Intracellular calcium signalling in Alzheimer’s disease

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Received: September 11, 2009; Accepted: November 9, 2009

Abstract

More than two decades ago, dysregulation of the intracellular Ca\(^{2+}\) homeostasis was suggested to underlie the development of Alzheimer’s disease (AD). This hypothesis was tested in numerous in vitro studies, which revealed multiple Ca\(^{2+}\) signalling pathways able to contribute to AD pathology. It remained, however, unclear whether these pathways are also activated in vivo, in cells involved in signal processing in the living brain. Here we review recent data analysing intracellular Ca\(^{2+}\) signalling in vivo in the context of previous in vitro findings. We particularly focus on the processes taking place in the immediate vicinity of amyloid plaques and on their possible role for AD-mediated brain dysfunction.

Keywords: hyperactivity • plaque vicinity • calcium dyshomeostasis • seizure • in vivo calcium imaging • two-photon microscopy

Introduction

Alzheimer’s disease (AD) is a progressive and irreversible neurodegenerative disorder, characterized by distinct neuropathological lesions. These include extracellular deposits of β amyloid (Aβ) in senile plaques, accumulation of intraneuronal neurofibrillary tangles and profound neuronal death (reviewed in [1–3]). Clinical symptoms include the inability to encode new memories as well as cognitive and behavioural impairments [4]. Most cases of the disease are sporadic, with advancing age being the major risk factor for developing AD. The prevalence of AD rises exponentially with age from approximately 1% at 65 years to 40% after the age of 90 [5–7]. Furthermore, individuals harbouring the e4 allele of apolipoprotein E have an increased risk for developing sporadic, late-onset AD [8, 9]. A small fraction of AD patients, however, have an inherited autosomal dominant form of the disease. These hereditary AD forms are characterized by an earlier onset and are typically caused by mutations in genes encoding human amyloid precursor protein (APP) or presenilin 1 (PS1) and presenilin 2 (PS2) [1, 3, 10]. The presenilins are part of the γ-secretase complex involved in the synthesis of Aβ, which is derived from APP by sequential enzymatic cleavage by β-APP cleaving enzyme and γ-secretase complex [10, 11].

Expressed in transgenic mice, APP and presenilins with familial mutations allow various aspects of AD neuropathology to be modelled. The mutant mice develop senile plaques and neurofibrillary tangles, exhibit dysregulation of the intracellular Ca\(^{2+}\) homeostasis, brain inflammatory response and memory impairment. However, they do not recapitulate the widespread neuronal loss seen in humans [12].

Accumulation of Aβ plays a crucial role in the genesis of AD [2, 3, 13]. Among the three forms of Aβ (Aβ\(^{38}\), Aβ\(^{40}\), Aβ\(^{42}\)), Aβ\(^{42}\) seems to be the most important for the pathogenesis of the disease because it more easily aggregates into oligomers and amyloid fibrils [14]. Mounting evidence suggests that the soluble oligomers (presumably dimers and trimers) are the neurotoxic species in AD [13]. Indeed, naturally secreted small Aβ oligomers have been shown to inhibit long-term potentiation (LTP, [15]), the
electrophysiological correlate of learning and memory [13, 16] and to induce a loss of hippocampal synapses [17–19]. Moreover, similar effects were caused by oligomers extracted from the cerebral cortex of AD patients. In wild-type rodents, human oligomers inhibited LTP, enhanced long-term depression, reduced dendritic spine density and interfered with the memory of a learned behaviour [20]. Interestingly, the soluble Aβ dodecamer (Aβ*56, [21]) also seems to impair memory. Thus, young rats injected intracranially with Aβ*56 purified from the brains of old AD mouse mutants showed a reduced performance in the Morris water maze test (a common test for spatial learning).

As a matter of course, formation of Aβ oligomers is abetted by Aβ accumulation within the brain. Interestingly, mutations associated with inherited forms of AD promote both accumulation and oligomerization of Aβ. Thus, familial APP mutations that flank or occur within the Aβ region alter the amount or aggregation properties of Aβ, whereas mutations within presenilins were found to increase the Aβ42/Aβ40 ratio [10, 13]. Another source of Aβ accumulation within the brain is the imbalance between its production and clearance caused by impaired degradation of Aβ (reviewed in [22, 23]). Aβ is cleaved by several proteases, including neprilysin [24, 25], insulin-degrading enzyme [25], endothelin-converting enzyme [26], plasmin [27] and cathepsin B [28]. Neprilysin [25, 29] and cathepsin B [28] overexpression in transgenic mice reduces total Aβ levels and plaque deposition, whereas their pharmacological blockade or genetic ablation increases Aβ load [28, 30, 31]. The activity/expression of neprilysin is down-regulated with aging and at the early stage of AD [28, 32–34], suggesting that decreased activity of Aβ-degrading enzymes may contribute to the sporadic form of the disease.

