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Neuronal Calcium Mishandling and the Pathogenesis of Alzheimer's Disease

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Abstract

Perturbed neuronal Ca^{2+} homeostasis is implicated in age-related cognitive impairment and Alzheimer's disease (AD). With advancing age neurons encounter increased oxidative stress and impaired energy metabolism, which compromise the function of proteins that control membrane excitability and subcellular Ca^{2+} dynamics. Toxic forms of amyloid β -peptide ($\text{A}\beta$) may induce Ca^{2+} influx into neurons by inducing membrane-associated oxidative stress or by forming an oligomeric pore in the membrane, thereby rendering neurons vulnerable to excitotoxicity and apoptosis. AD-causing mutations in the β -amyloid precursor protein and presenilins may compromise normal function of these proteins in the plasma membrane and endoplasmic reticulum, respectively. Emerging knowledge of the actions of Ca^{2+} upstream and downstream of $\text{A}\beta$ provide opportunities to develop novel preventative and therapeutic interventions for AD.

Neuronal Ca^{2+} Signaling in Healthy Brains and the Adverse Effects of Aging

Neurons use Ca^{2+} signals to control membrane excitability, to trigger release of a neurotransmitter, to mediate activity-dependent changes in gene expression and modulate neuronal growth, differentiation and transition to apoptosis¹. Neuronal Ca^{2+} signaling involves an intricate interplay between Ca^{2+} influx across plasma membrane through voltage-gated Ca^{2+} channels, NMDA receptors and TRP (transient receptor potential) channels, and Ca^{2+} release from intracellular Ca^{2+} stores via inositol triphosphate receptor (IP_3R) and ryanodine receptor (RyR) channels in the endoplasmic reticulum (ER). Intracellular Ca^{2+} release via IP_3R is triggered by second messenger IP_3 which is produced following activation of metabotropic receptors coupled to phospholipase C. Neuronal RyR function primarily as Ca^{2+} -activated Ca^{2+} channels which further amplify Ca^{2+} signals originating from other sources. Mitochondria also play an important role in shaping neuronal Ca^{2+} signaling by utilizing potent mitochondrial Ca^{2+} uptake mechanisms. Ca^{2+} uptake into mitochondria plays an important role in neuronal physiology by stimulating mitochondrial metabolism and increasing mitochondrial energy production. Excessive Ca^{2+} uptake into mitochondria can lead to opening of a permeability transition pore (PTP) and apoptosis². Owing to its importance for neuronal function, Ca^{2+} signaling in neurons is tightly compartmentalized and regulated within signaling microdomains which involve, for example, functional coupling between voltage-gated Ca^{2+} channels and intracellular Ca^{2+} release channels, or between ER Ca^{2+} release and Ca^{2+} uptake into mitochondria.

The major risk factor for Alzheimer's disease (AD) is advancing age; in the most common sporadic form of AD the individuals first manifest symptoms when they are in their 7th or 8th decades of life. But even those who inherit a disease-causing mutation in the β -amyloid precursor protein (APP) or one of the presenilins (PS1 and PS2) remain asymptomatic into their fourth or fifth decades³. Age-related alterations in specific Ca^{2+} -regulating systems in brain cells have been reported including: elevated intracellular Ca^{2+} levels; enhanced Ca^{2+} influx through voltage-dependent Ca^{2+} channels; impaired ability of mitochondria to buffer or cycle Ca^{2+} ; perturbed Ca^{2+} regulation in ryanodine and IP_3 -sensitive Ca^{2+} stores. Gene array and proteomic analyses suggest dysregulation of the expression of an array of Ca^{2+} -handling systems during aging^{4–7}. Many of the alterations in Ca^{2+} -handling described in normal aging can be reproduced by subjecting neurons to oxidative and metabolic stress in culture or in vivo, suggesting important contributions of these fundamental aging processes to the dysregulation of neuronal Ca^{2+} regulation in AD described below. Moreover, studies of brain tissue samples obtained from brains of AD patients and animal models of AD have revealed significant alterations in levels of proteins and genes directly involved in neuronal Ca^{2+} signaling^{8–10}. Many of the latest advances in understanding the roles of perturbed cellular Ca^{2+} handling in AD pathogenesis are described in the remainder of this article.

