

Calcium Signaling and Amyloid Toxicity in Alzheimer Disease*

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Intracellular Ca²⁺ signaling is fundamental to neuronal physiology and viability. Because of its ubiquitous roles, disruptions in Ca²⁺ homeostasis are implicated in diverse disease processes and have become a major focus of study in multifactorial neurodegenerative diseases such as Alzheimer disease (AD). A hallmark of AD is the excessive production of β -amyloid (A β) and its massive accumulation in amyloid plaques. In this minireview, we highlight the pathogenic interactions between altered cellular Ca²⁺ signaling and A β in its different aggregation states and how these elements coalesce to alter the course of the neurodegenerative disease. Ca²⁺ and A β intersect at several functional levels and temporal stages of AD, thereby altering neurotransmitter receptor properties, disrupting membrane integrity, and initiating apoptotic signaling cascades. Notably, there are reciprocal interactions between Ca²⁺ pathways and amyloid pathology; altered Ca²⁺ signaling accelerates A β formation, whereas A β peptides, particularly in soluble oligomeric forms, induce Ca²⁺ disruptions. A degenerative feed-forward cycle of toxic A β generation and Ca²⁺ perturbations results, which in turn can spin off to accelerate more global neuropathological cascades, ultimately leading to synaptic breakdown, cell death, and devastating memory loss. Although no cause or cure is currently known, targeting Ca²⁺ dyshomeostasis as an underlying and integral component of AD pathology may result in novel and effective treatments for AD.

Alzheimer disease (AD)² is an idiopathic neurodegenerative disease, and little is yet understood of its underlying causes or mechanisms. Certain diagnostic features are central to AD (amyloid plaques, neurofibrillary tangles, and elevated levels of soluble amyloids in the brain and cerebrospinal fluid), but their roles in the most devastating aspect of the disease, namely memory loss, are unclear. One common factor that underlies AD pathogenesis is neuronal Ca²⁺ dysregulation. In this mini-

review, we focus specifically on the pathogenic interplay between β -amyloid (A β) and Ca²⁺ signaling dysregulation. Ca²⁺ signaling is fundamental to cellular function, involving a multitude of entry and release channels, clearance mechanisms, and intracellular stores. Among these Ca²⁺-regulating entities, A β may interact with a critical subset as discussed below and enable AD progression by altering Ca²⁺ homeostasis and triggering downstream pathogenic signaling cascades (1–3).

Implications of Cellular Ca²⁺ Dysregulation

Sustained disruptions in Ca²⁺ signaling have significant implications for the health and functionality of neurons over the lifetime of an organism (4) and form the basis of the Ca²⁺ hypothesis of AD (5). Under resting conditions, cytosolic Ca²⁺ is maintained at low nanomolar concentrations by an array of pumps, buffers, and transport mechanisms. Ca²⁺ entry into the cytosol is rigorously regulated and originates from one of two major sources: the extracellular fluid via entry across the plasma membrane (through receptor-, voltage-, and store-operated channels and Ca²⁺ exchangers) and intracellular stores such as the endoplasmic reticulum (ER) and mitochondria (6).

Interactions between A β and intracellular Ca²⁺ are particularly relevant to AD pathogenesis, as Ca²⁺ perturbations are a causal factor in excitotoxicity, synaptic degeneration, and cell death, whereas reduced Ca²⁺ release is neuroprotective (7). Both neuroprotective and pathogenic Ca²⁺ cascades can be triggered sequentially: the cell attempting to first compensate for metabolic stress by up-regulating protective mechanisms and then succumbing to sustained insults and initiating pathogenic and/or apoptotic pathways. For example, excess Ca²⁺ release initially activates anti-apoptotic transcription factors such as NF κ B (8), which protects cells by inducing genes that promote cell survival and anti-apoptotic proteins (e.g. Bcl-2) and the cAMP response element-binding protein (CREB), which is Ca²⁺-dependent and plays a critical role in synaptic plasticity and neuronal survival (9). Among pathogenic responses, expression of C/EBP homologous protein (CHOP) inhibits protective proteins such as Bcl-2. Increased Ca²⁺ levels through A β -mediated mechanisms can also lead to mitochondrial Ca²⁺ overload, generation of superoxide radicals, and pro-apoptotic mitochondrial proteins such as caspases and cytochrome *c*, which are linked to cell death and neurodegeneration in several AD models (1).

