The Pathogenesis of Alzheimer's Disease: Is It a Lifelong "Calciumopathy"?
Grace E. Stutzmann

Neuroscientist 2007; 13; 546
DOI: 10.1177/1073858407299730

The online version of this article can be found at:
http://nro.sagepub.com/cgi/content/abstract/13/5/546

Published by:
SAGE Publications
http://www.sagepublications.com

Additional services and information for The Neuroscientist can be found at:

Email Alerts: http://nro.sagepub.com/cgi/alerts
Subscriptions: http://nro.sagepub.com/subscriptions
Reprints: http://www.sagepub.com/journalsReprints.nav
Permissions: http://www.sagepub.com/journalsPermissions.nav

Citations (this article cites 110 articles hosted on the SAGE Journals Online and HighWire Press platforms): http://nro.sagepub.com/cgi/content/refs/13/5/546
Alzheimer’s disease (AD) is a devastating and fatal neurodegenerative disorder that slowly destroys neurons and cognitive abilities. Although a variety of drug treatments can delay its onset or temporarily reduce its severity, there is currently no cure or effective long-term treatment. The hallmark features of AD—amyloid plaque deposits (Aβ), neurofibrillary tangles (NFT), cell death, and severe cognitive impairments—are all late-stage markers of the disease. One of the questions surrounding AD is if early signaling dysregulations can enable pathogenesis, insidiously working "below the radar" of physiological detection, long before the onset of marked histopathology or cognitive changes. Under normal conditions, intracellular calcium signals are coupled to effectors that maintain a healthy physiological state. Consequently, sustained up-regulation of calcium may have pathophysiological consequences. Indeed, upon reviewing the current body of literature, increased calcium levels are functionally linked to the major features and risk factors of AD: ApoE4 expression, presenilin and APP mutations, beta amyloid plaques, hyperphosphorylation of tau, apoptosis, and synaptic dysfunction. In turn, the histopathological features of AD, once formed, are capable of further increasing calcium levels, leading to a rapid feed-forward acceleration once the disease process has taken hold. The views proposed here consider that AD pathogenesis reflects long-term calcium dysregulations that ultimately serve an enabling role in the disease process. Therefore, “Calcinitists” do not necessarily reject βAptist or Tauist doctrine, but rather believe that their genesis is associated with earlier calcium signaling dysregulations.

KEY WORDS Calcium, Beta amyloid, Neuron, Plaques, Tangles, Presenilin, APP, Tau

Calcium as a Critical Regulator of Short- and Long-term Neuronal Health

Maintaining proper calcium homeostasis is critical for the viability of neurons, both acutely and throughout the lifetime of an organism (Berridge and others 1998; Berridge and others 2000; Toescue and Verkhratsky 2003). Sustained alterations in calcium homeostasis form the basis of the calcium hypothesis of AD, which proposes that sustained changes in intracellular calcium homeostasis provide the final common pathway for age-associated brain changes (Khachaturian 1987). Although the hypothesis here is referring to brain aging, the reference to calcium dyshomeostasis as playing a key role in brain aging is relevant to AD pathogenesis.

Calcium is involved in a wide array of vital functions, ranging from gene transcription (Mellstrom and Naranjo 2001; Berridge and others 2003), modulation of membrane excitability (Davies and others 1996; Stutzmann and others 2003), and synaptic plasticity (Nakamura and others 1999; Fujii and others 2000; Fig. 1). Calcium ions enter the cytosol from extracellular sources such as through voltage-gated or receptor-operated calcium channels in the plasma membrane, or via liberation from the vast endoplasmic reticulum (ER) stores through activation of inositol triphosphate (IP3) and/or ryanodine (RYR) receptors. Maintaining homeostasis requires stabilizing the large calcium reservoir in the ER, which
is estimated to reach concentrations up to 5 mM, roughly four to five orders of magnitude greater than the surrounding cytosol (Berridge and others 1999; Solovyova and Verkhratsky 2002; Ghribi 2006). Under normal conditions, the activation of IP₃, R and RyR channels is initially enhanced by cytosolic calcium, resulting in a regenerative process of calcium-induced calcium release. Upon reaching a threshold calcium concentration, this same calcium serves as a negative feedback regulator, suppressing subsequent calcium release and ensuring that potentially toxic levels are not sustained (Finch and others 1991; Friel and Tsien 1992; Yao and Parker 1992). The ER calcium stores extend throughout multiple neuronal compartments, from the soma and nuclear envelope, through dendritic arborizations, and into dendritic spine heads (Fig. 2). Based on the large concentration gradient maintained between the ER lumen and the cytosol, and the extensive ER network throughout the neuron, it becomes clear why intracellular calcium stores must be kept under tight regulatory control (Berridge and others 1998).