Amyloid β -Peptide Promotes Ca^{2+} Influx and Ca^{2+} -Mediated Excitotoxicity

Amyloid plaques, a histological hallmark of AD, are comprised of extracellular aggregates of the amyloid β -peptide ($\text{A}\beta$) a 40–42 amino acid peptide generated by successive enzymatic cleavages of APP by β - and γ -secretases (Fig. 1). $\text{A}\beta$ is believed to be a pivotal mediator of neuronal degeneration and impaired cognitive function in AD^{3,11}. Adverse effects of $\text{A}\beta$ on synaptic function and neuronal survival are mediated primarily by soluble protein oligomers¹². $\text{A}\beta$ interaction with the plasma membrane results in elevated intracellular (cytoplasmic) Ca^{2+} concentrations ($[\text{Ca}^{2+}]_i$) and increased vulnerability of the neurons to excitotoxicity¹³. Oligomeric forms of $\text{A}\beta_{42}$ cause Ca^{2+} -mediated toxicity in cultured cells¹⁴. Degenerative changes occur in neurites associated with $\text{A}\beta$ deposits in APP mutant mice, suggesting the involvement of Ca^{2+} -mediated $\text{A}\beta$ neurotoxicity in vivo¹⁵. In addition to increasing the production of $\text{A}\beta$, amyloidogenic processing of APP may perturb neuronal Ca^{2+} homeostasis by decreasing the production of a secreted form of APP ($\text{sAPP}\alpha$) that activates K^+ channels¹⁶, and by generating an APP intracellular domain that affects ER Ca^{2+} release by regulating the expression of genes involved in Ca^{2+} homeostasis (Fig. 1)¹⁷.

One mechanism by which $\text{A}\beta$ may cause Ca^{2+} influx is by inserting into the plasma membrane and forming ion-conducting pores¹⁸ (Fig. 1). Neurotoxic forms of $\text{A}\beta$ are oligomers that share structural and functional homology with pore-forming bacterial toxins and the cytotoxic lymphocyte protein perforin¹⁹. Interestingly, the ability of $\text{A}\beta$ to associate with membranes and form channels is enhanced by exposure of phosphatidylserine on the cell surface²⁰. Because cell surface exposure of phosphatidylserine is usually indicative of apoptotic or energy-deprived cells, it is possible that age-related mitochondrial impairments may increase surface phosphatidylserine levels in affected neurons and thereby facilitate $\text{A}\beta$ -mediated pore formation, Ca^{2+} influx and cell death (Fig. 1); indeed, neurons with reduced cytosolic ATP levels and elevated surface phosphatidylserine are particularly vulnerable to $\text{A}\beta$ toxicity²¹. The surface exposure of phosphatidylserine may also result from activation of a Ca^{2+} -sensitive phospholipid scramblase 1 (PLSCR1) which mediates a rapid trans-bilayer reorganization of plasma membrane phospholipids²² (Fig. 1).

A different mechanism by which $\text{A}\beta$ perturbs neuronal Ca^{2+} homeostasis is by inducing membrane lipid peroxidation¹¹. During peptide oligomer formation $\text{A}\beta$ generates hydrogen peroxide, a process enhanced by iron (Fe^{2+}) and copper (Cu^+)^{23,24}. Hydrogen peroxide is then converted to hydroxyl radical which initiates lipid peroxidation resulting in the generation of

toxic lipid aldehydes such as 4-hydroxynonenal which impair the function of ion-motive ATPases, and glutamate and glucose transporters resulting in Ca^{2+} overload, synaptic dysfunction, neuronal degeneration and cognitive impairment^{11,25}. Particularly striking, is the ability of $\text{A}\beta$ to increase the vulnerability of neurons to excitotoxicity mediated by the N-methyl-D-aspartate (NMDA) receptor^{11,13}. Because excessive and sustained Ca^{2+} elevations induce free radical production (by altering mitochondrial oxidative phosphorylation and activating oxygenases) it is likely that perturbed Ca^{2+} homeostasis contributes to the increased oxidative stress in neurons in AD, resulting in a self-amplifying cascade of free radical- and Ca^{2+} -mediated degenerative processes (Fig. 1). Lesser amounts of $\text{A}\beta$ may also be toxic to neurons. For example, exposure of rat organotypic hippocampal slice cultures to picomolar concentrations of $\text{A}\beta$ oligomers caused the loss of dendritic spines and decreased numbers of electrophysiologically active synapses; the spine loss was reversible and required NMDA receptor activity²⁶. $\text{A}\beta$ oligomers cause an increase in NMDA receptor activity, which may require direct association between $\text{A}\beta$ oligomers and NR1 subunit of the NMDAR. On the other hand, $\text{A}\beta$ oligomers may suppress activity of presynaptic P/Q-type voltage-gated Ca^{2+} channels²⁷. $\text{A}\beta$ also blocks the response of $\alpha 7$ -containing nicotinic acetylcholine receptors (nAChRs) in hippocampal neurons²⁸ and directly evokes sustained nAChR-mediated increases in presynaptic Ca^{2+} levels²⁹ suggesting a mechanism for impairment of cholinergic signaling in AD.