A β Physiology and Pathophysiology

A β is a 39–42-amino acid peptide produced by the proteolytic cleavage of the amyloid precursor protein (APP), an integral membrane protein involved in signal transduction pathways. Cleavage of APP by α - or β -secretases forms the C-terminal portion of A β , and subsequently, the remaining membrane-bound C-terminal fragment is cleaved within its transmembrane domain by the aspartyl protease complex γ -secretase, of which presenilin is a crucial component (10). Mutations in the genes encoding APP and presenilin are asso-

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² The abbreviations used are: AD, Alzheimer disease; A β , β -amyloid; ER, endoplasmic reticulum; APP, amyloid precursor protein; AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA, *N*-methyl-D-aspartic acid; IP₃R, inositol trisphosphate receptor; nAChR, nicotinic acetylcholine receptor; NMDAR, NMDA receptor; pS, picosiemens; RyR, ryanodine receptor.

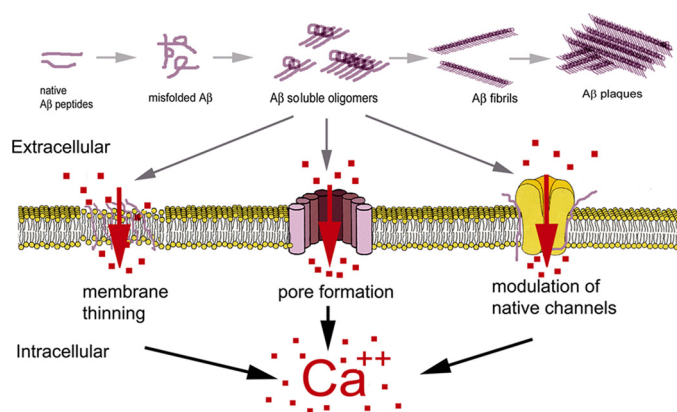


FIGURE 1. Schematic model for $A\beta$ monomers in which misfolding triggers self-aggregation into dimers, trimers, oligomers, fibrils, and fibrillar aggregates or plaques. The $A\beta$ aggregates formed by 2–50 monomers are considered the toxic species.

ciated with familial forms of AD and lead to increased $A\beta$ production, suggesting a causal relationship between $A\beta$ overproduction and AD pathogenesis (11). Moreover, the majority of mutations linked to early-onset AD cause increased production of $A\beta_{42}$ ($A\beta$ ending at position 42) relative to $A\beta_{40}$, and $A\beta_{42}$ appears to be the more toxic form of the peptide and more prone to undergo aggregation (12, 13).

$A\beta$ Aggregation States: Which Is the Toxic Species?

$A\beta$ plaques are the most obvious and characteristic feature of AD. However, increasing evidence suggests that they may not be primarily responsible for the neurological deficits but rather implicates small soluble oligomeric aggregation states of $A\beta$. Protein aggregation is an aberrant self-associating process that can produce macroscopic entities such as the extracellular aggregates of $A\beta$ peptide found in the brains of many AD patients. This process proceeds, in both *in vitro* and *in vivo* settings, through various intermediate aggregation states of $A\beta$ peptides, ranging from small soluble oligomeric species formed by 2–50 peptides to insoluble filamentous aggregates from which plaques are formed (Fig. 1) (14). Several studies have characterized these intermediates, which most likely represent the most toxic forms of $A\beta$ aggregates (15–18).

The monomeric form of $A\beta$ (either 1–40 or 1–42) has long been considered to be nontoxic or even protective and fails to evoke Ca^{2+} influx in *in vitro* experiments (18, 19, 20). From these monomers, up to 50 $A\beta$ subunits can form intermediate aggregates, termed “small oligomers.” These low molecular weight aggregates are found in the growth medium of $A\beta$ -secreting cells (21) and in extracts from human brain (17, 22). This category also includes $A\beta$ -derived diffusible ligands, a neurotoxic species of $A\beta$ aggregate formed by trimers through 24-mers secreted in *in vitro* preparations and found in murine and human brain extracts (16, 23–25). Small oligomers are reported to be the most toxic species of $A\beta$ and potentially disrupt cellular Ca^{2+} homeostasis (16, 18, 26). A different approach for classifying $A\beta$ toxicity has been recently proposed by Glabe (27) based on the use of conformation-dependent antibodies that recognize generic epitopes associated with distinct peptide aggregation state of peptides rather than specific amino acid sequence and number of peptides.

