Calcium dysregulation in Alzheimer's disease: From mechanisms to therapeutic opportunities

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Abstract

Calcium is involved in many facets of neuronal physiology, including activity, growth and differentiation, synaptic plasticity, and learning and memory, as well as pathophysiology, including necrosis, apoptosis, and degeneration. Though disturbances in calcium homeostasis in cells from Alzheimer's disease (AD) patients have been observed for many years, much more attention was focused on amyloid-β (Aβ) and tau as key causative factors for the disease. Nevertheless, increasing lines of evidence have recently reported that calcium dysregulation plays a central role in AD pathogenesis. Systemic calcium changes accompany almost the whole brain pathology process that is observed in AD, including synaptic dysfunction, mitochondrial dysfunction, presenilins mutation, Aβ production and Tau phosphorylation. Given the early and ubiquitous involvement of calcium dysregulation in AD pathogenesis, it logically presents a variety of potential therapeutic targets for AD prevention and treatment, such as calcium channels in the plasma membrane, calcium channels in the endoplasmic reticulum membrane, Aβ-formed calcium channels, calcium-related proteins. The review aims to provide an overview of the current understanding of the molecular mechanisms involved in calcium dysregulation in AD, and an insight on how to exploit calcium regulation as therapeutic opportunities in AD.

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Abbreviations: Aβ, amyloid-β; AchE, acetylcholinesterase; AD, Alzheimer’s disease; AICD, APP carboxy-terminal intracellular domain; AIF, apoptosis-inducing factor; AMPA, amino-3-hydroxy-5-methyl-4-isoxazol propionate; APP, amyloid precursor protein; CA2+/CaMII, CA2+/CaM-dependent protein kinase II; CALHM1, calcium homeostasis modulator 1; CCE, capacitative calcium entry; Cdk5, cyclin-dependent kinase 5; CypD, Cyclophilin D; ER, endoplasmic reticulum; FAD, familial Alzheimer’s disease; GSK3β, glycogen synthase kinase-3β; IP3R, inositol(1,4,5)-trisphosphate receptors; LTD, long-term depression; LTP, Long-term potentiation; MAPK, mitogen-activated protein kinase; mPTP, mitochondrial permeability transition pore; NF2, neurofibrillary tangles; NMDA, N-methyl-D-aspartate; NSAIDs, non-steroidal anti-inflammatory drugs; PKA, protein kinase A; PKC, protein kinase C; Pss, presenilins; Ptds, phosphatidylserine; ROCs, receptor-operated calcium channels; ROS, reactive oxygen species; RyR, ryanodine receptors; S-312-d, S-(+)-2,3-dihydro-[3,4]-cyclopentano-1,2,4-benzothiadiazine-1,1-dioxide; SAD, spordic Alzheimer’s disease; SERCA pump, sarco-/endoplasmic reticulum calcium ATPase; SOCCs, store-operated calcium channels; VDCC, voltage-dependent calcium channel; VGCCs, voltage-gated calcium channels.

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1. Introduction

Alzheimer’s disease (AD) is the most common cause of progressive dementia in aging human populations, and one of the most important medical, social, and economic problems confronting contemporary society, characterized by progressive disturbances of cognitive functions including memory, judgement, decision-making, orientation to physical surroundings and language (Nussbaum and Ellis, 2003). The growing number of elderly and the continuing expansion of life expectancy have led to a fast growing number of patients suffering from AD, reaching upwards of 30 million worldwide in 2004 (Selkoe, 2004). The neuropathological hallmarks of AD include selective neuronal and synaptic losses, extracellular neuritic plaques made by aggregated amyloid-β (Aβ) peptides, and intracellular neurofibrillary tangles (NFTs) composed of hyperphosphorylated forms of the tau protein in characteristic brain regions (Mattson, 2004).

Despite intense research into the causes of AD, only marginal clinical progress has been made and it remains incurable. Current medications that have passed FDA approval for the treatment of AD include acetylcholinesterase (AchE) inhibitors for mild to moderate cases; and memantine, an N‐methyl‐D‐aspartate (NMDA)‐receptor antagonist for the treatment of moderate to severe cases. However, these are symptomatic drugs with modest efficacy, and none of them are proved to reverse the underlying progression of the disease. Hence, there is enormous urgent medical need for the development of novel therapeutic strategies that target the underlying pathogenic mechanisms in AD.

Calcium is involved in many facets of neuronal physiology, including activity, growth and differentiation, synaptic plasticity, and learning and memory, as well as pathophysiology including necrosis, apoptosis, and degeneration (Bezprozvanny, 2009). The calcium hypothesis of aging and neurodegenerative disease was first proposed in the mid-1980s by Khachaturian, Landfield and Gibson (Khachaturian, 1987; Landfield, 1987; Gibson and Peterson, 1987). They postulated that sustained intracellular calcium disturbances are the proximal causes of aging and neurodegenerative disorders, including AD. Although disturbances in calcium homeostasis in cells from AD patients have been observed for more than 20 years (Peterson et al., 1985), much more attention was focused on amyloid hypothesis and hyperphosphorylated tau hypothesis as key causative factors for the disease. Nevertheless, increasing lines of evidence have recently claimed that calcium dysregulation plays a central role in AD pathogenesis (Bezprozvanny and Mattson, 2008; Bojarski et al., 2008a,b), indicating that targeting calcium signaling pathways need to be seriously considered for a therapeutic approach for AD prevention and treatment. Our review aims to provide an overview of the current understanding of the molecular mechanisms involved in calcium dysregulation in AD, and an insight on how to exploit calcium regulation as therapeutic opportunities in AD.

2. Mechanisms linking calcium dysregulation to AD pathogenesis

2.1. Calcium dysregulation and aging

Aging is the principal risk factor in AD and calcium dysregulation in aged brains is one of the molecular hypotheses of aging-dependent brain impairment (Thibault et al., 2007). Comparative studies of young and old rodents have indicated that neuronal calcium-regulating systems undergoes significant age-dependent changes, including elevated intracellular calcium levels, increased calcium influx through L-type voltage-gated calcium channels (VGCCs), enhanced calcium release from intracellular stores through inositol(1,4,5)-trisphosphate receptors (IP3R) and ryanodine receptors (RyR), reduced contribution of NMDA receptor-mediated calcium influx, impaired ability of mitochondria to buffer or cycle calcium, reduced cytosolic calcium buffering capacity and activation of calcineurin and calpains (Raza et al., 2007; Thibault et al., 2007). Recently, dysregulation of calcium homeostasis with aging has been shown to directly correlate with increased activity of calpains, which are intracellular, calcium-dependent cysteine proteases involved in regulation of intracellular signaling pathways and calpain-dependent apoptotic neuronal cell death (Hajieva et al., 2009). Interestingly, increased activity of calpains, which modulate the function and metabolism of amyloid precursor protein (APP) and tau, have also been involved in neuronal injury present in hippocampus and cerebral cortex during AD (Lebart and Benyamin, 2006). Age-related changes in the expression of calcium signaling systems have also been observed in gene microarray and proteomic analyses (Toescu et al., 2004; Mattson and Magnus, 2006). Some of these changes are directly caused by aging and some are compensatorymultiple levels. Alterations of calcium in normal aging can be reproduced by subjecting neurons to oxidative and metabolic stress in vitro or vivo, suggesting that these fundamental aging processes may also contribute to the calcium dysregulation in AD.

The mechanisms responsible for age-related neuronal calcium dysregulation are not clearly understood. One potential explanation is the age-induced defects in mitochondrial functions due to cumulative oxidative damage to mitochondria. Mitochondria are the principal cellular generators of reactive oxygen species (ROS) and their dysfunction leads to an age-related accumulation of oxidative damage. The mitochondria from aged neurons are depolarized and less efficient in handling calcium load (Leslie et al., 1985). The direct role of mitochondria in the refilling of intracellular calcium stores has also been proposed (Arnau et al., 2001). It was reported that localization of this organelle close to the sites of calcium release from the endoplasmic reticulum (ER) and mitochondria recycle portions of released calcium back to ER domains. Indeed, a defect in mitochondrial complex IV (cytochrome c oxidase) was shown to be associated with AD (Sheehan et al., 1997). As the consequence of mitochondrial dysfunction, elevation of cytosolic calcium levels and a delay in its recovery to baseline after IP3R stimulation was observed in cybrids bearing mitochondria from AD patients.

2.2. Calcium dysregulation and synaptic dysfunction

Synaptic dysfunction appears to be the earliest events of cognitive decline in AD, and has long been considered to be the best correlation of these two events (Coleman and Yao, 2003). Neuropathological studies suggest that synapse dysfunction (or loss), rather than cell death, is closely related to cognitive decline (Terry, 2000). Long-term potentiation (LTP) is extensively studied in the hippocampus as a model of activity-dependent, long-term
memories in the brain. At the CA1 and perforant path synapses, widely considered to form a basis for formation and storage of dependent and persistent changes in synaptic strength, which are neurons (Fig. 1) (Foster, 2007). LTD and LTP refer to activity-increase in the threshold frequency for induction of LTP in aged hippocampal synaptic function and plasticity. The resulting calcium-dependent signaling pathways play essential roles in AD have not been defined. Numerous studies have shown that dysfunction that is ultimately responsible for the cognitive decline in AD of human amyloid precursor protein (APP) (Chapman et al., 1999; Larson et al., 1999; Moechars et al., 1999).

