An alternative interpretation of the amyloid Aβ hypothesis with regard to the pathogenesis of Alzheimer's disease

Vincent T. Marchesi*

Department of Pathology, Boyer Center for Molecular Medicine, Yale University School of Medicine, New Haven, CT 06510

Contributed by Vincent T. Marchesi, April 20, 2005

Alzheimer's disease is a complex neurodegenerative process that is believed to be due to the accumulation of short, hydrophobic peptides derived from amyloid precursor proteins by proteolytic cleavage. It is widely believed that these Aβ peptides are secreted into the extracellular spaces of the CNS, where they assemble into toxic oligomers that kill neurons and eventually form deposits of senile plaques. This essay explores the possibility that a fraction of these Aβ peptides never leave the membrane lipid bilayer after they are generated, but instead exert their toxic effects by competing with and compromising the functions of intramembranous segments of membrane-bound proteins that serve many critical functions. Based on the presence of shared amino acid sequences containing GxxG motifs, I speculate that accumulations of intramembranous Aβ peptides might affect the functions of amyloid precursor protein itself and the assembly of the PS1, Aph1, Pen 2, Nicastrin complex.

neurodegeneration

It has been known for close to a century that plaque-like deposits of material are scattered throughout the brains of patients with advanced dementia, an observation first described by a German neurologist named Alois Alzheimer. We now know that these plaques are composed of many components, including small peptides generated by proteolytic cleavage of a family of transmembrane polypeptides known as amyloid precursor proteins (APPs). Two peptides that are widely regarded as major contributors to the pathology of Alzheimer's disease (AD) are known as Aβ peptides A40 and A42, and each has the amino acid sequence presented below. Both are derived from the putative transmembrane segments of APP molecules, with A42 having two additional nonpolar amino acids.

Cleavage of the APPs by different combinations of three proteolytic enzymes, which are commonly referred to as secretases, generates peptides with different biological properties (Fig. 1). The two Aβ peptides, A40 and A42, are generated when the β- and γ-secretases act in concert, and it is their overproduction (or underremoval) and subsequent aggregation, that is believed to lead to a cascade of reactions that are responsible for neuronal dysfunction, neuronal death, and plaque formation (1, 2).

The evidence that links Aβ peptides to the pathogenesis of AD is substantial, but the means by which these peptides exert their toxic effects, and where in neuronal cells they act, is far from clear. Many new treatments for AD, some quite revolutionary, such as attempts to vaccinate people with the Aβ peptides themselves, are based on the notion that one of these Aβ peptides, the A42 form, is primarily responsible for neuronal damage and cell death that is characteristic of the Alzheimer's syndrome. Although there is no question that Aβ peptides are present in Alzheimer's plaques, we still have no way of knowing how, or even whether, such deposits contribute to the earliest stages of the disease. Some investigators, convinced that the plaques accumulate long after the disease has progressed, believe that small aggregated forms of Aβ 42, often referred to as "soluble oligomers," are the pathogenic agents; but how they work is still a mystery. Some thoughts as to how they might be pathogenic are summarized below.

Mutant forms of APP and of the presenilin proteins (PS1 and PS2), which are now believed to be the γ-secretases, have been identified in a small subset of AD patients who suffer from early-onset disease, and these are associated with elevated levels of both types of Aβ peptides in the affected brains. Because <5% of AD patients have these mutant forms of APP or PS1, we assume that these mutations enhance the same pathogenic processes that also operate in the far more prevalent sporadic forms of AD in patients who lack these mutations. A significant fraction of such patients do express a specific allele of the gene that codes for apolipoprotein E, but how this enhances the onset of AD is still not clear.

