Review
Aβ ion channels. Prospects for treating Alzheimer’s disease with Aβ channel blockers

Nelson Arispe *, Juan C. Diaz, Olga Simakova

Department of Anatomy, Physiology and Genetics, and Institute for Molecular Medicine, Uniformed Services University School of Medicine, USUHS, 4301 Jones Bridge Rd, Bethesda, MD 20814, USA

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Abstract

The main pathological features in the Alzheimer’s brain are progressive depositions of amyloid protein plaques among nerve cells, and neurofibrillary tangles within the nerve cells. The major components of plaques are Aβ peptides. Numerous reports have provided evidence that Aβ peptides are cytotoxic and may play a role in the pathogenesis of AD. An increasing number of research reports support the concept that the Aβ–membrane interaction event may be followed by the insertion of Aβ into the membrane in a structural configuration which forms an ion channel. This review summarizes experimental procedures which have been designed to test the hypothesis that the interaction of Aβ with a variety of membranes, both artificial and natural, results in the subsequent formation of Aβ ion channels. We describe experiments, by ourselves and others, that support the view that Aβ is cytotoxic largely due to the action of Aβ channels in the cell membrane. The interaction of Aβ with the surface of the cell membrane may result in the activation of a chain of processes that, when large enough, become cytotoxic and induce cell death by apoptosis. Remarkably, the blockage of Aβ ion channels at the surface of the cell absolutely prevents the activation of these processes at different intracellular levels, thereby preserving the life of the cells. As a prospect for therapy for Alzheimer’s disease, our findings at cellular level may be testable on AD animal models to elucidate the potential role and the magnitude of the contribution of the Aβ channels for induction of the disease.

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* Corresponding author. Tel.: +1 301 295 9367; fax: +1 301 295 1715.
E-mail address: narispe@usuhs.mil (N. Arispe).

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1. Introduction

The discovery of the ion channel activity of amyloid-beta (Aβ) peptides, and its possible relevance to the pathogenesis of Alzheimer’s disease (AD), dates back to our discovery of Aβ channels in 1993 [1]. AD is a progressive neurodegenerative disorder characterized by irreversible cognitive and physical deterioration. The main pathological features in the Alzheimer’s brain are progressive depositions of amyloid protein plaques between nerve cells and neurofibrillary tangles within the nerve cells [2]. The major components of plaques are Aβ peptides of up to 43 amino acids. These peptides are produced from a transmembrane protein, the amyloid precursor protein (APP), by the activity of specific enzymes [3–5]. Numerous reports have provided evidence that Aβ peptides are cytotoxic and may play a role in the pathogenesis of AD [6–9]. Consequently, it has been hypothesized that the primary cause of cellular damage and degeneration in AD brain may be a toxic interaction between Aβ peptides and nerve cells [10]. However, Aβ is not a pathological product itself. Rather it is made continuously and normally by most cells in the body throughout life [11]. Aβ is also released from cultured cells during normal metabolism [3,12,13]. Although there is no question that the inappropriate accumulation of cerebral amyloid plaques is a hallmark of Alzheimer’s disease, the importance of this accumulation for the onset and progression of the disease is less clear. In fact, recent evidence suggests that the content of soluble oligomers of Aβ in the human brain is actually better correlated with the severity of the disease than is the occurrence of the classical macroscopic amyloid plaques containing insoluble Aβ deposits [14,15]. Furthermore, soluble oligomers, rather than insoluble plaque assemblies, are the apparent causes of learning and memory deficits in animal models of Alzheimer’s disease [16,17].

Fig. 1 schematically summarizes some of the major steps in the life of the enzymatically liberated Aβ peptides and their action on cells. Normally, Aβ is released into the body fluids where it can remain in the non-toxic non-aggregated state. However, in a cell culture medium or in an ionic solution, Aβ will convert from random coil and α-helical structure characteristic of the non-aggregated state of Aβ into small and large aggregates, protofibrils and fibrils. These aggregates are enriched in more toxic β-sheet structures. The schematic shows, as has been recently proposed, that the cellular damage and degeneration induced by external Aβ in Alzheimer’s disease may occur either by the formation of harmful Aβ ion channels [18,19], or by the interaction of the soluble oligomers of Aβ with the neuronal membrane [20].