**Calcium and AD-Linked Mutations**

The majority of AD cases are categorized as sporadic, with a relatively late detectable onset (65+ years) and an unknown, presumably heterogeneous, etiology. Certain environmental and genetic risk factors are associated with sporadic AD, such as education level, diet, injury or stroke, expression of apoE4 alleles, and cardiovascular disease (Blennow and others 2006, for review). Yet, these risk factors account for less than half of AD cases at best (Rogaeva and others 2006), so a confident identification of a root cause or initiating event is still elusive. A far less common but intensely studied variant is termed early-onset familial AD (FAD). This inherited form of AD is linked to mutations in the presenilin 1, presenilin 2 (PS1 and PS2) and amyloid precursor protein (APP) genes. The common endpoint of these mutations results in increased formation of the pathogenic Aβ₁₋₄₂ peptide, with the accumulation of intra- and extracellular Aβ plaques. Although the link between the mutant genes and AD is still under investigation, it is
established that their expression will lead to AD (Hutton and others 1996; LaFerla 2002).

ER calcium dysregulations have been shown consistently across a wide variety of mutant PS-expressing model systems, from transfected oocytes (Leissring and others 1999; Leissring and others 2001), fibroblasts from FAD patients (Etcheberrigaray and others 1998), and cultured neurons (Guo and others 1996; Smith and others 2005). Neurons from young, adult, and aged mutant PS1-expressing mice (ranging from 3 weeks to 2+ years) also demonstrate significant ER calcium signaling dysregulations, indicating the calcium disruptions are present throughout the organism’s lifetime (Stutzmann and others 2004; Stutzmann and others 2006). These PS-linked calcium alterations appear specific to intracellular ER stores and do not alter voltage-gated calcium entry through plasma membrane channels (Stutzmann and others 2003; Stutzmann and others 2004). This suggests that PS mutations are not causing a random or disorganized calcium dysregulation but rather are targeting specific signaling pathways that may be linked to later downstream manifestations of AD pathology. Presenilin proteins are embedded in the ER membrane, but how they influence luminal calcium release is unclear. Recent studies suggest that upregulation of the ryanodine receptor/channel (RyR) contributes to enhanced ER calcium release (Chan and others 2000; Smith and others 2005; Stutzmann and others 2006). A separate, but not necessarily mutually exclusive, hypothesis is that the wild-type PS protein normally serves as the elusive ER leak channel, maintaining luminal calcium homeostasis by releasing calcium and serving in opposition to SERCA (sarcoplasmic reticulum calcium ATPase) pumps actively bringing calcium into the ER. Mutant PS is impaired in this leak function and does not release the calcium overflow. Consequently, ER calcium stores become overfilled and result in increased calcium release due to the exceptionally high concentration gradient (Tu and others 2006). In conjunction, the sensitivity of the RyR increases with increased ER calcium levels, creating a type of feed-forward facilitation (Friel and Tsien 1992; Berridge 2002). This dynamic interaction creates a possible link between mutant PS, increased ER stores, and up-regulation of RyR activity.

