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Review article

The plasma membrane calcium pumps—The old and the new

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ABSTRACT

The plasma membrane Ca^{2+}-ATPase (PMCA) pumps play a critical role in the maintenance of calcium (Ca^{2+}) homeostasis, crucial for optimal neuronal function and cell survival. Loss of Ca^{2+} homeostasis is a key precursor in neuronal dysfunction associated with brain aging and in the pathogenesis of neurodegenerative disorders. In this article, we review evidence showing age-related changes in the PMCAs in synaptic plasma membranes (SPMs) and lipid raft microdomains isolated from rat brain. Both PMCA activity and protein levels decline progressively with increasing age. However, the loss of activity is disproportionate to the reduction of protein levels suggesting the presence of dysfunctional PMCA molecules in aged brain. PMCA activity is also diminished in post-mortem human brain samples from Alzheimer's disease and Parkinson's disease patients and in cell models of these neurodegenerative disorders. Experimental reduction of the PMCAs not only alter Ca^{2+} homeostasis but also have diverse effects on neurons such as reduced neuritic network, impaired release of neurotransmitter and increased susceptibility to stressful stimuli, particularly to agents that elevate intracellular Ca^{2+} $[Ca^{2+}]_i$. Loss of PMCA is likely to contribute to neuronal dysfunction observed in the aging brain and in the development of age-dependent neurodegenerative disorders. Therapeutic (pharmacological and/or non-pharmacological) approaches that can enhance PMCA activity and stabilize $[Ca^{2+}]_i$ homeostasis may be capable of preventing, slowing, and/or reversing neuronal degeneration.

1. Introduction

Calcium (Ca^{2+}) is an important second messenger molecule that plays a crucial role in regulating diverse neuronal functions such as release of neurotransmitters, signal transduction, induction of gene expression and synaptic plasticity [1–3]. The intracellular Ca^{2+} $[Ca^{2+}]_i$ needs to be tightly controlled in terms of time, space and amplitude to enable it to serve as a signaling molecule [4]. Upon stimulation, Ca^{2+} enters neurons via the voltage-gated Ca^{2+} channels (VGCC) and/or ligand-gated Ca^{2+} channels but within a matter of milliseconds, the 10,000-fold Ca^{2+} gradient that exists across the plasma membrane under resting conditions is restored. This enables the neuron to prepare for the next round of stimulation. The return to baseline $[Ca^{2+}]_i$ is the consequence of a complex interplay between several Ca^{2+} regulating systems including buffering by the Ca^{2+} binding proteins calmodulin (CaM) and calbindin, uptake into the mitochondria, sequestration into the endoplasmic reticulum by the sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA), and transport across the plasma membrane by the sodium Ca^{2+} exchanger (NCX) and the plasma membrane Ca^{2+}-ATPase (PMCA) [5,6].

The PMCA plays a critical role in fine tuning $[Ca^{2+}]_i$ and maintaining neuronal Ca^{2+} homeostasis [7]. It extrudes cytoplasmic Ca^{2+} to the extracellular milieu via active transport by using energy from ATP hydrolysis. The PMCA is an integral membrane protein with 10 transmembrane domains. The functional domains are present in the cytosol with the active site localized between the 4th and 5th transmembrane domains. This region forms an intracellular loop which houses the ATP binding site and the phosphorylation site. The PMCA is physiologically stimulated by the Ca^{2+} sensor protein CaM [8]. Under resting conditions (low cytosolic Ca^{2+}), the protein is in its auto-inhibited state in which the C-terminal auto-inhibitory domain interacts with and blocks the ATP binding site [9]. Increase in $[Ca^{2+}]_i$ is accompanied by the binding of Ca^{2+} to CaM, conformational changes in CaM, and interaction of the Ca^{2+}-CaM complex with the auto-inhibitory domain which then dissociates from the active site [9,10]. Exposure of the active site allows for ATP binding, phosphorylation of a conserved aspartate residue, and transport of Ca^{2+} to the extracellular environment. There are four PMCA isoforms each with their own set of splice variants [11,12]. Among these isoforms, PMCA1 is considered to be the housekeeping form being ubiquitously expressed in all tissue types. PMCA4 null mice are male sterile, suggesting a more specialized role for this isoform [13]. PMCA2 and PMCA3 are tissue-specific, being expressed exclusively in excitable cells such as neurons and skeletal muscle cells [14]. PMCA isoforms and their variants have different

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affinity for Ca^{2+} and CaM and are thus positioned to shape the dynamics of Ca^{2+} handling in a cell specific manner [15–17]. PMCA2 has the highest affinity for Ca^{2+} and, therefore, responds the fastest to increases in cytosolic Ca^{2+} even in the absence of CaM [18]. Not surprisingly, this isoform is abundant in excitable cells such as neurons.