When a suspension of Aβ peptides is added to cells in culture, Aβ molecules immediately interact with the surface of cells and remain tightly attached. The fluorescence images and associated histograms shown in Fig. 2 illustrate this phenomenon. The images in this figure are of PC12 cells that were exposed for a brief period of time to FITC-conjugated Aβ42. The Aβ42 was then removed from the medium, and cells were analyzed for bound ligand by fluorescent microscopy and flow cytometry. The images were taken either immediately or 24 h after a 2-h exposure to Aβ. For this experiment Aβ-FITC was removed from the external medium, and promptly washed three times.
2. Aβ interaction with membranes

2.1. Search for candidate membrane protein receptors for Aβ

The identity of a membrane protein receptor for Aβ has been a quest for many years [33,34]. Several candidate cell surface receptors have been considered, these include tachykinin receptors [35,36], serpin—enzyme complex (SEC) receptors [37,38], integrins; P75 neurotrophin receptor; and receptor for advance glycosylation end-products (RAGE) [39]. However, the available supportive evidence remains problematic. The data either question whether the interaction of Aβ with these receptors actually results in calcium influx and Aβ neurotoxicity, or whether additional neuronal binding sites are needed for the Aβ-neuron interaction [25–27,40]. Alternatively, it has been conjectured that Aβ may directly interact with existing voltage or ligand-gated calcium channels on the surface of the cell membrane, thereby potentiating the toxic effects of neuronal insults from excitatory amino acids [21,30,31]. However, this alternative has been questioned on two bases: firstly, the kinetic bases for the Aβ-induced intracellular calcium increase are inconsistent [20,22]. Secondly, cells exposed to Aβ continue to die even after surface calcium channels have been blocked by very specific and effective channel blockers. Such blockers include ω-conotoxin, nifedipine, verapamil, D-APV, bicuculline, MK-801, cAMP, 8-bromo-cAMP, and cGMP [24,41]. However, the debate has continued because of more recent reports that Aβ promotes stimulation of calcium influx into cortical synaptosomes via activation of L- and N-type voltage dependent calcium channels, and by increases in the amplitude of N- and P-type currents in cultured cortical neurons [42]. Yet, morphological changes leading to cell degeneration induced by fresh and globular Aβ42 cannot be prevented by Cd²⁺, which blocks voltage-sensitive Ca²⁺ channels [25].

2.2. Search for non-protein components of the plasma membrane that interact with Aβ

Alternative molecular mechanism(s) has been proposed to explain how the Aβ interaction with cells results in calcium
influx. These mechanisms consider direct interactions of Aβ with non-protein components of the plasma membrane with consequent disruption of membrane permeability [1,43,44]. One of these proposed mechanisms suggests that Aβ oligomers increase the area per molecule of the membrane-forming lipids, thus thinning the membrane, lowering the dielectric barrier and increasing the conductance [44]. Another proposed mechanism considers that following interaction with the membrane Aβ inserts into the membrane and forms well-defined pores which selectively increase the membrane ionic permeability [1]. The existing evidence for Aβ penetration into the membrane introducing a selective change of membrane conductance is so overwhelming that the alternative to explain the altering of the membrane conductance by Aβ due to destabilization without membrane penetration and formation of ion channels, could just be complementary and unspecific by nature. The possibility of Aβ insertion into the membrane to form well-defined pores was originated from the pioneering experiments performed by Arispe et al. (1993) [1,45] which showed the appearance of ionic current fluxes after Aβ incorporation into artificial membranes. The possibility of penetration and disruption of membrane permeability by Aβ has received additional support by numerous reports of induced ion channel activity after membrane exposure to Aβ [46,47,81–86,93,101,103–106].