In this paper we are going to review recent findings regarding the mechanisms of AD. In particular, we will focus on the role of calcium (Ca2+) signalling as well as the regulation of the intracellular Ca2+ homeostasis in AD, discussing data obtained in various AD mouse models, both in vitro and in vivo.

**Dysregulation of Ca2+ homeostasis in AD**

Numerous studies suggest that besides Aβ accumulation, dysregulation of the intracellular Ca2+ homeostasis might act as an important progenitor of AD. The Ca2+ hypothesis of AD is supported by the fact that many AD-related genes (e.g. those encoding APP, PS1 and PS2) are also involved in Ca2+ signalling [35]. The role of disturbed Ca2+ homeostasis as a proximal cause of brain aging and neurodegenerative processes like AD was postulated by Khachaturian more than 20 years ago [36, 37]. However, it is still debated whether the disturbed intracellular Ca2+ homeostasis is the cause or the result of altered Aβ and tau production [38]. Recent data reviewed below suggest a complex mutually potentiating interaction between Aβ accumulation and Ca2+ dyshomeostasis.

**Aβ accumulation causes Ca2+ dyshomeostasis**

*In vitro* studies have revealed several mechanisms by which Aβ can increase intracellular free Ca2+ concentration ([Ca2+]i). In lipid bilayers, for example Aβ causes the formation of cation-selective ion pores through which Ca2+ passes into the cytosol [39, 40]. The formation of the pores by Aβ oligomers is enhanced by the presence of phosphatidylserine, one of the earliest signs of apoptosis, on the cell surface [41]. Thus, an already suffering neuron with increased phosphatidylserine on the cell surface might show an enhanced vulnerability towards Aβ oligomers. Interestingly, specific blockage of ion-conducting Aβ-channels with the peptide NA-7 eliminates any signs of Aβ-induced apoptosis in cell culture models [42].

Studies in human neuroblastoma cells have revealed that oligomeric Aβ can non-selectively increase Ca2+-permeability of cellular membranes, thus increasing both Ca2+ influx from the extracellular space and Ca2+ leakage from the intracellular Ca2+ stores [43]. Furthermore, Aβ can interact with endogenous Ca2+-permeable channels in the cell membrane, thus increasing NMDA receptor-dependent Ca2+ influx [44] or causing free radical-mediated potentiation of Ca2+ entry through voltage-gated Ca2+ channels [45]. The finding that the blocker of L-type Ca2+ channels nimodipine reduces Aβ-mediated cell death [45] has suggested that neuronal death is directly related to Aβ-induced Ca2+ influx through voltage-gated Ca2+ channels. Other Ca2+-permeable channels, however, are inhibited by Aβ. It blocks, for example presynaptic P/Q-type voltage-gated Ca2+-channels [46] as well as Ca2+-permeable α7-containing nicotinic acetylcholine receptor-channels [47].

Membrane-associated oxidative stress represents another mechanism by which Aβ can impair intracellular Ca2+ homeostasis. Reactive oxygen species (H2O2 and hydroxyl radicals), generated during formation of Aβ oligomers, could attack cell membranes and initiate lipoperoxidation [48]. Membrane lipid peroxidation results in the generation of neurotoxic lipid aldehydes (such as 4-hydroxynonenal), which impair the function of membrane proteins involved in ion transport. The latter include ATPases (e.g. Na+K+-ATPase and Ca2+-ATPase) and glutamate and glucose transporters [49, 50]. The Aβ-mediated impairment of ion-motive ATPases was observed in both primary neuron cultures and synaptosomes from adult postmortem hippocampus [49]. It results in membrane depolarization and opening of NMDA receptor-channels as well as voltage-gated Ca2+ channels (see above), while impaired Ca2+-ATPase activity reduces the ability of the cell to extrude Ca2+ [49]. Impairment of glutamate transport results in increased extracellular glutamate and overstimulation of glutamate receptors, whereas the impairment of glucose transport causes ATP depletion and decreased activity of ion-motive ATPases [50]. Both processes might further increase intracellular Ca2+ levels (reviewed in [51]).

