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Dysregulation of calcium in Alzheimer's disease

Maria Brzyska and **Danek Elbaum**

Laboratory of Bio-Physical Methods, Nencki Institute of Experimental Biology, 3 Pasteur St., 02-093 Warsaw, Poland

Abstract. Multiple efforts has underlined importance of calcium dependent cellular processes in the biochemical characterisation of Alzheimer's disease (AD), suggesting that abnormalities in calcium (Ca^{2+}) homeostasis might be involved in the pathophysiology of the disease. Studies of the pathogenic mutations in presenilins 1 and 2 (PS1 and PS2) and amyloid precursor protein (APP) responsible for early onset familial AD have estabilished central roles for perturbed cellular Ca^{2+} homeostasis. Studies of apolipoprotein E (ApoE) neurotoxic effects in AD confirmed involvement of Ca^{2+}-mediated mechanisms. Futher consequences of Ca^{2+} alterations in AD underline the importance of the ER and mitochondria as the regulatory sites involved in the pathogenesis of neuronal degeneration. Alterations of Ca^{2+} homeostasis include cells from peripheral tissues, including lymphocytes and fibroblasts from AD donors.

The correspondence should be addressed to: M. Brzyska, Email: mbrzyska@nencki.gov.pl

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INTRODUCTION

Alzheimer's disease is a progressive neurodegenerative disorder and the most frequent case of dementia. It is characterised clinically as a decline of intellectual function of insidious onset, that is associated with changes in behavior and impairment of social and professional activities, and is reflected in every day life functions (Blass 1985). Memory loss is a major feature of the clinical syndrom. Gradual impairment of cognitive functions caused by neurodegeneration in selected regions of the brain: hippocampus, amygdala and neocortex is accompanied by two main pathological changes: presence of a large number of extracellular neuritic plaques and intracellular neurofibrillary tangles. Main constituent of neuritic plaques is beta-amyloid (βA), 40-42/43 amino acid peptide derived through the processing of APP, while the neurofibrillary tangles are intracellular lesions consisting of twisted filaments of aberratively phosphorylated cytoskeletal tau protein.

Alzheimer's disease does not have a simple etiology. Although the majority of cases of AD occur at late age as a "sporadic" event, there are families in which illness is inherited as an autosomal dominant disease of early onset. There are at least three genetic loci known to participate in the etiology of early AD onset: the APP gene on chromosome 21, the PS1 gene on chromosome 14, the PS2 gene on chromosome 1. ApoE gene on chromosome 19 and alpha2-macroglobulin (A2M) gene on chromosome 12 are regarded as additional risk factors (Hutton et al. 1998).

Numerous mechanisms for the neuronal cell death in AD have been proposed. Genetic, neuropathological and biochemical studies indicate an important role of βA in the pathogenesis. Although the mechanism of βA neurotoxicity is complex, disruption of calcium homeostasis may be a part of it.

Ca^{2+} plays an important role in regulating a great variety of brain processes. Like other cells, neurones use both extracellular and intracellular sources of calcium.

Reversible complex formation between Ca^{2+} and proteins belonging to the family of proteins called EF-hand proteins regulates its concentration inside the cell. They change their conformation after binding Ca^{2+} (essentially they become more hydrophobic), approach the cellular target and collapse around its binding domain. Representative members of EF-hand protein family are: parvalbumin, calcineurin, S100 family, sorcin. They may function as a committed separate subunit of a single (enzyme) protein or as a subunit that associates reversibly with different proteins (e.g. calmodulin - CaM). They may even be an integral portion of the enzyme sequence (e.g. calpain). Other protein that also carry Ca^{2+} signals are the annexins, gelsolin and proteins containing C2 domains (Carafoli 2002).

Among many other molecular targets of internal Ca^{2+} signaling, α, β and γ PKC isozymes are activated and translocated by combination(s) of Ca^{2+}, DAG and arachidonic acid (AA). Elevated Ca^{2+} also acts on Ca^{2+}/calmodulin-dependent (typeII) kinase(s) (CaM kinases) that in turn can regulate voltage-dependent K^{+} channels (Sakakibara et al. 1986), cholinergic control of neuronal responsiveness (Muller et al. 1992), smooth muscle contraction (McCarron et al. 1992), and synaptic transmission (Goldenring et al. 1984).

