COMMENTARY

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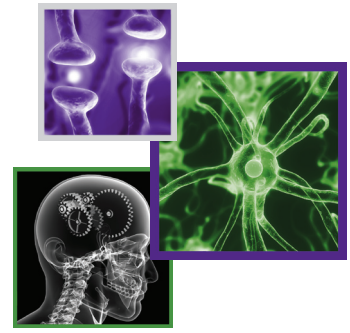
Neurodegenerative Disease Management



Restoring calcium homeostasis to treat Alzheimer's disease: a future perspective



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Alzheimer's disease (AD) is a neurodegenerative disorder that primarily compromises memory formation and storage. Several hypotheses regarding the pathogenesis of AD have been proposed; however, no cure is available to date. Here we describe the calcium hypothesis of AD, which is gaining popularity. We present data supporting this hypothesis and focus on a recently discovered calcium-signaling pathway that is dysregulated in AD and propose targets for the development of disease-modifying therapies.

Alzheimer's disease (AD) is the most common reason for dementia in the elderly. There is currently no cure for AD. Scientists and physicians worldwide are working diligently to pinpoint the problems in AD brains. There are many hypotheses regarding the pathogenesis of AD; in this article, we will summarize one of these, the calcium hypothesis. The calcium hypothesis states that disruption of Ca^{2+} signaling/homeostasis via abnormal functioning of calcium handling proteins such as ion channels underlies the pathogenesis of AD. This hypothesis has gained popularity in recent years as other hypotheses concerning AD (e.g., the amyloid hypothesis) have so far failed to yield disease-modifying therapies. Yet, there is evidence for important crosstalk between dysregulated calcium signaling and amyloid pathology in AD. It was proposed that Aβ peptides (the main constituent of amyloid plaques) form Ca^{2+} permeable pores and bind to and modulate NMDAR, AMPAR, mGluR5 and VGCC, leading to the overfilling of neurons with calcium ions (for review see [1]). It was also proposed that genetically inherited mutations in presenilins influence the production of Aβ42 and increase the Aβ42/Aβ40 ratio [2,3].

Increased cytosolic levels of Ca^{2+} in neurons have been observed in multiple studies involving cellular and mouse models of AD as well as in cells derived from AD patients. Moreover, Ca^{2+} accumulates in intracellular stores such as endoplasmic reticulum (ER) and mitochondria. What is the source of increased calcium content? The majority of early-onset familial AD (FAD) cases are caused by missense mutations in *PSEN1* and *PSEN2* genes. Around

KEYWORDS

• Alzheimer disease • Ca^{2+} signaling • mushroom spines • neuronal store-operated Ca^{2+} channels • synapse

"...the STIM2-nSOC-CaMKII pathway may constitute an attractive target for the development of Alzheimer's disease-preventing therapies."

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226 AD-causing mutations in *PSEN1* have been identified [4]. To explain neuronal Ca^{2+} overloading, it has been proposed that mutant PS decrease activity of intracellular ER Ca^{2+} release channels such as RyanRs [5] and IP3Rs [6], modulate the function of the SERC) [7], and/or affect store-operated Ca^{2+} influx [8,9]. Presenilins, together with nicastrin, anterior pharynx-defective 1 and presenilin enhancer 2, form the γ-secretase complex that cleaves several substrates including APP. Sequential cleavage of APP by β- and γ-secretases constitutes the amyloidogenic pathway that leads to production of toxic Aβ peptides. There was a long-standing debate on whether mutations in PS1 and PS2 proteins cause a loss or gain in γ-secretase function and on whether this loss or gain in function is full or partial [10,11]. This debate was continued recently by Shen, Kelleher and colleagues. They generated two novel presenilin knock-in (KI) models that express the FAD mutations L435F or C410Y [12]. Shen, Kelleher and colleagues report that these mutations cause complete loss of PS1 function *in vivo* and they propose to extrapolate their finding to the whole field of FAD saying that this may be the mechanism of the disease [12]. However, γ-secretase is not a simple molecule it has many different substrates as well as it exists in four different forms [13,14]. Development of AD preventing therapies based on modulation of γ-secretase function requires exquisite precision [14]. The unfortunate outcome of the recent semagacestat trial in Phase III [15] highlights the potential hazards associated with modulation of γ-secretase function, suggesting the need for further research to comprehensively delineate all γ-secretase functions at a precise, mechanistic level before adequate therapeutics can be developed that rely on targeting this multifaceted protease.