Despite the marked calcium alterations, PS-mutant mice fail to display the Aβ plaques and neurofibrillary tangles that are the signature hallmarks of AD. Several lines of mice expressing human mutant APP will generate Aβ plaques in the hippocampus and cortex at later ages (Dewachter and others 2000; van Dooren and others 2005; Smith and others 2005), and coexpressing mutant PS1 with mutant APP will greatly accelerate and exacerbate plaque deposition and neurofibrillary tangle formation (Samura and others 2006). Although this synergistic relationship between mutant APP and PS is complex, it is certainly possible that the increased plaque and tangle formation in the bigenic animals is a consequence of dysregulated calcium potentiating the histopathology. A triple transgenic mouse, 3xTg-AD, expressing mutant human PS1, APP, and tau, is also capable of developing Aβ plaques and neurofibrillary tangles in an age- and region-dependent fashion similar to human AD progression (Oddo and others 2003). Calcium imaging studies have demonstrated that cortical neurons from mutant PS1 and 3xTg-AD mice show exaggerated ER-calcium release (> fourfold over control neurons), likely through up-regulation of RyR expression and activity (Smith and others 2005; Stutzmann and others 2006). It appears that the additional calcium influx through the RyR can largely account for the total enhanced response, as blocking RyR with dantrolene or ryanodine normalizes the IP3-evoked calcium response back to NonTg levels (Fig. 3; Stutzmann and others 2006). In addition, the membrane hyperpolarization associated with ER-calcium release is enhanced in the mutant mice, leading to an interesting effect on neuronal excitability—expression of mutant PS1 increases the “excitability” of the ER, which in turn reduces spiking activity and responsiveness to synaptic inputs (Oddo and others 2003; Stutzmann and others 2006). The calcium signaling changes, reductions in neuronal excitability, and enhanced contribution of RyR expression levels are found throughout the organism’s lifetime and are present long before the formation of plaques and tangles (Fig. 3E; Stutzmann and others 2006).

**AβC’s of Aβ and Calcium**

Recently, much attention has focused on Aβ as the underlying cause of AD, even though plaque deposition is not detected until the mid- to late-disease stages and, cognitively normal aged adults will often express substantial Aβ plaque load (Snowdon 1997; Blennow and others 2006). Despite these ambiguities, a strong case has been made that Aβ plays a central pathogenic role in AD (Hardy and Higgins 1992; Hardy 2006). The amyloid cascade hypothesis proposes that Aβ is the root cause and common initiating factor in AD. Gradual accumulation of
aggregated Aβ, either by excess accumulation or reduced clearance mechanisms, initiates a complex pathological cascade that includes gliosis, inflammatory changes, neuritic/synaptic change, tangles, and transmitter loss, which ultimately results in cell death and severe cognitive impairments (Selkoe 2005). What is still open for investigation is the contribution of early preclinical processes that preexist the histopathology and may serve as accelerants or enablers of the disease process. Accumulating evidence points to disruptions in neuronal calcium signaling as an early contributing factor in AD, occurring prior to the development of detectable plaques, tangles, and cognitive

Fig. 3. Comparison of IP₃- and spike-evoked calcium signals in NonTg and 3xTg-AD cortical neurons. A, Left, two-photon image of fura-2 labeled NonTg neuron at rest. Middle, images of calcium changes evoked from flash photolysis of caged IP₃ or from a train of spikes. Traces (far right) represent IP₃-calcium signals evoked from a range of flash durations (10–100 ms) in NonTg (top) and 3xTg-AD neurons (bottom). B, Same sequence as in A, for the 3xTg-AD neurons. C,D, Spike-evoked calcium signals from NonTg and 3xTg-AD neurons. The IP₃-calcium signals in the 3xTg-AD neuron are up to 3× larger, whereas the spike signals are similar. E, Averaged maximal IP₃-evoked ER-calcium responses in 6-week, 6-month, and 1.5-year-old NonTg neurons (open bars) and 3xTg-AD neurons (black bars). * indicated significantly different at P < .05. F, top traces, IP₃-evoked calcium signals are reduced by the RyR blocker dantrolene in the Alzheimer's disease (AD) transgenic mice. Traces show calcium responses evoked by photolysis of caged IP₃ in control conditions (black) and in the presence of bath-applied dantrolene (gray) in representative NonTg (left) and 3xTg-AD (right) neurons. Bottom graph, Effect of dantrolene on the dose-response relationship of IP₃-evoked calcium signals. Points show measurements from NonTg neurons and pooled measurements from 3xTg-AD and PS1 KI neurons (Tg; circles) before (filled symbols) and after (open symbols) applying dantrolene. Data on the right show respective spike-evoked calcium signals. (Reproduced with permission from Stutzmann and others 2006.)
A relationship between Aβ peptides and calcium has been established in studies dating back to the early 1990s, demonstrating that Aβ peptides can increase intracellular calcium levels. For example, Aβ can form rudimentary cation-selective and calcium-permeable channels, as first shown in lipid bilayer preparations (Arispe and others 1993; Kawahara and Kuroda 2000), and then in a variety of cell lines including human fibroblasts and hypothalamic neurons (Kawahara and others 2000; Zhu and others 2000). The whole cell currents and calcium influx generated by the Aβ channels are sufficient to activate secretion of neurotransmitter-containing vesicles (Taylor and others 1999; Green and Peers 2001), demonstrating functional implications associated with these channels. Subsequent studies have suggested a more dysfunctional role of Aβ-generated channels, whereby the increased cytosolic calcium levels are a source of metabolic stress for the neuron (Zhu and others 2000; Lin and others 2001; Mattson and Chan 2003). At the time, these and other studies were not particularly focused on the exact configuration of the Aβ peptide—whether it was a monomer, dimer, oligomer, or fibril. More recent studies have shown that these distinctions form important dividing lines when assigning culpability in AD pathology. Fingers point to the oligomeric form as the toxic culprit leading to the neuronal devastation seen in AD (Fig. 4A). These oligomers are thought to increase membrane permeability to calcium, resulting in extracellular influx to the cytosol and release from intracellular stores (Demuro and others 2005; Glabe 2005; Deshpande and others 2006).