In cell Ca^{2+} is sequestered by various organelles such as sarcoplasmic/endoplasmic reticulum (SR/ER), mitochondria and extruded across the plasma membrane by energy-dependent transport systems. The versatility of Ca^{2+} signaling arises both from the ability of cells to employ a range of mechanisms to generate stimulus-induced Ca^{2+} signals with defined characteristics and the existence of many proteins binding Ca^{2+} that mediate the effects of Ca^{2+}. Extracellular Ca^{2+} concentration usually vary from 1 to 2 mM, while intraplasmatic level are much lower (50-200 nM). The large concentration gradient is maintained largely by removal of Ca^{2+} from the cytoplasm by plasma membrane and ER Ca^{2+}-ATPases. Ca^{2+} influx can occur in response to: 1) opening of plasma membrane voltage-dependent and ligand-gated Ca^{2+} channels, 2) activation of receptors coupled *via* GTP-binding proteins to phospholipase C and production of inositol 1,4,5-triphosphate (IP_3) and 3) other second messengers. The Ca^{2+} concentration gradient across the ER membrane is maintained by the sarco(endo)plasmic reticulum calcium ATPases (SERCA), which pump calcium ions into ER in an energy-requiring process against concentration gradient. ER contains two types of Ca^{2+} channels that regulate calcium release into the cytosol, the inositol 1,4,5-trisphosphate receptor (IP_3R) and the ryanodine receptor (RyR). Receptor-mediated activation of G proteins that induce phospholipase C (PLC) at the outer ER membrane cleaves phospatidylinositol biphosphate (PIP2) to generate 1,2-diacylglicerol (DAG) and second messenger IP_3 which activates the IP_3R-mediated release of Ca^{2+} from the ER. Alternatively, release can also occur through tyrosine-kinase receptors (Berridge et al. 2000). Endogennous signaling pathway(s) that acti-

vate RyR-mediated release of Ca^{2+} is less known. It depends on the level of Ca^{2+} already present in the cytosol, and cyclic ADP ribose (cADPR) activation. However, both the endogenous levels and initial signaling molecules for cADPR have not been elucidated. Ca^{2+} release and uptake by ER is also modulated by several different proteins, including these interacting with IP_3Rs and RyRs. This group comprises FKBP (FK506 binding protein, a 12kDa cytosolic protein associated with RyR - the receptor for FK506, immunosupressant drugs and rapamycin) (Brillantes et al. 1994, Cameron et al. 1997), the protein phosphatase calcineurin (Cameron et al. 1995), which interacts with IP_3Rs, the Ca^{2+} binding protein calmodulin (Yamada et al. 1995), which modifies IP_3R activity, ankyrin (Bourguignon and Jin 1995), a cytoskeleton-associated protein that is suggested to link actin filaments to ER, sorcin (Pickel et al. 1997), a 22 kDa Ca^{2+}-binding protein associated with RyR and PS1, a 49 kDa integral ER membrane protein that interacts with the RyRs and has been implicated in the pathogenesis of AD (Mattson et al. 1998).

Degenerating neurons in brains of AD patients showed increased level of calcium, as suggested by colocalization of neurofibrillary tangles with Ca^{2+} measurements (Murray et al. 1992) and increased activation of Ca^{2+}-dependent enzymes (Nixon et al. 1994).

Calcium dysregulation in AD includes also APP processing regulation, endoplasmic reticulum (ER) dysfunction, mitochondrial changes and gene expression alterations.

CALCIUM AND APP PROCESSING

There is a reciprocal relationship between APP and calcium in AD: calcium dyshomeostasis affects APP processing, and changed APP products can modify calcium homeostasis. Moreover, establishement which process is primary does not seem to be possible at this moment.

APP belongs to a type 1 transmembrane family of glycoproteins that is ubiquitously expressed in several types of cells. In neurones prevailing form of APP is APP695. The N-terminal moiety of APP is projected toward the extracellular domain or can be localized in the lumen of intracellular vesicles, such as those of the ER, Golgi apparatus, and intracellular endosomes (Neve and