A great step forward was achieved when it was realized that human forms of these mutated genes were able to promote the expression of large quantities of Aβ peptides in the brains of transgenic animals, creating potential animal models of the human disease. Many comparable experiments have been reported, using a variety of mutant forms of both APP and PS1, both singly and in combination. Animals bearing combinations of mutant forms of APP and PS1 develop large deposits of Aβ peptides in their brains, but the neurological defects cause by the expression of these mutant human genes have been variable. Some, but not all, affected animals develop significant neuronal degeneration, and some show evidence of learning defects. However, missing from the brains of these doubly transgenic animals were neurofibrillary tangles, one of the hallmarks of human AD. Neurofibrillary tangles are large protein aggregates composed of phosphorylated forms of tau proteins that are linked functionally with the microtubular network that is involved in axonal transport. Recently, a triple transgenic mouse bearing mutant forms of APP, PS1, and Tau was created that seems to have every pathologic feature of human AD, including neurofibrillary tangles (3). Although these triple transgenes had large extracellular deposits of Aβ peptides in their brains as the disease progressed, which were similar in appearance to the plaques seen in human AD, most of the immunologically detectable Aβ was first seen inside neurons, not in the extracellular spaces. These findings confirm what many previous investigators have noted, that the initial manifestations of Aβ accumulation begin inside neurons, with the earliest detectable material located inside membranous compartments,

*E-mail: vincent.marchesi@yale.edu.

Fig. 1. Amino acid sequences of peptides generated by proteolytic cleavage of the APP. The yellow background highlights some of the hydrophobic amino acids that are believed to reside within the lipid bilayer of the membrane. Positively charged residues are blue, and negatively charged residues are red.

which some have identified as endosomal or lysosomal (4–6). Although this latest triple transgene satisfies the criticisms directed to earlier transgene models that lacked one or more features of the human disease, we should remember that no patient has yet been described who has, as far as we know, a comparable genetic burden.

Although mutations in the two presenilin genes (PS1 and PS2) are found in more than half the cases of early onset familial AD, in which large amounts of Aβ 42 accumulate, it is still a mystery how PS1 mutations cause the disease, because >150 different mutations have been identified, and they are scattered throughout the entire polypeptide chain of PS1 molecule (7). If these mutations cause a "gain of function," as many have suggested, because more Aβ 42 accumulates in the brains of these patients, how can so many different mutations achieve the same result? This question has been asked by many workers in the field and still remains unanswered.

Both APP and Aβ Peptides Derived from APP Appear to Be Dimeric

An additional element of complexity, which now has to be considered in any interpretation of the intramembranous cleavage process, is the recent claim (8), not yet widely acknowledged, that APP molecules probably exist *in situ* in neuronal plasma membranes as homodimers. These APP dimers appear to be sequestered in specific domains of the lipid bilayer that are enriched in cholesterol and other sphingolipids. These domains, often referred to as "rafts," are likely to affect how protein–protein interactions are regulated, and may be part of the mechanism through which cholesterol could influence Aβ production (9, 10). Moreover, it has recently been reported that Aβ peptide dimers can also be found in such lipid raft regions (11), a remarkable finding that could imply that both polypeptides of an APP dimer are cleaved in some coordinated way. However, finding aβ dimeric peptides bound to membrane fragments derived from neuronal surface membranes has other implications, as discussed in detail below.

APP dimers are likely to be held together through noncovalent interactions between their transmembrane segments, as is the case with many other single pass transmembrane (TM) proteins of similar type. Glycophorin A (GPA), the major sialoglycoprotein of the human erythrocyte membrane (12) is one well studied example that has a TM domain with a characteristic stretch of GxxxG residues, which, when present in an α-helical fold, is believed to allow close packing of neighboring helical segments. It has been suggested that this might explain why the associations between glycophorin monomers are strong enough to resist dissociation by SDS (13). In this regard, it is interesting to compare the TM sequences of GPA and corresponding segments of both APP and two APP-like proteins, APLP1 and APLP2 (Fig. 2).

Although all three APP-related sequences have the characteristic stretch of 22 nonpolar amino acids found in single-pass TM proteins, APP and APLP1 also have the GxxxG sequence present in GPA, which might be an indication that their dimeric forms might also have glycophorin-like stability when present in a lipid bilayer, or when dissolved in SDS. Although it does appear that this GxxxG pattern is important for the stability of the GPA dimer, recent studies show that stable dimers can also form between other hydrophobic sequences (14–17), including some that have an AxxxG motif. It is also clear that the stability of intramembranous dimeric helices involves van der Waals interactions between amino acids that are distant from the GxxxG motif.