The possibility that Aβ can access and release ER calcium stores is particularly concerning for several reasons. Unregulated and persistent release of excess ER calcium can have dire consequences over time, such as increased activation of calcium-dependent signaling pathways, activation of ER stress responses and the unfolded-protein response, mitochondria energy mismetabolism, and activation of apoptotic pathways (Berridge and others 1998). The mechanism by which Aβ can increase intracellular calcium levels is not entirely clear and may involve more than one system. For example, Aβ can nonselectively increase membrane permeability to calcium, form new calcium pores, and regulate existing calcium channels such as voltage-gated calcium channels, NMDA receptors, and AMPA receptors (Demuro and others 2005; Glabe 2005; Deshpande and others 2006; Kelly and Ferreira 2006; Shemer and others 2006). And, interestingly, the Aβ1-42 fragment has been found to increase RyR expression and function in mutant APP Tg mice, providing a critical link between ER-specific calcium dysregulation and AD mutations (Supnet and others 2006).

The chicken or the egg conundrum arises with new data demonstrating that calcium can also facilitate the formation of pathogenic Aβ fibril formation (Fig. 4b; Isaacs and others 2006). Extracellular calcium can trigger and even accelerate Aβ formation and aggregation to the protofibrillar forms. And, increases in calcium levels specifically induce intracellular Aβ1-42 production, which seeds fibril formation and plaque deposition (Pierrrot and others 2004; McGowan and others 2005). Earlier studies also suggest that increased calcium, from Ry-sensitive stores in particular, enhances the production and release of Aβ peptides (Querfurth and Selkoe 1994; Querfurth and others 1997). The one-two punch of pathogenic Aβ peptides and neuronal calcium dysregulation is linked to several downstream physiological events that adversely affect neuronal function, such as impairments in synaptic proteins, altered synaptic plasticity, and cell signaling functions, and reduction in the total number active synapses and neuronal processes (Deshpande and others 2006; Glabe and Kayed 2006; Kelly and Ferreira 2006; Townsend and others 2006).
Tangling with Tau and Calcium

A second histopathological feature and prerequisite diagnostic marker in AD is the formation of intracellular neurofibrillary tangles (NFTs). These tangles are composed of insoluble paired helical filaments largely made of hyperphosphorylated tau protein. Tau tangles have been found to precede Aβ plaque deposition by as much as 20 years, suggesting that initiation of tau histopathology may be a more proximal cause of AD rather than an end-stage effect (Blennow and others 2006). Normally, tau is associated with microtubule assembly, which maintains structural integrity and axonal transport. Tau’s multiple phosphorylation sites are regulated by an array of kinases and phosphatases that work in concert to maintain phosphorylation activity at a functional level. Under normal working conditions, kinases such as GSK3β and cdk 5 phosphorylate tau at serine and threonin sites, whereas PP-1 and PP2A (calcineurin) are well-established phosphatases that work in balanced concert with kinase activity. However, when tau becomes hyperphosphorylated due to either increased kinase or blunted phosphatase activity, it sequesters normal tau and other microtubule-associated proteins, detaches from and subsequently breaks down microtubules, and impairs the axonal transport system. Hyperphosphorylated tau will also aggregate into insoluble fibrils and tangles, impair neuronal and synaptic function, and ultimately choke neurons to death (Iqbal and others 2005).