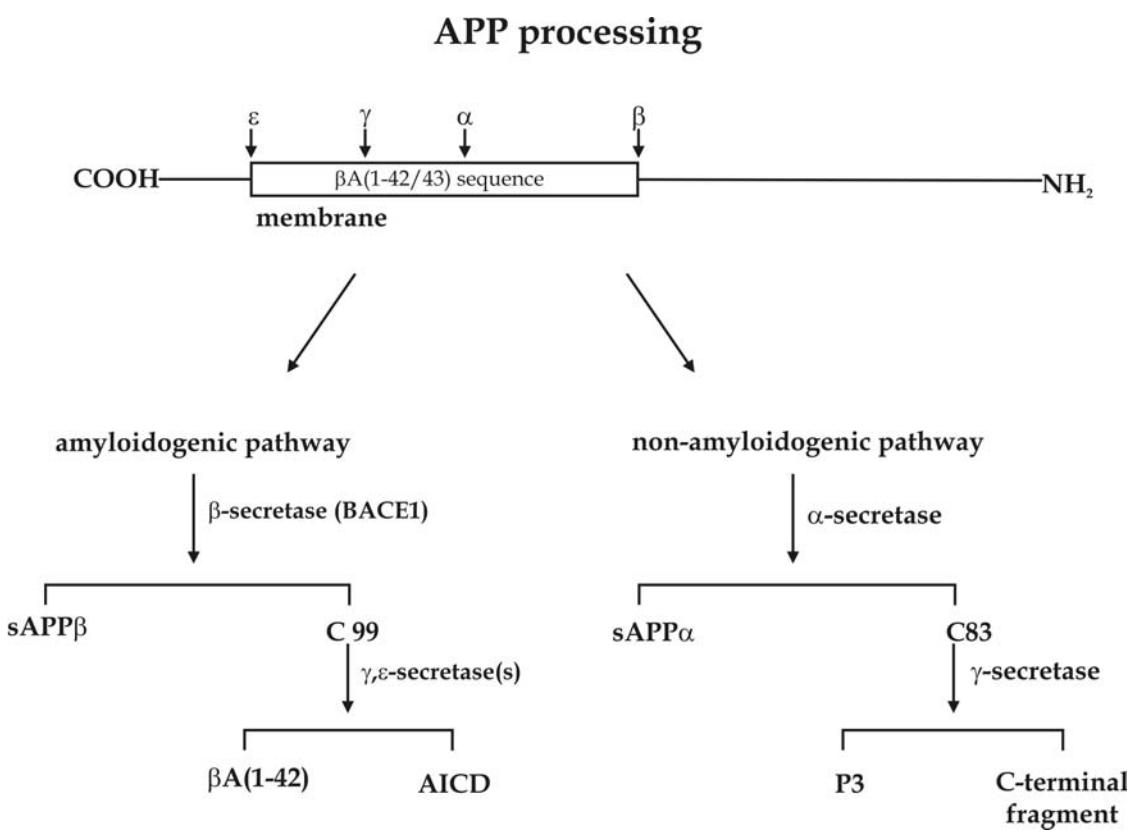

Fig. 1. Alternative pathways of APP processing.

McPhie 2000). The APP C-terminal region lies in the cytoplasmic domain (Kang et al. 1987). βA is formed from APP by intracellular cleavage at N-terminus of βA by β–secretase (BACE1), and subsequently, at the C-termini by γ–secretase(s). Cleavage by α-secretase - a metalloproteinase associated with membrane, occurs within the βA domain (between residues 16 and 17), thus preventing the formation of amyloidogenic peptide (Vassar et al. 1999). As shown in Fig. 1, independently of the metabolic pathway, there are two main products of APP processing: a larger, soluble N-terminal APP fragment (sAPPβα or sAPPβ, depending on the track leading to its generation) and shorter, C-terminal segment releasing βA(1-40/42) and analogous products of non-amyloidogenic processing.

EFFECTS OF APP DERIVATIVES ON CALCIUM DYNAMICS

Generally, it is believed that secreted N-terminal fragments of APP (sAPP), regarded as neuroprotective, were described to normalize Ca^{2+} levels. sAPPα was significantly more potent than sAPPβ in protecting hippocampal neurons against excitotoxicity, glucose deprivation and βA toxicity. sAPP moderates Ca^{2+} responses after exposure to glutamate, inducing an increase of cyclic GMP production, what promotes activation of K^+ channels and reduces Ca^{2+} levels (Barger et al. 1995). Using syntetic sAPP peptides and various truncated products generated by eucariotic and procariotic expression systems Furukawa and coauthors (1996) localized this activity of sAPPα to amino acids 519-612 at the C terminus. Stabilization of intracellular Ca^{2+} by sAPP can reverse apoptotic changes induced by mutations in PS1 (Guo et al. 1998a). After incubation of sAPP with apoE, which resulted in the formation of heteromeric complex, an enhancement of Ca^{2+}-lowering was observed. ApoE4 isoform, which accelerates AD onset, was less potent than apoE3 in modifying the activity of sAPP (Barger and Mattson 1997).