Not only Aβ itself but also other products of APP metabolism may affect the intracellular Ca2+ homeostasis. During APP processing, the amyloidogenic carboxy-terminal fragment is cleaved...
by γ-secretase, liberating the APP intracellular domain (AICD). AICD, too, was shown to influence intracellular Ca\(^{2+}\) signalling [52]. In mouse embryonic fibroblasts, inositol-triphosphate (IP\(_3\))-mediated Ca\(^{2+}\) release from the intracellular Ca\(^{2+}\) stores was significantly reduced both in the absence of presenilins (PS\(_1^{-/-}\)PS\(_2^{-/-}\) mice, γ-secretase inactive) and in the absence of APP (APP\(^{-/-}\) mice). Importantly, this functional deficit was rescued only by cotransfected fibroblasts with cDNA encoding either AICD itself, or AICD-containing parts of APP. Based on the finding that AICD can form a transcriptionally active complex [53], the authors suggested that AICD may affect Ca\(^{2+}\) signalling by regulating the expression of genes involved in Ca\(^{2+}\) homeostasis. This latter statement, however, caused controversial discussions within the AD community (for details see [54, 55]). On the other hand, the activation of the non-amyloidogenic secretory pathway (e.g. cutting APP by α-secretase) results in the generation of sAPP\(_\alpha\). This protein is neuroprotective because it activates \(K^+\) channels via cGMP, thus causing membrane hyperpolarization and reducing Ca\(^{2+}\) influx [56, 57].

### Ca\(^{2+}\) dyshomeostasis increases Aβ production

Several in vitro studies have shown that increased intracellular Ca\(^{2+}\) levels can trigger Aβ formation and aggregation to protofibrils, implicating Ca\(^{2+}\) dyshomeostasis as a possible causal factor in sporadic forms of AD (reviewed in [38]).

Initial evidence that APP processing is regulated by intracellular Ca\(^{2+}\) came from studies in non-neural cells (HEK-293) overexpressing human APP [58, 59]. These early studies have shown that a global increase in the cytosolic free Ca\(^{2+}\) concentration caused by Ca\(^{2+}\)-ionophore A23187 enhances Aβ production. Aβ levels were also increased when stimulating ryanodine receptors (Ca\(^{2+}\) release channels of endoplasmic reticulum [ER]) by caffeine [59]. Thus, Ca\(^{2+}\) release from intracellular Ca\(^{2+}\) stores can also contribute to the genesis of Aβ. Thapsigargin, a compound that inhibits uptake of Ca\(^{2+}\) into ER by sarco-/endoplasmic reticulum calcium ATPases (SERCA), thereby causing an increase in [Ca\(^{2+}\)], augmented the caffeine-stimulated release of Aβ [59]. In CHO cells overexpressing human APP, Buxbaum et al. [60] observed a more complex effect of thapsigargin: the formation of Aβ was stimulated at lower concentrations (10 nM) only and was inhibited at higher concentrations (20 nM). Later studies on neuronal cell cultures supported the finding that Ca\(^{2+}\) dyshomeostasis can influence APP processing. A depolarization-induced increase in [Ca\(^{2+}\)], for example specifically induced the production of large amounts of intraneuronal Aβ42, causing neuronal death [61]. In contrast to non-neural cells [59], however, it was found that Ca\(^{2+}\) release from the intracellular Ca\(^{2+}\) stores was not sufficient to induce generation of Aβ in neurons [61].

Very recently, Dreses-Werringloer et al. [62] identified a novel transmembrane glycoprotein with Ca\(^{2+}\) channel properties, which they called calcium homeostasis modulator 1 (CALHM1). Surprisingly, Ca\(^{2+}\) influx via CALHM1 decreased the total amount of extracellular Aβ. On the contrary, the CALHM1 polymorphism P86L increases Aβ levels by interfering with CALHM1-mediated Ca\(^{2+}\) permeability. These results are in contrast to the previous studies, which have shown that increased transmembrane Ca\(^{2+}\) influx enhances Aβ production (see above). The authors have also suggested that the CALHM1 polymorphism P86L is associated with an increased risk for late-onset AD [62]. This observation, however, was not confirmed in several recent studies analysing the potential association between AD risk and CALHM1 polymorphism in independent datasets of AD patients and control individuals [63, 64]. Thus, the role of CALHM1 as a risk factor in AD remains unclear.