When interactions between tau pathology and calcium signaling are examined, an independent, but analogous, feed-forward cycle becomes apparent that resembles the Aβ-calcium cycle. To skip to the finale—increases in intracellular calcium potentiate the phosphorylation of tau and fibrillary aggregation, and hyperphosphorylated or mutated tau can raise intracellular calcium levels (Fig. 5). The bottom line being that calcium-mediated phosphorylation is the focus of tau-based histopathology—once tau becomes hyperphosphorylated, it can fuel further calcium increases, and the cycle continues. The detailed mechanisms are still being worked out, but in the former scenario, increases in cytosolic calcium trigger calcium-activated kinases (such as glycogen synthase kinase 3β, cyclin-dependent kinase 5, mitogen-activated protein kinase/Par-1, and protein kinase C), which mediate the phosphorylation of tau (Avila and others 2004). Therefore, increases in calcium, regardless of the source, can lead to the initial development of the neurofibrillary tangles through the up-regulation of kinase activity. It is not clear if phosphatase activity is impaired as well, or if this pathway is merely overwhelmed in the later stages of AD.

Completing the cycle are recent studies in neuroblastoma cells demonstrating that exposure to phospho- or mutant tau results in the marked increase of intracellular calcium through cholinergic muscarinic receptor activation (Gomez-Ramos and others 2006). These findings parallel the calcium-mediated phosphorylation of APP on threonine 668, which results in the intracellular accumulation of the pathogenic Aβ1-42 fragment. Interestingly, fibrillar tangles do not have an effect (Pierrot and others 2006). It appears, therefore, that a persistent pattern of calcium dysregulation can activate signaling pathways that lead to the buildup of both hyperphosphorylated tau and Aβ1-42 fragments. This process may require years, if not a lifetime, to accumulate sufficient toxicity levels. However, once a neuronal threshold is reached, compensatory mechanisms may become overwhelmed and the feed-forward cycle of AD histopathology predominates.
Genetic Risk Factors and Calcium

Unlike the rare cases of FAD, which are causally linked to specific mutations in PS and APP, identifying the risk factors that contribute to sporadic or late-onset AD is particularly difficult. Despite widespread searches, the apoE gene is the only robustly replicated risk factor for AD with onset after 65 years of age and accounts for roughly half of the sporadic AD cases (Rogaeva and others 2006). Apolipoprotein E (ApoE) is a lipid-associated protein that binds and transports cholesterol-rich lipoproteins for internalization via the low-density lipoprotein receptor family. There are three major ApoE alleles, ApoE2, ApoE3, and ApoE4, and they confer markedly different vulnerability to AD. Inheriting a single allele of the ApoE4 gene increases the risk of developing AD by three times, and inheriting two alleles of the ApoE4 gene increases your likelihood by 15 times (Farrer and others 1997). In contrast, a double-dose of ApoE2 serves a protective role against AD (Corder and others 1993). The reason for these differences is not known, but ApoE4 is found in close approximation to Aβ plaques and neurofibrillary tangles, suggesting it is closely involved in Aβ formation and/or regulation (Namba and others 1991). By understanding the functional differences between the neuroprotective E2 and pathogenic E4 alleles, the hope is that new insights into both the mechanism of AD and the development of new therapies and treatments will be gained.

Consistent with the idea the AD may reflect a lifetime of accumulated calcium-related insults, the ApoE4 allele has also been found to alter intracellular calcium levels through a variety of pathways (Fig. 6). Application of physiological levels of ApoE4 (100 nm) to cultured hippocampal and cortical neurons can increase resting calcium levels by 70% (Veinbergs and others 2002; Qiu and Gruol 2003). In addition, truncated ApoE4 (a 22-kDa thrombin cleavage fragment) and an ApoE peptide consisting of a tandem repeat of residues 141-149 increase calcium levels as well. This increase in intracellular calcium levels is linked to downstream neurotoxicity, and later cell death. The toxicity and cell death associated with these ApoE4 fragments are greater than with the holoprotein and are unique to the E4 allele (Tolar and others 1997; Moulder and others 1999; Tolar and others 1999).