Proteolytic cleavage of APP dimers in neuronal membranes might also explain why dimeric forms of Aβ peptides are present in membrane fragments derived from neuronal surface membranes (11), a recently reported observation that has some surprising implications. Membrane fragments isolated from brains of Tg2576 transgenic mice were isolated by sucrose gradient flotation and found to contain remarkably high levels of peptides containing 40 and 42 amino acids (A40 and A42). Such membrane fragments, enriched in these Aβ peptides, were not found in control brain tissues, and their levels increased progressively as the transgenic mice aged. Furthermore, these investigators found similar Aβ dimer-enriched membrane fragments in brain samples derived from two patients with AD. The authors of this work considered these membrane-bound Aβ dimers to be part of the secretion pathway leading to the extracellular accumulation of Aβ oligomers, but it is also possible that some of these Aβ dimers were the products of intramembranous cleavage of AAP dimers that remained embedded in the lipid bilayer. The implications of this alternative pathway are discussed below.

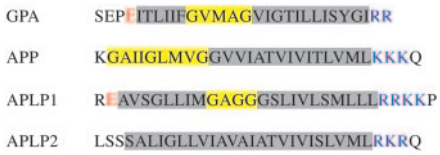

Fig. 2. Comparison of transmembrane domains of the APP family and human erythrocyte GPA. Gray highlighted residues are considered to be the transmembrane domains, with yellow highlighting the GxxxG motifs.

Proteolytic Cleavages of APPs: Many Paths to Aβ Generation

The number of proteolytic enzymes that have the ability to cleave APP and APP-like molecules continues to expand, and with this knowledge comes the opportunity to reconsider how aβ peptides might be generated in ways other than through the coordinated actions of the β and γ-secretases. As depicted below, the commonly accepted pathway for Aβ generation involves the cleavage of APP at sites within the lipid bilayer, which produce 40- and 42-aa peptides that are generally believed to exit from the membrane and collect in the extracellular space, or in the case of APPs on intracytoplasmic membranes, within some specific vesicular compartment. Because large amounts of such peptides are eventually found in the extracellular space, this assumption is reasonable, but one has to ask what fraction of APP molecules are cleaved in this way and whether every cleaved peptide necessarily exits from the membrane. The prevalent view that Aβ peptides jump from the membrane to the aqueous medium is based on the assumption that the polar and charged amino acids at the amino terminus of the Aβ peptides exert enough drag to pull the remaining hydrophobic amino acids and the carboxylated C terminus across the bilayer and into the aqueous medium. Even if a significant fraction of such peptide do partition into the extracellular medium, or inside cytoplasmic vesicles, peptides that are cleaved further, either by α-secretases or other ADAM-like proteases, would have shorter polar segments, and they may be more likely to remain within the lipid bilayer. Similarly, peptide fragments of

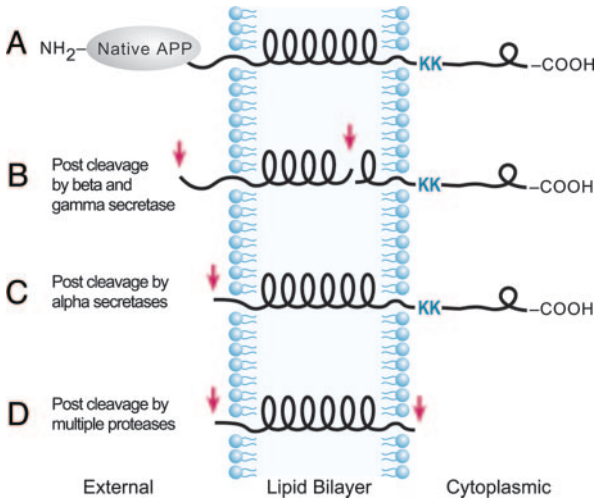

Fig. 3. Schematic diagram of the possible ways in which transmembrane APP molecules might be cleaved by different combinations of membrane-bound proteases. APP, believed to be dimeric in neuronal membranes, is depicted here as a monomer. The transmembrane orientation of APP would be reversed in intracytoplasmic membranes, with the amino terminus extending into the lumens of the ER and other cytoplasmic vesicles.