The insertion of protein-forming pores into membranes can be faithfully shown by following the changes in bilayer capacitance and resistance. Using a dual sinusoidal current, Vargas et al. [47] recorded Aβ-specific displacement currents across a planar lipid bilayer in response to sudden changes in the membrane potential after the membrane had been exposed to Aβ. These data indicated that the pore-forming Aβ had been inserted into the membrane. Most recent data from NMR spectra and high sensitivity circular dichroism (CD) spectroscopy on model membranes also confirms this finding [48]. These studies showed that Aβ interacts with the phospholipid head groups of the membrane where it can either remain in an unstructured associated state or incorporate within the lipid bilayer. When incorporated into the membrane Aβ adopts the most toxic β-sheet structure disrupting the membrane more severely than when unstructured Aβ is associated on the membrane [48]. The adoption of this preferred β-sheet structure when Aβ is incorporated into the membrane was one of the bases for Durell et al. [68] to develop theoretical models of the Aβ ion channel structure. The channel structures were designed as aggregates of peptide subunits in identical conformations. The secondary structure of the subunits was predicted to form a β-hairpin followed by a helix–turn–helix motif. One of these helices is embedded in the top head group layer of the membrane with the other helix inserted into the membrane core. The insertion of Aβ into cell membranes has also been modeled by Mobley et al. [49]. Using a Monte Carlo code which is specific at the amino acid level they examined the effect of mutations on the Aβ peptide’s insertion behavior. The results indicated that mutations affect peptide insertion causing the peptide to prefer (relative to wild-type) a conformation where those residues are in the upper lipid head region. This causes the peptide to hang up in the upper leaflet (the partially inserted conformation) and facilitates formation of channels. It is interesting to note that a channel structure suggested previously by Durell et al. (1994) has these mutated residues laying along the surface of the bilayer. Thus Mobley et al. [49] find that mutations increase the resemblance to the ion channel configuration. The formation of well-defined pores presumes that prior to the insertion in the membrane to form a pore the Aβ must first selectively interact with the components in the surface of the membrane. A series of findings indicate that the lipid composition of the membrane governs the outcome of Aβ interactions with cell membrane, and the effectiveness of this interaction will depend on the structural conformation of Aβ. Initially, the structural conformation of Aβ in solutions is characterized by a concentration-dependent equilibrium of mixture of β-sheet, α-helix and random coil conformations. In the presence of some lipids, the equilibrium may be shifted towards preferred states of conformation. For instance, the contact with neutral lipids does not affect the conformation of Aβ in solutions, and Aβ does not seem to interact with membranes formed of neutral phospholipids [47,50–52]. This assertion was validated by using compounds that decrease the effective negative charge of membranes [53]. The decrease in the negative charge of the membrane prevents the Aβ association as well as the subsequent cellular degeneration induced by Aβ. Therefore, these results suggest that negatively charged lipids are required to establish an effective Aβ–lipid interaction. These findings could be interpreted to indicate that Aβ toxicity is initiated by a nonspecific physicochemical interaction of Aβ with cell membranes. In any case, the Aβ–membrane interaction which precedes the insertion in the membrane would preferentially be a specific electrostatic interaction of Aβ with acidic phospholipids of negatively charge membranes [51,53,54]. Because positively charge aminoacids are necessary for the interaction with negatively charged phospholipids, it has been suggested that lysine residues in the sequence of Aβ may provide the site of association with the phospholipids in the cell membrane [55,56]. The sequence of Aβ possesses lysine residues at positions 16 and 28; therefore, it may be possible that Aβ molecules would interact with the membrane at these two possible sites. Compelling data suggest that the phospholipid phosphatidylserine is very likely to be the elusive membrane surface receptor site for Aβ binding [52,57,58]. However the possible association of the lysine residues in the Aβ molecule with membrane localized phosphatidyl serine as interactive site with the lipid matrix of the membrane is yet to be confirmed.

The interaction of Aβ with other non-protein components of the plasma membrane, such as monosialoganglioside GM1 and cholesterol has been a subject of intense investigation. These studies have addressed the fundamental question about the conversion of elemental units of Aβ into its toxic aggregates. Nevertheless, they have not yet been able to explain the molecular mechanism(s) by which Aβ interaction with cells could result in calcium influx. However, since we have proposed that when Aβ molecules assemble into conducting channels under the appropriate structural circumstances, the interaction between Aβ and the surface of the cell...
membrane may result in the formation of ion channels which consequently permit calcium influx. The findings, in summary, show that Aβ selectively recognizes a GM1 cluster in membranes and binds to and accumulates on GM1-rich domains in membrane in a time- and concentration-dependent manner [59,60]. Yanagisawa et al. (1995) [61] identified a unique Aβ peptide species in the AD brain which is characterized by its binding to GM1 ganglioside. This species of Aβ was shown to exhibit unique molecular characteristics with high aggregation potential. The discovery of this species led to the hypothesis that Aβ adopts an altered conformation by binding to GM1 and acts as a seed for the assembly of soluble Aβ [62].

Cholesterol may also contribute to Aβ activity. It has been shown that the ability of Aβ to insert into the membrane is critically controlled by the ratio of cholesterol to phospholipids [63]. Altering this ratio, by lowering the concentration of cholesterol, results in Aβ staying on the membrane surface region, mainly in a beta-sheet structure. In contrast, as the ratio of cholesterol to phospholipids rises Aβ can spontaneously insert, by its C terminus, into the lipid bilayer. A series of other studies have shown that intramembrane cholesterol also facilitates the formation of the GM1–Aβ complexes which have been suggested to be the endogenous seed for Alzheimer amyloid [59,61,62,64]. In fact, depletion of cholesterol in PC12 cells abolishes the formation of GM1-rich domains and significantly reduces binding of Aβ [60]. Consistently, these investigators propose that membrane binding by Aβ triggers its conformational transition from helix-rich to β-sheet rich structures [43,65,66], in contrast to membrane insertion which generates alpha-helix and removes almost all β-sheet structure [65]. Data obtained most recently by other investigators present a different structure for Aβ inserted into membranes [48]. Aβ incorporated into lipid bilayers, analyzed with Synchrotron radiation CD which possesses a higher sensitivity and better signal to noise ratio than conventional CD, has more β-sheet structure compared to Aβ just associated with membranes. In any case, all data suggest that the structure of Aβ aggregates in membranes may be different from that of the protein aggregated in solution [60,67]. We have proposed and modeled a polymeric structure for the membrane bound Aβ which we suggest might be the structure responsible for the formation of the Aβ ion channel and the consequent intracellular calcium increase [68,69].

