, enhancing the nonamyloidogenic pathway should prove to be a potential pharmacologic approach for the treatment of AD.

Effects of Aβ on Cerebral Endothelium

Cerebral endothelial cell (CECs) layer is a major component of the blood–brain barrier (BBB). CECs layer is consisted of high-density cells connected by tight junctions. CECs have a little number of endothelial pores, are rich in mitochondria, and have a very low content of the pinocytotic vesicles. The biomechanical properties of the CECs are critical to regulations of many cellular functions, such as adhesion, signaling, and morphology and play a vital role in the maintenance of the BBB permeability and brain parenchyma homeostasis.

In agreement with impairments of CECs structure and functions in AD, many studies have indicated the decrease in cerebral blood flow, reduced microvascular density, and low immunoreactivity of endothelial markers CD34 and CD31 in AD brains [131–136]. Light and electron microscopy studies have demonstrated decreased mitochondrial and increased pinocytotic vesicles content, swelling, and degeneration of endothelial cells in AD brain [137, 138]. In vitro studies have also shown the ability for Aβ to induce significant dysfunctions in the CECs. Specifically, physiologic concentrations of soluble Aβ (10^{-9}–10^{-6}M) induced dose-dependent reduction of NO production, altered cellular calcium level by forming calcium-permeable channels in the membranes, initiated albumin transfer across EC monolayer, and impaired EC glucose uptake [139–142]. Higher concentrations of Aβ have been demonstrated to induce mitochondria dysfunction, nuclear chromatin condensation, DNA fragmentation, and significant CECs death [140, 141, 143].

There is increasing evidence that oxidative stress is a major mechanism leading to a cerebrovascular dysfunction in AD. Several studies of transgenic mice overexpressing APP have demonstrated oxidative damage of CECs, up-regulation of superoxide dismutase (SOD) around brain microvessels, and significant impairment of the cerebrovascular functions [144–146]. In vitro, the treatment of CECs with Aβ increased free radical production, and this effect was attenuated by free radical scavengers [144]. Aβ-induced oxidative stress in CECs, in turn, initiated a cascade of redox reactions leading to apoptosis and neurovascular inflammation [141, 143, 147–150]. ROS also cause CECs membrane depolarization, dysfunction of membrane binding proteins, and alteration of membrane structure and functions [151–154].

In AD brains, the increased deposition of Aβ in the cerebral vasculature has been found to correlate with the accumulation of monocytes in the vessel walls and activated microglia cells in the adjacent parenchyma [155–157]. There is evidence that peripheral monocytes can migrate across the BBB and differentiate into microglia [158]. In vitro studies have demonstrated that Aβ deposition at the endothelial cell layer enhances the transmigration of monocytes [159–162]. Since primary capture of the monocytes to endothelium and rolling are mediated by tethering on selectin–ligand interactions [163–165], mechanical properties of the membranes and membrane–cytoskeleton connectivity as well as the expression of adhesion molecules are critical for transmigration [166–171]. To study the direct effects of oligomeric Aβ on mechanical properties of CECs, atomic force microscopy (AFM) and quantitative fluorescent microscopy (QIM) were applied (Askarova et al., unpublished data). QIM studies have demonstrated that Aβ promoted expression of adhesion molecules (P-selectin) and increased actin polymerization. Consistent with QIM results, AFM data demonstrated that oligomeric Aβ increased cell stiffness and the probability of adhesion, but decreased the apparent rupture force of selectin–ligand bonding, probably due to dissociation of connectivity between the cytoskeleton and the bilayer membrane (Askarova et al., unpublished data).

The tight junctions of high electrical resistance and close cell–cell contacts are also critical biomechanical factors maintaining brain homeostasis and impermeability of BBB for the blood cells. Tight junction is a complex of transmembrane proteins (occluding, claudins, junctional molecule-1) and submembrane molecules connected to actin network. In vitro studies have demonstrated that exposure of CECs to Aβ altered expression of occluding and claudin-1, disrupted plasma membrane subunits of claudin-5, and led to relocation of the submembrane protein ZO-2 to the cytoplasm [172]. Several studies have shown that oligomeric Aβ also altered actin polymerization within neurons and CECs [173, 174]. These findings suggest that the effects of Aβ on actin and tight junction protein complexes may cause the alteration of endothelial layer integrity and contribute to the enhanced transmigration of monocytes across the BBB.

Consequently, chronic neurovascular dysfunctions and degeneration of endothelium are observed in all stages of AD and may even precede neuron degeneration in AD brains [175, 176]. In vivo and In vitro studies have demonstrated that vascular deposition of Aβ induces oxidative stress in cerebral vasculature, triggers inflammatory processes and apoptosis, promotes expression of adhesion molecules, affects tight junctions, and changes mechanical properties of the CECs membranes in a manner favoring transmigration of immune cells across BBB. Continuous degeneration of CECs is likely to impair BBB permeability, leading to leakage of blood plasma components and neurotoxic substances into the brain parenchyma. Breakdown of BBB functions drives the disease development toward exacerbation of oxidative and inflammatory conditions characteristic of the AD brain and contributes to further progression of the disease. Understanding the early molecular and biophysical mechanisms of the CECs alteration may offer new approaches to diagnosis and treatment of AD.

Conclusion

Increased deposition and accumulation of Aβ in the brain parenchyma and cerebral blood vessels and Aβ-altered cell membranes are the major physiologic events in AD. Here, we have reviewed the effects of Aβ on neuronal cells, astrocytes, and CECs with the focus on cell membrane properties. Strong evidence has shown that Aβ–membrane binding causes the disturbance of the biochemical, biophysical, and functional parameters of the plasma, intracellular, and mitochondria membranes. An alteration of membrane properties and changes of membrane-related protein activity in neurons and astrocytes can disrupt calcium metabolism and, by leading to mitochondria dysfunctions, can trigger downstream cellular pathways causing oxidative stress and neurodegeneration. In the brain endothelium, Aβ interaction with endothelium increases ROS generation and apoptosis in the CECs induces inflammation and recruitment of immune cells from a bloodstream, enhances cell stiffness, and weakens adhesion between membranes and cytoskeleton.

On the other hand, membrane physical and chemical properties may influence APP processing, Aβ binding, and permeabilization to membrane. Therefore, understanding different molecular mechanisms underlying Aβ–membrane interactions should provide new insights into the develop-

ment of preventive and treatment strategies for cerebrovascular and neurodegenerative disorders.

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