However, the cellular mechanisms involved in the synaptic dysfunction that is ultimately responsible for the cognitive decline in AD have not been defined. Numerous studies have shown that calcium-dependent signaling pathways play essential roles in hippocampal synaptic function and plasticity. The resulting changes in neuronal calcium dynamics lead to augmented susceptibility to induction of long-term depression (LTD) and an increase in the threshold frequency for induction of LTP in aged neurons (Fig. 1) (Foster, 2007). LTD and LTP refer to activity-dependent and persistent changes in synaptic strength, which are widely considered to form a basis for formation and storage of memories in the brain. At the CA1 and perforant path synapses, induction of LTP is initiated by calcium influx into the postsynaptic dendritic spine via NMDA receptor channels (Nicoll et al., 1988). Inhibition of NMDA receptors or associated channels prevents LTP induction or even causes depression of the synapse (Cummings et al., 1996). Moreover, Ca\textsuperscript{2+}/CaMKII-dependent protein kinase II (Ca\textsuperscript{2+}/CaMKII) (Lledo et al., 1995) and protein kinase C (PKC) (Ben-Ari et al., 1992), have been crucially implicated in early LTP. Phosphorylation of proteins by these two kinases can either enhance the function of amino-3-hydroxy-5-methyl-4-isoxazol propionate (AMPA)-type glutamate receptors (Lee et al., 2000a) or inhibition of activity-dependent CaMKII autophosphorylation and 

![Diagram of synaptic related proteins](image_url)

**Fig. 1.** The postulated mechanisms linking calcium dysregulation to AD pathogenesis. Sequential cleavages of the amyloid precursor protein (APP) by \( \beta \)-secretase (\( \beta \)) and \( \gamma \)-secretase (\( \gamma \)) generate sAPP\( \beta \) and sAPP\( \gamma \); these cleavages likely occur in the plasma membrane (PM) and ER membrane. A\( \beta \) enhances calcium ion influx into the cell by the formation of Ca\textsuperscript{2+}-permeable channels. The association of A\( \beta \) oligomers with the PM is facilitated by binding to surface phosphatidylserine (PtdS); age and Ca\textsuperscript{2+}-related mitochondrial impairment might trigger the exposure of PtdS on the cell surface. A\( \beta \) can also interact with Fe\textsuperscript{3+} and Cu\textsuperscript{2+} to generate reactive oxygen species (ROS) during the aggregation process, resulting in lipid peroxidation (LP) which generates the neurotoxic aldehyde 4-hydroxynonenal (HNE). 4HNE covalently modifies membrane transporters (ion-motive ATPases, a glucose transporter and glutamate transporter), GTP-binding proteins, and also ion channels (VDCC, NMDA-R) and thereby impairs their function. In addition, A\( \beta \) acts on mitochondria to cause mitochondrial oxidative stress and Ca\textsuperscript{2+} dysregulation, resulting in impairment of the electron transport chain, increased production of superoxide anion radical and decreased production of ATP. Reduction in ATP levels and loss of membrane integrity causes membrane depolarization, which leads to facilitation of Ca\textsuperscript{2+} influx through NMDA-R and voltage-gated Ca\textsuperscript{2+} channels (VGCCs). A\( \beta \) oligomers can also affect activity of NMDA-R, AMPA-R and VGCCs directly. AICD migrates to the nucleus, interacts with transcription regulators, such as Fe65 and Tip60, and modifies gene transcription in ways that perturb Ca\textsuperscript{2+} homeostasis. Presenilins are located within the ER membrane but are also found at the PM and other subcellular locations, and studies have implicated that they function as ER Ca\textsuperscript{2+} leak channels and FAD-associated mutants impair this Ca\textsuperscript{2+} leak-channel function, as a consequence, enhancing Ca\textsuperscript{2+} release through RyR and IP3R channels. There is also evidence that both wild-type and FAD-associated mutant presenilins can interact directly or indirectly with IP3R, RyR and SERCA (smooth endoplasmic reticulum Ca\textsuperscript{2+}-ATPase) pump to alter ER Ca\textsuperscript{2+} release and uptake. CALHM1 is located mainly in the ER and appears to associate with a passive Ca\textsuperscript{2+} leak channel. Calcium influx through CALHM1 promotes the \( \alpha \)-secretase pathway (\( \alpha \)) and reduces A\( \beta \) generation. The SOCCs localized in the plasma membrane are responsible for cell Ca\textsuperscript{2+} refilling via CCE. Elevated cytosolic Ca\textsuperscript{2+} levels result in the activation of Ca\textsuperscript{2+}-related proteins and lead to facilitation of LTD, inhibition of LTP, modification of neuronal cytoskeleton, synaptic loss, oxidative damage, excitotoxicity and cellular apoptosis/necrosis. Elevated cytosolic calcium level can also enhance A\( \beta \) production and tau hyperphosphorylation. Excessive cytosolic Ca\textsuperscript{2+} is taken up by mitochondria through mitochondrial Ca\textsuperscript{2+} uniporter (MCU), resulting in calcium overload of mitochondria, eventually leading to opening of mitochondrial permeability-transition pore (mPTP), which damages mitochondrial ultrastructure, inhibits mitochondrial ATP production and other energy-dependent functions, and releases calcium stored in mitochondria, thereby further deregulating neuronal calcium signaling. An accumulation of A\( \beta \) in mitochondria and its direct interaction with CypD also promotes calcium-induced mPTP opening.
protein kinase A (PKA) and mitogen-activated protein kinase (MAPK) have been implicated (Abel et al., 1997; Rosenblum et al., 2002). Inhibition of calpains (calcium-dependent enzymes) can also restore normal synaptic function both in hippocampal cultures and in hippocampal slices from the APP/PS1 mouse, an animal model of AD (Trinchese et al., 2008). Recent studies have demonstrated that Aβ-induced synaptic dysfunction is linked with altered calcium signaling in hippocampal neurons (Xie, 2004). Consistent with this view, the L-type VGCCs blocker verapamil reverses Aβ-induced LTP deficits in vitro and in vivo (Freir et al., 2003), and blocking calcineurin, a calcium- and calmodulin-dependent protein phosphatase, activity prevents inhibition of dentate LTP by Aβ (Chen et al., 2002).

2.3. Calcium dysregulation and mitochondrial dysfunction

Neurons and glia in the central nervous system contain abundant mitochondria which have a crucial role in energy metabolism and maintaining homeostasis. Clinical and experimental studies have provided increasing evidence for the hypothesis that mitochondrial dysfunction plays an important role in the pathogenesis of AD (Mancuso et al., 2006). On one hand, mitochondrial DNA abnormalities due to age-related accumulation of mutated mitochondrial DNA or germ-line transmitted mitochondrial DNA variants might increase cellular vulnerability to the detrimental effects of Aβ and even susceptibility to AD (Hutchinson and Cortopassi, 1995; Davis et al., 1997). On the other hand, Aβ can gain access to mitochondria and interact with specific intramitochondrial targets leading to direct dysfunction of this organelle (Casperson et al., 2005).

Mitochondria also participate in calcium homeostasis. They serve as a high-capacity, low-affinity transient calcium store. Changes in cytosolic calcium concentration ([Ca2+]i) in response to hormones and neurotransmitters induce an increase in the mitochondrial matrix calcium concentration ([Ca2+]m). Calcium ions enter the inner mitochondrial membrane following its electrochemical gradient when [Ca2+]i levels reach a submicromolar threshold (Nicholls, 2005). In addition, it has been reported that Ca2+ can enter mitochondria through a “rapid mode” mechanism (Sparagna et al., 1995), on a millisecond timescale, as well as through a mitochondrial isoform of RyR described in some excitable cells (Beutner et al., 2005). The calcium accumulated in mitochondria during the rising phase of the [Ca2+]i signal is subsequently released during the declining phase of the signals via a Na+/Ca2+ exchanger (Scaloni et al., 2000). Excessive calcium taken up into mitochondria has been shown to increase ROS production, inhibit ATP synthesis, induce the opening of the mitochondrial permeability transition pore (mPTP), and release of cytochrome c and apoptosis-inducing factor (AIF) triggering initiation of apoptosis, from the mitochondrial intermembrane space into the cytoplasm (Fig. 1) (Brustovetsky et al., 2003; Jiang et al., 2001). Released cytochrome c binds apoptotic protease activating factor 1 (Apaf-1) and activates the caspase cascade (Hengartner, 2000). Such alterations in mitochondrial functions have been proposed as a potential mechanism in the development and pathogenesis of AD (Moreira et al., 2007).