APP that are generated only by β-secretase action would be expected to remain within the bilayer, as would peptide fragments created by the combined actions of multiple proteases that cleave the APPs at each membrane interface. What happens to peptides like those depicted in Fig. 3*D* is anybody's guess, but there is no reason why they cannot remain within the bilayer for long periods of time, perhaps, in the case of nondividing neurons, even indefinitely.

Interactions Between Aβ Peptides and Cell Membranes: Do They Act on the Outside or from Within the Lipid Bilayer?

Aβ peptides are often referred to as being amphipathic in the sense that their carboxyl terminal segments (either 12 or 14 aa) are nonpolar and, in most cases, hydrophobic. It is this segment that is assumed to interact most strongly with membrane lipids, and indeed, recent theoretical studies using molecular dynamics simulations (18) predict that Aβ peptides can either insert into lipid bilayers or remain there once inserted. Schematic models taken from this study show how different versions of Aβ peptides can assume a transmembrane configuration, even with half the peptide made up of a number of polar and charged residues. The native transmembrane APP molecule is believed to be oriented in neuronal surface membranes as depicted schematically in Fig. 3*A*, with a ≥600-aa segment extending into the extracellular space. Although APP is believed to be dimeric *in situ*, for simplicity, only monomeric versions are shown here.

Sequential cleavage by both β- and γ-secretases generates the fragments depicted in Fig. 3*B* that are the well characterized A40 and A42 fragments considered to be the neurotoxic peptides. Each Aβ peptide (sequences shown above) has a relatively polar amino terminal segment linked to 9 or 11 hydrophobic residues that represent its intramembranous domain. The intramembranous site of cleavage by γ-secretase is marked by one of the red arrows.

If APP molecules are cleaved by either α- or β-secretases, but without subsequent γ cleavage, peptides like those depicted in Fig. 3*C* would be generated, and presumably would remain anchored firmly in the membrane bilayer. Inactive mutant forms of PS1 might be responsible for such partial cleavages. Peptides stripped of most of their charged and polar amino acids, through the actions of multiple proteases, some like the ADAMs acting at the external interface and others like the δ- and ε-secretases (19) acting at the cytoplasmic interface, are shown schematically in Fig. 3*D*. Such peptides would be extremely hydrophobic and likely to remain within the lipid bilayer. They might be confined to lipid raft regions, as some have suggested, or they could move within the membrane, with the potential to affect membrane functions in a number of ways, as described below.

Because an average lipid bilayer, composed primarily of phospholipids, has polar, water-containing domains that cover both surfaces, positively charged amino acids could be stabilized in these domains, and it is the combination of these electrostatic interactions with the surface domains and the stability of the hydrophobic residues within the interior that is believed to account for their ability to insert into membranes. According to this line of reasoning, once these peptide are inserted in the membrane, they are likely to remain there. This idea was exploited experimentally by a number of investigators who attempted to show that Aβ peptides that are secreted into extracellular fluids could reinsert themselves into model lipid bilayers, and once there, could associate together to create what were believed to be channel-like structures that modified the electrical properties of the membrane. This, they suggested, could be the basis for the ability of Aβ peptides to induce neuronal apoptosis by allowing lethal concentrations of calcium to enter cells. Although this is a credible hypothesis that has some experimental support (20, 21), one has to ask whether Aβ peptides can actually do this *in vivo*.

There are many examples of well documented, channel proteins that are composed of β peptides that are arranged in conformations known as "β barrels," but these barrel-like structures are composed of continuous polypeptide chains and are not assembled from individual β strands, as would be the case if the Aβ peptides were to create similar channels. Another complication is the fact that the transmembrane segments of APP molecules, from which the Aβ peptides are derived, have an α-helical conformation, which explains their thermodynamic stability within the membrane environment. Because Aβ peptides present in the extracellular fluid are believed to convert to a hairpin-like β-strand structure after they exit from the membrane, one wonders why they insert back into the lipid bilayer as β-strands, rather than the more stable helical coils. How multiple β-strands can assemble into ordered, stable, functioning channels is another question that remains to be answered.