However, as shown by Ca^{2+} measurements, neurones expressing the membrane-bound form of APP, showed greater responsiveness to applied glutamate than non-expressing control ones (Tominaga et al. 1997) which suggests that only secreted forms of APP have protective properties. Moreover, attenuation of the increase of Ca^{2+} evoked by glutamate and sAPP695 was also described at early stages of hippocampal neurones development (Koizumi et al. 1998). A clue towards possible explanation of this effect may be a result of inverse relationship between APP695 and IP_3R gene expression at both the mRNA and protein level during differentiation (Murray and Igwe 2003). Elevation of Ca^{2+} levels were observed in the presence of C-terminal parts of APP, including βA. Overexpression of C-100 in transfected PC12 cells increased cellular vulnerability to calcium ionophore A23187 (McKeon-O'Malley et al. 1999). Pretreatement of SK-N-SH and PC12 cells with C105 fragment increased intracellular Ca^{2+} concentration, and rendered the cells vulnerable to excitotoxicity. This effect was not observed in U251 cell line, originated from glioblastoma (Kim et al. 2000). C105 was found to inhibit endoplasmic reticulum Mg^{2+}-Ca^{2+}-ATPase, diminishing the ability to sequester calcium, suggesting the contribution of this effect to disrupt calcium homeostasis (Kim et al. 1998). C terminus of APP inhibited IP_3R-mediated intracellular Ca^{2+} release in a dose dependent manner and inhibited Na^+/Ca^{2+} exchanger activity (Kim et al. 1998, 2002). AICD, 50 amino acid C-terminal fragment of APP released by γ-secretase in the amyloidogenic pathway, interacts with transcription factors, participating in gene regulation (La Ferla 2002).

Similarly, a growning number of reports suggests that elevated levels of extracellular βA(1-40/42) and its fragment (25-35) alter Ca^{2+} regulation in different cell types in mammalian brain (see for review: Mattson 1994). ER as well as mitochondria contribute to βA produced intracellular Ca^{2+} increase (He et al. 2002). Stimulatory effects of βA peptide on Ca^{2+} influx were blocked following exposure to IL-1β, suggesting that IL-1β may regulate neuronal responses to βA by regulating Ca^{2+} homeostasis (MacManus et al. 2000).

Potentially, several mechanisms can be responsible for elevation of intracellular Ca^{2+} levels: ability of βA to form Ca^{2+} permeable channels and to generate the oxidative damage to membrane constituents including enzymes, channels, receptors and other Ca^{2+} transporting and buffering systems (Gibson et al. 2002). Participation of existing Ca^{2+}-channels in deregulated Ca^{2+} ions influx into the cell was also studied. βA (1-40/42) and (25-35) fragment induced Ca^{2+} influx in culture *via* Ca^{2+}-channel (Rovira et al. 2002). The effect of βA(25-35), in contrast to 1-40/42 was blocked by nifedipine, a L-type voltage-dependent Ca^{2+}-channel, suggesting that each fragment of βA increase Ca^{2+} concentration by different mechanism: the shorter form by potentiation of L-type Ca^{2+}-channel, while longer pep-

tide by compromised N-type Ca^{2+}-channel (Kasparova et al. 2001). Moreover, Aβ fragments (1-40) and (25-35) bind to IP_3 and IP_4 and could affect intracellular Ca^{2+} levels this way (Cowburn et al. 1995). Stimulation of Ca^{2+}-dependent enzymes and proteins (like phospholipases A, C and D) by βA may be another way of Ca^{2+} dysregulation of (Singh et al. 1997, 1998, Strosznajder et al. 1999). However, responsiveness of neurones to IL-1β presence suggests involvement of more complicated mechanisms connected with neuronal--glia relationships.

EFFECTS OF CALCIUM ON APP PROCESSING

Despite the obvious importance of this process, it has not been elucidated in details. It was shown that APP proteolysis is metal-dependent event, enhanced by calcium (Chen et al. 2000). However, existing data are rather contradictory. First reports revealed that βA formation can be modulated by calcium. Elevated intracellular calcium levels, as well as releasing of calcium from ER stores due to opening of RyRs, were described to increase the production of βA (Querfurth and Selkoe 1994, Querfurth et al. 1997). Glutamate induced secretion of sAPPα, with the parallel Ca^{2+} increase was also described (Jolly-Tornetta et al. 1998). Other works report that irreversible inhibition of SERCA pump by thapsigargin diminished the formation of βA (Buxbaum et al. 1994). Moreover, exposure to calcium ionophore significantly decreased the level of APP mRNA (Westmark and Malter 2001) and secretion of soluble β-secretase cleaved APP without affecting secretion of total sAPP (Sennvik et al. 2001). Secretory cleavage of APP is stimulated by the activation of muscarinic receptors coupled to phosphoinositide hydrolysis. The signaling pathways involved in the releasing process exhibit both protein kinase C- and protein tyrosine phosphorylation-dependent components (Petryniak et al. 1996, Slack et al. 1997). Complexity of APP processing, requiring cooperation of many factors, can be a basis for those discrepancies. More data are necessary to create universal hypothesis unifying all obtained observations.