In concert, all of these mechanisms have a common trajectory towards the formation and activation of Aβ ion channels. This molecular mechanism was proposed by Arispe et al., in 1994 and is known as the β-amyloid calcium channel hypothesis. According to this hypothesis a selective increase of the membrane ionic permeability involves Aβ interaction and insertion into the membrane to form a selective cation channel [1,18,19,45,46,70,71]. The hypothesis proposed that Aβ forms minimal polymeric structures, possibly only hexamers, which may directly interact with the neuronal plasma membrane and form a conducting pore. We suggested that these pores might be the basis for loss of neurons in Alzheimer disease.

3. Aβ forms calcium channels in artificial and natural membranes

3.1. Aβ channels formed in planar lipid bilayers

The capacity of Aβ molecules to form ion channels was originally proposed by Arispe et al. (1993) [1,45] from the analysis of records of ionic current in artificial lipid membranes exposed to solutions of Aβ. The idea that the Aβ peptide could acquire the appropriate configuration to form ion channels in membranes was instigated by two basic findings. First, it had been shown that Aβ increased intraneuronal [Ca2+]i and disrupted calcium homeostasis [8]. Cells exposed to Aβ experienced a peak increase in the intracellular calcium concentration that depended on the presence of calcium in the extracellular medium. Second, it was observed that the first 16 residues of the Aβ molecule had the characteristic feature of alternating charged or neutral residues with hydrophobic residues capable of forming amphipathic beta-sheets. This residue arrangement was consistent with amphipathic beta sheet secondary structures, as occurs in other molecules that had been shown to form ion channels. This was the case of molecules such as porin/VDAC and shaker K [72,73], and some protein members of the annexin gene family such as lipocortin I (ANXA1), endonexin II (ANXA5) and synexin (ANXA7) [74–80] which generate highly selective calcium channels.

The procedure to record current activity from membrane-incorporated Aβ channels consisted of first incorporating Aβ molecules from a freshly prepared water solution into a suspension of pure phosphatidylserine liposomes. Proteoliposomes were used as a vehicle to facilitate the fusion with the artificial phosphatidylserine-containing lipid bilayer, and to prompt the incorporation of the Aβ ion channel in the membrane. The liposomes containing the Aβ molecules were added to one of the compartments of a chamber holding two solutions separated by a planar lipid membrane made by a mixture of artificial phospholipids dissolved in decane. Fig. 3A schematizes the experimental arrangement and the ensuing electrical currents that appear after a liposome-containing an Aβ channel is fused with the planar lipid membrane. The Aβ channel incorporated into the dividing planar lipid membrane allows ions to flow between the two solutions, based on the channel permeability and the ionic concentration gradient. When a driving force is present, such as a membrane electrical potential difference or an ionic concentration gradient, ions move between the solutions and discrete current changes, characteristic of ion channel activity, are observed. The Aβ channels have been identified as cation channels by measuring the direction of the shifts in the reversal potential of the ionic currents when the ionic concentrations in the solutions are changed, and the permeability sequence for cations was established as P_Ca>P_Li=P_K>P_Na [1]. Although the Aβ channel was found to be permeable to different cations, the ion permeation ceased if calcium in the micromolar range were added. This property is characteristic of classical calcium channels; therefore, the Aβ ion channel was regarded to be a functional calcium channel. Nevertheless, the Aβ calcium
channel differentiates from the classical calcium channels in its pharmacological characteristics. For example, Aβ channels are insensitive to blockage by nifedipine, and are selectively sensitive to blockage by zinc ions and tromethamine [45].

Membrane-free Aβ molecules in solutions, as one would expect Aβ to be found in the body fluids, can also spontaneously interact and form cation selective channels with both intracellular and extracellular aspects of both artificial [45,81–83] and natural plasma membranes [84]. The planar lipid membrane technology, as it was originally applied to describe the ion channel properties of Aβ, is based on the use of artificial membranes prepared from a mixture of phospholipids dissolved in decane. This mixture is then applied in a small orifice separating two ionic solutions. This procedure generates the concern that some of the organic solvent used to dissolve the phospholipid remains in the artificial membrane and may affect the natural configuration of the channel-forming molecules. During the discovery of the ability of Aβ to form ion channels, unnatural molecular configurations of Aβ were ruled out by the application of the pipette double dip method to form the bilayer membranes [45]. This method consisted in forming a phospholipid bilayer membrane at the tip of a patch pipette which is double dipped into a solution with a monolayer film made of synthetic phospholipids spread upon it. Fig. 3B schematizes this procedure and shows the type of ionic channel activity that appears in solvent-free membranes after the addition of Aβ to the bathing solution. The ion channel activity is similar both in character and in properties to the channel activity observed from bilayers formed with phospholipids in organic solvents. The results demonstrated beyond any doubt that Aβ in solution can assume a conformation that enables the molecule to enter the bilayer membranes and form cation selective channels.

