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Evidence for a role of plasma membrane calcium pumps in neurodegenerative disease: Recent developments

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Abstract

Plasma membrane Ca^{2+} ATPases (PMCAs) are a major system for calcium extrusion from all cells. Different PMCA isoforms and splice variants are involved in the precise temporal and spatial handling of Ca^{2+} signals and the re-establishment of resting Ca^{2+} levels in the nervous system. Lack or inappropriate expression of specific PMCAs leads to characteristic neuronal phenotypes, which may be reciprocally exacerbated by genetic predisposition through alleles in other genes that modify PMCA interactions, regulation, and function. PMCA dysfunction is often poorly compensated in neurons and may lead to changes in synaptic transmission, altered excitability and, with long-term calcium overload, eventual cell death. Decrease and functional decline of PMCAs are hallmarks of neurodegeneration during aging, and mutations in specific PMCAs are responsible for neuronal dysfunction and accelerated neurodegeneration in many sensory and cognitive diseases.

Graphical Abstract

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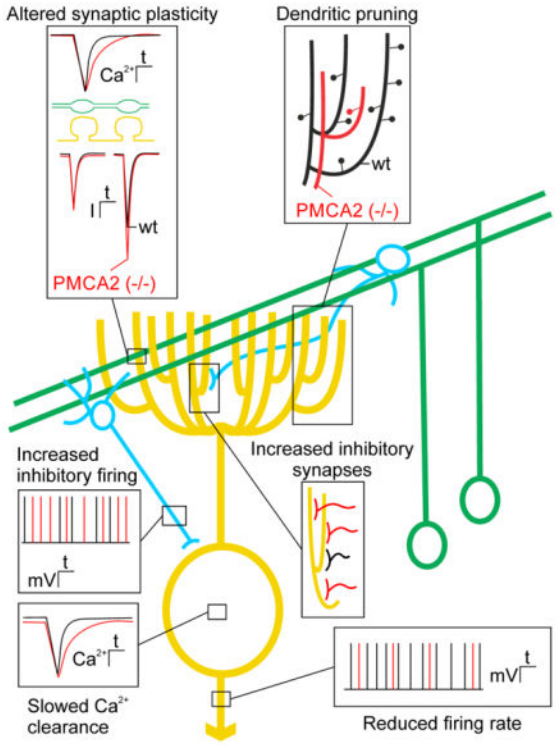

Keywords

Calcium homeostasis; cerebellar ataxia; excitotoxicity; neurodegenerative disease; plasma membrane calcium ATPase

Introduction

Communication in the nervous system is dependent on the generation, propagation, and decoding of signals. Signal transmission at pre- and post-synaptic sites generally involves calcium in its ionized form (Ca^{2+}) as a universal and versatile signaling agent. Ca^{2+} is crucial not only for neuronal development, differentiation, synaptic strengthening and disassembly, but also plays a central role in neuronal cell death [4]. Consequently, the intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ must be precisely controlled in space and time to enable and sustain proper neuronal function [4, 72]. In turn, aberrant Ca^{2+} regulation results in neuronal dysfunction and is an early event in the development and progression of major neurodegenerative disorders including Alzheimer's disease and multiple sclerosis [3, 60, 69].

Because Ca^{2+} cannot be synthetized or destroyed, changes in its cellular concentration require specific uptake and extrusion mechanisms in the cell membrane. While Ca^{2+} influx is mediated by a large number of voltage-, ligand- or mechanically-gated channels and transporters, Ca^{2+} expulsion is dependent on Ca^{2+} pumps and Na^+/Ca^{2+} (or Na^+/Ca^{2+}-K^+) exchangers [5, 50]. Among these, the plasma membrane Ca^{2+} pumps (also known as plasma membrane Ca^{2+} ATPases or PMCAs) are the major high-affinity Ca^{2+} extrusion system

capable of exporting Ca^{2+} ions out of the cell against a large concentration gradient. These pumps are thus important gatekeepers of overall Ca^{2+} homeostasis and are also involved in spatially and temporally defined Ca^{2+} signaling events [14, 98].