ROLE OF PRESENILINS IN CALCIUM REGULATION

PS1 and PS2 have 67% identity at the amino acid level. They are integral membrane proteins containing eight transmembrane domains, widely expressed in various tissues, where they are located primarily in the ER of cells. PS1 is widely expressed in neurons, glia as well as in lymphocytes (Mattson and Guo 1997). More than 50 mutations in PS1 and 2 mutations in PS2 have been linked with familial cases of AD (Mattson et al. 2001). The normal functions of PS are linked to notch signaling (Berezovska et al. 2001) and APP processing. Furthermore, PS1 interacts with glycogen synthase kinase (GSK3b), one of the critical protein kinases involved in tau phosphorylation (Takashima et al. 1998, 2001).

Modulation of APP processing connects the PSs with Ca^{2+} regulation. PS1 and PS2 have been shown to form stable complexes with APP and are involved in normal APP processing, therefore mutations leading to PS1 and PS2 with different altered sites appear to be responsible for the erroneous cleavage of APP and generation of βA(1-42/3), the most aggresive variant for plaque deposition in the human brain (Xia et al. 1997). Disruption of APP traficking presumably underlies the pathogeneticity of PSs mutations, although its precise mechanism is still to be elucidated. Neurons from PS1 knockout mice show normal α– and β–secretase activity but absent γ–secretase activity for human APP, suggesting that PS1 may regulate γ–secretase (De Strooper et al. 1998). Although intracellular generation of βA(1-40) is affected in PS1/PS2 deficient neurones, they do not affect βA(1-42) form. This may suggest presence of βA pools formed without the PSs participation (Wilson et al. 2002).

Another way of calcium regulation by PSs is connected with ER receptors. PS1 and PS2 stimulate the IP_3R and this effect is more pronounced for the mutants (Leissring et al. 1999a,b). Moreover, in PC12 cells with PS1 mutation, as well as in primary hippocampal neurons from PS1 mutant knocking mice, RyR expression and function were significantly increased (Chan et al. 2000). Similarly, the release of calcium from ER stores after treatement with thapsigargin, an irreversible inhibitor of the SERCA pump, was also elevated in cells that expressed physiological levels of mutant PS1 (Leissring et al. 2000a). Intracellular Ca^{2+} increase after glucose deprivation and chemical hypoxia is higher in neurons from PS1 mutant mice and the Ca^{2+} release may involve both IP_3Rs and RyRs activation (Mattson 2002). The treatement of PS1 mutant cells with: 1) intracellular chelators, 2) inhibitors of ER calcium channels, such as xestospongin that blocks IP_3Rs or dantrolene, that inhibits RyRs and 3) overexpression of Ca^{2+}-binding proteins protect cells from βA toxicity (Cedazo-Minguez et

al. 2002, Chen et al. 2000). Mutant PS1 knockin cells exhibited a marked potentiation of the Ca^{2+} transients amplitude evoked by antagonist stimulation. These cells also showed significant impairments in capacitative calcium entry (CCE, also known as store-operated calcium entry), an important signaling pathway where depletion of intracellular calcium stores triggers influx of extracellular calcium into the cytosol (Leissring et al. 2000a). In addition, PS1 mutations cause a marked increase in basal protein levels of the pro-apoptotic transcription factor gadd153 and decrease levels of the anti-apoptotic protein Bcl-2 (Milhavet et al. 2002). Cells expressing PS1 exhibit increased sensitivity to death induced by DNA damage, correlated with increased intracellular Ca^{2+} levels, mitochondrial membrane depolarization and activation of ER-associated caspases (e.g. caspase-12) (Chan et al. 2002).

PS1 and PS2 interact with Ca^{2+}-binding proteins: calsenilin, calmyrin (Stabler et al. 1999), μ-calpain (Shinozaki et al. 1998) and sorcin (Pack-Chung et al. 2000), that binds only to PS2. Calsenilin has an ability to diminish the elevated Ca^{2+} release caused by PSs mutations (Leissring et al. 2000b) and probably to change proteolytic processing of PSs (Buxbaum et al. 1998). As well, the expression pattern of calsenilin, which is similar to that of presenilins, suggests that the common locations of these two proteins provide an opportunity for physical interaction *in vivo* (Zaidi et al. 2002). Moreover, calsenilin mediates calcium-mediated apoptosis that is regulated by PS1 (Jo et al. 2003).