Enter the Presenilins

Presenilins (PS1 and the structurally and functionally related PS2) are integral membrane proteins. The holoprotein form of presenilins is located in the ER. Both PS1 and PS2 holoproteins undergo endoproteolysis in the cytosolic loop between the 6th and 7th transmembrane domains, resulting in the generation of amino-terminal and carboxy-terminal fragments, which remain associated with each other. Cleaved presenilins assemble with nicastrin, Aph-1 and Pen-2, exit the ER and translocate into the Golgi apparatus and eventually to plasma membrane. A mature complex of cleaved presenilins, nicastrin, Aph-1 and Pen-2 possesses aspartyl protease activity and functions as the γ -secretase enzyme that cleaves APP to generate $\text{A}\beta$ ^{3,11}. Many mutations in presenilins that cause familial (dominantly inherited) AD (FAD) increase the production of the long aggregation-prone form of $\text{A}\beta$ ($\text{A}\beta 42$) or reduce the production of a short soluble form $\text{A}\beta 40$, and therefore one way in which presenilin mutations may perturb neuronal Ca^{2+} homeostasis is by elevating $\text{A}\beta 42:\text{A}\beta 40$ ratio and activating the $\text{A}\beta$ oligomer-mediated mechanisms described above.

Additional roles for presenilins in modulating Ca^{2+} homeostasis are suggested by data linking two other presenilin substrate cleavage products, the AICD and the Notch intracellular domain (NICD), to Ca^{2+} -mediated neuroplasticity and cell death. AICD may translocate to the nucleus and therein regulate the expression of genes encoding proteins involved in Ca^{2+} homeostasis¹⁷. Notch, a membrane receptor activated by cell surface-associated ligands such as Jagged and Delta, plays fundamental roles in regulating the proliferation and differentiation of neural progenitor cells in the developing and adult brain³⁰. Upon ligand binding, γ -secretase cleaves Notch to release the NICD which translocates to the nucleus where it regulates gene transcription. Recent findings suggest potential roles for Notch and NICD in synaptic plasticity, learning and memory and Ca^{2+} -mediated cell death³¹.

A γ -secretase-independent connection between presenilins and Ca^{2+} signaling was initially suggested in Ca^{2+} imaging experiments with fibroblasts from FAD patients containing PS1-A246E mutation³². It was then shown that cultured neural cells expressing AD PS1 mutations exhibit increased amounts of Ca^{2+} released from the ER when exposed to ligands that stimulate IP_3 production or activation of RyR^{33,34}. Similar results were obtained in Ca^{2+} imaging experiments with *Xenopus* oocytes injected with cRNA encoding PS1-M146V and PS2-N141I

FAD mutants³⁵, in experiments with synaptosomes and cortical neurons from PS1-M146V mutant mice^{36,37}, and in hippocampal neurons from PS2-N141I transgenic mice³⁸. In vitro and in vivo studies demonstrated that exaggerated ER Ca^{2+} signaling resulting from FAD mutations in presenilins leads to sensitization of PS-FAD neurons to $\text{A}\beta$ and excitotoxic cell death via a Ca^{2+} -dependent mechanism involving excessive Ca^{2+} release from the ER^{36–39}.

Biochemical and functional interactions have been uncovered between presenilins and several ER Ca^{2+} -regulating proteins including ryanodine receptors⁴⁰, sorcin⁴¹, the myristoylated calcium-binding protein calmyrin⁴² and calsenilin⁴³. Presenilins may also modulate SERCA Ca^{2+} pump activity⁴⁴. Presenilin-2 has been reported to associate with IP_3R and to enhance IP_3R activity⁴⁵. Specific effects of FAD mutants PS1-M146V and PS2-N141I on sensitivity of IP_3R to activation by IP_3 have been recently discovered in patch-clamp experiments⁴⁶. A significant increase in ryanodine receptor expression levels has been reported in brains from PS1 mutant mice⁴⁷, an alteration that increases as the mice age, providing a potential link between AD pathogenesis and aging.