In this review we will start with a brief overview of the PMCA isoforms, their basic functional and regulatory properties, and their tissue- and cell-specific expression in the nervous system. In the following sections, the emerging role of the PMCAs in neuropathology will be discussed with an emphasis on recent findings combining *in vitro* studies with animal model-derived data and genetic studies obtained in human populations. The sections are divided according to major neuronal functions, systems or diseases, however, we note that these distinctions are somewhat arbitrary as the PMCAs affect Ca^{2+} regulation in all neuronal tissues, and PMCA dysfunction may be a common trait in all of these conditions.

Overview of the PMCAs in the nervous system

PMCAs are members of the large family of ion-transport ATPases responsible for vectorial movement of ions across biological membranes [79]. They are P-type ATPases characterized by the formation of a phosphorylated aspartate intermediate during the ion-transport reaction cycle [81]. PMCAs are membrane proteins of about 1200 amino acids with calculated molecular masses of 125–140 kDa. Based on biochemical and structural studies the bulk of their mass, including their N- and C-terminal tail, faces the cytosol. PMCAs have ten membrane-spanning segments, with major cytosolic loops between membrane-spanning segments 2–3 and 4–5 (Figure 1). Based on their homology to the SERCA (sarco/endoplasmic reticulum Ca^{2+} ATPase) pumps, for which detailed structural information in different functional states is available [77, 102], the PMCAs are thought to undergo similar conformational changes and operate by a similar mechanism during the ion transport cycle. Structural and mechanistic aspects of the PMCAs have been covered in several recent reviews [13, 14, 78] and will not be discussed further in this contribution.

Four genes encode mammalian PMCAs, giving rise to PMCA isoforms 1–4. Each gene contains alternatively spliced exons whose differential usage results in PMCA variants differing in two specific regions of the protein: the first intracellular loop (splice site A) and the C-terminal tail (splice site C). Over 20 different PMCA splice variants have been identified at the transcript and/or protein level [99]. A schematic overview of the PMCA structure and the major human splice variants is presented in Figure 1. Alternative splicing at site A results in the inclusion or exclusion of a short peptide sequence in the first cytosolic loop, which may affect the membrane targeting of the PMCA isoform [1, 27, 53]. Alternative splicing at site C affects the length and sequence of the C-tail, which thus varies significantly among different PMCA isoforms. Importantly, the C-tail contains the binding site(s) for calmodulin (CaM), the major regulatory protein affecting PMCA activity [99]. Some C-terminal alternative splice variants also differ in their ability to interact with PDZ (PSD95/Dlg/ZO-1) domain-containing signaling and scaffolding proteins, affecting the type of multi-protein complex to which these PMCA variants can contribute [97].

As essential regulators of cellular Ca^{2+} homeostasis, PMCAs are found in all animal cells. PMCA expression in the mammalian brain has been studied in multiple species, showing that all four PMCA isoforms and virtually all known splice variants are expressed in the nervous system, albeit at highly variable levels and with distinct tissue- and cell-specificity [11, 16, 94, 95, 109]. PMCA1, often referred to as “housekeeping” PMCA isoform, is expressed early in (mouse) embryonic development [111] and is present in all tissues, including the nervous system, throughout adulthood. PMCA4, which is also expressed in most tissues, shows differential expression patterns in different regions of the brain. PMCA2 and PMCA3 are predominantly found in excitable tissues and highly enriched in specific cell types such as cerebellar Purkinje cells. In the adult rat brain, PMCA1 is enriched in the cortex and hippocampus and present at lower levels in the cerebellum. PMCA2 and PMCA3 are abundant in the cerebellum and forebrain, and PMCA4 is found at higher levels in the superficial layers of the cortex than in the hippocampus and cerebellum [16].