Emerging evidence suggests that the PMCAs play a key role as regulators of Ca^{2+}-dependent signal transduction pathways. By partnering with other proteins, PMCA confines them to a low-Ca^{2+}-microdomain thus modulating the protein's activity as seen with inhibition of the nitric oxide (NO) pathway after the interaction of PMCA with neuronal and endothelial nitric oxide synthase (nNOS and eNOS, respectively) [19–21]. Other studies have shown that the PMCAs interact with the membrane-associated guanylate kinase (MAGUK) family of proteins known to be involved in scaffolding, signaling, and synaptic regulation. Isoform PMCA4b interacts with a MAGUK called the Ca^{2+}/CaM-dependent serine kinase (CASK), a transcription coactivator that regulates synapses by reducing local $[Ca^{2+}]_I$ [22]. PMCA2b (but not PMCA4b) interacts with another protein called the Na^+/H^+ exchanger regulatory factor 2 (NHERF2) [23]. We and others have shown that the PMCA is compartmentalized into lipid rafts, cholesterol-enriched microdomains in the neuronal plasma membrane known to serve as local sites for the orchestration of signal transduction pathways [24,25]. Quantitative mass spectrometric analysis of synaptic plasma membrane (SPM) preparations showed that 60% of the total SPM PMCAs (including all 4 isoforms) are localized in raft microdomains while the remaining 40% are associated with the phospholipid-enriched non-raft domains [26]. Although the physiological significance of the two pools remains unclear, the raft-associated PMCA is uniquely sensitive to its lipid environment. Depletion of cholesterol and gangliosides achieved by exposure of cells to lovastatin, and D-*threo*-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP), respectively, dramatically reduced PMCA activity suggesting a unique mechanism for selective modulation of this pool [24,27]. Marques-da-Silva et al. [28] demonstrated the close association of both NCX and PMCA with Ca^{2+} influx systems such as the L-type voltage-gated Ca^{2+} channels (VGCC), N-methyl-D-aspartate (NMDA) receptors, and redox molecules such as nNOS and cytochrome b_5 reductase within the same lipid rafts. Dynamic sub-microcompartments of high Ca^{2+} were documented near the plasma membrane in neurons exposed to L-glutamate. The presence of both Ca^{2+} influx and efflux proteins within the same membrane microdomain strongly suggests that these entities serve as compartments for organizing and regulating local Ca^{2+} signaling events and promoting crosstalk between Ca^{2+} signaling and redox signaling [28].

2. Disruption of neuronal Ca^{2+} homeostasis in brain aging and neurodegeneration

In the early 1980s, Zaven Khachaturian proposed a largely theoretical hypothesis that brain aging is associated with alterations in neuronal Ca^{2+} homeostasis [29]. A later version of the "Calcium Hypothesis of Aging" asserted that sustained changes in the molecular mechanisms that regulate cellular Ca^{2+} homeostasis play a pivotal role in many of the neural dysfunctions underlying chronic brain disorders associated with aging [30]. It goes on to add that disrupted Ca^{2+} homeostasis serves as a "*necessary precursor and driver*" not only to age-associated neuronal dysfunction but also to the molecular mechanisms underlying neuronal degeneration [30]. Since then, the Ca^{2+} hypothesis has gained universal support and the backing of a large body of scientific evidence. The development of new experimental tools, technologies, and paradigms has allowed the unraveling of the myriad ways in which neuronal Ca^{2+} regulation is modified during normal aging and in age-dependent neurodegenerative pathologies [31–36]. Disruption in the precise regulation of $[Ca^{2+}]_i$ is considered to be a final common pathway leading to neuronal dysfunction and cell death [37–41].