Taken together, the in vitro data strongly suggest that Aβ accumulation causes Ca\(^{2+}\) dyshomeostasis and, vice versa, Ca\(^{2+}\) dysregulation causes Aβ overproduction. Furthermore, increases in [Ca\(^{2+}\)]\(_i\) may trigger Ca\(^{2+}\) -activated kinases, which mediate the phosphorylation of tau [65, 66] thereby facilitating the development of neurofibrillary tangles. These neuropathological changes may worsen disease symptoms and ultimately may lead to neuronal death. The ‘chicken or the egg’ conundrum (i.e. whether Ca\(^{2+}\) acts upstream or downstream of Aβ), however, is very difficult to resolve, since altered Ca\(^{2+}\) homeostasis affects the metabolism of the AD-related pathological proteins (Aβ and tau) and, conversely, the accumulation of these proteins further disturbs the Ca\(^{2+}\) metabolism.

### Presenilins and Ca\(^{2+}\) homeostasis

Presenilins play a double role in the pathogenesis of AD. First, presenilins are the part of the γ-secretase complex, which generates Aβ through APP cleavage (see above). Familial AD-linked presenilin mutations were shown to elevate the concentration of the aggregation-prone form of Aβ (Aβ42) in expense of Aβ40 (reviewed in [10]). As a consequence, the presenilin-mediated elevation of the Aβ42/Aβ40-ratio activates Aβ-dependent mechanisms of Ca\(^{2+}\) dyshomeostasis (see section ‘Aβ accumulation causes Ca\(^{2+}\) dyshomeostasis’). Secondly, presenilins can directly alter intracellular Ca\(^{2+}\) homeostasis. They interact with three key components of the Ca\(^{2+}\) signalling cascade: IP\(_3\) receptors (reviewed in [67, 68]), ryanodine receptors [69–71] and SERCA pumps (Fig. 1, [72]). Already early studies in fibroblasts from AD patients [73, 74] have revealed the altered properties of IP\(_3\) receptor-mediated Ca\(^{2+}\) release from the intracellular Ca\(^{2+}\) stores. Subsequently, potentiation of IP\(_3\)-mediated Ca\(^{2+}\) signals by presenilin mutations has been documented in different experimental systems ranging from Xenopus oocytes to cells from transgenic animals [75, 76]. While the above studies have suggested that exaggerated [Ca\(^{2+}\)]\(_i\) responses in cells expressing mutant presenilins are caused by overfilling of intracellular Ca\(^{2+}\) stores (discussed in [55, 67]), Cheung et al. [68] recently discovered a mechanism that can account for potentiated IP\(_3\)-mediated Ca\(^{2+}\) signalling in the...
absence of elevated Ca\(^{2+}\) within the ER. The authors have shown that presenilins can physically interact with the IP\(_3\) receptor-channel and thereby stimulate its gating activity (Fig. 1). Expression of two different presenilins with familial mutations (PS1 (M146L) and PS2 (N141I)) sensitized the IP\(_3\) receptor-channel to IP\(_3\) and enhanced IP\(_3\)-mediated Ca\(^{2+}\) release from the intracellular Ca\(^{2+}\) stores. In addition to potentiating agonist-induced Ca\(^{2+}\) transients, this sensitization enabled Ca\(^{2+}\) release even at very low resting concentrations of IP\(_3\), causing continuous IP\(_3\) receptor-mediated 'leak' of Ca\(^{2+}\) from the ER.

As shown by studies in human SH-SYSY neuroblastoma cells, presenilins may also regulate IP\(_3\) production by influencing basal activity of the IP\(_3\)-producing enzyme phospholipase C (PLC) (Fig. 1, [77]). Familial PS1 mutations (PS1-ΔE9 and PS1 M146V) enhance PLC activity, thereby increasing IP\(_3\) levels within the cell which, in turn, mediate enhanced Ca\(^{2+}\) release from the intracellular Ca\(^{2+}\) stores [77, 78].

In addition to interaction with the IP\(_3\) receptor-mediated signalling, PS1 and PS2 were shown to interact with the ryanodine receptor at the cytoplasmic side of the ER membrane. For example, they increase Ca\(^{2+}\) flux through brain-type ryanodine receptor-channels incorporated into lipid bilayers (Fig. 1, [70, 71]). As documented in a recent study [79], this interaction seems to be important for the regulation of neurotransmitter release in the hippocampus. Using a genetic approach for selective inactivation of presenilins both in presynaptic and in postsynaptic neurons of the Schaeffer-collateral pathway, Zhang et al. demonstrated that presynaptic inactivation of presenilins impairs synaptic glutamate release and LTP. The underlying mechanism involves ryanodine receptor-mediated Ca\(^{2+}\)-induced Ca\(^{2+}\) release from the ER. Presenilins seem to control this Ca\(^{2+}\) release, thereby modulating the release probability of neurotransmitters.