The final stage of $A\beta$ peptide aggregation is represented by amyloid plaques in the brains of AD patients. Although plaques are a hallmark of AD, their density does not correlate well with the degree of neuronal or cognitive deficits (28). On the contrary, it has been proposed that plaques may contribute to the removal and inactivation of the smaller soluble toxic species (17, 29), rendering the insoluble plaque deposits as potentially neuroprotective, particularly in the early stage of the disease.

As detailed below, numerous publications studying possible molecular mechanisms of $A\beta_{40/42}$ oligomers have proposed diverse modalities of action. We believe that many of the apparently contradictory results in the literature may be attributed to different experimental methods and inconsistencies in preparation of $A\beta$ oligomers, resulting in variability in the initial structure and aggregation state of the peptide, the presence of different solvents, heterogeneous nucleation, pH, and starting concentrations of the peptide (30).

$A\beta$ and Membrane Ca^{2+} Permeability

A major mechanism by which $A\beta$ is believed to alter cellular Ca^{2+} homeostasis involves disruption of membrane Ca^{2+} permeability. It is widely accepted that application of $A\beta$ to cultured cells triggers unregulated flux of Ca^{2+} through the plasma membrane (5, 18, 26). However, the precise molecular mechanism of $A\beta$ toxicity remains to be determined. Here, we outline the three major proposed mechanisms of $A\beta$ interaction with cell membranes, involving interactions with endogenous Ca^{2+} -permeable channels, disruption of membrane lipid integrity, and formation of Ca^{2+} -permeable channels by $A\beta$.

Actions of $A\beta$ on Endogenous Plasmalemmal Ion Channels—Interactions of $A\beta$ with various Ca^{2+} -permeable channels have been established (31, 32), including voltage-gated Ca^{2+} channels (N, P, and Q), nicotinic acetylcholine channels (α_7 and $\alpha_4\beta_2$), glutamate receptors (AMPA and NMDA), dopamine receptors, serotonin receptors (5-hydroxytryptamine type 3), and intracellular inositol trisphosphate receptors (IP₃Rs). Several lines of evidence point to complex dynamics between $A\beta$ and both the cholinergic and glutamatergic neurotransmitter systems during the progression of AD (33, 34). Receptor subtypes within these two receptor families, such as α_7 -nAChRs and AMPA and NMDA glutamate receptors, are all Ca^{2+} -permeable and expressed in brain regions supporting higher cognitive functions such as the neocortex and hippocampus (35). Moreover, neuronal loss during the course of the disease occurs predominantly in these brain areas (36). These observations, together with the discovery of substantial neocortical deficits in choline acetyltransferase and reduced choline uptake in AD animal models, led to the “cholinergic hypothesis of AD,” wherein the degeneration of cholinergic neurons and loss of cholinergic neurotransmission significantly contribute to cognitive deterioration (37). This hypothesis has been strengthened by positive correlations between nAChR α_7 - and α_4 -subunit expression and neurons that accumulate $A\beta$ and by the colocalization of α_7 -nAChRs with plaques (38). However, $A\beta$ affects nAChR functioning with conflicting results describing $A\beta$ as either an agonist or antagonist of nAChRs (33). Importantly, $A\beta$ has been shown to bind with high affinity to α_7 - and $\alpha_4\beta_2$ -nAChRs (respective K_i of ~ 5 pM and 30 nM) in cortical

and hippocampal synaptic membrane preparations, suggesting that $A\beta$ peptide accumulation in the synaptic cleft of cholinergic synapses may promote the formation of $A\beta$ - α_7 -nAChR complexes that seed plaque formation (39).