Recent studies have presented compelling evidence that the interaction between Aβ and Cyclophilin D (CypD) promotes the opening of the mPTP, thereby causing neuronal injury and a decline in cognitive functions in the AD animal model (Fig. 1) (Du et al., 2008). Aβ forms a complex with CypD in vivo and in vitro and that formation of this complex in vitro makes mitochondria more susceptible to mPTP opening. Finally, energy deficit and release of proapoptotic proteins from damaged mitochondria results in neuronal injury. Furthermore, genetic ablation of CypD renders their brain mitochondria more resistant to mPTP opening and also substantially improves their cognitive abilities in a mouse model of AD. As the mPTP opens upon excessive calcium accumulation by mitochondria, which may result from the elevated cytosolic calcium levels induced by soluble Aβ, dysregulation of calcium-regulated signaling pathways may also involve in the underlying mechanisms. Calcium overload of mitochondria resulted from the accumulation of Aβ promotes calcium-induced mPTP opening, which damages mitochondrial ultrastructure, inhibits mitochondrial ATP production and other energy-dependent functions, and releases calcium stored in mitochondria, thereby further deregulating neuronal calcium signaling (Starkov and Beal, 2008). Moreover, a series of non-steroidal anti-inflammatory drugs (NSAIDs), including salicylate, sulindac sulfide, indomethacin, ibuprofen and R-flurbiprofen, have been shown to protect neurons against Aβ1-42 oligomers toxicity by inhibiting mitochondrial calcium overload induced by Aβ oligomers (Sanz-Blasco et al., 2008).
underlying mechanism that accounts for enhancing ER calcium release (Fig. 1) (Cheung et al., 2008). Recently, overexpression of presenilins was found to accelerate calcium clearance and exaggerate IP3-mediated calcium liberation by enhancing the activity of SERCA pump (Green et al., 2008). It had also been reported that exaggerated calcium responses in cells expressing FAD mutant PS were associated with enhanced expression or activities of RyR calcium release channels (Chan et al., 2000; Smith et al., 2005b; Stutzmann et al., 2006). In AD-transgenic mouse cortical neurons, exaggerated responses to IP3 were mediated in part by RyR activated by calcium released through IP3R (Stutzmann et al., 2006), suggesting that exaggerated RyR responses could be a secondary effect. Since calcium signaling can influence IP3R and RyR expression (Cai et al., 2004; Genazzani et al., 1999), it was possible that mutant presenilins mediated enhanced IP3R calcium signaling drives transcriptional programs, with RyR expression up-regulated as a result. Moreover, Aβ exposure increased RyR expression in mouse cortical neurons (Supnet et al., 2006), suggesting that changes in RyR expression may be more downstream compared with a more proximal IP3R-mediated process. Alternately, presenilins and RyR may functionally interact similar to the PS-IP3R interaction. In addition, familial (dominantly inherited) AD (FAD) mutants PS1-M146V and PS2-N141I mutation recently have been shown to interact with the IP3R calcium release channel and exert profound stimulatory effects on its gating activity that result in exaggerated calcium signaling in intact cells, including brain neurons in patch-clamp experiments (Cheung et al., 2008). A recent study suggested that presenilins could form calcium leak channels in the ER (Fig. 1) (Tu et al., 2006) and were an integral part of intracellular calcium homeostasis via passive leak from the ER stores.

Given that presenilins FAD mutations enhance calcium release from the ER via the IP3R, the RyR, and through endogenous calcium leak channels as described above, it was hypothesized that these results could all be explained by an increase in ER calcium load (Fig. 1). ER calcium stores overload, which would generate a greater driving force to allow more calcium efflux once the IP3-sensitive ion channels open the activation of IP3R and RyR channels (Leissring et al., 2000a), had been widely accounted for exaggerated calcium release in mutant PS-expressing cells (LaFerla, 2002; Mattson and Chan, 2003; Stutzmann, 2005). The mechanism leading to the overload of ER calcium stores in PS mutants remains unclear. Recent results suggest that presenilins function as ER calcium leak channels in cells, a balance between SERCA pump activity and presenilin-mediated passive calcium leak determines the steady-state resting ER calcium levels in cells, and that mutations in presenilin have been reported to disrupt the formation of ER leak channels, thereby preventing passive calcium leak, which then leads to store overfilling (Tu et al., 2006; Nelson et al., 2007). Although this calcium overload hypothesis has been widely invoked, many studies have also observed either no alteration or reduced ER calcium stores in FAD PS-expressing cells (Giacomello et al., 2005; Lessard et al., 2005; Zatti et al., 2004, 2006; Kasri et al., 2006). Kasri et al. reported that ER calcium content in presenilin double knockout (DKO) cells (lacking both PS1 and PS2) assayed with ER targeted aequorin was decreased, and the increase of IP3R expression in the R1 subtype correlated with the enhanced calcium leak from the ER in the same cells (Kasri et al., 2006). Moreover, decreased levels of calcium ions in the ER and Golgi apparatus were reported recently for several PS1 and PS2 FAD mutants (Zatti et al., 2006). In addition, calcium stores were diminished in presenilin-deficient cells (Leissring et al., 2002). Enhanced spontaneous calcium release activity has also been observed in PS1-M146L-expressing DT40 cells reduced ER calcium (Cheung et al., 2008). Regardless of ER calcium overload or not, all researchers have consistently measured increased calcium release form ER with FAD mutations. The absence of elevated ER calcium suggests that exaggerated calcium release may be affected by many variables, including activities and expression of release channels, amount of ER calcium, calcium buffering, and more.

The most direct consequence of ER calcium stores overfill is the attenuation of CCE (Leissring et al., 2000a; Yoo et al., 2000) – a refilling mechanism that regulates the coupled process of IP3-mediated release of ER calcium and the replenishment of intracellular calcium through plasma-membrane channels, as their calcium stores do not require replenishing as much as those of normal cells. Cells that lack PS1 or express a dominant-negative PS1 mutant show a potentiation of CCE, whereas FAD-linked PS variants attenuate CCE (Leissring et al., 2000a; Yoo et al., 2000; Hermes et al., 2003). The attenuation of CCE is a direct effect of PS1 mutants and is independent on APP metabolism in APP knockout cells (Hermes et al., 2003). On the other hand, the PS1-driven inhibition of CCE influences APP processing leading to the increase of Aβ1-42 generation (Yoo et al., 2000). Calcium influx by way of CCE in PS1–/– but not in wild-type neurons is sufficient to trigger LTP in hippocampal slice preparations, suggesting a physiological role for PS1 regulation of CCE in neuronal synaptic transmission (Ris et al., 2003).

Additional role for presenilins in modulating calcium homeostasis is the γ-secretase-dependent connections. First, FAD-associated presenilin mutations increase the production of the long aggregation-prone form of Aβ1-42 (Aβ1-42) or reduce the production of a short soluble form, Aβ1-40, and therefore one way in which PSSs can perturb neuronal calcium homeostasis is by elevating Aβ1-42/Aβ1-40 production ratio and activating the Aβ-mediated mechanisms described below. Second, changes in γ-secretase-mediated production of the APP carboxy-terminal intracellular domain (AICD) are responsible for aberrant calcium signaling observed in cells expressing FAD mutant presenilin (Leissring et al., 2002). A decreased release of calcium from the intracellular stores was observed in cells with genetic ablation of the presenilins or pharmacological inhibition of γ-secretase activity (and thereby AICD production). The disturbances in calcium signaling were rescued only by an overexpression of AICD-containing APP fragments and not by those lacking the AICD sequence. These results indicated a tight link between presenilins dependent γ-secretase activity and calcium homeostasis alterations. This function of AICD may be linked to its properties as a co-regulator of the expression of genes encoding proteins involved in calcium homeostasis. It was shown to migrate to the nucleus and interact with the adapter protein Fe65 and the histone acetyltransferase Tip60, potent transcription stimulators (Fig. 1) (Cao and Sudhof, 2001; Kinoshita et al., 2002).

Moreover, many biochemical and functional studies demonstrate that presenilins can interact with several calcium-related proteins including calsenilin (Buxbaum et al., 1998), sorcin (Pack-Chung et al., 2000), the myristoylated calcium-binding protein calmyrin (Stabler et al., 1999), the calcium-dependent thiol protease µ-calpain (Shinozaki et al., 1998).

2.5. Calcium dysregulation and Aβ

Aβ is the principal constituent of the hallmark amyloid plaques found in AD and is now almost universally accepted as a central pathophysiological role in the disease (LaFerla et al., 2007). Aβ is produced by endoproteolysis of the parental APP, which is achieved by the sequential cleavage of APP by groups of enzymes or enzyme complexes termed α-, β- and γ-secretases. APP is a type 1 transmembrane family of glycoproteins with the sequence of Aβ partly embedded in the plasma membrane. The cleavage and processing of APP can be divided into two classes: a non-amyloidogenic pathway and an amyloidogenic pathway. In the
normalizes cytosolic calcium levels, Aβ-nositide-mediated calcium signaling through a transgenic mice, an age before plaque deposition, there was no appreciable calcium overload. In addition, Aβ, a soluble amino-terminal part of the protein (sAPPβ) as well as a slightly larger amyloidogenic carboxy-terminal a slightly larger C-terminal fragment (C99), retained within the membrane. The cleavage of C99 occurs first at the γ-site and liberates the AICD (Fig. 1). This is followed by a cut of membrane remaining fragment at the γ-site and the release of Aβ40 or Aβ42 (Suh and Checler, 2002).

Studies have revealed that metabolites of APP metabolism are involved in calcium signaling. Firstly, AICD regulates phosphoinositide-mediated calcium signaling through a γ-secretase-dependent signaling pathway, as described above (Leissring et al., 2002). Then, the sAPP, which has been shown to exhibit neuroprotective properties, were demonstrated to normalize calcium levels. It moderates calcium responses after exposure to glutamate, inducing an increase of cyclic GMP production, which promotes activation of K+ channels and reduces calcium levels. It moderates calcium responses after exposure to glutamate (Mattson, 1994). Stabilization of intracellular calcium by sAPP can reverse apoptotic changes in cell bearing proapoptotic mutant of PS1 (Guo et al., 1998a). After incubation of sAPP with apoE, sAPP exhibited an enhanced [Ca2+]i-lowering activity and enhanced protection against excitotoxicity in rat primary hippocampal neurons (Barger and Mattson, 1997). The decreased level of sAPP that results from altered APP processing may contribute to the disruption of calcium homeostasis and neuronal degeneration in AD (Mattson and Chan, 2003). Moreover, in human AD and transgenic mice expressing familial APP mutant (K670N, M671L, and V717F), there is a significant depletion of the calcium-binding protein, calbindin-D28k, in hippocampal dentate granule cells, which correlates with the relative abundance of Aβ1–42 in the brain (Palop et al., 2003). Also, calbindin knockout mice increase dendritic spine basal calcium and develop abnormal spine morphologies (Vecellio et al., 2000).