APOE AND CALCIUM LEVELS

The three common apoE alleles differentially contribute to the risk of Alzheimer's disease. While apoE2 genotype diminishes susceptibility to disease expression, individuals with apoE4 allele have the highest risk for developing AD. A tandem dimer repeat apoE peptide (ApoEdp) derived from the receptor binding domain of apoE, caused mobilization of intracellular Ca^{2+} *via* G-protein-linked phospholipase C (PLC) pathway as well as influx of extracellular Ca^{2+} by Ca^{2+}-channel (Wang and Gruenstein 1997). Further studies revealed that apoE dose-dependently increases intracellular free Ca^{2+} in order of isoforms E4>E3>E2, demonstrating an isoform specific activation of P/Q type Ca^{2+}-channels (Muller et al. 1998). Finally, studies with transgenic mice model confirmed that the neurotoxic effect of apoE4 is mediated by alteration of Ca^{2+} homeostasis (Veinbergs et al. 2002). Moreover, in contrast to apoE3, apoE4 stimulates the transcriptional activity of cAMP-response element-binding protein (CREB) by activating the intracellular signal-regulated kinase (ERK) cascade and inhibition of Ca^{2+} elevation attenuate the activation of CREB (Ohkubo et al. 2001).

CONSEQUENCES OF Ca^{2+} DYSREGULATION IN ER

Disruption of Ca^{2+} homeostasis is one of the factors that create an ER dysfunction and stress. As ER is not only Ca^{2+} storage and signaling compartment but also patricipates in protein processing and folding, and many of these processes are Ca^{2+}-dependent. Ca^{2+} dysregulations may thus affect all metabolic events occuring in ER. According to the hypothesis that binds ER dysfunction with stress responses, the organellum reacts in three possible ways: 1) unfolded protein response (UPR), 2) ER overload response (EOR), and 3) ER-associated degradation (ERAD) (see for review: Paschen and Frandsen 2001). It seems that in AD the elements of all three ways of response to ER stress were suggested, although at this moment most data document involvement of the UPR pathway (Mattson et al. 2000). Normal cells respond to ER stress by increasing transcription of genes encoding ER-resident chaperones such as grp78/BiP, grp94 and protein disulfide isomerase to facilitate protein folding. Grp78 binds to APP and decreases Aβ(1-40/42) (Yang et al. 1998). In the brains of AD patients grp78 levels are reduced (Katayama et al. 1999). PS1 mutation downregulates the UPR and leads to vulnerability to stress. The mechanisms by which mutant PS1 affects the ER stress response are attributed to the inhibited activation of ER stress transducers such as IRE1, PERK and ATF6 (Imaizumi et al. 2001). Recently, it was suggested that mutant PS1 empedes general translational attenuation regulated by PERK and eIF2alpha, resulting in an increased load of newly synthesized proteins into the ER (Yasuda et al. 2002). PS2 splice variant found in AD brains and induced by hypoxia or Ca^{2+} ionophore exposure has been shown to interfere with the UPR signaling pathway by blocking IRE1p phosphorylation (Sato et al. 2001). Activation of gadd153 expression, characteristic for ER dysfunction, upregulated in conditions of DNA damage was observed in PS1 mutant cells (Milhavet et al. 2002). Although activation of NF-κB transcription factor, leading to an up-regulation of the genes encoding pro-inflamatory proteins, described in

response to Ca^{2+} increase in AD was not studied in relation to ER dysfunction response (Barger and Mattson 1996), it is also a characteristic feature of EOR. Inhibition of proteosome, and ubiquitin-proteosome system in AD (Checler et al. 2000, Lam et al. 2000) may implicate the participation of ERAD response in the pathogenesis of AD.

MITOCHONDRIAL CALCIUM DESTABILIZATION

It has been shown that sustained elevation of cytoplasmic calcium levels can promote mitochondrial oxyradical (superoxide) production, membrane depolarization and ATP depletion (Guo et al. 1998b, 1999), suggesting a contribution of altered calcium homeostasis to mitochondrial dysfunction in AD. Conversely, impairment of mitochondrial function can result in the increased cytoplasmic calcium levels. Studies of experimental models of AD suggest that mitochondrial impairment may promote a dysregulation of neuronal calcium homeostasis (Keller et al. 1998, Kruman et al. 1998). DNA-depleted cells, called ρ^0, repopulated with mitochondria from Alzheimer's disease patients, show increased basal Ca^{2+} levels and enhanced IP3-mediated calcium release accompanied by a decreased mitochondrial calcium sequestration (Sheehan et al. 1997); in the mitochondrial calcium influx participated channels blocked by ADP and cyclosporin A (Shevtzova et al. 2001). Moreover, ryanodine receptor was involved in ER-mediated mitochondrial calcium changes (Mungarro-Menchaca et al. 2002). Recent studies have suggested that βA may be directly toxic to mitochondria (Casley et al. 2002) and it also causes loss of cytochrome c oxidase activity in neurons in culture (Kim et al. 2002). Apoptotic proteins such as p53, Bax, and Par-4 induce mitochondrial membrane permeability changes resulting not only in the release of cytochrome c, but also activation of proteases such as caspase-3 (Mattson 2003).