While the studies described above suggest that FAD mutations in presenilins act by altering a normal function of other Ca^{2+} -regulating proteins, recent findings indicated that presenilins themselves may play a direct role in Ca^{2+} signaling. It is well established known that the ER membrane is “leaky” for Ca^{2+} , but the exact identity of the putative “ Ca^{2+} leak channel” was previously unknown. Recent results suggest that presenilins function as ER Ca^{2+} leak channels in cells, and that a balance between SERCA Ca^{2+} pump activity and presenilin-mediated passive Ca^{2+} leak determines the steady-state resting ER Ca^{2+} levels in cells⁴⁸. The ER Ca^{2+} leak function of presenilins does not involve γ -secretase activity and is not supported by a cleaved form of presenilins; instead many FAD mutations in presenilins result in “loss of function” for ER Ca^{2+} leak activity^{48,49} resulting in excessive Ca^{2+} accumulation in the ER (Fig. 1). Although most tested FAD mutants in presenilins compromised its ER Ca^{2+} leak function, the PS1- ΔE9 mutant was unique and appeared to act as a “gain of function” leading to “superleaky” channels⁴⁸. The “gain of Ca^{2+} leak function” phenotype of PS1- ΔE9 mutant is consistent with an earlier observation of elevated basal Ca^{2+} levels in neuronal cells transfected with PS1- ΔE9 expression construct⁵⁰. Thus, the cells expressing PS1- ΔE9 mutants are expected to be exposed to constitutively elevated cytosolic Ca^{2+} levels and partially depleted ER. This is in contrast to cells expressing “loss of ER leak function” PS FAD mutants, which are expected to have normal steady-state cytosolic Ca^{2+} levels and overloaded ER. Interestingly, the PS1- ΔE9 mutation is associated with a unique cotton wool plaques and spastic paraparesis clinical phenotype (CWP/SP), which is not observed for most other FAD PS1 mutations⁵¹. It will be very important to determine if other FAD mutations in PS1 associated with CWP/SP phenotype may also be associated with “gain of function” for the ER Ca^{2+} leak activity. If such a correlation is established, it would support a causal connection between ER Ca^{2+} dyshomeostasis and $\text{A}\beta$ pathology in AD.

Calcium and the cytoskeletal pathology in AD

Neurofibrillary tangles, the most overt manifestation of cytoskeletal abnormalities in AD, consist of intracellular fibrillar aggregates of hyperphosphorylated forms of the microtubule-associated protein tau¹¹. Tau is normally located in axons where it maintains microtubules in a polymerized state, but in AD tau dissociates from microtubules resulting in microtubule depolymerization and the accumulation of tau in the cell body. Studies of AD patient brain tissue samples suggest an association between elevated $[\text{Ca}^{2+}]_i$ and neurofibrillary pathology. For example, neurons prone to neurofibrillary tangle formation are enriched in type II calcium/calmodulin-dependent protein kinase⁵², and calpains (Ca^{2+} -dependent proteases that cleave cytoskeletal proteins) are elevated in vulnerable neuronal populations early in the disease process⁵³. Overactivation of glutamate receptors in hippocampal neurons can cause Ca^{2+} -

mediated changes in tau and microtubules similar to those seen in neurofibrillary tangles⁷² suggesting a possible cause-effect relationship between aberrant increases in $[Ca^{2+}]_i$ and tangle formation (Fig. 2). In addition, Ca^{2+} can cause AD-like tau phosphorylation and intracellular A β accumulation in neurons⁵⁴. Conversely, tau mutations that cause tangle formation in frontotemporal lobe dementia alter the function of voltage-dependent Ca^{2+} channels in a manner that increases Ca^{2+} influx⁵⁵ and may contribute to the cell death process in this disease.

Calcium Actions Upstream of Amyloidogenesis

The placement of A β at the apex of the amyloid cascade hypothesis belies the fact there must be changes that occur during aging and AD that result in increased production and aggregation of A β . Evidence suggests that Ca^{2+} may be such an upstream factor. Environmental factors that inhibit amyloidogenesis (caloric restriction, cognitive stimulation and antioxidants) stabilize neuronal Ca^{2+} homeostasis, whereas factors that enhance amyloidogenesis disrupt Ca^{2+} homeostasis. In addition to these kinds of circumstantial evidence, direct evidence that Ca^{2+} influences APP processing has been reported. Exposure of cultured neurons to Ca^{2+} ionophores increases their production of A β ⁵⁶, as do conditions such as ischemia that cause sustained elevations of $[Ca^{2+}]_i$ ⁵⁷. On the other hand, physiological Ca^{2+} transients (as occur during LTP, for example) increase α -secretase cleavage of APP and may thereby decrease A β production^{58,59}.