Similar findings have been reported for the glutamatergic system. The NMDAR is highly Ca^{2+} -permeable (single channel conductance of ~ 60 pS, with $>10\%$ of the current carried by Ca^{2+}) (40) and is therefore a highly studied target of $A\beta$ - Ca^{2+} interactions. $A\beta$ peptides affect neuronal function in brain regions where NMDARs are the principal excitotoxic mediators and underlie cell loss during the disease progression (41). Moreover, $A\beta$ oligomers trigger increases in NMDAR-mediated Ca^{2+} influx, which disrupts neuronal transmission. In critical and vulnerable brain regions such as the hippocampus, impaired neurotransmission could further impact learning and memory mechanisms (17, 42).

Although several studies have examined the effects of amyloid on NMDAR function and Ca^{2+} influx, the results are not consistent. These differences may partially reflect the distinct effects of different $A\beta$ species on cellular activity, as well as experimental differences in acute *versus* chronic exposures. For example, short-term incubation of neuronal cultures with $A\beta$ oligomers has been shown to increase Ca^{2+} influx through NMDA channels. In turn, this is linked to downstream pathogenic effects, such as dynamin 1 degradation, increased reactive oxygen species production, and aberrant calpain activation (43), all of which can impair synaptic integrity. Acute treatment studies applying $A\beta_{1-40}$ and $A\beta_{25-35}$ peptides have demonstrated similar patterns of enhanced NMDA currents (44). In contrast, sustained exposure of neurons to $A\beta$ oligomeric peptides reduces NMDA cell-surface expression, Ca^{2+} influx, and glutamatergic currents (17, 45, 46). Spine density loss, reduced AMPA currents, and impaired synaptic plasticity are resulting consequences and likely involve alterations in calcineurin, a Ca^{2+} -sensitive phosphatase, and cofilin, a cytoskeleton-regulating protein that is activated by calcineurin-mediated dephosphorylation (47).

Another major source of cytosolic Ca^{2+} entry in neurons is through voltage-gated plasmalemmal Ca^{2+} channels. Ca^{2+} entry through the high threshold, low conductance N- and T-type channels (8–13 pS) and high conductance L-type channels (25 pS) (48) is thought to be increased by amyloid peptides ($A\beta_{1-40}$) (31, 49), resulting in increased postsynaptic Ca^{2+} responses. In contrast, the high threshold, predominantly presynaptic P/Q-type channels (15–17 pS) are *suppressed* by $A\beta$ oligomers (50), which serves to reduce synaptic vesicle release, neurotransmission, and plasticity.

Disruption of Membrane Lipid Integrity— $A\beta$ peptides interact with membrane lipids such as phosphoinositides (51), phosphatidylglycerol (52), phosphatidylcholine (53), and gangliosides (54). A direct interaction of $A\beta$ with cell membranes was initially proposed by Cotman and co-workers (55), who showed that D- and L-stereoisomers of a truncated form of $A\beta$ induced similar toxicity levels in cultured hippocampal neurons, suggesting that $A\beta$ toxicity does not involve a specific ligand-receptor interaction. Fluorescence spectroscopy measurements indicate that $A\beta$ interaction with the synaptic plasma membrane causes substantial changes in the membrane fluidity both

in the bulk lipid milieu and in proximity to integral membrane proteins. This may account for the effects of $A\beta$ peptides in increasing membrane permeability to Ca^{2+} , Na^+ , and K^+ ions as well as larger molecules such as dyes (56, 57). However, different groups have shown varying results, reporting increases (58), decreases (59), or no effect (60) of $A\beta$ peptides on membrane fluidity.

More recently, using uniform preparations of $A\beta$ peptides (in their monomeric, oligomeric, and fibrillar forms), Sokolov and co-workers (19, 61) reported increases in conductance of lipid bilayer and patch-clamped mammalian cell membranes exclusively by the oligomeric form of $A\beta_{1-42}$. Because the $A\beta$ -induced conductance showed no selectivity between anionic and cationic probes and was apparent only in membranes formed from soft highly compressible lipids, the authors suggested that $A\beta$ oligomers thin the membrane, thereby lowering the dielectric barrier and increasing its conductance. However, this mechanism has been challenged. Capone *et al.* (62) proposed that the membrane thinning was due to the residual solvent (hexafluoroisopropanol) used during $A\beta$ oligomer preparation and was independent of the peptide itself.