While AICD and sAPP seems to be neuroprotective and normalizes cytosolic calcium levels, Aβ-containing fragments disrupt intracellular calcium homeostasis. Existing evidence has shown that exogenous application of synthetic or aggregates Aβ leads to a rapid and sustained rise in [Ca2+]i in cultured cells (Demuro et al., 2005; Guo et al., 1999; Mattson et al., 1992). Recently, using transgenic mouse models of AD together with multiphoton imaging, Aβ deposition was observed to be required to induce calcium overload in vivo (Kuchibhotla et al., 2008). The calcium overload was coupled to the deposition of senile plaques and was most pronounced in the immediate vicinity of senile plaques. However, in contrast to the APPswt/PS1–ΔE9 mouse line and the Tg-2576 (APPSwe) line, only mutant PS1 (PS1–ΔE9 or PS1–M146V), though important in accelerating plaque deposition when coupled with mutant APP, does not play a role in the calcium overload in neurites. Furthermore, in 3.5-month-old APP/PS1 transgenic mice, an age before plaque deposition, there was no appreciable calcium overload. In addition, Aβ oligomers are observed to induce mitochondrial calcium overload (Sanz-Blasco et al., 2008), and calcium-regulated signaling pathways demonstrate to involve in the Aβ-induced synaptic dysfunction (Xie, 2004).

One mechanism by which Aβ disrupts calcium homeostasis is by inserting into the plasma membrane and forming ion-conducting pores (Fig. 1) (Table 1). Since the initial study by Arispe et al. (1993a), Aβ peptides of various lengths, including Aβ25–35, Aβ1–40 and Aβ1–42, have been observed to elicit cation-selective currents when reconstituted into lipid bilayers (Arispe et al., 1993b, 1996; Hirakura et al., 1999; Mirzabekov et al., 1996). When reconstituted in liposome prepared with mixture of natural phospholipids such as phosphatidyl choline and phosphatidyl serine, Aβ1–42 and Aβ1–40 allow significant uptake of calcium ions in a dose-dependent manner (Lin et al., 1999; Rhee et al., 1998). Interestingly, exposure of phosphatidylserine (PtdS) on the cell surface enhances the ability of Aβ to associate with the membrane and form channels (Fig. 1) (Lee et al., 2002). Because cell-surface exposure of phosphatidylserine is usually indicative of cells in conditions of energy deficit and apoptosis, it is possible that age and AD-related mitochondrial impairment might trigger flipping of PtdS from the inner portion of the plasma membrane to the cell surface in affected neurons and set them up for Aβ-mediated channel formation, calcium influx and cell death (Fig. 1). In fact, neurons with reduced cytosolic ATP levels and elevated surface PtdS levels are particularly vulnerable to Aβ toxicity (Simakova and Arispe, 2007). The formation of ion channels by Aβ in the liposome membrane was confirmed by specifically blocking the channels with zinc ions, tromethamine and an antibody raised against the amino-terminal domain of Aβ, which had been shown to inhibit Aβ ion channels (Arispe et al., 1996; Lin et al., 1999). Aβ molecules in solution plug into ion channels in natural membranes. The excised membrane patches from a cell line derived from hypothalamic neurons have been shown to produce new ion-channel activity when exposed to Aβ molecules. Patch-clamp experiments were performed in membrane patches pulled from cultured neurons, and membrane currents under voltage clamp conditions were recorded. The Aβ-induced channel activity was observed after Aβ interacted with either side of isolated patches of membranes (Kawahara et al., 1997). These channels would allow excessive calcium influx and disrupt the normal cellular calcium homeostasis. Direct evidence that Aβ may form channels in the membrane has also been reported (Arispe, 2004; Bhatia et al., 2000; Lin et al., 2001; Lal et al., 2007; Quist et al., 2005). Bhatia and co-workers used atomic force microscopy, laser confocal microscopy, and calcium imaging, which allow the real-time and acute effects of cellular morphological changes on a submicrometre scale, to show that Aβ1–42, at nanomolar concentrations, could formed calcium-permeable pores that induced an elevated cellular calcium level and the degeneration of endothelial cells (Bhatia et al., 2000). Lin et al. found that small 8–12 nm annular structures could be observed by atomic force microscopy when Aβ1–42 was fused with planar lipid bilayers of 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (Lin et al., 2001). These annular structures were suggested to be the Aβ channels predicted by previous electrophysiological studies. In reconstituted membranes, various amyloid molecules, including Aβ1–40, α-synuclein, Aβri, ADan, serum amyloid A, and amylin, have been observed to form morphologically compatible ion-channel-like structures and elicit single ion-channel currents which would destabilize cellular ionic homeostasis; and hence induce cell pathophysiology and degeneration in amyloid diseases (Quist et al., 2005). The ability of Aβ oligomers to form calcium-permeable channels in neuronal plasma membranes is consistent to recent in vivo calcium-imaging experiments performed with APP transgenic mice, by showing that resting calcium levels were significantly elevated in approximately 35% of neurites located in the immediate vicinity of Aβ plaques (Kuchibhotla et al., 2008). The probable explanation for these results is that a high local concentration of Aβ oligomers in the area surrounding amyloid plaques causes the formation of calcium-permeable ion channels in the neuronal plasma membrane.

Another mechanism by which Aβ can disrupt calcium homeostasis is related to its ability to form ROS that may induce
Table 1