Along with Ca\(^{2+}\) release channels, Ca\(^{2+}\) pumps are the key component of the Ca\(^{2+}\) regulatory system. Since many presenilin...
mutations lead to enhanced filling of Ca^{2+} stores, presenilins might also regulate Ca^{2+} pumps. Indeed, using gain-of-function and loss-of-function approaches in both mammalian cell culture and Xenopus oocyte models, Green et al. [72] have demonstrated that presenilins physically associate with SERCA pumps and are necessary for their proper function (Fig. 1). Furthermore, modulating SERCA activity in CHO cells altered the amount of Aβ produced by these cells [72]. These data suggest that dysregulation of SERCA pumps (caused by AD-relevant presenilin mutations) may contribute to the pathogenesis of AD

Presenilins not only modulate the function of the other Ca^{2+} regulating proteins, but also seem to form Ca^{2+}-permeable ion channels themselves (Fig. 1). In a recent study, Tu et al. [80] report that in planar lipid bilayers, wild-type presenilins form low-conductance Ca^{2+}-permeable channels, which account for ~80% of passive Ca^{2+} leak from the ER. Notably, this ability of presenilins is disrupted by two AD-relevant presenilin mutations, PS1-M146V and PS2-N141I [80]. These results were confirmed by a subsequent study demonstrating that out of 6 familial AD mutations tested, five mutations abolished Ca^{2+} leak function of presenilins [81]. The consequence of an impaired Ca^{2+} leak from the ER would be again an overfilling of the intracellular Ca^{2+} stores [82] as well as deficits in capacitative calcium entry [83, 84].

It has to be mentioned that ER is not the only Ca^{2+} store within the cell. Intracellular Ca^{2+} is also buffered by mitochondria (for review see [85]). However, mitochondrial dysfunction in AD seems to be linked to Aβ rather than to presenilins and has recently been discussed in several review articles (see, e.g. [86, 87]). We would like to refer the reader to those papers for further details on this issue.

Taken together, in vitro data suggest that presenilins control the functional state of the intracellular Ca^{2+} stores through interaction with both SERCAs and multiple Ca^{2+} release mechanisms. These complex interactions might explain why presenilin mutations have such widespread effects on intracellular Ca^{2+} signalling. However, it remains unclear to what extent these processes also occur in vivo, in the intact brain tissue.

**Dysregulation of Ca^{2+} homeostasis in vivo**

Recently, three in vivo studies have investigated Ca^{2+} dynamics in the brains of AD mouse mutants. Using an adenoviral-based expression of the genetically encoded Ca^{2+} indicator Yellow Cameleon 3.6, Kuchibhotla et al. [88] observed increased resting intracellular Ca^{2+} levels in dentrites and dendritic spines of APPSwe/PS1-ΔE9 (APP/PS1) transgenic mice. This ‘Ca^{2+} overload’ was extremely evident in close proximity to amyloid plaques. Within 25 μm of a plaque, more than 30% of all neurites exhibited strongly elevated intracellular Ca^{2+} levels (yellow-red neurites in Fig. 2). Interestingly, the Ca^{2+} overload observed in APP/PS1 mice was associated with morphological neuritic alterations. These were mediated, at least in part, by activation of the Ca^{2+}/calmodulin-dependent phosphatase calcineurin [88]. These data support previous studies from the Bacska/Hymans group [89] identifying plaque vicinity as a noxious factor in AD. However, the exact mechanism by which amyloid plaques affect [Ca^{2+}]i in spines and neurites remains unclear.

Notably, in the absence of human APP mutations, mutant PS1 transgenic mice (PS1-ΔE9 or PS1M146V) did not exhibit any neuritic Ca^{2+} overload, suggesting that these mutations alone are not sufficient to induce Ca^{2+} overload in vivo [88]. This result is surprising in view of several in vitro studies pointing to a key role of presenilins in Ca^{2+} dyshomeostasis (see above).