$A\beta$ Pore Formation—A different mechanism of action posits that $A\beta$ peptides incorporate into the cell membrane and reorganize to form nonselective high conductance cation pores (63–65). Electrophysiological recordings using artificial lipid membranes exposed to $A\beta$ demonstrated cation channels with the permeability sequence $P_{Cs} > P_{Li} > P_{Ca} = P_K > P_{Na}$ (66), which were blocked by Zn^{2+} . These $A\beta$ channels exhibit several different conductances, with spontaneous transition between levels ranging from 400 pS up to 5 nS (63). Channel formation has been proposed as a molecular mechanism for $A\beta$ toxicity because ionic leakages of Na^+ , K^+ , and Ca^{2+} through such high conductance channels could rapidly disrupt cellular homeostasis (63, 67). The pore-forming mechanism for amyloid proteins has been further supported by studies employing atomic force microscopy (64), electron microscopy (68, 69), and theoretical modeling (70, 71). Moreover, high resolution transmission electron microscopy has revealed the presence of $A\beta$ pores distributed *in situ* in the cell membrane of post-mortem brains of AD patients but not in healthy patients (72).

In a search for a specific blocker, Arispe (73) further strengthened the $A\beta$ channel hypothesis by designing short peptides complementary to the putative mouth of the $A\beta$ channel that potently and selectively blocked $A\beta$ channels and inhibited $A\beta$ cytotoxicity. More recently, Arispe and co-workers (74) also showed that two small enantiomeric molecules, MRS2481 and MRS2485, were both blockers of $A\beta$ channels in the micromolar range and exhibited protective behavior against $A\beta$ neurotoxicity in neurons.

Intracellular Ca^{2+} Sources and $A\beta$

In addition to extracellular Ca^{2+} sources, the ER constitutes a large reservoir of sequestered Ca^{2+} that is liberated via IP_3 Rs (whose activation requires binding of the second messenger IP_3) and ryanodine receptors (RyRs). Both of these receptor/channel types are activated by Ca^{2+} itself in a regenerative process termed Ca^{2+} -induced Ca^{2+} release. Numerous studies have linked up-regulation of ER Ca^{2+} release with presenilin

mutations in early stages of AD progression, prior to the onset of $A\beta$ plaques, neurofibrillary tangles, or cognitive impairment (1, 75), yet evidence exists that $A\beta$ also influences intracellular Ca^{2+} signaling at later disease stages subsequent to histopathology onset (6). For example, exposing RyRs to $A\beta_{1-42}$ peptides in lipid bilayers increases the channel open probability and alters gating kinetics, resulting in increased Ca^{2+} flux (76). Likewise, $A\beta$ exposure enhances the IP_3 R-evoked Ca^{2+} response in neurons (77). More subtle interactions of $A\beta$ with Ca^{2+} -regulating G-protein-coupled membrane proteins have also been uncovered. Preincubation with the $A\beta_{1-40}$ peptide enhances both the expression of G_q -coupled metabotropic glutamate receptor 5, which generates the Ca^{2+} -mobilizing messenger IP_3 , and the intracellular Ca^{2+} response to the group I metabotropic glutamate receptor agonist (*S*)-3,5-dihydroxyphenylglycine (78). $A\beta$ also interferes with the interplay between the APP haloprotein and G_o , which leads to G-protein-coupled Ca^{2+} activation and eventually cell death (79).

New on the neuronal Ca^{2+} channel list is CALHM1, which is highly permeable to Ca^{2+} and localized to the ER and plasma membranes. Notably, a CALHM1 polymorphism has been associated with AD and leads to increased $A\beta$ formation by interfering with Ca^{2+} permeability (80). In addition to being relevant from a Ca^{2+} signaling/amyloid perspective, the discovery of this polymorphism association with AD adds another possible marker to the short list of genetic risk factors (including *ApoE4* allele expression) linked to sporadic AD, thereby permitting early intervention for patients with an otherwise idiopathic neurodegenerative disease.