<table>
<thead>
<tr>
<th>Channels affected</th>
<th>Form and state of used</th>
<th>Particular effect</th>
<th>Model used</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMDA receptors</td>
<td>Aβ₁₋₄₂</td>
<td>Down-regulation of NMDA receptors</td>
<td>Cortical neurons</td>
<td>Snyder et al. (2005)</td>
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<tr>
<td>NMDA receptors</td>
<td>Aβ₁₋₄₂ globulomer</td>
<td>Dysregulation of NMDA receptors function</td>
<td>Hippocampal neurons</td>
<td>De Felice et al. (2007)</td>
</tr>
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<td>AMPA receptors</td>
<td>Aβ₁₋₄₀ and Aβ₁₋₄₀</td>
<td>Inhibitory effects on synaptic AMPA receptors</td>
<td>Hippocampal CA1 pyramidal neurons and artificial lip bilayers</td>
<td>Parameshwaran et al. (2007)</td>
</tr>
<tr>
<td>L-type VGCCs</td>
<td>Aβ₂₋₃₅</td>
<td>Increase in I_{Ca,L} associated with a rise in [Ca^{2+}]_{i}</td>
<td>Rat hippocampal neurons</td>
<td>Borson et al. (1995)</td>
</tr>
<tr>
<td>L-type VGCCs</td>
<td>Aβ₂₋₃₅ (40 μM)</td>
<td>Increase in I_{Ca,L} associated with a rise in [Ca^{2+}]_{i}</td>
<td>Human resting microglial cells</td>
<td>Sile et al. (1999)</td>
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<tr>
<td>L-type VGCCs</td>
<td>Aβ₂₋₃₅</td>
<td>Increase in calcium influx through I_{Ca,L} associated with a rise in [Ca^{2+}]_{i}</td>
<td>Rat cortical and hippocampal neurons</td>
<td>Ueda et al. (1997)</td>
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<tr>
<td>L-type VGCCs</td>
<td>Aβ₂₋₃₅ (20 μM)</td>
<td>Increase in I_{Ca,L} and induction of cell death</td>
<td>SK-N-SH cells</td>
<td>Ba et al. (2004)</td>
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<tr>
<td>L-type VGCCs</td>
<td>Aggregated both Aβ₁₋₄₀ and Aβ₂₋₃₅</td>
<td>Increase in I_{Ca,L} through MAPK phosphorylation</td>
<td>Cultured SH-SY-5Y human neuroblastoma cells (22–40 μM) and mice cultured cortical neurons (10–25 μM)</td>
<td>Elkinci et al. (1999)</td>
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<tr>
<td>L-type VGCCs</td>
<td>Aβ₁₋₄₀, Aβ₁₋₄₂ and Aβ₂₋₃₅</td>
<td>Increase in I_{Ca,L}</td>
<td>PC12 cells</td>
<td>Green and Peers (2001)</td>
</tr>
<tr>
<td>L-type VGCCs</td>
<td>Aβ₁₋₄₀ (1 μM)</td>
<td>Increase in calcium influx through I_{Ca,L} and I_{Ca,P/Q}</td>
<td>Cultured rat cortical neurons</td>
<td>Ramsden et al. (2002)</td>
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<tr>
<td>N- and P/Q-type VGCCs</td>
<td>Unaggregated Aβ₁₋₄₀ (1 μM)</td>
<td>Selective suppression of presynaptic I_{Ca,P/Q} at both glutamatergic and GABAergic synapses</td>
<td>Hippocampal neurons</td>
<td>Nimmrich et al. (2008)</td>
</tr>
<tr>
<td>VGCCs</td>
<td>Oligomeric Aβ₁₋₄₀ and Aβ₂₋₃₅</td>
<td>Increase in total I_{Ca}</td>
<td>Rat primary hippocampal cell cultures</td>
<td>Li et al. (2000)</td>
</tr>
<tr>
<td>VGCCs</td>
<td>Aggregated Aβ₁₋₄₀ (1 μM)</td>
<td>Increase in total I_{Ca}</td>
<td>Cortical neurons</td>
<td>Ferreira et al. (2004, 2008)</td>
</tr>
<tr>
<td>RyR</td>
<td>Aβ₁₋₄₀</td>
<td>Selectively increase RyR-3 expression and ER calcium release</td>
<td>Primary cortical neurons, TgCRND8 mice, CRND8 cortical neurons</td>
<td>Supnet et al. (2006)</td>
</tr>
<tr>
<td>mPTP</td>
<td>Aβ₁₋₄₀ and Aβ₁₋₄₂</td>
<td>Formation of a complex with CypD and release of calcium stored in mitochondria</td>
<td>Cortical mitochondria of transgenic mAPP mice</td>
<td>Du et al. (2008)</td>
</tr>
<tr>
<td>Aβ ion channels</td>
<td>Aβ₁₋₄₀ (about 5 μg in liposomes)</td>
<td>Formation of cation selective channels</td>
<td>Synthetic planar bilayers</td>
<td>Arispe et al. (1993a)</td>
</tr>
<tr>
<td>Aβ ion channels</td>
<td>Aβ₁₋₄₀ (about 1 μM)</td>
<td>Formation of cation selective channels; blocked by zine</td>
<td>Synthetic planar bilayers</td>
<td>Arispe et al. (1993b, 1996)</td>
</tr>
<tr>
<td>Aβ ion channels</td>
<td>Aβ₁₋₄₀ (about 5 μg in liposomes)</td>
<td>Reveals that ion channels are probably transmembrane annular polymeric structures</td>
<td>Synthetic planar bilayers</td>
<td>Arispe (2004)</td>
</tr>
<tr>
<td>Aβ ion channels</td>
<td>Soluble Aβ₁₋₄₀ (2 mg/ml in liposomes)</td>
<td>Formation of multimeric channel-like structures</td>
<td>Synthetic planar bilayers</td>
<td>Lin et al. (2001)</td>
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<tr>
<td>Aβ ion channels</td>
<td>Aβ₂₋₃₅ (20–100 μg/ml)</td>
<td>Formation of weakly selective, voltage-dependent, ion-permeable channels</td>
<td>Synthetic planar bilayers</td>
<td>Mirzabekov et al. (1996)</td>
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<tr>
<td>Aβ ion channels</td>
<td>Soluble Aβ₁₋₄₀</td>
<td>Formation of cation selective channels</td>
<td>Cell membranes from hypothalamic neurons</td>
<td>Kawahara et al. (1997)</td>
</tr>
<tr>
<td>Aβ ion channels</td>
<td>Aβ₁₋₄₀ (2 μM) and Aβ₁₋₄₀ (21 μM)</td>
<td>Formation of slightly cation selective, voltage-independent ion channels</td>
<td>Synthetic planar bilayers</td>
<td>Hirakura et al. (1999)</td>
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<tr>
<td>Aβ ion channels</td>
<td>Aβ₁₋₄₀</td>
<td>Formation of calcium-permeable channels</td>
<td>Unilamellar lipidosome membrane</td>
<td>Rhee et al. (1998)</td>
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<tr>
<td>Aβ ion channels</td>
<td>Aβ₁₋₄₀</td>
<td>Formation of calcium-permeable channels</td>
<td>Unilamellar lipidosome membrane</td>
<td>Lin et al. (1999)</td>
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<tr>
<td>Aβ ion channels</td>
<td>Aβ₁₋₄₀</td>
<td>Formation of calcium-permeable channels in plasma membrane</td>
<td>Cultural endothelial cells</td>
<td>Bhatia et al. (2000)</td>
</tr>
<tr>
<td>Potassium channels</td>
<td>Aβ₁₋₄₀ and Aβ₂₋₃₅</td>
<td>Inhibition of I_{K_A} and I_{K_C}, leading to increase calcium influx</td>
<td>Dissociated cholinergic neurons</td>
<td>Jhamandas et al. (2001)</td>
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<tr>
<td>Potassium channels</td>
<td>Aβ₁₋₂₈, Aβ₁₋₃₀, Aβ₁₋₄₀ and Aβ₁₋₄₀</td>
<td>Inhibition of I_{K_A} and I_{K_C}, leading to increase calcium influx</td>
<td>Hippocampal neuronal</td>
<td>Good et al. (1996)</td>
</tr>
</tbody>
</table>

Aβ, amyloid-β; AMPA, amino-3-hydroxy-5-methyl-4-isoxazol propionate; [Ca^{2+}]_{i}, cytosolic calcium concentration; I_{Ca,L}, calcium-activated K+ current; I_{K_A}, delayed rectifier K+ current; I_{K_C}, K+ current type A (fast-activating and -inactivating current); I_{Ca,P/Q}, L-type voltage-dependent Ca^{2+} current; I_{Ca,K} and I_{Ca,P/Q}, N- and P-type voltage-dependent Ca^{2+} currents; IP3R, inositol(1,4,5)-trisphosphate receptors; LTP, long-term potentiation; NMDA, N-methyl-D-aspartate; RyR, ryanodine receptors; VGCCs, voltage-gated calcium channels.
membrane lipid peroxidation, which causes alterations in membrane properties and affects the function of membrane transporters and ion channels leading to an elevation of intracellular calcium levels (Fig. 1) (Mattson, 2004). Interactions of Aβ oligomers and Fe²⁺ and Cu²⁺ generate hydrogen peroxide and hydroxyl radicals (Morgan et al., 2000). During the process of Aβ aggregation at the cellular membrane, membrane-associated lipid peroxidation is initiated and results in the consequent generation of toxic lipid aldehydes such as 4-hydroxynonenal, a neurotoxic aldehyde that covalently modifies proteins on cysteine, lysine and histidin residues. The process impairs the function of ion-motive ATPases, glucose and glutamate transporters, GTP-binding proteins, and also ion channels (VDCC, voltage-dependent chloride channel; NMDA receptor) as the result of covalent modification of the proteins by the aldehyde 4-hydroxynonenal (Mattson, 1997). The membrane-associated oxidative stress also involves in mitochondrial membrane and contributes to the dysfunction of them. Ion-motive ATPases are intimately involved in regulating intracellular calcium elevations (such as the calcium-ATPase) (Mark et al., 1995). Studies using PC12 cells have also demonstrated that Aβ can cause inhibition of respiratory chain complexes with a concomitant reduction in cellular ATP levels (Casley et al., 2002), which will also result in a dramatic alteration in intracellular calcium handling (Bernridge et al., 2003). Antioxidants that inhibit lipid peroxidation, such as vitamin E, estrogens and uric acid prevent impairment of the membrane transporters, stabilize cellular calcium homeostasis, and prevent death of neurons exposed to Aβ (Keller et al., 1997, 1998; Ba et al., 2004).

In addition to the lipid peroxidation hypothesis, there are many studies reporting that Aβ can stimulate calcium uptake through endogenous receptors or ion channels (Fig. 1) (Table 1). Aβ1–40 or Aβ25–35 could induce ER calcium release through IP3R and RyR, leading to cytosolic calcium overload and activating the mitochondrial apoptotic pathway (Ferreiro et al., 2004, 2008). Aβ1–40 shows to selectively elevate RyR-3 expression levels in vitro and in vivo, and the elevation of RyR-3 functionally facilitates the increase of intracellular calcium levels following ryanodine or glutamate treatment (Supnet et al., 2006). Aβ1–40 and Aβ1–42 also shows to form a complex with CypD in vivo and in vitro and that formation of this complex in vitro makes mitochondria more susceptible to mPTP opening, leading to release calcium stored in mitochondria (Du et al., 2008). Aβ oligomers have also been shown to be responsible for calcium influx mediated by NMDA receptors. De Felice et al. found that Aβ oligomers induced a rapid and transient increase in intracellular calcium levels in mature hippocampal neurons, which was blocked by both an open channel NMDA-R antagonist memantine and anti-NR1 subunit of the NMDA receptor antibody. Domingues et al. (2007) showed that Aβ-induced toxicity in HEK293 cells expressing NMDA receptor subunits was blocked by a noncompetitive NMDA receptor antagonist. Snyder et al. have proposed that effects of Aβ on NMDA receptors may be mediated by a direct action on α7 nicotinic acetylcholine receptors (Snyder et al., 2005). However, this possibility has been disputed by Small et al. (2007), who could not find any evidence for a direct interaction between Aβ and the α7 nicotinic acetylcholine receptors. Parmeshwaran and co-workers shows that Aβ1–42 closely interacts with and exhibits inhibitory effects on synaptic AMPA receptors and may contribute to the memory impairment observed in AD (Parmeshwaran et al., 2007). Other studies support the view that VGCCs are altered by Aβ (Good et al., 1996; Ekinci et al., 1999; Li et al., 2000; Green and Peers, 2001; Ramsden et al., 2002; Rovira et al., 2002; Xie, 2004). Brorson and colleagues have demonstrated that micromolar concentrations of Aβ fragments induce a rapid increase in intracellular calcium levels that can be attenuated by the addition of L-type VGCC antagonists (Brorson et al., 1995). Ho et al. examined calcium uptake in SH-SYS5Y human neuroblastoma cells (Ho et al., 2001). They found that nime bordeline, an L-type calcium channel inhibitor blocking calcium uptake, whereas MK-801, an inhibitor of NMDA receptors, did not block uptake. In addition to the effects of Aβ on L-type VGCCs, Aβ has also been shown to enhance calcium current through the N- and L-type VGCCs in synaptosomes and the N- and P-type VGCCs in cortical neurons (MacManus et al., 2000). Furthermore, it was reported that Aβ can block a fast-inactivating K⁺ current, potentially leading to prolonged cell depolarization and increased calcium influx and intracellular accumulation (Good et al., 1996; Jamandras et al., 2001). Studies on other neurotoxic amyloidogenic proteins also support the view that Aβ can activate VGCCs. Silei et al. showed that activation of microglia by prion protein (PrP) and Aβ raised intracellular calcium through L-type channels (Silei et al., 1999). Hou et al. found that amylodigenic transthyretin could induce calcium entry through L-type channels in SH-SYS5Y neuroblastoma cells (Hou et al., 2007). In addition, extracellular application of Aβ has been shown to cause a dramatic increase in IP3R-mediated or RyR-mediated calcium release from ER stores (Smith et al., 2001; Ferreiro et al., 2004). Studies by Ye et al. (1997) suggest that calcium may enter through a nonselective cation channel in response to Aβ. Aβ1–42 globulomer has also been shown to disrupt synaptic plasticity by impairing presynaptic P/Q-type calcium currents at both glutamategic and GABAergic synapses (Nimrichter et al., 2008).