Fig. 3. (A) Aβ forms ion channels in planar lipid bilayers. The drawing schematizes the incorporation of an Aβ channel molecule into a planar phospholipid bilayer. The incorporation is produced by the fusion of an Aβ-carrying vesicle with the bilayer. The right panel shows actual current traces to illustrate the characteristic Aβ channel activity. The chambers at the bottom schematize the flow of charged particles through the open Aβ channel. This flow of charged particles, driven by an electrochemical gradient, generates the electrical current traces shown on the right panel of the figure. (B) Aβ forms ion channels in solvent-free planar lipid bilayers. The drawing schematizes the pipette double dipping procedure to form a solvent-free bilayer membrane. After the membrane is formed at the tip of the pipette, Aβ is added to the external solution to allow for spontaneous incorporation and formation of Aβ ion channels. The current traces on the right are actual current traces after the incorporation of an Aβ channel. The traces illustrate the characteristic current in response to pulsed changes in the bilayer electrical potential of increasing magnitude. The contribution of the current activity from the Aβ channel is shown after specifically blocking the Aβ channel. The remaining currents are the unspecific membrane leakage currents. (C) Aβ forms ion channels in patches of natural membranes. Aβ forms ion channels in detached inside-out patches of cell membrane after Aβ addition to either the solution inside the pipette or to the external medium. The current traces at the right (a) are actual current activity which appears after the addition of Aβ to the medium bathing an inside-out patch of membrane detached from a GT1-7 cell. To confirm the source of the current the effective Aβ channel blocker zinc was added to the external medium (b). The current from the Aβ channel reappears after the blocker is removed from the solution (c).

3.2. Aβ channels formed in liposomes

Three dimensional systems such as unilamellar liposomes have provided a very convenient way to confirm the formation of ion selective pathways by Aβ. They have also provided a convenient platform to study the pharmacological sensitivity of the Aβ channel pathways, and the influence of the membrane phospholipid content. Experiments performed on liposomes prepared with mixture of natural phospholipids such as phosphatidyl choline and phosphatidyl serine, showed significant uptake of calcium ions after the liposomes were exposed to solution containing Aβ molecules [85]. The formation of ion
channels by Aβ in the liposome membrane was confirmed by specifically blocking the channels with zinc ions and tromethamine, which had been shown to inhibit Aβ ion channels [1,70]. Similar liposome experiments using an antibody raised against the amino-terminal domain of Aβ showed that the antibody prevented the calcium uptake by liposome reconstituted with Aβ, thus confirming the molecular identity of Aβ channels [86]. Dose-dependent Na+ and Ca2+ entry has also been observed following the incorporation of Aβ molecules into the membrane of vesicles of phosphatidyl serine loaded with fluorescent ion indicators [52]. The stimulation of cation influx by Aβ required that the liposomes be composed of at least a small amount of acidic phospholipid. These results are in accord with previous studies in planar lipid bilayer, and in neuronal membrane patches which have demonstrated preferential formation of Aβ channels in negatively charged membranes [1,84].

Changes in the mechanical properties of bilayers, such as the Critical Lysis Tension of vesicles [87], accompanied by changes in permeability to fluorescent probes have also been observed in liposomes exposed to Aβ [88]. Vesicles of different lipid composition loaded with fluorescence probes and exposed to Aβ exhibit a decrease in fluorescence intensity caused by a permeability change and consequent leak of the fluorescent probe trapped inside the vesicles. When incorporated into the membrane Aβ also induces a strong destabilization of the vesicle membrane, as detected by a dramatic decrease in the critical tension needed for vesicle rupture. These changes denote the ability of Aβ to associate with and incorporate into the membrane, to reduce the lateral membrane cohesive forces and to induce permeability changes [88]. NMR spectra of bilayers with incorporated Aβ also show that Aβ incorporation into the bilayer has a more dramatic effect on the lipid bilayer integrity than when Aβ simple associates with the membrane. The Aβ incorporation produces a reduced stability of the bilayer with the greatest effect observed in the hydrocarbon core [48].