Consistent with data from Kuchibhotla et al. [88], our Ca^{2+} imaging study on double-transgenic mice overexpressing APPSwe and mutant PS1 (G384A) revealed a profound functional impairment in 50% of layer 2/3 neurons [90]. Almost half of these neurons (21% of the total population) were ‘hyperactive’, i.e. displayed increased frequencies of spontaneous Ca^{2+} transients. The other 29% of cells were ‘silent’, showing no Ca^{2+} transients over a 6-min.-long recording period (Fig. 2). Interestingly, the hyperactive cells were found only in close proximity to plaques (~<60 μm from the plaque border; yellow-red neurons in Fig. 2). In contrast to our initial expectation that Ca^{2+} transients in hyperactive cells would result from spontaneous Ca^{2+} release from overfilled intracellular Ca^{2+} stores (see above), these Ca^{2+} transients were tetrodotoxin-sensitive and thus caused by action potential firing. Further analyses have shown that these Ca^{2+} transients are of synaptic origin because they are completely and reversibly blocked by the glutamate receptor blockers CNQX (6-cyano-5-nitroquinoxaline-2,3-dione) and APV (D,L-2-amino-5-phosphonovaleric acid). It remains, however, to be established whether and to what extent the increased frequency of Ca^{2+} transients in hyperactive cells causes the Ca^{2+} overload observed by Kuchibhotla et al. [88].

In the latest in vivo Ca^{2+} imaging study, Bacska and colleagues quantitatively determined resting [Ca^{2+}]i in astrocytes of APP/PS1 mice and observed a global astrocytic response to plaque deposition [91]. Compared to wild-type mice, [Ca^{2+}]i was globally increased in the astrocytic network of 6- to 8-month-old mutant mice (yellow-green astrocytes in Fig. 2). Furthermore, astrocytes in mutant mice exhibited a significant increase in spontaneous activity (see also [92]). Sometimes this activity was correlated over long distances (up to 200 μm), thus forming an intracellular Ca^{2+} wave. While the increase in [Ca^{2+}]i and in the frequency of astrocytic Ca^{2+} transients was independent of plaque proximity, the pacemaker-astrocytes initiating the waves (yellow-red astrocytes in Fig. 2) were located 24.8 ± 7.8 μm from the three-dimensionally nearest amyloid plaque. These data suggest that plaques or plaque-associated bioactive species trigger these Ca^{2+} waves. Notably, increased astrocyte activity was not affected by blocking neuronal activity with tetrodotoxin. Therefore, increased astrocytic activity is not a simple reflection of neuronal hyperactivity in the plaque vicinity.

Taken together, these studies reveal a complex and widespread pattern of dysregulation of the intracellular Ca^{2+} homeostasis.
in vivo, involving several cellular/synaptic mechanisms in two different cell types. Some of these pathological changes are condensed in the plaque vicinity while others, like those in the astrocytic network, are diffusely distributed all over the brain.

AD-mediated hyperactivity and synaptic network dysfunction

The observation that amyloid plaques in vivo are surrounded by hyperactive cells [90] came as a surprise because of the wealth of data showing that AD is associated with synaptic dismantling and 'synaptic failure' [93]. Already early histological studies of brain tissue from AD patients were able to reveal neuronal loss, shrinkage of dendritic trees and a decrease in the density of synapses [94–98]. Further studies in mutant mice have shown that elevated Aβ levels result in spine loss [17, 99–101], reduction in the number of excitatory synapses [102] and depressed glutamatergic synaptic transmission [103, 104] accompanied by glutamate receptor endocytosis [104, 105]. In cultured cortical neurons, application of Aβ induced internalization of NMDA receptors [105], whereas Aβ overexpression in organotypic hippocampal slices promoted AMPA receptor removal followed by the loss of dendritic spines and NMDA responses [104]. In double knock-in mice carrying human mutations in the genes for APP and PS1, only AMPA receptors were down-regulated, while NMDA receptors remained unaffected [106]. In agreement with these data, electrophysiological studies of triple transgenic mice harbouring PS1M146V, APPSwe and tauP301L [107] revealed deficits in LTP. The Aβ-mediated LTP blockade was also observed in vivo [16]. This elegant study demonstrated that inhibition of hippocampal LTP is attributable specifically to oligomers, not monomers or fibrils, of naturally secreted human Aβ.

