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QUARTERLY

Review

Alzheimer's disease: Its origin at the membrane, evidence and questions★✪

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Numerous results on membrane lipid composition from different regions of autopsied Alzheimer's disease brains in comparison with corresponding fractions isolated from control brains revealed significant differences in serine- and ethanolamine-containing glycerophospholipid as well as in glycosphingolipid content. Changes in membrane lipid composition are frequently accompanied by alterations in membrane fluidity, hydrophobic mismatch, lipid signaling pathways, transient formation and disappearance of lipid microdomains, changes in membrane permeability to cations and variations of other membrane properties. In this review we focus on possible implications of altered membrane composition on β-amyloid precursor protein (APP) and on proteolysis of APP leading eventually to the formation of neurotoxic β-amyloid (Aβ) peptides, the major proteinaceous component of extracellular senile plaques, directly involved in Alzheimer's disease pathogenesis.

Alzheimer's disease is a progressive neurodegenerative disorder that is clinically characterized by the presence of extracellular senile plaques, intracellular neurofibrillary tangles formed by cytoskeletal protein tau in the neuronal cell body, and neuropil threads in dendrites, as well as by synapse and selective neuronal cell loss [1–3]. β-Amyloid (Aβ) pep-

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Abbreviations: Aβ, β-amyloid; APP, β-amyloid precursor protein; sAPP, secreted β-amyloid precursor protein.

tides represent the major component of senile plaques (so-called amyloid plaques) and are generated during endoproteolysis of a large transmembrane protein, β-amyloid precursor protein (APP). The detailed etiopathology of Alzheimer's disease is still unclear and is likely to be multifactorial with various genetic and environmental causes at distinct levels for each individual [4]. Several hypotheses have been proposed to explain the degeneration of neurons in Alzheimer's disease, including the "amyloid cascade hypothesis" which states that an increased level of 42 to 43 amino acid long Aβ peptides (due to mis-sense mutations in the APP and presenilin genes, age-related defects or environment) leads to aggregation of these peptides and formation of amyloid plaques, resulting in progressive neurodegeneration [5]. In addition to this hypothesis, several factors were invoked to contribute to the progressive neurodegenerative disease such as: selective vulnerability of cholinergic neurons in the basal forebrain, mitochondrial dysfunction, oxidative stress, viral agents, toxic material deposits, deficiency of particular nutrients, overstimulation of excitatory amino acid receptors, and altered phospholipid metabolism [6–9]. Especially the latter factor gained recently the attention of investigators due to the observations that the development of Alzheimer's disease is accompanied by changes in the levels of neuronal membrane phospholipids: phosphatidylserine, phosphatidylethanolamine, and phosphatidylinositol [10, 11]. In addition, Ginsberg *et al.* [12] found that decreased ratio of plasmalogen to nonplasmalogen ethanolamine glycerophospholipids in the temporal cortex of Alzheimer's disease individuals may result in membrane instability. This would follow the development of the so-called hydrophobic mismatch in the membranes, i.e., the difference in length between the hydrophobic part of membrane spanning proteins, such as APP, and the hydrophobic region of the membrane lipid bilayer. Such a hydrophobic mismatch can strongly affect protein and lipid organization [13]. In this review, we will discuss about possible effects of lipid compositions on i) APP endoproteolysis and on ii) ion channel properties of Aβ peptides in relation to their neurotoxicity.

BASIC CHARACTERISTICS OF β-AMYLOID PRECURSOR PROTEIN

Aβ peptides, the major components of amyloid plaques, are produced during endoproteolysis of a large type-I transmembrane protein, so-called β-amyloid precursor protein (APP). This protein is derived by differential splicing of a single gene transript located on the long arm of chromosome 21. In this respect, trisomy of chromosome 21 (Down syndrome) leads to the overexpression of APP and to the formation of precocious senile plaques. The predominant isoforms, APP770, APP751, and APP695 (numbers indicate the number of amino-acid residues in each isoform), are expressed with some tissue specificity [1]. The two longer isoforms of APP, APP751 and APP770, contain a 56 amino acid long ectodomain homologous to the Kunitz family of serine protease inhibitors. It has been postulated that the secreted form of APP (sAPP, see next paragraph) could function as a circulating protease inhibitor [14]. In addition, the secreted and membrane forms of APP may be involved in neurite adhesion, neurite extension *via* a neurotropic effect and may play a protective role against excitoxicity [1]. The secondary structure of the secreted and membrane forms of APP is largely α-helical, since these proteins contain 40–45% of α-helix and only 15–20% of β-sheet structures [15].