Using isoform- and splice variant-specific antibodies in high-resolution immunohistochemistry and immunogold electron microsopy, several PMCA isoform variants have been localized more precisely to specific neuron types and to subcellular areas such as dendrites, spines and pre- or post-synaptic membranes within single cells. For example, PMCA1x/a – the major PMCA1 splice variant expressed in the mature brain – is localized in a punctate pattern in the soma and dendrite membranes of pyramidal neurons in the somatosensory cortex and hippocampus [59]. In the rat cerebellar cortex PMCA2 and PMCA3 are prominent in the molecular layer, but whereas PMCA2 is concentrated in postsynaptic spine membranes of Purkinje cells, PMCA3 is present in somata of basket/stellate cells and co-localized with VGLUT1 in what appear to be presynaptic axon terminals of parallel fibers [18]. PMCA2w (likely PMCA2w/b) has been shown to concentrate at the postsynaptic density in hippocampal spines [17]; in contrast, PMCA2a was specifically localized in parvalbumin-positive GABA-ergic presynaptic terminals of inhibitory neurons in areas including the cortex and hippocampus [19].

The distinct expression and cellular localization of PMCA isoforms in different regions and cell types of the brain suggests that these pumps perform specific functions uniquely adapted to the physiological requirements of the particular neurons. PMCA isoforms and splice variants vary greatly in their basal calcium pumping activity and in the kinetics of activation by CaM as well as their regulation by phosphorylation, phospholipid interaction, and proteolysis [14, 78, 97]. Depending on the cellular context and the dynamic interactions between cells, different PMCAs may be needed to control bulk cytosolic [Ca^{2+}] and to regulate spatially and temporally limited Ca^{2+} signals. Considering the importance of precise Ca^{2+} regulation in the proper function of the nervous system, it is not surprising that physiological, genetic, and biochemical studies have suggested an involvement of the PMCAs in neuronal dysfunction including several types of neurodegenerative disease [15].

The role of PMCAs in excitotoxicity

Excitotoxicity, the excessive activation of receptors for the neurotransmitter glutamate, damages neurons as a result of excessive Ca^{2+} influx. Most neurodegenerative disorders have an excitotoxic component and in some, like stroke and epilepsy, glutamate plays a

major role. PMCAs are important in this process because they help neurons cope with the increased $[Ca^{2+}]_i$ and because they are vulnerable to Ca^{2+}-mediated toxicity. Changes in PMCA expression affect sensitivity to glutamate in several excitotoxicity models. Increased expression of message for PMCA2b and c was found in cerebellar granule cells that survived a glutamate insult, suggesting that increased expression enhanced survival [106]. Kainate-induced seizures decreased expression of PMCA isoforms 1 and 2 in hippocampal principal neurons, possibly contributing to the reduced survival of these cells [42]. The importance of PMCA2 in the protection against (excitotoxic) cell death was also demonstrated in spinal cord neurons: siRNA-mediated reduction of PMCA2 by about 50% caused significant cell death within 48 to 72 h *in vitro*, whereas administration of an AMPA receptor antagonist rescued PMCA2 expression and promoted cell survival in a mouse model of experimental autoimmune encephalopathy [64]. Acute excitotoxic increases in Ca^{2+} activate proteases that alter PMCA structure and function. Activation of caspases following excitotoxic insult leads to PMCA2 degradation, Ca^{2+} overload and death [91]. The loss of PMCA2 accelerated the cell death process to “seal the cell’s fate” [91]. Thus, caspase-mediated degradation of PMCAs may confer Ca^{2+} sensitivity to cell death processes not initially triggered by increases in $[Ca^{2+}]_i$, linking apoptotic to necrotic cell death pathways. In contrast, caspase 3 increases the activity of PMCA4b, indicating that functional changes are specific to the PMCA isoform [80]. Glutamate induced increases in $[Ca^{2+}]_i$ activate calpain with a subsequent decrease in PMCA-mediated Ca^{2+} efflux rate [83]. This might seem at odds with studies showing that calpain constitutively activates PMCA4 by cleaving the autoinhibitory domain on the C-terminal of the pump [56]. However, an initial proteolytic increase in PMCA function followed by degradation, either by continued digestion or increased susceptibility to other proteases, is a plausible sequence of events. Ca^{2+} influx via NMDA receptors is more detrimental to PMCA function than comparable $[Ca^{2+}]_i$ increases mediated by voltage-gated Ca^{2+} channels [38]. The source specificity of glutamate neurotoxicity is thought to result from the selective localization of Ca^{2+}-sensitive signaling cascades near the mouths of certain NMDA receptor subtypes [88]. Of note, reciprocal immunoprecipitation experiments showed that PMCA2b associates via the postsynaptic density protein PSD95 with the NR1 and NR2a subunits of the NMDA receptor in the rat brain [43]. The close proximity of PMCA2 and NMDA receptors allows tight spatio-temporal control of postsynaptic Ca^{2+} signaling and its modulation. Cross-talk between the calcium extrusion (PMCA) and calcium influx (glutamate receptor) systems is important for physiological synapse plasticity; for example, PMCA-mediated Ca^{2+} extrusion is reduced in an activity-dependent manner in rat hippocampal spines via Ca^{2+}-mediated inactivation of PMCA function [89]. While important for normal synapse function and plasticity, the intimate relationship between PMCAs and glutamate receptors at excitatory synapses carries significant risk for a catastrophic outcome if one or both of the systems are faulty. Hence even relatively minor disturbances of PMCA function or prolonged stimulation of Ca^{2+} influx may result in excitotoxic insults leading to neuronal death. In general, increased PMCA expression or activation attenuates glutamate toxicity. However, cellular changes in PMCA function in response to an excitotoxic challenge are unique to the specific insult and the particular PMCA isoforms involved.