Fig. 2 Pathological changes in brain parenchyma in the vicinity of amyloid plaques. Schematic drawing illustrating an amyloid plaque (purple) surrounded by neurons (circles), dendrites (branches) and astrocytes (branched cells). Three different types of neurons are illustrated: normally active neurons (white-green), hyperactive neurons (yellow-red) and silent neurons (white-blue; [90]). Dendritic branches with an increased intracellular Ca²⁺ concentration are coloured yellow-red while the ones with normal resting [Ca²⁺]i appear green [88]. The pacemaker astrocytes initiating propagating [Ca²⁺]-waves are shown in yellow-red and the rest of the astrocytes (having an increased resting [Ca²⁺]i) are shown in yellow-green [91]. Note that most of the changes in the intracellular Ca²⁺ homeostasis are restricted to the immediate plaque vicinity (an area situated less than 60 μm from the plaque border, coloured in pink).
increase in neuronal activity. By means of prolonged video-EEG monitoring, the authors observed generalized, sharp synchronous discharges in the cortex and in the hippocampus of mutant mice. Interestingly, some of the hippocampal discharges remained focal, with minimal spread to the neocortex [108]. Subsequently, similar epileptiform activity was observed in other AD mouse models, including Tg2576, hAPP/PS1 and APdE9 mice [109, 110]. Our data, obtained in the cortex of APPSwe/PS1 mice, additionally revealed a population of cells with augmented neuronal activity. Compared to control mice, the fraction of such hyperactive neurons in AD-mutants increased 16-fold [90]. Since application of the GABA_\text{A} agonist diazepam reduced activity of hyperactive neurons to normal levels, and application of the GABA_\text{A} antagonist gabazine was less effective in hyperactive cells compared to normal ones, our results support the hypothesis that hyperactivity is caused, at least in part, by impaired synaptic inhibition. The fact that some neurons become hyperactive and others silent [90] suggests that anatomical remodelling of both excitatory and inhibitory synapses underlies the changes in neuronal activity in AD. This conclusion is supported by the data of Palop et al. [108], showing that spontaneous epileptiform activity in cortical and hippocampal networks is accompanied by GABAergic sprouting in the dentate gyrus. Such abnormal sprouting, this time of excitatory entorhinal axons, was also observed by Phinney et al. [111] within the hippocampus and the thalamus of APP23 mice [112].

According to recent data, increased synaptic transmission enhances APP endocytosis in vivo via clathrin-mediated recycling of synaptic vesicles [113]. This, in turn, increases A_β generation and its release into the brain interstitial fluid. Thus, neuronal hyperactivity is very likely to result in enhanced A_β production and increased conversion of soluble A_β into oligomers and/or plaques. This process may sustain a vicious cycle by further increasing neuronal hyperactivity.

Neuronal hyperactivity: implications for humans

Several studies have shown that AD patients are more susceptible to epileptic seizures than the control population. It is estimated that 7–21% of patients with sporadic AD will develop at least one clinically apparent seizure, which is about 5 to 10 times the risk for non-demented aged people [109, 114]. Patients with the early-onset (familial) form of the disease, in particular those carrying a mutation in the presenilin genes, have a dramatically higher risk for developing seizures. Snider et al. [115], for example, have analysed 18 families with three or more family members exhibiting very early-onset AD (40 years or younger; 106 individuals in total). Out of these 18 families, 12 were analysed with respect to epileptic seizures. The latter were found in 75% of all cases, clearly indicating a close relation between the early-onset AD and seizure activity. Furthermore, the risk of developing seizures is substantially increased in patients with Down syndrome (who have three copies of the APP gene) [116]. Epileptiform activity has also been reported in non-demented carriers of the apolipoprotein E4, a known genetic risk factor of sporadic AD [117].

In many additional cases, neuronal hyperactivity may occur in a milder form (e.g. as interictal epileptiform discharges), thus remaining clinically undetected. Indeed, many clinical symptoms of AD, such as amnestic wandering, agitation, disorientation and, in particular, episodic fluctuations in functionality could be explained by sporadic hyperactivity of respective neuronal networks. Consistent with this hypothesis, a recent fMRI study in humans has reported the failure of task-induced inactivation of default network activity and hippocampal hyperactivation in asymptomatic and minimally impaired older individuals [118]. As revealed by simultaneous Pittsburg compound B imaging, this hyperactivity was associated with significant accumulation of fibrillar amyloid β-protein in the examined brain regions.

Taken together, data obtained in AD patients and mutant mice point towards a strong association between neuronal hyperactivity and AD. This suggests that neuronal (and glial) hyperactivity may represent a mechanism causally related to AD-mediated cognitive impairments.

Plaque vicinity

Although accumulation of cerebral amyloid plaques is the major hallmark of AD, the key question – how exactly do amyloid plaques impair brain function in AD patients – has not been answered. The principal argument against amyloid plaques as the primary toxic species stems from studies showing that the number of amyloid plaques does not correlate well with the severity of cognitive impairments. Indeed, several studies identified patients who had no overt symptoms of dementia antemortem, but post-mortem were found to have many plaque deposits [119, 120]. Other studies indicate that the severity of the cognitive decline in AD patients is better correlated with the concentration of soluble A_β oligomers than with the density of amyloid plaques [121, 122]. In vivo studies in mouse models of AD also confirmed that plaque formation is not necessary for learning and memory deficits [123, 124], thus suggesting that soluble A_β oligomers (and not plaques) are the primary toxic species (reviewed in [2]).