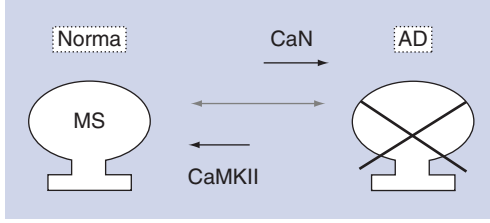

Figure 1. Schema representing the CaMKII/CaN balance in dendritic spines. If the balance is shifted toward favoring CaN activity then mushroom spines are eliminated, synapses are destroyed and cognitive dysfunctions start to appear. If CaMKII activity is maintained and outweighs CaN activity, then stability of mushroom spines is preserved and cognitive functions are normal.
AD: Alzheimer's disease MS: Mushroom spine.

Are there any other functions of presenilins? We recently discovered a new function, namely the formation of ER membrane-delimited ion channels with a passive, low conductance Ca^{2+} leak. Many, but not all, FAD mutations selectively disrupt this function, causing overfilling of ER with Ca^{2+} [16,17]. In support of our hypothesis, the archaeal homolog of presenilin 1 (PSH1) was recently crystallized, revealing a water-filled hole within the protein that traverses the lipid bilayer and presumably permits the flow of Ca^{2+} ions [18]. Notably, the ER leak channel function involves the holoprotein form of PSs, whereas γ-secretase function requires a cleaved form of PSs [16]. Because the FAD-causing M146V mutation disrupts the PS leak function [16,17], we used the PS1-M146V-knockin (KI) mouse model of AD to study the role of dysregulated ER Ca^{2+} homeostasis in AD pathology. We observed that the ER was overfilled with Ca^{2+}, in line with a diminished ER Ca^{2+} leak. To compensate and reduce excessive ER Ca^{2+} loading, KI neurons downregulate STIM2-dependent neuronal store-operated Ca^{2+} entry (nSOC) [19], which may be related to cognitive decline, because STIM2 knockout mice harbor striking spatial learning deficits [20].

What is the function of nSOC in neurons? There is limited information regarding this question. Our laboratory was among the first to demonstrate that nSOC regulates the stability of dendritic spines [19]. Dendritic spines are protrusions on the dendritic shaft where synapses form. There are three groups of spines that can be classified morphologically. Mushroom spines have a big head and thin neck, thin spines have a small diameter head and thin neck and stubby spines have no distinguishable borders between head and neck. Mushroom spines are stable structures that make functionally stronger synapses and are therefore responsible for memory storage [21]. Thin spines are suggested to be 'learning spines' since they scale with increases and decreases in synaptic activity [21]. The function of stubby spines is unclear. It was recently observed that hippocampal spines are less stable [22] in comparison to cortical spines [23]. What underlies the enhanced plasticity of hippocampal spines? How does this influence memory formation and storage? These questions remain unanswered, but we and others have proposed that cognitive decline in AD

reflects the preferential elimination of hippocampal mushroom spines during the progression of the disease. We have demonstrated that tonic nSOC is necessary for stability of mushroom spines [19]. Consistent with this, hippocampal STIM2 downregulation is correlated with worse mini-mental state exam scores in sporadic AD patients [19].

How does nSOC regulate mushroom spine maintenance? We suspect that nSOC regulates the stability of mushroom spines via the activity of Ca^{2+}/CaMKII. CaMKII activity is associated with the formation of long-term potentiation and is highly expressed in synaptic spines. During synaptic loss, decreased CaMKII phosphorylation appears to coincide with increased CaN activity. Indeed, CaN phosphatase activity is enhanced in aging neurons and plays an important role in increased long-term depression [24,25]. We observed that the STIM2-nSOC-CaMKII mushroom spine maintenance pathway is also disrupted in recently developed APP-KI mouse models of AD [26], in conditions of amyloid toxicity [27], in aging neurons and in sporadic AD brains [19]. Intriguingly, pharmacological or genetic rescuing of this pathway in KI mice restores mushroom spines as well as expression of synaptic proteins such as pCaMKII and postsynaptic density protein 95 [19,26,27]. Therefore, we think that the formation and stability of mushroom spines is regulated by the balance of CaMKII and CaN activity. Accordingly, insufficient CaMKII activity and/or disproportionate CaN activity drives mushroom spine elimination, synapse loss and the manifestation of cognitive deficits **(Figure 1)**. On the other hand, if CaMKII activity outweighs CaN activity then the stability of mushroom spines is preserved and cognitive functions are normal. We therefore propose that the STIM2-nSOC-CaMKII pathway may constitute an attractive target for the development of AD-preventing therapies.

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