Cerebral ischemia evokes an excitotoxic response that is further compounded by metabolic changes. PMCAs are exquisitely sensitive to changes in cellular ATP and extracellular pH. PMCAs in cerebellar and sensory neurons preferentially consume ATP generated through glycolysis [48, 55]. This reliance on anaerobic metabolism confers upon PMCAs a resistance to anoxia relative to other ATP-dependent ion pumps [52]. During cerebral ischemia PMCA1 immunoreactivity decreases [66] and following an initial recovery in function a subsequent decline in PMCA expression contributes to the delayed neuronal death of those neurons that survive the initial insult [75]. Overall, the reliance on ATP generated by glycolysis underlies an acute reduction in PMCA mediated Ca^{2+} extrusion during hypoxia/hypoglycemia followed by changes in PMCA expression that contribute to delayed neurotoxicity. Exposure to a brief non-lethal period of ischemia generates tolerance to a subsequent lethal ischemic insult. An increase in PMCA activity following preconditioning contributes in part to this protection [76]. Perhaps pharmacological treatments that increase PMCA expression might form the basis for neuroprotective strategies [29].

The PMCAs have been studied in animal models of epilepsy both after acute induction of seizure activity and in chronic epilepsy. A reduction in PMCA expression in the hippocampus within 1–2 days after seizure induction likely reflects cell loss in the areas affected by acute excitotoxicity. In the chronic stages of epilepsy long-term changes in calcium regulatory mechanisms are observed. Basal $[Ca^{2+}]_i$ levels were increased in hippocampal CA1 neurons, and the recovery time to baseline after an induced Ca^{2+} spike was longer than in control neurons [86]. In agreement with an altered Ca^{2+} homeostasis in surviving neurons in the epileptic brain, Bravo-Martinez et al. [12] showed that PMCA1 transcripts were increased whereas PMCA3 expression was decreased one month after epileptogenic stimulation. This altered expression pattern of the PMCA isoforms likely contributes to the different excitable properties of the epileptic neurons.

The role of PMCAs in altered sensory function

PMCA function is critically important to hearing, vision and nociception. The prominent role of PMCAs in sensory function is exemplified by defects in the ATP2B2 gene encoding PMCA2 that produce dramatic defects in hearing and vision due to the lack of redundancy for the highly specialized roles of this PMCA isoform in cells of the inner ear and retina [47, 115]. A prominent role for PMCAs in primary sensory neurons of the dorsal root ganglion (DRG) is revealed by marked changes in pump function and expression following nerve injury [36].