Functional Evidence for $A\beta$ and Ca^{2+} Interactions in Brain

Attempts to establish causative links between $A\beta$ histopathology and AD memory deficits have been tenuous, with little direct correlation between plaque load and cognitive decline (81, 82). However, recent evidence demonstrates functional associations between dense core plaques and Ca^{2+} signaling alterations in AD mouse models. A series of *in vivo* imaging studies show that $A\beta$ deposits result in intracellular Ca^{2+} dysregulation in neurons and glia (83, 84) and a structural breakdown of dendritic processes in later stages of AD pathology (85). Utilizing fluorescent live cell imaging techniques in plaque-bearing APP transgenic mice, increased resting Ca^{2+} levels have been observed in neurites in close proximity ($\sim 20 \mu m$) to dense core plaques (83), suggesting that plaques exert a direct pathogenic effect on steady-state Ca^{2+} levels in dendrites and spines, regions critical for electrochemical signal transmission. In concert, the compartmentalization of Ca^{2+} signals between spine heads and the neighboring dendritic branch is lost. These alterations would likely have implications for signal transduction and synaptic transmission, which are reliant on precise spatial and temporal Ca^{2+} signaling.

Possibly related to the above findings is the observation of increased spontaneous Ca^{2+} transients in the soma of cells close to plaques, perhaps resulting from reduced inhibitory input through reduced GABAergic tone (86). Alterations in Ca^{2+} transients may exert global alterations in intracellular function and affect long-range coordination among cells mediated by intercellular Ca^{2+} waves. This phenomenon is not lim-

ited to neurons, as increased Ca^{2+} activity and synchronized Ca^{2+} waves are observed across networks of astrocytes (84). Interestingly, astrocytic Ca^{2+} signals differ from those in neurons in that they are independent of proximity to plaques. Although these *in vivo* studies detailed above provide some of the most direct evidence for pathogenic $A\beta$ and Ca^{2+} interactions in intact brains, they are limited in their interpretation because only dense core plaques were visualized, and the role and localization of other $A\beta$ species, notably oligomeric forms, could not be identified in these preparations.

Structural abnormalities in neurites have also been attributed to the activity of calcineurin, a Ca^{2+} -sensitive phosphatase whose many functions include regulation of cofilin, which maintains neuronal cytoarchitecture. These findings may relate to the breakdown of synapses attributed to fibrillar and oligomeric $A\beta$ (87) in that aberrant Ca^{2+} levels can disrupt glutamate receptor trafficking and Ca^{2+} /calmodulin-dependent protein kinase II and calcineurin activity and alter spine head geometry (88, 89).

$A\beta$ oligomers have also been found to disrupt synaptic function at the circuit level. Associations between naturally produced $A\beta$ oligomers and AD pathology were made by Selkoe and co-workers (90), who identified a naturally secreted species of $A\beta$ aggregates capable of disrupting neuronal plasticity. Soluble $A\beta$ oligomers extracted from AD patients inhibit long-term potentiation, enhance long-term depression, and trigger dendritic spine reduction in rodent hippocampus (17, 91). These pathological effects were shown to be specifically attributable to $A\beta_{1-42}$ dimers.

A Vicious Spiral in AD: $A\beta$ and Ca^{2+} Go Round and Round

Increased Ca^{2+} levels are functionally linked to most of the major features and risk factors of AD: presenilin and APP mutations, ApoE4 expression, CALHM1 mutations, $A\beta$ plaques, Tau hyperphosphorylation, apoptosis, and synaptic dysfunction (1). In many of these interactions, a pathogenic feed-forward cascade evolves, wherein Ca^{2+} facilitates a pathogenic state, which in turn increases Ca^{2+} levels. For example, Ca^{2+} can facilitate the formation of pathogenic $A\beta$ fibril formation (92), and in parallel, $A\beta$ can form Ca^{2+} -permeable channels, interfere with existing Ca^{2+} channels, and increase RyR function (43, 93, 94). Apoptosis can also be triggered by Ca^{2+} -sensitive cell death pathways via caspase and calpain activation and vice versa. Ca^{2+} dysregulation may then reflect a lifetime of episodic and slowly accumulating insults that favor the aggregation and deposition of pathogenic $A\beta$ peptides, trigger apoptosis via ER and mitochondrial stress responses, and impair synaptic morphology and membrane function. The culmination of these downstream Ca^{2+} -mediated events may ultimately lead to the devastating loss of memory and deteriorating cognitive functions (Fig. 2).