Several recent observations support an alternative interpretation of how Aβ peptides might alter the properties of neuronal cell membranes. Rather than assuming that they damage cells by inserting into surface membranes after they accumulate in the extracellular spaces, one has to consider the possibility that they might exert their potentially toxic effects only if they remain within the lipid bilayer after proteolytic cleavage. All of the arguments offered to support the claim that they enter membranes from the outside can equally well support the notion that they need not necessarily exit from the membrane after cleavage. Although we know that Aβ

peptides do eventually accumulate outside cells as the disease progresses, how they do so may be more complicated than their simply being expelled as a result of the cleavage process. The recent demonstration, described above, that dimeric forms of the Aβ peptides exist in neuronal membrane fragments, offers powerful support for this idea. By far the simplest interpretation of this finding is that APP dimers are cleaved by the secretases as dimers, and a significant fraction that contain the Aβ 42 fragment are retained in surface membranes. The Aβ dimers identified in this study are likely to have been derived by cleavage of existing APP dimers, but reassociation of cleaved monomers, or fragments of them, is also possible.

One can only imagine how the accumulation of Aβ peptides, or fragments of them, within surface membranes might affect neuronal functions. Intact, uncleaved APP dimers are undoubtedly anchored to specific sites along the membrane through links to cytoskeletal elements, but no obvious restraints would prevent Aβ peptides from diffusing within the plane of the membrane. We have no idea how long such intramembranous fragments would remain within the membrane interior, but if even low levels persist over time, they could have a significant impact on the health of long-lived, nondividing neurons. It is easy to imagine how such peptides could influence the behavior of intramembranous segments of receptors or channels, or even intramembranous segments of enzymes like the presenilins and other secretases.

Lessons From GPA That Might Apply to Amyloid Aβ Peptides

GPA was one of the first TM proteins to be sequenced (12), and it has proved to be a good model system to explore the conformation and behavior of intramembranous segments of single-pass TM proteins (22, 23). The evidence is substantial that its TM domain is a 22-aa segment composed largely of hydrophobic amino acids that fold into a classical α-helical conformation. Moreover, this helical segment retains its helicity in the presence of high concentrations of SDS, and in common with amyloid Aβ peptides, it also is able to oligomerize (or dimerize) in the presence of SDS. When this was first described decades ago (22), the ability of GPA to form stable dimers in SDS was considered a puzzling observation, because SDS was then believed to disrupt all noncovalent interactions between polypeptide chains. Because of its ability to dissociate proteins into their individual subunits, SDS/PAGE was at that time, and still is, routinely used to identify and determine the approximate sizes of subunits of all classes of protein molecules. To explain the ability of GPA dimers to resist disruption by SDS, Engelman and coworkers (13) proposed that the amino acid sequence GxxxG in the TM domain of GPA accounts in part for their stability. They reasoned that glycines on the same side of an α-helix permit closer packing of the polypeptide chains, thereby enhancing van der Waals forces between neighboring helices. This, they believe, complements the stability of the hydrogen bonds that link helical segments together. Although these observations were carried out on chimeric GPA peptides in the presence of SDS, it is reasonable to assume that GPA dimers also exist in the hydrophobic milieu of a lipid bilayer, an environment that enhances hydrogen bonding.

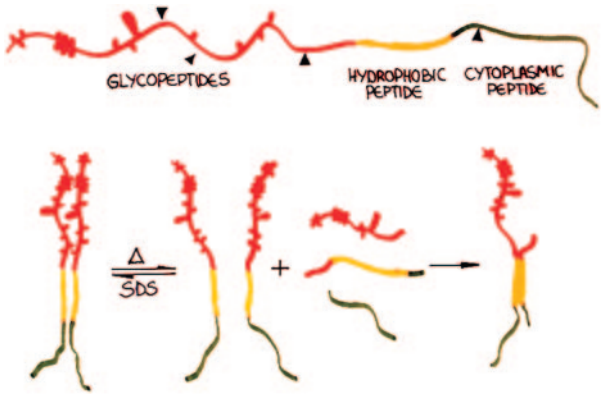

Fig. 4. Glycophorin dimers, normally stable in SDS, are dissociated at high temperature. Under these conditions, peptide fragments generated by proteolytic cleavage of the transmembrane domain are able to associate with glycophorin monomers, creating chimeric pseudodimers. [Reproduced with permission from ref. 22 (Copyright 1976, American Chemical Society, Washington, DC).]