Not only does ApoE4 raise resting calcium levels but activity-dependent calcium influx through the glutamate NMDA receptor is increased as well. Preincubation with the activity-dependent NMDA antagonist, MK-801, blocks both the calcium rise and subsequent neurotoxicity and cell death. Blockers of the L-type and P/Q-type voltage-gated calcium channels also inhibit the ApoE4-triggered calcium influx, but likely this is mediated indirectly through the NMDA-initiated depolarization, which subsequently activates the voltage-sensitive channels (Ohkubo and others 2001). Other glutamate receptor subtypes, such as AMPA and kainate receptors, do not seem to be involved (Tolar and others 1999; Qiu and Gruol 2003).

Beyond the short-term insults inflicted by drastic, although phasic, rises in intracellular calcium levels, ApoE4 inflicts longer term effects by stimulating the activation of calcium-dependent transcription factors, such as pCREB, cfos, and Bcl-2. Significant increases in calcium influx via the NMDA receptor can activate ERK, which activates transcriptional cascades including CRE production (Rajadhyaksha and others 1999). Bcl-2, whose expression is triggered by CRE, is a neuroprotective protein that counters apoptotic signaling molecules (Freeland and others 2001). Although up-regulation of Bcl-2 may be sufficient to maintain neuronal function in the early stages of one’s life, over a period of decades, this and other neuroprotective agents may not be able to ward off the accumulating insults associated with increasing calcium flux. Additional effects induced by ApoE4, but not seen with other ApoE alleles, is a slowing in vesicular transport and a reduction in the number of transported vesicles (Dekroon and Armstrong 2002). Once the cellular functional transport pathways are impaired, marked disruptions in signaling, structural integrity, and repair mechanisms are forthcoming (Fig. 6B).

The mechanism by which ApoE4 alters intracellular calcium is thought to involve a cell-surface LDL receptor-mediated process. The increased calcium influx from extracellular sources most likely reflects activity-dependent activation, and not a steady-state up-regulation, which would too quickly become neurotoxic. A phasic or transient rise in excessive calcium may be more conducive for supporting a slow and insidious disease process, as seen in AD. The ApoE4-mediated calcium dysregulation is not likely acting in isolation, and there is evidence of recruitment of intracellular stores, leading to a feed-forward cycle of calcium influx through plasma membrane channels (including the recruitment of the voltage-sensitive channels) and activation of intracellular stores from the endoplasmic reticulum through the ryanodine receptor (Ohkubo and others 2001).

Calcium as a Harbinger of Cell Death and Cell Survival

Although AD is believed to have a heterogeneous etiology, the death of neurons and breakdown of functional synapses are considered the fundamental events underlying the tragic cognitive and behavioral dysfunctions (Culmsee and Landshamer 2006). The morphological and biochemical signatures underlying cell death in AD point to apoptosis, or programmed cell death, rather than necrosis (Cotman and Anderson 1995; Masliah and others 1998; Wu and others 2005). This indicates a highly choreographed and organized shutdown of the neuron to prevent lysing of the cell’s contents into the surrounding environment. There are several intrinsic triggers for apoptosis in AD, although it is not clear if a single initiating event or a culmination of multiple signals is required. These include Aβ peptides, glutamate-induced excitotoxicity, mitochondrial/ER/oxidative stress, and increased intracellular calcium levels (Mattson and Chan 2003, for review). In addition, reductions in calcium-binding proteins and buffers can also lead to unregulated calcium levels and ultimately trigger the apoptotic signaling pathway (Wu and others 2005).
Once apoptosis is triggered, several pro-apoptotic proteins kick into action, such as p53, Bax, Bid, Bad, and Par-4, and induce changes in mitochondrial permeability. As a result, cytochrome c and apoptosis-inducing factor are released from the mitochondria and fan out to activate caspase-cleavage events in the apoptotic cascade. Cytochrome c also binds to IP3 receptors on the ER membrane and inflicts two rather devious insults. First, cytochrome c stimulates additional calcium release from the ER and then blocks the IP3-R negative feedback function that normally is triggered in the presence of high calcium levels (Bezprozvanny and others 1991). Thus, ER calcium flux essentially becomes unregulated in the presence of sufficient cytochrome c levels. The subsequent overall increase in cytosolic and mitochondrial calcium synchronize to induce a massive release of cytochrome c from mitochondria throughout the cell (Boehning and others 2003; Boehning and others 2004). This positive feedback loop sustains ER calcium release, which can quickly reach pathological levels and initiate additional calcium-dependent cascades. In turn, the cytochrome c–induced cascade activates caspase 9, a cysteine protease. Caspase 9 can then activate additional caspases, which are responsible for cleaving substrate proteins and killing the cell from within (Fig. 7).