3.3. Aβ channels formed in natural membranes

Aβ molecules in solution also form ion channels in natural membranes. Kawahara et al. [84] showed that excised membrane patches from a cell line derived from hypothalamic neurons produced new ion channel activity when exposed to Aβ molecules. Patch-clamp experiments were performed in membrane patches pulled from cultured neurons, and membrane currents under voltage clamp conditions were recorded. The Aβ-induced channel activity was observed after Aβ interacted with either side of isolated patches of membranes. Observation occurred sooner when the Aβ interacted with the phosphatidylserine-richer inner side of the membrane. Fig. 3C illustrates the experimental procedure and the ion channel activity. For the formation of ion channels from the inner side of the membrane, Aβ molecules were added to the external cell medium of inside-out cell membrane patches. For the formation of ion channels on the outer side of the membrane, the Aβ molecules were placed inside the pipette solution. Both types of experiments lead to the generation of ion channel activity due to successful incorporation of Aβ molecules. However, the faster observation of channel activity from the phosphatidylserine-richer inner side of the membrane is considered highly meaningful since it adds to the increasing data endorsing the hypothesis that phosphatidylserine may be the possible membrane surface receptor site for Aβ binding [57]. Remarkably, the properties of the Aβ channels formed in natural membrane are similar to those observed in artificial bilayer membranes. For example, the Aβ channels in natural membranes also show a cationic selectivity, frequent transitions among different levels of conductance and sensitivity to zinc ions. Previously we showed that zinc ions are very effective blockers of the ionic flow through Aβ channels [70].

Although the formation of Aβ channels has been convincingly demonstrated in artificial and natural patches of membranes, technical considerations limit the unquestionable demonstration of single channel activity from Aβ channels incorporated in the membrane of a cell. The amount of current flowing through a single channel is so small, less than 1 pA/pF, that it is difficult to detect in a whole cell recording configuration. However, compelling evidence for Aβ channel formation in cells has been reported by selectively blocking the calcium currents induced by Aβ in cells with specific Aβ channels blockers. Peers and colleagues [89,90] found that periods of chronic hypoxia, which induce enhancement of calcium channels, led to the appearance of novel calcium-conducting channels in PC12 cells. These channels could not be prevented by Cd2+. However, they were blocked either by Congo red or by an antibody raised against the N-terminal region of Aβ. Similar intracellular calcium increases induced by Aβ in fibroblasts and in GT1–7 hypothalamic neurons, were selectively blocked by a specific antibody against Aβ [24,26]. In these cells, transient calcium currents persisted after addition of Aβ to media containing of a variety of channel blockers, including TTX, D-APV, nifedipine, and bicuculline. However, Aβ calcium currents could not be observed when the antibody to Aβ was also present in the media.

While the activity of small conductance Aβ channels formed in the cell membrane can be managed by the cellular homeostatic mechanisms, large conductance channels can be deleterious to cells and generate increases in the intracellular calcium levels associated with profound cytotoxicity [91]. In our previous studies of Aβ channels in planar lipid membranes we showed that Aβ channel conductance can spontaneously interconvert among levels in both the picosiemens and the nanosiemens range [45]. One might expect then that conversions to the large nanosiemens conductance levels after an Aβ channel has been formed in a cell membrane may have profound consequences for cytosolic homeostasis.

4. Effect of specific Aβ ion channels blockers on Aβ channel activity

4.1. Blockage of Aβ ion channels in planar lipid bilayers

The contribution of Aβ channels to the cytotoxic properties of Aβ has been compellingly confirmed by specifically
blocking Aβ channels with specific Aβ channel blockers. Based in the chemistry of the regions around the putative mouth of the polymeric membrane-bound Aβ channel model, we have designed a series of compounds that selectively block Aβ channels incorporated in artificial membranes [69]. Additionally, we have successfully tested them as blockers of Aβ channels and Aβ cytotoxicity in cells [92,93]. The selectivity of these compounds as blockers of Aβ channels arises from the fact that they possess the complementary chemistry necessary to establish an effective and selective interaction with Aβ. The recordings shown in Fig. 4A illustrate the blocking effect of one of these compounds to an Aβ channel was reconstituted in a planar lipid bilayer. After developing a stable ion channel activity, the specific blocker NA7 was added to the experimental chamber. The chemical structure of NA7 partially copies the sequence of the putative mouth of the Aβ channel [69]. We have shown that peptides with sequence homologous to the hairpin region of the model did not interfere with the Aβ aggregation process [69]. Soon after NA7 was added to the chamber the current flowing through the Aβ channel vanished.

The amplitude histograms of the current events from the current records before and after the addition of NA7 show the progressive reduction of the current events, trending towards zero until the electrical recording becomes silent.