The APP also has a similar GxxxG segment within its transmembrane domain, as do many other transmembrane segments, as discussed below, and many have speculated that APP also exists as a dimer in the plasma membrane. Although the evidence favoring a dimer structure in native membranes is still circumstantial, it is worth noting that many single-pass TM proteins have been shown to be dimeric, and in every case, their TM segments are comparable to those of both GPA and all members of the APP family. If APP is dimeric when embedded in a membrane, and it is cleaved by the secretases while dimeric, one would expect to find dimeric forms of the Aβ peptides in residual membrane fragments, and this is precisely what has been reported, as described above. In those studies, substantial amounts of SDS-stable dimeric forms of both Aβ-40 and Aβ-42 peptides were found in membrane fragments prepared by detergent extraction and sucrose gradient flotation, which indicated that the Aβ dimers were associated with so-called lipid raft domains of the membrane.

The simplest interpretation of these findings is to assume that a substantial amount of uncleaved APP molecules exists as dimers in brain membranes, and when they are cleaved by the sequential actions of the β- and γ-secretases, their intramembranous domains remain associated as dimeric Aβ peptides, as they were in the intact APP molecules. This interpretation differs from the widely held view that Aβ peptides are released into the extracellular space through secretory mechanisms, and as their local concentration increases, they form a series of oligomers and eventually higher forms that become amyloid fibrils (24, 25). What is proposed here is that a fraction of the cleaved Aβ peptides never leave the membrane, but remain instead within the phospholipid bilayer. The effects of these intramembranous peptides are likely to depend on where they are generated and how long they remain within the bilayer. What follows below are speculations as to how Aβ peptides might influence vital membrane functions depending on whether they accumulate in neuronal surface or ER membranes or at synaptic clefts.

Aβ Peptides Might Affect the Cleavage of APP Itself

If Aβ peptides accumulate in high enough concentrations in neuronal cell membranes, they should be able to displace full-length APP monomers and create chimeric dimers, with their associations mediated by their common GxxxG segments. We make this assumption based on earlier studies of GPA, in which it was shown that peptides derived from the transmembrane domain of

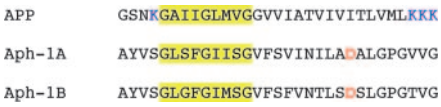

Fig. 5. A comparison of the amino acid sequences of the transmembrane segments of APP and two isoforms of Aph, a critical protein involved the assembly of the PS1/γ-secretase complex. Yellow highlighting defines the GxxxG motifs.

GPA, which contain the GxxxG motif, readily form SDS-stable chimeras with intact GPA molecules. This is shown schematically in Fig. 4. A similar mechanism is proposed for the interactions between Aβ peptides and full-length APP molecules.

Because chimeric dimers, composed of a full-length APP molecule linked to an Aβ fragment, are not normally seen in membranes, both subunits of APP must be cleaved with great efficiency, implying that partially cleaved APP dimers are better substrates for the secretases than uncleaved dimers. If this is so, APP/Aβ chimeras would be preferentially cleaved, thereby generating ever increasing amounts of intramembranous Aβ. Because this cascade effect obviously does not happen in normal neuronal membranes, regulatory mechanisms must exist to either limit the amount of Aβ generated or reduce the amount that is freely diffusible within the bilayer. The PS1/γ-secretase complex is a likely regulator of Aβ production, but as will be described below, its ability to regulate the cleavage process may also be compromised by excessive intramembranous Aβ.