The influence of calcium dysregulation on the metabolism and production of Aβ is not as thoroughly studied as the role of Aβ in calcium signaling. Nevertheless, several studies have illustrated that changes in calcium levels and dynamics alter the metabolism and production of Aβ, implicating calcium dysfunction as a possible causative factor in sporadic AD (SAD) (Fig. 1) (Green et al., 2007). An elevated cytosolic calcium concentration, either by influx through calcium channels of the plasma membrane (Querfurth and Selkoe, 1994; Pierrot et al., 2004) or by release from ER stores (Querfurth et al., 1997), can increase the production of the Aβ peptide, indicating that a sustained high concentration of cytosolic calcium is required to induce the production of intraneuronal Aβ from human APP. Calcium-mediated transient phosphorylation is involved in the underlying mechanism (Pierrot et al., 2006). By contrast, some reports indicated that the inhibition of SERCA pump with thapsigargin, which elevate cytosolic calcium levels by blocking the reuptake of calcium into the ER, diminished Aβ production (Buxbaum et al., 1994; Green et al., 2008). One possible explanation is that depleting ER stores of calcium has a stronger effect on preventing Aβ production than the effects of CCE activation (Green et al., 2008). Curiously, it has been reported that either stimulation (Pierrot et al., 2004) or inhibition (Yoo et al., 2000) of CCE leads to increased production of Aβ1–42.

Interestingly, a novel calcium-conducting channel, named calcium homeostasis modulator 1 (CALHM1), with polymorphisms was recently discovered to be associated with increased risk for the development of SAD (Dreses-Werringloer et al., 2008). Critically, endogenous overexpression of CALHM1 was observed to decrease Aβ production and be accompanied by increases in sAPPa (Fig. 1). The effects of CALHM1 on the regulation of Aβ and sAPPa levels are in line with its effect on cytosolic calcium, indicating that CALHM1 controls APP proteolysis in a calcium-dependent manner. In addition, environmental factors that inhibit amyloidogenesis (caloric restriction, cognitive stimulation and antioxidants) stabilize neuronal calcium homeostasis, whereas factors that enhance amyloidogenesis disrupt calcium homeostasis.

### 2.6. Calcium dysregulation and Tau phosphorylation

Intracellular accumulation of an abnormally phosphorylated protein tau leading to the formation of NFTs is another pathological...
hallmark of AD (Mattson, 2004). It has been reported that hyperphosphorylated tau found in NFTs induced cytotoxicity to neurons. Several lines of evidence indicate that the state of Tau phosphorylation is tightly regulated and seems to be calcium-dependent. Cyclin-dependent kinase 5 (CdK5) and glycogen synthase kinase-3β (GSK3β) are involved in AD-like phosphorylation of tau (Flaherty et al., 2000; Ahlijanian et al., 2000). A transient increase in intracellular calcium concentration was previously demonstrated to induce a GSK3β-mediated phosphorylation of tau (Hartigan and Johnson, 1999). On the other hand, a calcium-dependent proteolytic cleavage of p35 generates p25, leading to aberrant CdK5 activation (Lee et al., 2000b; Lew et al., 1994). In transgenic mice overexpressing p25 in the postnatal forebrain, endogenous tau was hyperphosphorylated and neurofibrillary tangles developed in the brain (Cruz et al., 2003). The biphasic effect of calcium influx on Tau phosphorylation was also observed in SH-SYSY cells (Shea and Ekinici, 1999), which indicates that net dephosphorylation or phosphorylation is dependent upon the extent and/or rate of calcium influx (Fig. 1). A recent study indicates that membrane depolarization of cultured neurons induced a calcium-mediated transient phosphorylation of tau mediated by GSK3β and CdK5 kinases, followed by a dephosphorylation of this protein mediated by calcineurin (Pierrot et al., 2006). Moreover, many calcium-related or dependent proteins, including calcium/calmodulin-dependent protein kinase II (Yoshimura et al., 2003), calpains (calcium-dependent proteases that cleave cytoskeletal proteins) (Chen et al., 2008), calcineurin (Fleming and Johnson, 1995), transglutaminase (a calcium-activated enzyme) (Miller and Johnson, 1995) and a novel EF-hand domain-containing calcium-binding protein (Vega et al., 2008) have been indicated to involve in tauopathy. Furthermore, inhibiting NMDA receptor with memantine demonstrate to reverse the Alzheimer type abnormal hyperphosphorylation of tau (Li et al., 2004). Conversely, tau mutations reducing its microtubule-binding ability alter the function of VGCCs in a manner that increases calcium influx (Furukawa et al., 2003) and might contribute to the cell death process in this disease.

3. Calcium regulation as a therapeutic approach for AD: present and future

Intracellular levels are maintained by receptor-operated, voltage-gated, or store-operated calcium channels in the plasma membrane and by ER-resident channels. Altered calcium signaling could impinge on the increase of susceptibility of cells to apoptosis, synaptic plasticity, mitochondrial dysfunction, membrane excitability, oxidative stress, APP processing, and Tau phosphorylation as described above. Large body of evidence for the involvement of disrupted cellular calcium homeostasis in AD pathogenesis suggest that therapeutic approaches that stabilize neuronal calcium homeostasis by targeting the calcium signaling pathways may be capable of preventing or treating neuronal degeneration in AD, and several drugs have demonstrated potential therapeutic opportunities in vitro and in vivo (Table 2).

3.1. Targeting calcium channels in the plasma membrane

3.1.1. Targeting receptor-operated calcium channels

Although many different drugs that target calcium influx have demonstrated efficacy in animal models of AD, very few studies have been successful in clinical trials. However, significant beneficial effects of the NMDA open channel blocker memantine (Bullock, 2006) in AD patients have shown that calcium-regulating systems are viable targets. Memantine is the first and only medication that has been approved by European, US and Canadian regulatory agencies for the treatment of moderate-to-severe AD. It is a non-competitive antagonist of NMDA receptor that works to prevent excitotoxicity and cell death, which are mediated by the excessive influx of calcium during a sustained release of glutamate. In addition to blocking the NMDA receptor, memantine has demonstrated to decrease the basal level of intracellular calcium, increase the calcium content of the intracellular calcium store, and thus increasing agonist-induced intracellular calcium release, and increase agonist-induced calcium entry by potentiating store-operated calcium entry (Blanchard et al., 2008). Many well-designed clinical studies have demonstrated that it benefits in cognitive, functional, and behavioral outcomes in patients with moderate to severe AD, either as monotherapy or in combination with the AChE inhibitor donepezil (Emre et al., 2008). Although memantine has not yet been approved for the treatment of earlier stages of this disease, emerging data suggest its potential benefits in patients with mild to moderate AD (Cosman et al., 2007). In addition, the NMDA receptor 2B (NR2B)-specific antagonist EVT-101 (Evotec AG, Hamburg, Germany; http://www.evotec.com/) was recently developed for AD treatment. The Phase I AD trial of EVT-101 (NCT00529698) has been completed and a Phase II AD trial of EVT-101 is anticipated soon.

AMPA receptors are the other important receptor-operated calcium channels (ROCCs) that may involve in the pathogenesis of AD. When activated by glutamate or AMPA, the AMPA receptor allows Na⁺ and Ca²⁺ ions to flow through the channel into the cell. This activation of AMPA receptors have been implicated in a slower form of synaptic plasticity, termed homeostatic synaptic scaling, in which the total synaptic strength of a neuron is modified to regulate its excitability (Turrigiano and Nelson, 2004). Aβ1–42 can closely interact with and exhibit inhibitory effects on synaptic AMPA receptors and may contribute to the memory impairment observed in AD (Parmeshwaran et al., 2007). Recently, the study of double knockin mice carrying human mutations in the genes for APP and presenilin-1 demonstrates a decrease in AMPA receptor efficacy (Chang et al., 2006), indicative of synaptic downscaling at the onset of AD pathology, which indicates that memory decline in AD could be mitigated by positive AMPA receptor modulators and such drugs are currently being studied for their potential utility in AD. In rats, LY451395, a positive AMPA receptors modulator, shows to reverse memory deficits induced by pharmacologic agents, reverse age-associated memory deficits, and increase performance in a water maze test and a radial arm maze test. However, recently, an 11-week, double-blind and placebo-controlled clinical trial failed to support the utility of LY451395 in the treatment of cognitive dysfunction in AD (Chappell et al., 2007). One possible reason for a lack of clinical efficacy is that the doses was too low and did not allow concentration for a drug benefit to manifest, and another possible reason is that the duration of treatment was too short and did not allow time for a drug benefit to manifest (Chappell et al., 2007). (S)-2,3-Dihydro-[3,4]-cyclopentano-1,2,4-triazole-1-dioxide (S18986), another positive AMPA receptors modulator, has also been proved to improve behavioral performance and alleviate age-related deficits in oxidative stress status in the prelimbic cortex and hippocampus (Kelly et al., 2009).