4.2. Effect of specific blockade of Aβ ion channels and Aβ cytotoxicity

The categorical results on the prevention of Aβ cytotoxicity observed after the use of the highly effective and very selective Aβ channels blockers, added further corroboration to the formation of Aβ channels in the surface membrane of cells, and appraised the participation of these channels in the generation of those changes [92]. The experimental data plotted in Fig. 4B clearly demonstrate that blocking the Aβ channel also prevents the reduction in cell viability induced by Aβ. The viability of PC12 cells was determined after 3 days of incubation in the presence of Aβ (15 μM) and Aβ plus the peptides NA7 or NA3. NA3 had previously been shown not to affect Aβ channels incorporated in planar lipid bilayer [69].

Fig. 4. (A) Blockage of Aβ ion channels in planar lipid bilayers. Activity from an Aβ ion channel incorporated into a planar lipid bilayer. The current activity was recorded before and after the addition of the specific Aβ channel blocker NA7. An electrical potential gradient of 20 mV was imposed on the membrane to enhance the driving force on the ions. The effect of the blocker on the channel activity is recorded 1 and 3 min after the addition of the blocker (middle and bottom current trace). The current amplitude histograms from the channel activity displayed in the above current traces show the blocking effect on the different channel conductance levels.

(B) Specific blockage of Aβ ion channels prevents the reduction in cell viability induced by Aβ. (a) The viability of PC12 cells was determined after 3 days of incubation in the presence of Aβ (15 μM) and Aβ plus the peptides NA3 and NA7. Cell viability was measured using a colorimetric XTT assay (Cell Proliferation Kit II from Roche Molecular Biochemicals). Aβ plus peptide NA7 completely protected the cells from Aβ toxicity. By contrast, Aβ plus peptide NA3 showed cell viability levels significantly similar to the one obtained with Aβ alone. (b) Dose–response experiment to evaluate the capacity of protection of the two different peptides against Aβ cytotoxicity. Peptide NA7 was able to fully protect PC12 cells against Aβ toxicity at concentrations higher than 4 μg/mL, and with 50% protection at less than 1 μg/mL.
The bar histograms in Fig. 4Ba show that Aβ alone considerably reduces the cell viability. As expected NA3, in combination with Aβ, was unable to protect cells from Aβ toxicity. By contrast, Aβ plus native sequence NA7 completely protected the cells from Aβ toxicity. The results of a dose–response experiment are shown in Fig. 4Bb. The data indicate that peptide NA7 fully protects PC12 cells from Aβ toxicity at concentrations higher than 4 μg/mL. The 50% protection level was seen at NA7 concentrations of less than 1 μg/mL. These and other data [92] indicate that prevention of Aβ cytotoxicity can be achieved by selective blockade of the Aβ channels.

A compelling demonstration that Aβ ion channel activity initiates the mechanisms that generate increase in intracellular calcium are provided by experiments in which Aβ is allowed to form channels in the surface membrane of the cells in the presence and absence of specific Aβ channel blockers. The records of intracellular calcium levels taken from GT1–7 hypothalamic neurons are displayed in Fig. 5, and illustrate the presence and absence of specific Aβ channels.

Fig. 5. Blockage of Aβ ion channels in the cell surface membrane stops Aβ-induced intracellular calcium increase. Cells were plated on glass coverslips and loaded with calcium-sensitive probes using media containing FURA-2AM (Molecular Probes). Changes in the emission from Fura-2AM were observed by blocking Aβ channels in the presence of Aβ. The 50% protection level was seen at NA7 concentrations of less than 1 μg/mL. These and other data [92] indicate that prevention of Aβ cytotoxicity can be achieved by selective blockade of the Aβ channels. The specific Aβ channel blockers [69,93]. It would be expected that blocking the Aβ channels the events that characterize the development of apoptosis can be prevented. A number of independent assays have been developed to evaluate the distinct mechanisms that characterize apoptosis. The loss of Phosphatidylinositol (PS) membrane asymmetry is one of the earliest signs of apoptosis while DNA fragmentation, a process that results from the activation of endonucleases, is one of the later steps. The activation of some members of the caspase family of proteases also mediates events associated with apoptosis. Cell death by apoptosis has also been evaluated by the quantification of plasma membrane damage, such as the release of lactate dehydrogenase (LDH) from the cytosol into the media, which estimates the integrity of the cell membrane. Our published data [92] demonstrated that in addition to morphological changes and intracellular calcium increase, cells exposed to Aβ show reduction in the number of metabolically active cells, increased release of lactate dehydrogenase (LDH) from the cytosol, loss of PS membrane asymmetry, and activation of caspase-3/7. The involvement of the Aβ channels in the mechanisms that activate all these intracellular events was demonstrated by the specific blockage of Aβ channels. The compiled series of results shown in Fig. 6 are evidence of this statement. The specific Aβ channel blocker NA7 [67] was used in these experiments. We have shown that this type of peptide blocker is totally innocuous to cells and do not participate in any other manipulations that induce apoptosis (92). PC12 cells were exposed for 24 h to Aβ in the presence and in the absence of NA7 in the culture medium and assays to detect the release of lactate dehydrogenase (LDH) from the cytosol into the media, the activity of caspase 3/7, the presence of PS in the external surface of the cell membrane and the fragmentation of DNA, were applied. The extraordinary elimination of any sign of activation by Aβ of the characteristic mechanisms of apoptosis, after the specific blockage of the Aβ channels, remarkably and unquestionable demonstrate the critical role played by Aβ channels in the characteristic cytotoxicity of Aβ.