Synapses: the Weakest Link

Studies of patients with mild cognitive impairment and AD suggest that synaptic dysfunction and degeneration may occur relatively early in the disease process, and studies of AD mouse models uniformly support this tenet¹¹. Synaptic terminals are particularly vulnerable to Ca^{2+} -mediated degeneration because they experience repeated bouts of Ca^{2+} influx and have unusually high energy requirements to support their ion-homeostatic and signaling systems. APP is actively transported to presynaptic terminals and considerable evidence suggests that A β is produced and accumulated primarily in synaptic regions⁶⁰. A β can directly disrupt Ca^{2+} homeostasis in synaptic terminals by causing membrane-associated oxidative stress¹¹. Consistent with a major role for A β in synaptic damage in AD are data showing loss of dendritic spines in dendrites associated with A β deposits in APP mutant mice⁶¹. A β also causes down-regulation of expression of calcineurin, a Ca^{2+} -activated phosphatase known to play fundamental roles in synaptic plasticity⁶². A β oligomers caused a rapid decrease in membrane expression of NMDA and EphB2 receptors, followed by abnormal dendritic spine morphology and degeneration of spines⁶³. The latter effects of A β are prevented by treatment with an NMDA receptor antagonist suggesting a major role for Ca^{2+} influx in the dendritic dystrophy. Moreover, A β immunotherapy prevented synaptic dysfunction and restores cognitive function in a mouse model of AD⁶⁴.

Electrophysiological analyses of synaptic activity in hippocampal slices from APP and PS1 mutant mice have revealed abnormalities in several aspects of Ca^{2+} -mediated synaptic function. APP mutant mice exhibit abnormal excitatory neuronal activity and compensatory remodeling of inhibitory circuits in the hippocampus⁶⁵. Expression of mutant PS1 in cultured hippocampal neurons results in a significant depression of the amplitude of evoked AMPA and NMDA receptor-mediated synaptic currents, and a lower frequency of spontaneous miniature synaptic currents⁶⁶. A β impairs spike-timing-dependent synaptic potentiation at excitatory synapses on neocortical layer 2/3 cortical pyramidal cells in APP mutant mice, which was associated with a decrease in AMPA but not NMDA receptor-mediated currents⁶⁷. A β may also perturb Ca^{2+} handling in neural stem cells resulting in impaired hippocampal neurogenesis and a compromised ability to form and integrate new neurons from endogenous stem cells⁶⁸.

PS1 mutations have a local adverse effect on synaptic Ca^{2+} regulation that may contribute to mitochondrial dysfunction and synaptic degeneration in AD. Thus, synaptosomes from PS1 mutant transgenic mice which exhibit enhanced elevations of cytoplasmic Ca^{2+} levels following exposure to depolarizing agents, $\text{A}\beta$, and a mitochondrial toxin compared with synaptosomes from nontransgenic mice and mice overexpressing wild-type PS1³⁹. Two-photon imaging studies revealed a 10-fold enhancement in RyR-mediated Ca^{2+} release in spines of PS1-M146V mutant-expressing mice, indicating a major alteration in synaptic ER Ca^{2+} handling in this AD model (Beth Stutzmann, personal communication). Agents that buffer cytoplasmic Ca^{2+} or that prevent Ca^{2+} release from the ER protected synaptosomes against the adverse effect of PS1 mutations.

Polymorphisms in the apolipoprotein E (ApoE) gene affect one's risk for late onset AD. Of the three isoforms (E2, E3 and E4), E3 is the most common and E2 the least common. The three isoforms differ at residues 112 and 158; E3 has Cys-112 and Arg-158, whereas E4 has arginine in both positions and E2 has cysteine in both positions. Inheritance of the allele for E4 isoform is associated with increased risk and earlier age of onset of the sporadic AD whereas E2 reduces risk⁶⁹. Several studies indicate a potential link between ApoE and synaptic Ca^{2+} signaling. As mentioned above, ApoE2, but not ApoE4, can inhibit $\text{A}\beta$ association with phosphatidylserine in the membrane, providing a potential explanation for protective effects of ApoE2 in AD. A different line of research demonstrated that application of low levels of ApoE4 to cultured neurons induces NMDAR-mediated Ca^{2+} influx and causes neuronal toxicity⁷⁰. In addition, it was recently demonstrated that reelin can activate neuronal NMDAR via a src-family tyrosine kinase (SFK)-mediated mechanism and that reelin association with ApoE receptor 2 (ApoER2) was necessary for activation of NMDAR⁷¹. Marked changes in reelin expression levels were observed in brains from AD patients and AD mouse models^{72, 73} further implicating a potential importance of reelin signaling pathway in AD.