PROCESSING OF β-AMYLOID PRECURSOR PROTEIN

Newly synthesized APP matures in the secretory pathway by the addition of O-glycosyl

and N-glycosyl residues as well as tyrosine sulfation in the *trans*-Golgi network [14]. At least two distinct pools of APP appear to be present in primary neuronal cultures: the major pool of APP is characterized by a short half-life in the range of 30–60 min and a minor full-length, transmembrane pool of APP by a longer half-life [4]. It has been proposed that the major pool of APP with the rapid turnover is in part secreted out of the cell in the form of soluble APP (sAPPα), while the minor pool of full-length APP remains at the surface of the neurite. The latter localization of APP is consistent with a role for this protein in the stabilization of cell–matrix or cell–cell interaction [4]. Further processing of APP *via* the nonamyloidogenic or amyloidogenic pathways is depicted in Fig. 1. APP is more likely to be cleaved after O-glycosylation, indicating that the cleavage of APP occurs either throughout the Golgi complex (site of glycosylation) or in compartments subsequent to *trans*-Golgi in the APP processing pathway [16]. In the major processing pathway of APP, i.e., the nonamyloidogenic pathway, this protein is cleaved within the Aβ domain (between Lys16 and Leu17; numbering according to the primary sequence of Aβ peptides) preventing the formation of Aβ peptides. During this cleavage by the protease called α-secretase, a soluble ectodomain of APP (sAPPα) is released and a 10-kDa C-terminal fragment (p3CT) remains within the membrane (Fig. 1). This cleavage may occur in the *post*-Golgi compartment [14], at the surface of a neuronal cell [17] or within specific membrane microdomains, caveolae [18], suggesting that distinct pools of APP may coexist within the cell [4, 19]. The soluble peptide derived from APP, sAPPα, is detected in plasma and cerebrospinal fluid and may have neuroprotective roles [1, 14]. In the amyloidogenic pathway that is a minor route, APP is cleaved by β-secretase at the N-terminus of the Aβ domain, producing a soluble protein (sAPPβ) shorter than sAPPα and a C-terminal

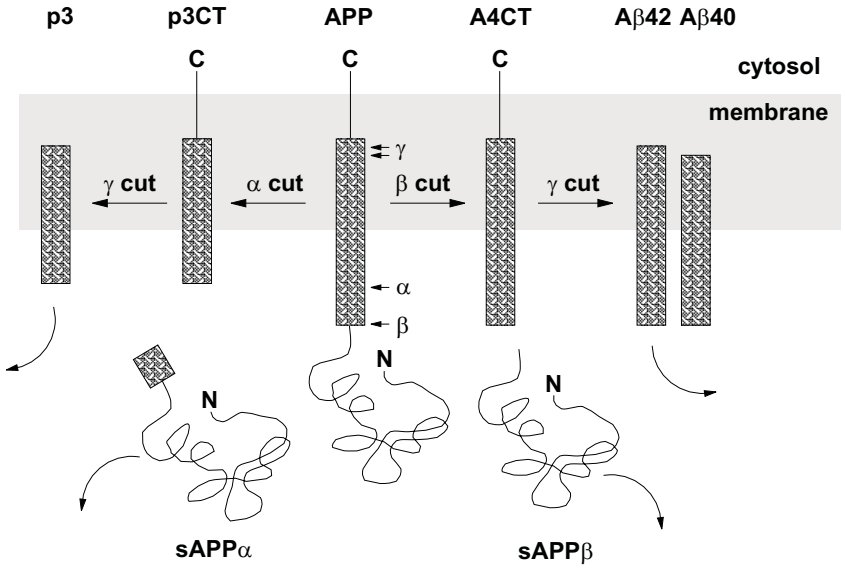

Figure 1. Schematic representation of APP isoforms and their processing by α-, β- and γ-secretases. APP is processed *via* either the nonamyloidogenic (toward the formation of less neurotoxic p3 fragment) or amyloidogenic pathways (toward the formation of more neurotoxic Aβ42 or Aβ40 fragments).