Dimebon is an old Russian antihistamine compound that has been claimed to exert neuroprotective effects at picomolar concentrations and yield promising results in Phase II AD clinical trials based on cognitive-outcome measures (Doody et al., 2008). Coincidently, it has been claimed to be able to stabilize calcium signaling by blocking NMDA receptor and VGCCs (Grigorov et al., 2003; Wu et al., 2008). Interestingly, dimebon has also shown to prevent opening of mPTP (Bachurin et al., 2003).

3.1.2. Targeting voltage-gated calcium channels

Although increasing lines of evidence suggest that calcium influx through VGCCs plays an important role in the pathogenesis
Table 2

<table>
<thead>
<tr>
<th>Targets</th>
<th>Model used</th>
<th>Drugs</th>
<th>Mechanism</th>
<th>Effect</th>
<th>References</th>
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<tr>
<td>NMDA-R</td>
<td>Hippocampal slices</td>
<td>Memantine</td>
<td>Antagonist</td>
<td>Reverses hyperphosphorylation of tau</td>
<td>Li et al. (2004)</td>
</tr>
<tr>
<td>NMDA-R</td>
<td>E-18 hippocampal neurons</td>
<td>Memantine</td>
<td>Antagonist</td>
<td>Protects synapses against Aβ oligomers-induced neuronal oxidative stress</td>
<td>De Felice et al. (2007)</td>
</tr>
<tr>
<td>AMPA-R</td>
<td>Rats</td>
<td>S18986</td>
<td>Antagonist</td>
<td>Improves behavioral performance and alleviates age-related deficits</td>
<td>Kelly et al. (2009)</td>
</tr>
<tr>
<td>L-type VGCCs</td>
<td>Cortical or hippocampal neurons</td>
<td>Nimodipine</td>
<td>Antagonist</td>
<td>Inhibits Aβ25–35 neurotoxicity or the production of intraneuronal Aβ1–42</td>
<td>Ueda et al. (1997) and Pierrot et al. (2004)</td>
</tr>
<tr>
<td>L-type VGCCs</td>
<td>PS1-deficient neurons</td>
<td>Nifedipine</td>
<td>Antagonist</td>
<td>Rescues the neurons from H2O2-induced death</td>
<td>Nakajima et al. (2001)</td>
</tr>
<tr>
<td>L-type VGCCs</td>
<td>Wistar rats and hippocampal slice</td>
<td>Diltiazem</td>
<td>Antagonist</td>
<td>Fails to reverse the Aβ25–35-induced depression of LTP</td>
<td>Freir et al. (2003)</td>
</tr>
<tr>
<td>L-type VGCCs</td>
<td>Older rabbits</td>
<td>MEM 1003</td>
<td>Antagonist</td>
<td>Enhances learning</td>
<td>Rose et al. (2007)</td>
</tr>
<tr>
<td>L-type VGCCs</td>
<td>Cortical and hippocampal neurons</td>
<td>Omega-agatoxin IVA</td>
<td>Antagonist</td>
<td>No effects</td>
<td>Ueda et al. (1997)</td>
</tr>
<tr>
<td>L-type VGCCs</td>
<td>PS1-deficient neurons</td>
<td>Omega-conotoxin GVA</td>
<td>Antagonist</td>
<td>No effects</td>
<td>Nakajima et al. (2001)</td>
</tr>
<tr>
<td>L-type VGCCs</td>
<td>Cortical neurons</td>
<td>Xestospongin C</td>
<td>Antagonist</td>
<td>Prevents partially Aβ25–35 or Aβ1–40-induced neuronal apoptosis</td>
<td>Ferreiro et al. (2004)</td>
</tr>
<tr>
<td>L-type VGCCs</td>
<td>Neuronal PC12 cells</td>
<td>MRS2481 and MRS2485</td>
<td>Antagonist</td>
<td>Protects neurons from Aβ toxicity</td>
<td>Diaz et al. (2009)</td>
</tr>
<tr>
<td>N-type VGCCs</td>
<td>Hippocampal neurons</td>
<td>Roscovitine</td>
<td>Enhancer of P/Q currents</td>
<td>Reverses Aβ1–42 globulomer-induced synaptic impairments</td>
<td>Nimmrich et al. (2008)</td>
</tr>
<tr>
<td>P/Q-type VGCCs</td>
<td>Cortical and hippocampal neurons</td>
<td>Xestospongin C or dantrolene</td>
<td>Antagonist</td>
<td>Prevents partially Aβ25–35 or Aβ1–40-induced neuronal apoptosis</td>
<td>Ferreiro et al. (2004)</td>
</tr>
<tr>
<td>P/Q-type VGCCs</td>
<td>Neuronal PC12 cells</td>
<td>Thapsigargin</td>
<td>Antagonist</td>
<td>Diminishes Aβ production</td>
<td>Buxbaum et al. (1994) and Green et al. (2008)</td>
</tr>
<tr>
<td>Aβ ion channels</td>
<td>Neuronal PC12 cells</td>
<td>Ni(2+), imidazole, His, NAHIS01, NAHIS02, and NAHIS04</td>
<td>Antagonist</td>
<td>Protection of cells from the Aβ cytotoxicity</td>
<td>Arispe et al. (2008)</td>
</tr>
<tr>
<td>Aβ ion channels</td>
<td>Neuronal PC12 cells</td>
<td>MRS2481 and MRS2485</td>
<td>Antagonist</td>
<td>Blocks the most known cellular responses to Aβ</td>
<td>Arispe and Doh (2002) and Simakova and Arispe (2006)</td>
</tr>
<tr>
<td>Calcineurin</td>
<td>APP/PS1 transgenic mice</td>
<td>FK-506</td>
<td>Inhibitor</td>
<td>Prevents neuritic beading and severe calcium overload</td>
<td>Chibhobhatla et al. (2008)</td>
</tr>
<tr>
<td>Calcineurin</td>
<td>Tg2576 APP transgenic mice</td>
<td>FD-506</td>
<td>Inhibitor</td>
<td>Improves memory function</td>
<td>Dineley et al. (2007)</td>
</tr>
<tr>
<td>Calpain</td>
<td>Neuronal PC12 cells</td>
<td>MRS2481 and MRS2485</td>
<td>Antagonist</td>
<td>Protects neurons from Aβ toxicity</td>
<td>Diaz et al. (2009)</td>
</tr>
<tr>
<td>Calpain</td>
<td>Neuronal PC12 cells</td>
<td>Thapsigargin</td>
<td>Antagonist</td>
<td>Protection of cells from the Aβ cytotoxicity</td>
<td>Arispe et al. (2008)</td>
</tr>
<tr>
<td>Calpain</td>
<td>Hippocampal cultures and in hippocampal slices from the APP/PS1 mouse</td>
<td>Mitochondria depolarization</td>
<td>Antagonist</td>
<td>Prevents neuron cell death induced by Aβ1–42 oligomers</td>
<td>Sanz-Blasco et al. (2008)</td>
</tr>
<tr>
<td>Intracellular calcium</td>
<td>PS1-deficient neurons</td>
<td>BAPTA-AM</td>
<td>Chelator</td>
<td>Rescues the neurons from H2O2-induced death</td>
<td>Nakajima et al. (2001)</td>
</tr>
</tbody>
</table>

Aβ, amyloid-β; AMPA, amino-3-hydroxy-5-methyl-4-isoxazol propionate; APP, amyloid precursor protein; BAPTA-AM, 1,2-bis(2-aminoophenoxo)ethane-N,N,N’,N”-tetraacetic acid tetra(acetoxymethyl ester); FCCP, carbonyl cyanide p-(trifluoromethoxy)phenylhydrazone; FK-506, tacrolimus; IP3R, inositol(1,4,5)-trisphosphate receptors; MDL28170, calpain inhibitor 3; NAHIS01, Ac-His-CONH2; NAHIS02, Ac-His-His-CONH2; NAHIS04, Ac-His-His-His-His-CONH2; NMDA, N-methyl-D-aspartate; NSAIDs, non-steroidal anti-inflammatory drugs; PS, presenilin; RyR, ryanodine receptors; S-312-d, S-(+)-methyl 4,7-dihydro-3-isobutyl-6-methyl-4-(3-nitro-phenyl)thieno[2,3- b]pyridine-5-carboxylate; STIM1, stromal interaction molecule 1; S18986, (S)-2,3-dihydro-[3,4]-cyclopenteno-1,2,4-benzoiodiazine-1,1-dioxide; SERCA pump, sarco-/endoplasmic reticulum calcium ATPase; VGCCs, voltage-gated calcium channels.
of AD, there are still only a few potential therapeutic compounds directed against their activation. Some clinical trials indicate the possibility that calcium signaling through L-type VGCCs is impaired in the AD brain. Nimodipine, an L-type channel inhibitor that is able to cross the blood–brain barrier, has been reported to have some benefits in clinical trials with AD patients (Fritze and Walden, 1995). In vitro, it was reported that nimodipine could inhibit Aβ1–42 neurotoxicity or the production of intraneuronal Aβ1–42 specifically induced by an increase of cytosolic calcium concentration in rat cortical neurons (Ueda et al., 1997; Pierrot et al., 2004). Interestingly, a meta-analysis of data from epidemiological studies suggests that nimodipine may slow progression of the disease rather than rapidly improve clinical manifestations (Lopez-Arrieta and Birks, 2002). It is worth to note that there is no clear evidence from clinical trials. Recently, nimodipine was reported to mimic, to some degree, the effects of several FAD-associated mutations by selectively stimulating secretion of Aβ1–42 (Facchini et al., 2006). The effect of nimodipine was independent of blockade of L-type channel currents and capacitative calcium entry. This may, perhaps, in part explain why there is no convincing clinical evidence that nimodipine is a useful treatment for AD. Experimental studies have shown that an analog of nimodipine (MEM 1003) may reduce age-related cognitive impairment in an animal model (Rose et al., 2007). A selective L-VSCC blocker, S(-)-methyl-4,7-dihydro-3-isobutyl-6-methyl-4-[3-nitro-phenyl]thieno[2,3-b]pyridine-5-carboxylate (S-312-d), significantly rescued cortical neurons from Aβ-induced cell death (Yagami et al., 2004). Recently, a Phase II trial of MEM 1003 (NCT00257673) has been completed, although the results of this trial have not officially been published. As the Phase II trial of MEM 1003 lasted only 12 weeks, this period of treatment may be too short to see any clear effect on disease progression. On this basis, therefore, further clinical trials of L-type VGCCs antagonists are warranted.