CALCIUM AND NEUROFIBRILLARY TANGLES

Overactivation of glutamate receptors, as well as other conditions that result in a sustained elevation of intracellular Ca^{2+} levels, can induce alterations in the neuronal cytoskeleton similar to those seen in neurofibrillary tangles (Mattson 1990, Stein-Behrens et al. 1994). Levels of calcium/calmodulin-dependent protein kinase II are increased in neurons prone to neurodegeneration (McKee et al. 1990), and this kinase plays a role in the formation of neurofibrillary tangles as it can associate directly with paired helical filaments. In addition, transglutaminase, a calcium activated enzyme, is increased in AD (Johnson et al.1997), and has been shown to induce crosslinking of tau protein, the major component of neurofibrillary tangles (Miller and Johnson 1995). Although contradictory results suggested that calcium influx caused by βA induced tau hyperphosphorylation which occurs by other mechanism than calcium increase (Shea et al. 1997), recent data revealed that tau phosphorylation induced by βA is concomitant with an increase in both p25 to p35 ratio and Cdk5 activity (without protein levels) and abolished by blockade of L-type Ca^{2+} channels (Town et al. 2002).

CALCIUM OUTSIDE CENTRAL NERVOUS SYSTEM

Alterations in calcium homeostasis implicated in Alzheimer's disease are not confined to neurons. Abnormalities in calcium regulation in the peripheral cells including lymphoblasts (Gibson and Toral-Barza 1992), lymphocytes (Adunsky et al. 1991), platelets (Zubenko et al. 1987) and fibroblasts (Peterson et al. 1985) have been reported. Several aspects of Ca^{2+} homeostasis were investigated in peripheral model.

Signal transduction in peripheral AD cells is changed. Although basal IP_3 levels are similar in AD and control cells, the bradykinin-stimulated increase in IP_3 levels was much greater in AD fibroblasts than in controls. Elevated IP_3 production in response to bradykinin in AD fibroblasts is positively correlated with an increase in the receptor number (Huang et al. 1991). Changes in total cell calcium during ageing and further elevation in AD was demonstrated in fibroblasts (Peterson et al. 1986) and lymphocytes (Hartmann et al. 1994). Cytosolic free Ca^{2+} concentration is also increased in platelets (Le Quan Sang et al. 1993). In addition, lymphocytes showed elevated mitogen-induced calcium responses after exposure to βA (Eckert et al. 1994). Increasing calcium with ionophore elevates production of βA in fibroblasts and this increase depends on external Ca^{2+} (Querfurth and Selkoe 1994).

Exposure of erythrocytes and lymphocytes to β-amyloid peptides generated disruptions of cellular membranes, confirming the hypothesis of pore forming

abilities of the peptide, that could influence ion homeostasis (Mattson et al. 1997).

APP as well as PS1 and PS2 mutations resulted in changed expression of the relative protein products in fibroblasts and lymphocytes. Several abnormalities in lymphocyte calcium signaling have been reported in Alzheimer's disease and Down's syndrom patients with presenilin mutations (Grossmann et al. 1993). In addition, lymphocytes from presenilin mutant mice (PS1) exhibited increased level of intracellular calcium concentrations and a higher calcium response to phytohemagglutinin thus suggesting contribution of PS1 to calcium homeostasis (Eckert et al. 2001).

In addition to their value as an investigational tool for AD molecular and cellular abnormalities, peripheral cells were studied as a potential source of markers suitable for identification of the disease progress and examining the response to future treatement. To investigate the diagnostic potential of peripheral cells, several agonists were applied to induce changes in cytosolic calcium concentration:

a) bradykinin which stimulates calcium release through endoplasmic reticulum IP3 receptor

b) bombesin, a peptide capable to induce G-protein phospholipase C-mediated IP3 generation

c) thapsigargin, an inhibitor of calcium ATPase in ER

d) tetraethylammonium (TEA), a blocker of potassium channels capable to open the K^+ channels in majority of excitable and non exitable cells, leading to the Ca^{2+} influx (see Fig. 2). Abnormal K^+ channel functioning in AD was postulated (Etcheberrigaray et al. 1994), although the data are still contradictory. Our results indicated that TEA responses were delayed in AD fibroblasts with respect to normal controls (Brzyska et al. 2002).