In the nonamyloidogenic pathway α-secretase cleaves APP isoforms within the Aβ domain releasing a large soluble fragments of APP (sAPPα) and a membrane-bound fragment (p3CT). Then, eventually, p3CT fragment can be cleaved by a γ-secretase, releasing the C-terminal p3 peptide. In the amyloidogenic pathway, β-secretase produces the membrane-bound A4CT fragment and releases the soluble sAPPβ. Further processing of the A4CT peptide by γ-secretase generates the Aβ40 or Aβ42 peptides [1]. The filled rectangle within APP indicates the Aβ42 peptide and its approximate location within the membrane bilayer. The Greek letters α, β and γ beside the rectangle show the cleavage sites for the respective secretases.

peptide residing in the membrane (A4CT). The A4CT fragment is the precursor for Aβ peptides (Fig. 1). β-Secretase cleaves APP either within the endocytic pathway following reinternalization of cell-surface APP or within the endoplasmic reticulum and Golgi. The existence of various sites of action of β-secretase is consistent with the existence of distinct pools of APP within the nervous system [4, 19]. The membrane-bound fragments of APP, A4CT and p3CT (Fig. 1), can be cleaved by γ-secretase, an unusual protease that seems to cut within the transmembrane domain of C-terminal fragments of APP [20], releasing respectively the 40 and 42 amino acid long Aβ peptides (Aβ40 and Aβ42) and the shorter p3 peptide [21]. The Aβ40 peptide is the major type of Aβ peptides secreted into normal human cerebrospinal fluid, while the more pathological Aβ42 peptide is the minor species.

Recently, Kosik [18] noted that the precise site of cleavage of APP by γ-secretase could be related to changes of cholesterol content within the membrane. More precisely, as APP transits from the endoplasmic reticulum to the plasma membrane, the cholesterol content within membrane increases, inducing an increase of membrane flexibility and thickness. It has been suggested that unusual membrane flexibility could permit differential access of γ-secretase to the cleavage site when APP is in the endoplasmic reticulum, Golgi or plasma membrane [18]. Indeed, it has already been observed that the preferred site for the production of the Aβ42 peptide is in the endoplasmic reticulum/intermediate compartment, while the preferred site for the production of the Aβ40 peptide is Golgi apparatus and beyond [18].

SECRETASES ARE MEMBRANE PROTEINS

The identification of proteases involved in APP processing is of particular importance since they are good candidates for drug design to prevent the Aβ peptide formation. Although α-secretase has not been isolated yet, this protease appears to be an integral membrane protein that is inhibited by hydroxamic acid-based zinc metalloproteinase inhibitors [22]. Moreover, Vassar *et al.* [23] have cloned a transmembrane aspartic protease, BACE, which has characteristics of β-secretase. Independently, other groups purified and cloned β-secretase obtaining the same sequence as for BACE (also called Asp 2) [24–26]. Recently, Lin *et al.* [27] isolated a human aspartic protease, memapsine 2, which cleaves at the β-secretase site of APP. In the case of γ-secretase, several authors reported that presenilins are either essential for γ-secretase activity or are themselves γ-secretases [18, 28–31]. Recently, presenilin I was identified as a γ-secretase, an intramembrane aspartyl protease [32]. It has to be stressed that α-secretase, β-secretase and presenilin (alias γ-secretase) are all transmembrane proteins, implying that subtle changes in lipid composition could modulate their activities.