Fig. 6. ApoE4 increases calcium levels in neurons through plasma membrane channels and intracellular stores. A, ApoE peptide induces a rapid and sustained elevation of calcium in neurons that is blocked by the activity dependent NMDA receptor antagonist, MK-801. B, Images of intracellular-free calcium levels in hippocampal neurons before and 1.5, 3, and 10 min after exposure to ApoE4. Calcium levels are significantly increased within 1.5 min after exposure to the peptide. By 10 min of calcium exposure, blebbing of the neuritis and structural breakdown of the microtubules occurs. (Reprinted with permission from Tolar and others 1999.)
In addition to the mitochondrial-based pathways, ER stress induced by calcium overload leaves neurons vulnerable to apoptosis via other mechanisms (Guo and others 1996; Guo and others 1999; Mattson and Chan 2003). Not only will ER stress lead to an uncontrolled feed-forward cycle of calcium release into the cytosol, but the unfolded-protein response becomes compromised. Normally, the ER ensures that only correctly folded proteins are forwarded to the Golgi and unfolded or misfolded proteins are retained and ultimately degraded. Perturbation in calcium levels can disrupt ER homeostasis, induce stress to the ER, and lead to toxic accumulation of unfolded or misfolded proteins in the ER lumen (Zhang and Kaufman 2006a, 2006b). Prolonged ER stress and substantial accumulation of misfolded proteins are sufficient to up-regulate genes and protein products, which will initiate apoptosis (Paschen and Frandsen 2001; Lindholm and others 2006). Another means by which increased calcium levels can trigger cell death responses and synaptic degradation is by direct activation of cell-signaling molecules and transcription factors. For example, the calpain family of calcium-dependent cysteine proteases are under complex cellular regulation and are implicated in cellular apoptotic processes (Raynaud and Marcilhac 2006). Indeed, members of the Bcl-2 family of cell death regulators, nuclear transcription factors (p53),
and several caspases are processed by calpains. As a major effector of calcium signals, calpain activity may reflect disturbances in calcium homeostasis and provoke pathologic consequences in response, such as aggregation of Aβ and tau (Nixon and others 1994; Yamashima 2004; Raynaud and Marcilhac 2006).

In addition, calcium-activated transcription factors, immediate-early genes, and signaling molecules offer intriguing targets for early involvement in AD pathogenesis. Transcription factors activate (or inactivate) gene families to produce proteins necessary for optimal cellular functioning, often in response to environmental changes, growth, recent activity, or stress. For example, excess ER calcium release activates the antiapoptotic transcription factor nuclear factor kappaB (NF-κB; Pahl and Baueuerle 1996, 1997). In turn, NF-κB protects cells by inducing genes that promote cell survival such as those encoding for antioxidants (manganese superoxide dismutase; Mattson and others 1997), calcium buffering agents (calbindin D28K; Cheng and others 1994), and antiapoptotic proteins (Bcl-2, Bcl-XL) (Mattson and others 1997; Tamatani and others 1999; Tamatani and others 2000). Increasing evidence suggests that the family of NF-κB transcription factors plays an important role in synaptic plasticity and long-term memory formation (O’Riordan and others 2006), which ties in well with AD pathology. Among other calcium-sensitive apoptotic responses is expression of C/EBP homologous protein (CHOP; Oyadomari and Mori 2004), a transcription factor that inhibits protective proteins such as Bcl-2 (DeGracia and others 2002). Suppression of ER calcium release inhibits CHOP expression and protects neurons from Aβ-mediated death (Mattson 2000; Suen and others 2003; Ferreiro and others 2004). Cyclic-AMP response element-binding protein (CREB) is another calcium-activated transcription factor that plays a critical role in long-term synaptic plasticity and activity-dependent neuronal survival. Altered expression of CREB and similar proteins may therefore have negative implications for related learning and memory processes, and long-term viability (Bito and others 2003; Zhang and others 2006).