Future Directions

In light of the ubiquity of Ca^{2+} signaling in neurons and glia and its complex reciprocal interactions with $A\beta$ in the pathogenesis of AD, research is likely to progress in parallel along multiple paths. Below, we highlight just a few areas that we believe most promising.

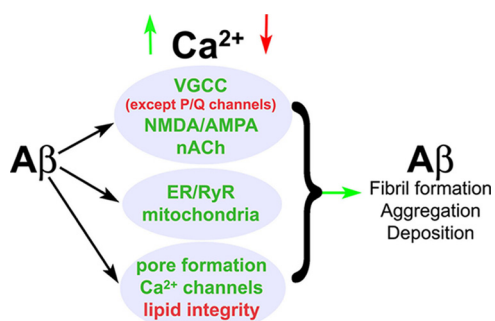


FIGURE 2. **Feed-forward $A\beta$ peptide and Ca^{2+} signaling interactions.** $A\beta$ peptides interact with a variety of Ca^{2+} channels and sources (center, blue), often serving to up-regulate or aberrantly generate Ca^{2+} flux within the cell. This increase in Ca^{2+} from direct calcium channel alteration or through compromised lipid barriers can then serve to accelerate $A\beta$ generation, thereby sustaining a pathogenic cycle. VGCC, voltage-gated Ca^{2+} channel.

Consistency of $A\beta$ Preparations—Studies of $A\beta$ toxicity are confounded by inconsistencies in oligomeric state of the peptide, a factor that likely accounts for widely varying and sometimes contradictory reports in the literature. There is therefore a clear need to resolve the effects of the various $A\beta$ species by systematically examining the effect of uniformly prepared and characterized $A\beta$ aggregates.

Mechanisms of $A\beta$ Ca^{2+} Toxicity—Disruption of membrane integrity and the resulting unregulated Ca^{2+} flux are now well established as major factors underlying $A\beta$ oligomer toxicity. There is strong evidence that $A\beta$ itself forms cation pores in the membrane, but actions on the lipid bilayer and on endogenous membrane channels may also contribute. Elucidation of the mechanism(s) by which $A\beta$ acts on surface and intracellular membranes is crucial, as this represents a selective and most attractive therapeutic target. Experiments have thus far been limited largely to *in vitro* systems, but developments in techniques for optical imaging of single channel Ca^{2+} flux (95) offer considerable potential for extending these studies to intact cell systems (96).

Ca^{2+} Signaling as a Therapeutic Target—Because Ca^{2+} signaling impinges upon nearly every characteristic feature, genetic cause, and major risk factor in AD, it is an obvious target for potential therapeutic strategies. Compounds that normalize dysregulated Ca^{2+} levels or specifically block Ca^{2+} -regulated pathogenic signaling cascades could, in theory, prevent or reduce many of the histopathological and cognitive components of AD. Indeed, the few effective treatments currently available for early-to-mid-stage AD directly or indirectly include some aspect of Ca^{2+} modification. Memantine is a low affinity NMDAR Ca^{2+} channel antagonist that prevents excessive Ca^{2+} influx while maintaining glutamatergic transmission sufficiently to support synaptic transmission and plasticity (97). Another example is dimebon, which was suggested in clinical trials to sustain cognitive function in AD patients. Although the mechanism is unclear, the neuroprotective effects of dimebon may lie in its ability to inhibit L-type Ca^{2+} channels and NMDAR and protect against mitochondrial stress (98). Another target, though not yet in clinical trials, is the RyR, an intracellular Ca^{2+} release channel that is up-regulated in an initially neuroprotective manner in response to $A\beta_{1-42}$ exposure (94) and that shows increased expression and Ca^{2+} flux in

certain familial forms of AD (99, 100). Given the ubiquity of Ca^{2+} signaling, a caveat with these approaches is the potential to disrupt normal neuronal function. The design of novel compounds to block Ca^{2+} -permeable pores formed by $A\beta$ thus holds particular promise (73, 74).

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