On the other hand, blockers of other VOCCs such as the P/Q-type calcium channels have also indicated protective efficacy in AD models. Nimmrich and co-workers provide evidence that Aβ1–42 globulomerly strongly impairs presynaptic P/Q-type calcium currents at both glutamatergic and GABAergic synapses (Nimmrich et al., 2008). In addition, roscovitine, which has been shown to enhance P/Q-type calcium currents by slowing the deactivation kinetics of the channel (Yan et al., 2002), was shown to reverse the globulomerly-induced deficits on vesicle release in hippocampal neurons by enhancing the P/Q-type calcium current, which suggests that P/Q current-enhancing compounds could at least in part offset synaptic deficits in AD. Furthermore, additional studies need to confirm that P/Q current-enhancing compounds can reverse cognitive deficits in animal models of AD. However, N-type VGCCs blocker, omega-conotoxin, shows no protective effect in PS1-deficient neurons (Ueda et al., 1997; Nakajima et al., 2001).

1.3.3. Targeting store-operated calcium channels

The store-operated calcium channels (SOCs) localized in the plasma membrane are responsible for cell calcium refilling via CCE, the attenuation of which is the most often observed alteration of calcium homeostasis in cells bearing FAD PS1 mutations as described above. The coupling mechanism between ER calcium stores and SOCCs in the plasma membrane as well as the identity and nature of these ion transporters has remained unknown for a long time. Our understanding of SOCCs has increased over the past 4 years with the identification, using primarily siRNA technology, of two groups of proteins, the ER calcium sensors stromal interactions molecule 1 (STIM1) and the plasma-membrane calcium channel Orai1 (Lewis, 2007). Calcium store depletion is sensed by STIM1, causing it to accumulate in junctional ER adjacent to the plasma membrane. At the same time, Orai1 accumulates in regions of the plasma membrane directly opposite the STIM1 clusters. The colocalization of STIM1 and Orai1 restricts channel activation and calcium entry to these sites (Lewis, 2007). The cellular level of STIM proteins changed in either mouse embryonic fibroblasts lacking presenilins or human B lymphocytes with FAD mutations in endogenous PS1 (Bojarski et al., 2008a,b). It is reasonable to target calcium store-operated signalling pathway, such as STIM1 and Orai1 to open up a possible new therapeutic approach for AD in the future.

2. Targeting calcium channels in the endoplasmic reticulum membrane

The overload of ER calcium stores is the principal alterations in calcium homeostasis caused by presenilins FAD mutations. Calcium release from intracellular stores in the ER occurs through two channels in the ER membrane: the IP3R is activated by the second messenger IP3 and the RyR is activated by cytosolic calcium. The overload of ER calcium stores was correlated with an increase of susceptibility of cells to apoptosis (Chan et al., 2000). The treatment of PS FAD cells with pharmacological inhibitors of ER calcium channels, such as dantrolene blocking RyR, or xestospongin inhibiting the IP3R, protects cells from proapoptotic agents, including Aβ (Cedazo-Minguez et al., 2002). Dantrolene and xestospongin C have also been shown to be able to prevent the increase in intracellular calcium and the activation of caspase-3 and to protect partially against apoptosis induced by treatment with Aβ25–35 or Aβ1–42 (Ferreiro et al., 2004). Indeed, Cheung et al. found that presenilin mutant-induced enhancement of Aβ secretion was abolished in IP3 receptor knockout cells (Cheung et al., 2008). By contrast, Nakajima and co-workers reported that both dantrolene and xestospongin C failed to rescue the PS1-deficient neurons from H2O2-induced death in calcium-dependent manners (Nakajima et al., 2001).

The SERCA pump has the highest affinity for calcium removal from the cytosol into ER stores, determine the resting cytosolic calcium concentration at rest. The calcium load is replenished in the ER through the action of SERCA pump. Recently, studies suggest that the interaction between SERCA pump and presenilin is a very important interaction that serves to regulate sequestration of calcium into the ER stores (Green et al., 2008). Furthermore, both the pharmacological treatment with thapsigargin and loss-of-function genetic means that inhibits or decreases SERCA pump function, which depletes ER stores, lead to diminish Aβ production (Buxbaum et al., 1994; Green et al., 2008).

3.2. Targeting calcium channels in the endoplasmic reticulum membrane

As it has been described above that calcium conducted in the target neurons by the hypothesized Aβ channel might be responsible for the neurotoxic properties of Aβ. Aβ channel blockers may also serve as AD therapeutics targets (Arispe et al., 2007). In fact, nonspecific Aβ channel blockers such as trimethamine (Tris) and Zn2+ have been reported to inhibit Aβ neurotoxicity (Arispe and Doh, 2002; Simakova and Arispe, 2006). More recently, some highly selective Aβ channel blockers, NA4, MRS2481 and its enantiomer MRS2485, have been demonstrated to potently block the Aβ calcium channel and protect neurons from Aβ toxicity (Simakova and Arispe, 2006; Diaz et al., 2009). Additionally, the compounds that are known to have histidine association capacity, including Ni2+, imidazole, His, and a series of His-related compounds (NAHIS01, NAHIS02, NAHIS04), also demonstrated to be effective at blocking both Aβ channel and preventing Aβ cytotoxicity (Arispe et al., 2008).

Several calcium-related proteins have also been shown to serve as potential therapeutic targets for AD. The overexpression of calcium-binding proteins such as calbindin-D markedly attenuates
Aβ-induced elevations in intracellular calcium, and completely blocks the enhanced calcium response in mutant PS1 cells (Guo et al., 1998b). The enhancement of calcium release can also be suppressed by overexpressing the calcium-binding protein calseenilin (Buxbaum et al., 1998; Leissring et al., 2000b). Recent evidence suggests that calcineurin activation is directly linked to dendritic structural degeneration and neuritic beading (Zeng et al., 2007). In behavioral studies, calcineurin inhibition with FK-506 partially restored learning and memory in Tg-2576 transgenic mice (Dineley et al., 2007). In morphological studies, inhibiting calcineurin with FK-506 prevents neuritic beading and severe calcium overload in APP/PS1 transgenic mice (Kuchibhotla et al., 2008). Calpains, cysteine-calcium-activated cysteine proteases, are another important calcium-related proteins that have been linked to AD (Nixon, 2003). Trinchese and co-workers found that the abnormal synaptic function and memory in APP/PS1 mice could be largely reversed by either of 2 calpain inhibitors, E64 and BDA-410 (Trinchese et al., 2008). Moreover, calpain inhibitor ML-28170 has been shown to decrease tau hyperphosphorylation, and to improve neurological function after spinal cord hemisection in rats (Hung et al., 2005).

4. Conclusion

There is now ample evidence from a variety of experimental and human systems to confirm that calcium dysregulation plays an important and proximal pathological role in AD. The precise contribution of calcium dysregulation to the pathogenesis of this disease remains unclear, but it indicates that systemic calcium changes accompany almost the whole brain pathology process that is observed in AD. Calcium dysregulation was not only observed to be secondary to some AD pathology such as the accumulation of Aβ oligomers or expression of FAD mutants in presenins, but also observed in early in the AD process and contribute to upstream of amyloidogenesis, APP metabolism or tauopathy. Hence, it is hypothesized that a pathologic cycle of calcium dysregulation and cell damage, whereby, the effect (calcium dysregulation) can reciprocate to further enhance the cause (AD pathology), is one possible mechanism for AD progression.

Given the early and ubiquitous involvement of calcium dysregulation in AD pathogenesis, it is logically to consider the calcium channels as potential therapeutic targets for AD prevention and treatment as described above. Numerous drugs that target calcium signaling pathways have been demonstrated efficacy in many cellular or animal models of AD, and a few have been successful in clinical trials such as memantine (Emre et al., 2008), dimebon (Doody et al., 2008) and nimodipine (Lopez-Arrieta and Birks, 2002). However, all these treatments target plasma-membrane calcium channels rather than the process of intracellular calcium signaling pathways that appears to be the principal locus of calcium dysregulation in AD. Optimistically, as the development of pharmacology and we learn more of the molecular mechanisms linking calcium dysregulation to AD pathology, it is likely that more specific strategies that target store-operated channels, intracellular calcium-release channels (RyR and IP3R), the SERCA pump, Aβ-formed calcium channels, calcium-related proteins or the mitochondrial calcium-handling system will provide more new potential opportunities in the future.

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