Several cell-signaling pathways are also tightly coupled to calcium levels and may likely be up-regulated, or even desensitized, in response to episodic increases in intracellular calcium release. Increase in kinase activity, such as calcium-calmodulin kinase II, protein kinase C, and protein kinase G, would result in increased phosphorylation of receptor subunits, ion channels, and other second messengers, and thereby create sustained changes in neuronal activity. Calcineurin, or protein phosphatase 2b, can negatively modulate IP3R activity and binds the antiapoptotic factor Bcl-2 in a calcium-dependent manner (Erin and others 2003). Homer1a protein expression increases with calcium activity and can also negatively modulate RyR and Gq-coupled receptors, thereby serving to reduce evoked ER calcium release (Xiao and others 2000; Paschon and Mengesdorf 2003; Westhoff and others 2003). These are all calcium-dependent neuronal signaling factors that, if subtly altered over sustained periods, may contribute to the long-term cellular and synaptic pathology seen in AD. It is possible that the neuroprotective pathways may become activated at early time points in response to the increased calcium levels. Yet, with chronic, life-long up-regulation, the effectiveness of the protective mechanisms decline and ultimately succumb to the more pathogenic pathways, resulting in the later stage markers of AD and onset of measurable pathology.

Conclusions

Perturbations to the delicate calcium balance are linked to functional declines in normal aging processes and to the development of neurodegenerative diseases such as AD (LaFerla 2002; Mattson and Chan 2003; Stutzmann and others 2004; Toescue and others 2004). A critical gap in our understanding is if neurodegenerative diseases reflect the same calcium-related changes observed in normal aging, or if they are the result of aberrant calcium processes that present novel pathogenic insults. The current evidence suggests that the calcium dysregulations seen in AD are not merely accelerated or amplified signaling changes inevitable in old age but, rather, are novel and pathogenic changes to fundamental calcium signaling patterns (Stutzmann 2005; Verkhratsky 2005; Stutzmann and others 2006). It is still not clear how to link the early dysregulated calcium signaling to the later onset of histopathological or cognitive symptoms characteristic of AD. The cytosolic calcium increases via intracellular release or influx through plasma membrane channels are not continuously present but are released in a transient manner. Calcium-induced pathogenesis may then reflect a lifetime of episodic and slowly accumulating insults that favor the aggregation of Aβ peptides and tau, trigger apoptosis via ER and mitochondrial stress responses, and negatively affect synaptic morphology and membrane function (Mattson and others 1993; Mattson and others 2001; Oddo and others 2003; Stutzmann and others 2004; Ghribi 2006; Stutzmann and others 2006). The early convergence and accumulation of multiple calcium-related cell signaling disruptions may initiate a feed-forward cascade and ultimately enable the onset of AD pathogenesis (Fig. 8). Of interest to Calcinists, therefore, is examining the therapeutic implications of normalizing aberrant calcium signaling prior to the feed-forward cascade stage, and determining if this can reduce the histopathology and devastating cognitive decline of AD.

Acknowledgments

The author would like to thank Dr Robert Marr (Rosalind Franklin University) for insightful commentary and coining of “Calcinites,” Dr Ian Parker (University of California, Irvine) for coining of “Calciumopathy,” Ivan Goussakov (Rosalind Franklin University) for helpful comments, and Shreaya Chakroborty (Rosalind Franklin University) for help in the preparation of this manuscript.
**Fig. 8. Convergent and enabling pathways for calcium involvement in the early pathogenesis of Alzheimer’s disease (AD).** Extracellular calcium entry through the plasma membrane or intracellular release from endoplasmic reticulum (ER) stores have the ability to induce or facilitate many of the principal features of late-stage AD. And many of these same features can potentiate further calcium release, supporting a feed-forward pathological cycle. Calcium signaling dysregulations may be present long before the onset of measurable symptoms, slowly accumulating cellular insults over one’s lifetime, until the cell’s compensatory mechanisms are overwhelmed and the pathological cascade predominates.

**References**


Rajadhyaksha A, Barczak A, Macias W, Leveque JC, Lewis SE, Konradi C. 1999. L-Type Ca$^{2+}$ channels are essential for hypothalamicpituitary adenylate cyclase cascade.