Calcium and the Selectivity of Neuronal Vulnerability in AD

Differential production and deposition of $\text{A}\beta$ and the resulting disruption of Ca^{2+} homeostasis is one likely determinant of selective neuronal vulnerability because neurons in brain regions with high $\text{A}\beta$ loads (entorhinal cortex, hippocampus, inferior parietal cortex) degenerate, whereas neurons in regions with little or no $\text{A}\beta$ accumulation (cerebellum, striatum, motor cortex) typically do not⁴. However, it is clear that there are additional factors at work because within a vulnerable brain region (in the presence of similar amounts of $\text{A}\beta$) some neurons degenerate in AD, whereas others do not. Populations of neurons that degenerate in AD typically express high levels of NMDA receptors and have relatively low levels of some Ca^{2+} -binding proteins compared to resistant neurons². Although hippocampal dentate and CA1 neurons each express NMDA receptors, the dentate neurons express high amounts of calbindin, whereas CA1 neurons do not. Experimental findings suggest that calbindin buffers Ca^{2+} loads and protects neurons against excitotoxicity; in the hippocampus calbindin-positive neurons are relatively preserved in AD patients with moderate plaque and tangle content, but in severe cases the calbindin-positive pyramidal cells are also lost, suggesting the possibility that calbindin protects neurons in the early stages of AD⁷⁴. Basal forebrain cholinergic neurons may become depleted of calbindin during aging, which may increase their vulnerability to degeneration in AD⁷⁵. However, in the entorhinal cortex calbindin- and parvalbumin-positive non-principal neurons exhibit degenerative changes early in AD, whereas calretinin- and calbindin-positive pyramidal neurons are relatively preserved⁷⁶. Changes in the expression of glutamate receptors may also contribute to altered neuronal Ca^{2+} handling in AD; as AD progresses the levels of NR1/2B subunits in hippocampal neurons decrease, while the NR2A subunit levels remain unchanged⁷⁷. Other factors that may contribute to selective neuronal vulnerability in AD by perturbing Ca^{2+} homeostasis are neuron-specific differences in energy metabolism, antioxidant systems and neurotrophic factor support⁴.

Optimizing Neuronal Calcium Homeostasis as a Therapeutic Approach for AD

Because aging is the major risk factor for AD, it follows that interventions that counteract the aging process would protect neurons against Ca^{2+} dysregulation and AD (Fig. 3). Epidemiological and experimental evidence suggests that exercise, dietary energy restriction and cognitive stimulation may retard aging processes and protect against AD¹¹. Indeed, environmental enrichment⁷⁸, exercise⁷⁹ and dietary energy restriction⁸⁰ suppress the disease process and enhance cognitive performance in mouse models of AD. These beneficial environmental factors may act, in part, by inducing the expression of neurotrophic factors such as BDNF, that stabilize neuronal Ca^{2+} homeostasis⁸¹. Antioxidants and cellular energy-promoting agents might also be expected to stabilize neuronal Ca^{2+} homeostasis and protect against AD. Because increased $\text{A}\beta$ production and accumulation at synapses is of major importance in AD pathogenesis, treatments that reduce $\text{A}\beta$ production or enhance its clearance from the brain are being vigorously pursued. One of the most promising anti- $\text{A}\beta$ approaches that is currently being tested in patients is immunization with $\text{A}\beta$ or treatment with purified $\text{A}\beta$ antibodies⁶⁴. By removing $\text{A}\beta$ from the brain, immunization would be expected to prevent or reverse $\text{A}\beta$ -induced neuronal Ca^{2+} dysregulation.

Drugs that inhibit β - or γ -secretases are another viable approach for reducing $\text{A}\beta$ production and associated Ca^{2+} -mediated neurotoxicity⁸². Drugs that target specific Ca^{2+} -regulating systems (downstream of age- and $\text{A}\beta$ -related disruption of Ca^{2+} homeostasis) provide another approach. Indeed, the only drug thus far shown to slow disease progression in AD patients is the NMDA receptor open channel blocker memantine⁸³. Beneficial effects have also been reported in AD clinical trials of Dimebon⁸⁴, a drug that has been claimed to stabilize Ca^{2+} signaling by blocking NMDAR and voltage-gated Ca^{2+} channels⁸⁵. As with other major age-related diseases (cardiovascular disease, diabetes and cancers) risk reduction for AD may be achievable by dietary moderation and exercise combined with dietary supplements (omega-3 fatty acids and folic acid, for example). For individuals at high risk for AD (ApoE4 genotype and family history, for example) prophylactic approaches may be prescribed including anti-inflammatory drugs and immunization.