Recent data, however, seem to challenge this hypothesis. Thus, in transgenic mouse models, loss of dendritic spines, shaft atrophy of dendrites and the development of large axonal varicosities were only observed inside and within 15–50 μm of amyloid plaques [99]. Furthermore, repeated two-photon imaging in vivo has shown that plaque deposition precedes neuritic deformation. Indeed, dystrophic neurites become visible only 3 to 4 days after the first appearance of a new plaque [89]. Further studies have shown that (i) increased resting Ca^{2+} concentration in neurites [88], (ii) synaptically driven neuronal hyperactivity [90], (iii) initiation of intercellular Ca^{2+} waves in astrocytes [91] and (iv) the
already mentioned loss of dendritic spines [101] and excitatory synapses [102] are predominantly observed in close proximity to amyloid plaques (Fig. 2). Furthermore, the in vivo data in humans mentioned above [118] show a strong correlation between plaque-associated amyloid deposition and dysfunction of neural systems supporting the formation of new memories. Taken together, these recent in vivo data establish plaques as a critical mediator of cellular/network pathology in AD.

The mechanisms of this plaque-mediated toxicity remain unclear. On the one hand, the concentration of diffusible Aβ oligomers is increased within and in the immediate vicinity of amyloid plaques (about 6.5 μm from the edge of the plaque, [102]). The oligomer-rich volume is 180% larger than the volume of the dense core of the plaque and corresponds to the area with severe decrease in synapse density. Based on this observation, Koffie et al. [102] have suggested that senile plaques act as a potent local reservoir of oligomeric Aβ, which in turn acts as a toxic moiety to synapses in the cortex.

However, Aβ oligomers are not the only potentially toxic species in the plaque vicinity. As revealed by histological studies [125, 126], glial cells, namely activated astrocytes and microglia (the major immunocompetent cells in the brain), cluster around sites of amyloid deposition. These cells produce a wide variety of potentially neurotoxic substances, such as reactive oxygen and nitrogen species, inflammatory cytokines (e.g. interleukins, tumour necrosis factor-α and transforming growth factor-β), prostaglandins, complement system proteins and other inflammatory mediators [127]. Each of these factors may, alone or in concert, contribute to cellular/network dysfunction in the plaque vicinity.

Interestingly, a recent in vivo study has shown that acute application of lipopolysaccharides (the major toxins of gram-negative bacteria inducing inflammatory response in the central nervous system [for review see [128]]) induces aberrant neuronal activity in the rat somatosensory cortex [129]. Notably, lipopolysaccharide-induced pathology manifested itself in a potentiation of somatosensory evoked potentials as well as epileptiform discharges and seizures. This aberrant cortical excitability was prevented by an interleukin-1 receptor antagonist, suggesting that interleukin-1, released by activated microglia, provokes neuronal hyperactivity.

Thus, an amyloid plaque, surrounded by synchronized hyperactive neurons and pacemaker astrocytes, represents a grain of hyperactivity within the brain parenchyma (Fig. 2). Such grains of hyperactivity may trigger a variety of pathological activities, including paradoxically increased fMRI signals [118], interictal epileptiform discharges and epileptic seizures.

Conclusions

Recent high-resolution analyses of cortical function in mouse models of AD revealed a marked dysregulation (mainly potentiation) of intracellular calcium homeostasis in vivo. This is reflected in (i) increased resting Ca$^{2+}$ levels in neurons and astrocytes and (ii) increased frequency of spontaneous Ca$^{2+}$ waves in neighbouring hyperactive neurons and in astrocytic networks. Notably, many of these pathological changes are either restricted to or governed from the immediate vicinity of amyloid plaques. Taken together, these new data identify hyperactive neurons and glia themselves as well as the ‘hyperactive’ plaque vicinity as important vicious species in AD.

Does the ‘hyperactive’ plaque vicinity play a major causal role in AD pathology? Cumulative evidence suggests that this may be the case, although more direct experiments are needed to definitively answer this question. The good news, however, is that this question is relatively easy to address because plaque vicinity can be targeted pharmacologically (e.g. by the drugs recognizing a fibrillar form of Aβ).

Acknowledgements

The authors thank A. Weible for the graphical assistance. This work was supported by grants from the Deutsche Forschungsgemeinschaft (SFB 596, GA 654/1–1).

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