Aβ Peptides Might Destabilize the PS1/γ-Secretase Complex

Recent reports highlight the functional importance of GxxxG motifs in the TM segments of APh1 (26, 27), and these findings raise the possibility that Aβ peptides might also affect the proteolytic processing of APP molecules by interfering with the assembly of the presenilin1/γ-secretase complex. The sequences of the two isoforms of Aph-1 that contain the GxxxG motifs are shown in Fig. 5 and compared with the TM sequence of APP.

Aph1 is a stabilizing element that links together nicastrin and Pen2 with presenilin I to generate γ-secretase action. The role of Aph1 as a necessary component of the PS1/γ-secretase activity was first discovered in *Caenorhabditis elegans* (26), and a mutant form, which had a loss-of-function phenotype, was found to have an amino acid substitution (aspartic) for a critical glycine residue in the fourth transmembrane segment of the molecule. Follow-up studies confirmed that mutated glycines in GxxxG domains of mammalian cells block the ability of APH-1/Nicastrin subcomplexes to stabilize the PS1/γ-secretase complex (27). One has to ask whether excessive amounts of Aβ peptides, which contain similar GxxxG domains, can modify in some way the capacity of GxxxG-containing segments of Aph1 to assemble functional multiprotein complexes. Aβ peptides might compete with the natural binding partners of Aph-1–GxxxG segments or possibly even associate directly with homologous segments of Aph-1 itself. We have no way at present to predict how much Aβ might accumulate inside the membrane, but if it is confined to the two dimensional bilayer, its diffusion would be restricted and its effective concentration would be higher than would be the case for extracellular material.

It may seem a stretch to propose that the Aβ peptides might influence associations between peptides that have similar, but nonidentical, amino acid sequences, but many have noted that the amino acid sequences of TM domains of proteins are not strictly conserved, and substitutions can be made in noncritical residues and still retain intersubunit packing. This has been shown most dramatically in several elegant studies comparing the high-affinity associations between GPA monomers and the lower-affinity homodimers of the M12 coat protein (MCP) (28, 29). Although there are numerous differences in the amino acid sequences of their TM domains, heterodimers are readily formed if they both contain extended peptide segments of 13 hydrophobic amino acids containing the GxxxG motif and a number of β-branched amino acids (isoleucine, valine) that appear to enhance intersubunit packing. It has been suggested, on the basis of studies with several model systems, that the canonical sequence LxxxGxxxGxxxT/S is a characteristic feature of TM domains that can form stable oligomers. If we compare the relevant sequences of Aβ and Aph-1, we see that these parameters are largely satisfied.

Although they both lack an amino terminal leucine, they have the GxxxG

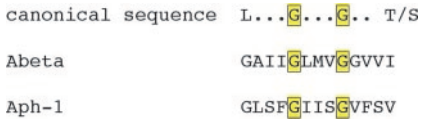

Fig. 6. Both the Aβ peptides and an Aph-1 peptide have amino acid sequences that are similar to the canonical sequence LxxxGxxxGxxxT/S, thought to be a characteristic feature of transmembrane segments that are able to form stable oligomers.

anchoring point and an abundance of valines and isoleucines (Fig. 6). We might also predict that the affinity between the TM segments of Aβ and Aph-1 would be lower than that between GPA dimers, because the hydroxyl side chain of serine[75] (or threonine) enhances the packing of GPA dimers (30), and Aβ and APh-1 have valine or isoleucine at that position. Low-affinity associations between Aβ/Aph-1 heterodimers would be appropriate if their interactions were part of a dynamic regulatory mechanism.

Mutant forms of PS1 have long been associated with the increased production of Aβ peptides in patients with early onset AD, a finding that has been difficult to explain because numerous different mutations seem to generate the same result. If the PS1/γ-secretase complex has a more complex regulatory role than we now realize, a partial loss of an inhibitory function could explain the increased Aβ generation if the mechanism proposed above plays any role *in vivo*.