ROLE OF MEMBRANE MICRODOMAINS IN APP PROCESSING

The localization of APP and its fragments in distinct membrane microdomains is still controversial due to the differences in procedures used to isolate these microdomains. It has been reported that APP does not colocalize to a detectable extent with glycosylphosphatidylinositol (GPI)-anchored proteins and inositol 1,4,5-triphosphate receptor, suggesting that APP is not present in detergent-insoluble membrane domains with caveolae-like properties [33]. The results of similar investigations indicated that APP is indeed not present in abundance in caveolae or caveolae-like domains [34]. This is in accordance with the fact that caveolae are not abundant structures in the nervous system. Although these results

are convincing, it has been suggested that a part of APP can be transported to caveolae-like domains from other membrane domains for further processing [34]. In line with this observation, it has been found that APP is present in a detergent-insoluble glycolipid-enriched fraction, but does not behave as a typical detergent-insoluble membrane protein [35]. APP was also localized to a unique cholesterol-rich domain different from caveolae or caveolae-like domain [34], and it has been reported that depletion of cholesterol content inhibits the production of the Aβ peptide [36]. These results would suggest that APP processing may occur in specific membrane microdomains [37]. In addition, Aβ peptides have been found in the detergent-insoluble membrane compartment [38]. Consistent with these results, it was inferred that presenilin (alias γ-secretase) is present in detergent-insoluble membrane microdomains [39].

THE EFFECTS OF β-AMYLOID PEPTIDES ON MEMBRANE STRUCTURE AND PERMEABILITY AS RELATIONED TO THEIR NEUROTOXICITY

The mechanism of neurotoxicity of the Aβ peptides is not yet completely established. It is based on the conformation of the peptides. Aggregated Aβ peptides contain more intermolecular β-sheet structures than the soluble ones. The correlation between neurotoxicity, aggregation and β-sheet structures has been observed for different types of Aβ peptides in neuronal cells [40–43]. Aggregation of Aβ peptides into insoluble fibers appears to be a nucleation event which can be induced by various types of factors and molecules. For example, there are numerous pieces of evidence that even lipids may induce Aβ peptides aggregation, as has been described in the case of interactions of Aβ peptides with phosphatidylinositol [44], ganglioside-containing membranes [45, 46] or with phosphatidylserine [47]. It was proposed that membrane perturbation by aggregated Aβ peptides constitutes the molecular basis of the peptide neurotoxicity [43]. One likely mechanism of neurotoxicity of Aβ peptides is the formation of calcium-permeable, zinc-sensitive ion channels from aggregated Aβ peptides [48, 49].

CONCLUDING REMARKS

On the basis of the observation that membrane lipids are targets for oxidative damage and that membrane lipid composition changes during aging, leading to progressive membrane defects, the origin of sporadic Alzheimer's disease could be at the lipid level of neuronal cell membrane [10–12]. The content of phosphatidylethanolamine and phosphatidylinositol, but not that of phosphatidylcholine, decreases significantly in the Alzheimer's brains in comparison with control brains [10, 11]. It has been suggested that oxidative stress could contribute to the selective loss of these phospholipid classes. Altered membrane composition could also affect signal transduction and induce neurodegeneration [11]. In addition, lipid composition of plasma membranes of neuronal cells could modify the stability and function of the minor pool APP bound to cell surface, as well as APP processing. Oxidative stress may also perturb the structural integrity of intracellular organelles, altering the processing of the major pool of APP within *trans*-Golgi, or within the subsequent endosomal, lysosomal and endoplasmic reticulum compartments. Changes in lipid composition could affect the function of secretases within the membrane and their activities towards APP. For example, depletion of cholesterol inhibits the generation of the Aβ peptides [36]. Membrane stability depends on the plasmalogen/non-plasmalogen ratio. Plasmalogen deficiency could, therefore, cause membrane instability [12], contributing to cell death, either inde-

pendently or cooperatively with amyloidogenesis [12]. It is also possible that various classes of lipids may favor the conversion of soluble Aβ peptides into aggregated ones. Phosphatidylinositol [44], phosphatidylserine [47], phosphatidylglycerol [47], and ganglioside [45, 46] favor the conversion of soluble Aβ peptides into the aggregated Aβ form. In contrast, *myo*-inositol [44] stabilizes soluble Aβ micelles, while phosphatidylcholine [47] slows down the aggregation process. These observations suggest that subtle lipid changes within the membranes of neuronal cells could induce the aggregation process and production of ion channels by Aβ peptides, inducing their neurotoxicity. Finally, it appears from the accumulated experimental evidence that membrane lipid composition, which can be affected during aging, could contribute to the pathology of sporadic Alzheimer's disease.

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