Although it is very tempting to postulate the potential diagnostic value of the above studies, the actual practical tests based on the abnormal cellular calcium changes remain to be proven.

CONCLUSIONS

Calcium is a very important signaling molecule. The ion plays a pivotal role in neuronal signaling. Abnormalities in calcium regulation have been reported in several neurodegenerative diseases. The data described above suggest that calcium homeostasis has been impli-

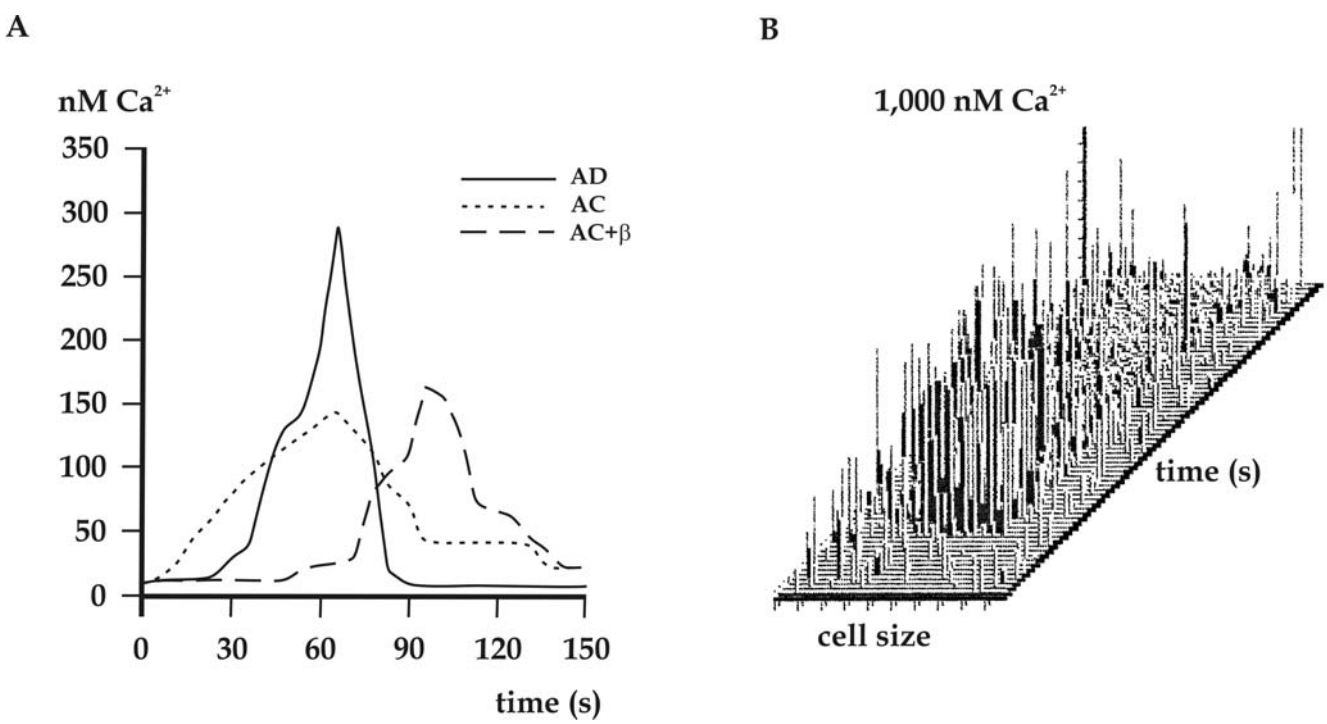

Fig. 2. Opening of K^+ channels by TEA results in Ca^{2+} influx from outside into the cell. A, delayed Ca^{2+} responses to TEA in AD fibroblasts in comparison to control (AC). Control cell incubation with βA(1-40) (1,000 nM, 24 hours) resulted in delayed response in Ca^{2+} influx by K^+ channels. B, visualization of delayed Ca^{2+} influx into an AD fibroblast.

cated in the pathophysiology of AD. The molecular and biochemical nature of the defect observed in the pathology remains not elucidated. The information on the pathogenic mechanisms of calcium metabolism could contribute directly to effective diagnostic, preventive and pharmacological strategies. The above subject is of key importance in ageing and AD research and due to fragmented knowledge about structure-function of the various calcium transporters it deserves futher consideration.

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