Conclusion and Future Directions

The ability of neurons to regulate the influx, efflux and subcellular compartmentalization of Ca^{2+} is compromised in AD as the result of age-related oxidative stress and metabolic impairment in combination with disease-related accumulation of $\text{A}\beta$ oligomers. $\text{A}\beta$ may promote cellular Ca^{2+} overload by inducing membrane-associated oxidative stress and by forming pores in the membrane. Mutant forms of presenilins that cause many cases of early-onset FAD cause ER Ca^{2+} overload, apparently by impairing the normal ER Ca^{2+} leak channel function of the presenilins. Synapses are particularly sensitive to the adverse effects of $\text{A}\beta$ and presenilin mutations, and environmental factors and therapeutic agents that promote synaptic Ca^{2+} homeostasis may be effective in preventing and treating AD. Key remaining questions include: does perturbed Ca^{2+} occur early in the AD process and contribute to altered APP processing and $\text{A}\beta$ production?; is there a cause-and-effect connection between abnormal neuronal Ca^{2+} signaling and amyloid plaque accumulation in AD brains?; what is a connection between “amyloid toxicity” and “ Ca^{2+} toxicity” in AD?; what are the weakest links in the various cellular Ca^{2+} -regulating systems in AD?; what is a role played by mitochondria in AD pathogenesis?; how, at the molecular and cellular levels, do risk factors for AD impact neuronal Ca^{2+} homeostasis?; can specific Ca^{2+} -regulating mechanisms be targeted for therapeutic intervention? Answering these and related questions will clarify a possible role of abnormal Ca^{2+} signaling in AD pathogenesis and may open a door to development of new classes of therapeutic agents targeting neuronal Ca^{2+} signaling pathways.

Box 1 Calcium Stability in the Face of Adversity

Neurons possess multiple defenses against the Ca^{2+} -destabilizing forces of aging and AD-specific pathogenic abnormalities (Fig. 3). Activity-dependent neurotrophic factor signaling plays a major role in stabilizing neuronal Ca^{2+} homeostasis as is evident from the abilities of BDNF, NGF, bFGF and others to protect neurons against excitotoxic, oxidative and metabolic insults relevant to AD⁸¹. The neurotrophic factors protect against sustained elevations of $[\text{Ca}^{2+}]_i$ by modifying the expression of Ca^{2+} -binding proteins, glutamate receptor subunits, antioxidant enzymes and mitochondrial membrane-stabilizing proteins such as Bcl-2. In addition, transcription factors that mediate adaptive stress responses, including NF- κ B and Nrf-2 may be activated oxidative and metabolic stress resulting in the up-regulation of antioxidant and phase 2 enzymes. Two organelles in which it is particularly important to maintain Ca^{2+} regulation are the ER and mitochondria. Evidence suggests that the ER is under stress in neurons affected in AD and may contribute to perturbed cellular Ca^{2+} homeostasis⁸⁶. Three proteins that have been shown to stabilize ER Ca^{2+} homeostasis and protect neurons against insults relevant to AD are Bcl-2 which stabilizes membranes and GRP-78 (glucose-regulated protein 78) which guards against protein misfolding and Herp (homocysteine-inducible ER protein⁸⁷. Depletion of Herp by RNA interference sensitizes neural cells to apoptosis induced by ER stress, whereas Herp overexpression promotes survival by a mechanism involving stabilization of ER Ca^{2+} levels, preservation of mitochondrial function and suppression of caspase 3 activation. Mitochondrial Ca^{2+} handling may be impaired in AD. For example, cyclical fluctuations in mitochondrial membrane potential, which are mediated by Ca^{2+} and likely represent coupling of membrane potential to ATP production, are reduced in AD cybrid cells⁸⁸. Mitochondrial uncoupling proteins (UCPs) may play important roles in stabilizing mitochondrial and cellular Ca^{2+} homeostasis as suggested by studies showing that UCP-4 stabilizes total cellular Ca^{2+} homeostasis (including ER and plasma membrane systems) which is associated with reduced mitochondrial oxidative stress and resistance of neurons to death⁸⁹.

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Figure 1.

Mechanisms that may result in perturbed neuronal Ca^{2+} homeostasis in Alzheimer's disease. Sequential cleavages of the β -amyloid precursor protein (APP) by β -secretase (β) and γ -secretase (γ) generates the amyloid β -peptide (A β). A β forms oligomers which can insert into the plasma membrane and form pores through which Ca^{2+} passes into the cytoplasm. The interaction of A β with the plasma membrane may be facilitated by binding to phosphatidylserine (PtdS); age/AD-related mitochondrial impairment (ATP depletion) may trigger flipping of PtdS from the inner portion of the plasma membrane to the cell surface. The PtdS flipping may also result from Ca^{2+} influx or release from the endoplasmic reticulum (ER) or mitochondria which can activate a phospholipid scramblase (PLSCR1). A β can also interact with Fe^{2+} and Cu^{2+} to generate hydrogen peroxide and hydroxyl radical ($\text{OH}\cdot$) resulting in membrane lipid peroxidation which generates toxic aldehydes that impair the function of membrane ion-motive ATPases (Na^{+} and Ca^{2+} pumps). As a result the membrane becomes depolarized and glutamate receptor channels (NMDAR, N-methyl-D-aspartate receptor) and voltage-dependent Ca^{2+} channels (VDCC) open and flux toxic amounts of Ca^{2+} into the cytoplasm. In addition, A β acts on mitochondria (either directly or indirectly by causing elevated cytoplasmic Ca^{2+} levels and oxidative stress) to cause increased free radical (superoxide anion radical; $\text{O}_2^{\cdot-}$) production, Ca^{2+} overload and decreased ATP production. Amyloidogenic processing also generates an intracellular APP domain (AICD) which can translocate to the nucleus and modify gene transcription in ways that perturb Ca^{2+} homeostasis. Studies of the presenilins (PS) have implicated ER Ca^{2+} mishandling in AD. PS functions as an ER Ca^{2+} leak channel and FAD PS mutations impair this Ca^{2+} leak channel function resulting in excessive accumulation of Ca^{2+} in the ER and, as a consequence, enhances Ca^{2+} release through ryanodine receptor (RyR) and IP_3 receptor (IP_3R) channels. There is also evidence that PS can interact directly or indirectly with RyR and SERCA (smooth endoplasmic