Work done by several groups in the last three decades has clearly demonstrated age-dependent changes in the handling of neuronal Ca^{2+}. Neurons from aged hippocampus show elevations in $[Ca^{2+}]_i$ during repetitive activation [34]. Elevated Ca^{2+} transients have also been recorded in the spines of young adult mice with Alzheimer's disease (AD) pathology [31]. Specific proteins that regulate $[Ca^{2+}]_i$ have also shown to be altered (*reviewed in* [34,36,42–44]). Brain aging is associated with increased activity and protein levels of VGCC, disruption of mitochondrial Ca^{2+} handling, reduced Ca^{2+} buffering capacity, oxidative inactivation of SERCA and CaM, and reduced activity of the NCXs and the PMCAs [45–47]. Given that the Ca^{2+} signal is controlled by the coordinated activity of multiple systems, it is unclear if deficits in a single Ca^{2+} regulatory protein are sufficient to impair Ca^{2+} regulation or whether sustained deficits in several proteins need to occur prior to an overall disruption of neuronal Ca^{2+} homeostasis.

A recent review by the Alzheimer's Association Calcium Hypothesis Workgroup has put forth a framework for integrating old and new evidence into a comprehensive theory underlying neuronal dysfunction in brain aging and the pathogenesis of age-dependent diseases [48]. Defects in Ca^{2+} handling may be the consequence of altered cellular bioenergetics, aberrant cellular metabolism, increase in oxidative and proteotoxic stress, compromise in degradation pathways, and the triggering of immune cascades in brain cells [49–56]. It is interesting to note that these adverse cellular events directly, or indirectly, exacerbate the dysfunction of proteins that regulate $[Ca^{2+}]_i$ thus creating a vicious cycle. The downstream effects of these events manifest as the classical molecular signatures of neurodegeneration such as reduced synaptic function, loss of dendrites and synapses, cytoskeletal pathologies, aggregation of proteins, mitochondrial dysfunction and inflammation [31,49,51]. A significant number of studies have shown a pre-symptomatic role of aberrant neuronal Ca^{2+} homeostasis in promoting the development of the aforementioned molecular signatures thus affirming their role as being the cause and not the consequence of such changes. Emerging research is now focusing on interventions such as dietary calorie restriction, physical exercise, intellectual activity and social interactions as potential neuroprotective strategies that may offset the development of neurodegenerative disorders such as AD [57]. Pharmacological approaches or "Ca^{2+}-targeted therapies" directed at three general targets (Ca^{2+} channels, receptors coupled to Ca^{2+}-regulating proteins, and downstream effectors of Ca^{2+} signals) are also being actively developed with the end goal of preserving Ca^{2+} homeostasis [48]. Future strategies (pharmacological, nutritional, life style and/or behavioral changes) that can boost the function of specific Ca^{2+} regulatory proteins would be of great benefit in alleviating neuronal dysfunction and preventing the development of neurodegenerative diseases.

3. The PMCAs in brain aging and neurodegeneration

The Michaelis group [58–63] was the first to experimentally demonstrate age-associated changes in the PMCAs in SPMs isolated from Fisher 344 rats. The SPMs from aged rats had significantly lower levels of PMCA activity and ATP-dependent Ca^{2+} transport compared to their younger counterparts [61]. Similar findings were observed independently in female Wistar rats [64]. In subsequent studies, we further confirmed the age-dependent decrease in PMCA activity in the longer lived Fisher 344/Brown Norway rats [63,65], a hybrid strain with fewer age-associated pathologies. PMCA activity in SPMs monitored in rats at five different ages representing young adults, middle-aged and aged animals declined progressively with increasing age. Decline in PMCA activity was associated with a statistically significant decrease in V^{max} (~45% reduction at 34 months, the highest age we tested, compared to 5 months) with no appreciable change in the affinity of the enzyme for Ca^{2+} or K_{act}) [63]. One of the possible factors that may contribute to lowered enzyme activity is altered protein levels. Both immunoblot and ELISA analyses showed an approximately 20% reduction in PMCA protein in SPMs from 34 month animals compared to the 5 month young adults [63]. A similar disproportionate decline in