5. Concluding remarks

This review summarizes a set of experiments, by us and others, that compellingly suggest that Aβ is cytotoxic largely due to the action of Aβ channels in the cell membrane. When
extracellularly released Aβ molecules assemble under the appropriate structural configuration, the interaction with the surface of the cell membrane results in the activation of a chain of processes that, when large enough, become cytotoxic and induce cell death by apoptosis [94–98]. In this interaction the membrane of the cell plays a major role in providing the necessary "receptor(s)" to facilitate the effective Aβ–membrane interaction. Most of the searches to this moment identify non-proteinaceous membrane components as the most likely candidates for receiving and lodging Aβ within the cell membrane [52,55–58,61,62,66]. Moreover, an increasing number of research reports are supporting the concept that the Aβ–membrane interaction event is followed by the insertion of Aβ into the membrane in a structural configuration which may form an ion channel [1,18,19,25–27,45,46,70,81,83,85,86,99–106]. The consequent activity of this channel is the activation of processes that, when uncontrolled, will conduct the cells to the irreversible degeneration. Experiments shown here and elsewhere [92,93] confirm that six of the most relevant cellular processes when activated by Aβ lead the cell to die by apoptosis. On the contrary, none take place when the ion channels formed by Aβ on the cell surface membrane are selectively blocked. The schematic described in Fig. 7 summarizes some routes to activate these classical pre-apoptotic processes which eventually produce irreversible cell damage. Remarkably, the blockage of Aβ ion channels at the surface of the cell absolutely prevents the activation of these processes at different intracellular levels, thereby preserving the life of the cells despite the extracellular presence of the toxic Aβ spicis. It is our hypothesis that the formation of Aβ ion channels in the cell surface membrane contributes to the creation of a toxic ionic imbalance, and that the failure of the intracellular calcium homeostasis initiates the activation of the intracellular events leading to cell degeneration.

A number of therapeutic approaches to reduce the burden imposed by the presence of Aβ and its toxic forms in the Alzheimer’s brain have been tested. Some of them include immuno-therapy agents, procedures to prevent Aβ generation...
from its precursor protein APP employing secretase modulators, clearance of existing Aβ from critical areas of the brain, and prevention of fibril formation by Aβ. At one time, it seemed likely that the selective inhibition of aggregation, and fibril and plaque formation by Aβ would provide a rational pharmacological tool for the design of drugs useful for the treatment of Alzheimer’s disease. However, the results from the application of these procedures to AD have not been encouraging [107–109]. Indeed, recent experiments show persistent amyloidosis following suppression of Aβ production in a transgenic AD animal model [110]. This suggests that the clearance procedure may not be applicable to relief of AD patients from human amyloid since all patients are believed to enter therapies with at least existing brain Aβ plaque burden. Moreover, it has been shown that rodents made transgenic for human Aβ develop amyloid deposits but do not lose the neurons that are central to Alzheimer’s disease [111]. Furthermore, irreversible structural changes [25] and early apoptosis [92] have been observed in cells exposed to fresh Aβ, long before Aβ forms fibrillar aggregates. These results suggest that other mechanisms in addition to Aβ aggregation are required to initiate the cascade of neurotoxic events that characterize the possible role of Aβ in AD. Despite intense effort, as of yet, no clinically relevant neuroprotective strategy has emerged by following this approach.

Based on our own studies, prevention of the toxic effects of Aβ on cells following exposure to Aβ, cannot be achieved by removing Aβ from the solution bathing the cells [92]. Rather it is necessary to selectively prevent the ionic fluxes through newly formed Aβ channels. Relevantly, if the Aβ channel blockers have been present in the cell culture media prior the formation of Aβ channels, long-term cytotoxic effects fail to occur. We have successfully developed effective and specific Aβ ion channels blockers that have permitted us to disclose the contribution of Aβ ion channels in cell degeneration induced by Aβ. As a prospect for therapy for Alzheimer’s disease, our findings at cellular level remain to be extended into reliable AD animal models to elucidate the role and the magnitude of the contribution of the Aβ channels in induction of the disease.

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References


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