Implications

If Aβ peptides, or fragments of them, are toxic to neurons because they concentrate within the lipid bilayer, we have to consider alternative ways that neurons might be damaged, as well as entirely different approaches to therapy. The idea that Aβ peptides compete with natural APP dimer formation is only one obvious way in which competing peptides might interfere with normal APP functions. It is now clear that many enzymatic reactions occur within lipid bilayers, including a series of proteolytic cleavages now referred to as regulated intramembranous cleavage (RIP) (31). A variety of signal transduction mechanisms seem to be controlled by RIP activity, including, but not limited to, notch processing, which requires an intact γ-secretase mechanism. Koo and Kopan (32) point out that the PS1/γ-secretase complex is now emerging as an important generator of neuronal dysfunction independent of its role as a generator of excessive Aβ. PS1/γ-secretase insufficiency could result if, as we postulate, Aβ peptides are able inhibit Aph-1's ability to assemble and maintain the PS1/γ-secretase complex.

It may seem a daunting prospect to try to design agents to inhibit interactions between hydrophobic peptides within the lipid bilayer, but a number of attempts have been made to inhibit receptor functions by adding synthetic peptides that mimic parts of transmembrane segments of the target receptor (33, 34). In a remarkable series of experiments involving growth factor recep-

tors, two sets of investigators have found that peptide segments corresponding to their intramembranous domains were able to alter the activities of the cognate receptors. Small hydrophobic peptides were able to either induce dimer formation in the case of the platelet-derived growth factor receptor (35) or inhibit dimerization of the Erb B receptor (36), and both were believed to act by associating with the corresponding intramembranous domains of the transmembrane molecules.

It may also be possible to modify transmembrane subunit interactions by agents that modify properties of the lipid bilayer itself. Compounds, like the sterols that naturally intercalate within the lipid bilayer, are known to affect many membrane functions. Recently, many investigators have reported that a class of polycyclic compounds known as nonsteroidal antiinflammatory drugs (NSAIDS) block the production of Aβ peptides in animals models of AD and cultured cells (37–39). It has been claimed that NSAIDS block Aβ production by affecting the conformation of PS1 (40), but it is also conceivable that NSAIDS might in some way modify interactions between intramembranous peptide segments.

Fundamental to our understanding of the Aβ cascade hypothesis is the still unanswered question: what are the physiological functions of the Aβ peptides in normal neurons? Although substantial amounts of Aβ peptides are present in blood and cerebrospinal fluid of normal people, we know nothing of their physiological role. The effect that hydrophobic segments of these peptides might have on neuronal cell membranes is even more problematical. If a fraction of the Aβ peptides that are generated under normal conditions do indeed partition within the lipid bilayer, one presumes they would be freely diffusible in the membrane and able to interact with intramembranous segments of other proteins. These could be parts of ion channels or pumps or critical receptors, with the Aβ fragments serving possibly as allosteric activators or inhibitors. In common with conventional chaperone proteins, which populate the cytosol in great numbers, hydrophobic segments of the Aβ peptides could associate with other hydrophobic peptide segments through multiple, low-affinity interactions. One might even imagine that they might function as a special class of chaperones that operate within the interior of the cell membrane.

Conclusions

The purpose of this essay is to suggest new ways that peptides derived from the proteolytic cleavage of a special family of cell membrane proteins might contribute to the pathogenesis of AD. As predicted by the amyloid Aβ hypothesis, large amounts of such peptides accumulate in the brains of affected people, and they almost certainly play a major role in the pathogenesis of the disease. How they do this is under intense investigation, but there is no clear consensus. Many new therapies designed to reduce Aβ levels in AD patients are being proposed, but efforts to reduce peptide levels in the blood and tissue spaces may not target the most toxic factors. To guide new therapeutic initiatives, more effort should be devoted to the study of normal functions of both the APP protein and the Aβ peptides derived from it. One idea, proposed here, is that hydrophobic segments of Aβ peptides might normally reside within the lipid bilayers of neuronal cell membranes, with consequences for normal physiology and pathogenesis yet to be explored. Although the intent of this paper is to focus attention to alternative ways in which Aβ peptides might contribute to neurodegeneration, one should also acknowledge that other mechanisms not involving Aβ peptides may be important in the pathogenesis of AD.

I have received helpful comments and suggestions from Drs. Pietro DeCamilli, Larry Goldstein, Ron Kaback, and Jon Morrow.

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