reticulum Ca^{2+} -ATPase) to alter ER Ca^{2+} release and uptake. Interaction of the protein reelin with the apolipoprotein E receptor (ApoER2) enhances Ca^{2+} influx through NMDA receptor channels by a mechanism involving a src family tyrosine kinase (SFk); ApoE can block this effect of reelin. Finally, amyloidogenic APP processing may prevent α -secretase (α) cleavage of APP which would otherwise generate a secreted form of APP (sAPP α). sAPP α is normally produced in response to synaptic activity and is known to activate a signaling pathway involving cyclic guanosine monophosphate (cGMP) that activates K^+ channels, thereby hyperpolarizing the membrane and reducing Ca^{2+} influx. The mechanisms illustrated here include abundant targets for therapeutic intervention (see also Fig. 3) and one drug that targets the NMDA receptor (memantine) is currently used to treat AD patients.

Figure 2.

Interactions of Ca^{2+} and the neuronal cytoskeleton in AD pathogenesis. **A.** In healthy neurons the axon contains relatively high amounts of microtubules which are stabilized by the protein tau. Microtubule dynamics in axons play pivotal roles in organellar (mitochondria, for example) and protein transport to presynaptic axon terminals. Dendrites receive synaptic inputs in postsynaptic structures called spines whose shape is controlled by actin filaments and various scaffolding proteins. Ca^{2+} influx during synaptic activity modifies the dynamics of actin and microtubules in ways that allow the neuron to adapt to environmental demands. **B.** During the course of AD tau becomes hyperphosphorylated and dissociates from microtubules which then depolymerize. The hyperphosphorylated tau self-aggregates and accumulates in the cell body where it forms paired-helical filaments (neurofibrillary tangles). As a consequence of accumulation of A β at synapses, Ca^{2+} regulation is impaired, the dendritic spines atrophy and the underlying cytoskeletal scaffold is disrupted resulting in synaptic degeneration. In these ways, cytoskeletal abnormalities underlie cognitive impairment in AD.

Healthy Brain Aging

Alzheimer's Disease

Figure 3.

Plausible mechanisms for healthy brain aging and AD. Healthy brain aging can be promoted by regular exercise, moderation in caloric intake and engaging in intellectually challenging activities. These lifestyle factors may stabilize neuronal Ca^{2+} homeostasis and counteract aging by inducing the production of neurotrophic factors, cellular antioxidant defense systems and cell survival-promoting proteins. Specific dietary factors may also reduce the risk of AD including omega-3 fatty acids, folic acid and possibly phytochemicals¹⁰⁶. By stabilizing neuronal Ca^{2+} homeostasis the healthy lifestyle and dietary factors can preserve synaptic and cognitive function during aging. Lifestyle factors that may accelerate aging and AD pathogenesis include lack of exercise, overeating and intellectually unchallenging daily

activities. The risk of developing AD is reduced by the presence of ApoE2 allele and increased by ApoE4. Genetic mutations in presenilins and APP lead to early-onset familial AD. Two disorders that increase the risk for AD are diabetes and depression. Diets high in saturated fats and cholesterol may also adversely affect the brain by increasing oxidative stress and perturbing cell membranes. As a result of the aging process, and genetic and lifestyle factors, neurons suffer from increased oxidative stress, metabolic impairment and the accumulation of aggregated proteins including A β and tau. All of the latter alterations perturb neuronal Ca²⁺ homeostasis resulting in synaptic dysfunction, amyloidogenic APP processing, tau pathology, neuronal death and cognitive dysfunction. Treatments that target A β , including immunization and β - and γ -secretase inhibitors may stabilize neuronal Ca²⁺ homeostasis by preventing the adverse actions of A β on membranes and associated Ca²⁺-regulating proteins (see Fig. 1). Drugs that modulate ion channels involved in Ca²⁺ influx and release from internal organelles have the potential to counteract neurodegenerative processes in AD.