PMCA2 is localized to the stereocilia of cochlea and vestibular hair cells [26, 53]. The pump transports Ca^{2+} to the endolymph following entry via Ca^{2+} permeable mechanotransducer (MT) channels activated by sound-, acceleration- or gravity-induced deflection of the stereocilia [47]. Elevated $[Ca^{2+}]_i$ within the stereocilia desensitizes the MT channels and is responsible for the adaptation process [8]. Maintenance of endolymph Ca^{2+} levels is critical for regulating Ca^{2+} binding proteins in the tip-links that couple hair cells to each other and enable bending of the stereocilia to gate MT channels [41]. Depletion of endolymph $[Ca^{2+}]$ leads to degeneration of the tip-links, consistent with the hearing loss and impaired balance observed in PMCA2 knockout mice [63]. PMCA isoform 2 splice variant *w/a* is the only

PMCA isoform expressed in the stereocilia of hair cells [49]. This lack of redundancy makes hearing and balance highly susceptible to mutations in PMCA2 that reduce Ca^{2+} transport [39]. Mutations that impair catalytic activity [10, 25] or reduce delivery of functional pump to the stereocilia [96, 101] impair hearing and balance. A reduced rate of Ca^{2+} clearance from the stereocilia would be expected to prolong adaptation and reduce endolymph [Ca^{2+}] altering mechanotransduction [67, 107]. PMCA2 mutations that produce a functional null display haploinsufficiency [70]. Interestingly, heterozygotes expressing a Gly^{283} to Ser mutation exhibit approximately 30% of wild type PMCA2 function [82] and display hearing and balance comparable to control animals. This may explain why PMCA2 mutations without obvious phenotype when expressed alone, potentiate the deafness phenotype of co-existing mutations of other hearing related genes and sensitize individuals to noise-induced hearing loss [62, 90]. Thus, PMCA hypomorphs impair normal hearing and balance and predispose to inner ear toxicity induced by genetic and sensory insults. We speculate that PMCA mutations in general may potentiate the severity of neurodegenerative diseases mediated by Ca^{2+} overload.

PMCAs play a prominent role in synaptic transmission within the retina. They are the dominant mechanism for removal of Ca^{2+} from the presynaptic terminals of bipolar cell ribbon synapses [117]. PMCAs regulate intraterminal $[Ca^{2+}]_i$ at rest and following stimulation, modulating luminance coding and adaptation to background illumination [105]. PMCA2 is expressed both presynaptically in rod terminals and postsynaptically in bipolar cells [30]. Rod spherules employ high affinity PMCAs to maintain low $[Ca^{2+}]_i$ in darkness, which increases their sensitivity and signal-to-noise ratio [58]. The sensitivity of transmission at the rod output synapse is reduced by approximately 50% in deafwaddler mice [30], consistent with a presynaptic site of action. Thus, PMCAs serve as key modulators of scotopic visual signaling.

Increased PMCA activity contributes to the increased excitability of sensory neurons in response to injury. Bradykinin and ATP, mediators of local inflammation, activate PMCA4 in DRG neurons via protein kinase C-mediated phosphorylation [104]. The increased Ca^{2+} clearance rate decreases the activation of Ca^{2+} activated K^+ channels to reduce the amplitude of the action potential after hyperpolarization. Similarly, nerve injury alters Ca^{2+} signaling in primary sensory neurons by increasing PMCA function in axotomized mice [44]. This increase in Ca^{2+} clearance rate results from an increase in the extracellular matrix protein thrombospondin-4 which activates protein kinase C [51]. Short oligosaccharides derived from another element of the extracellular matrix, hyaluronic acid, are also produced by injury. Short oligosaccharides were found to inhibit a CD44-activated kinase cascade in sensory neurons, accelerating PMCA4-mediated Ca^{2+} clearance with a subsequent decrease in the activity of Ca^{2+}-activated K^+ channels [46]. Thus, degradation of the extracellular matrix provides an indirect mechanism via PMCA stimulation to increase the excitability of sensory neurons following injury. Brief intense increases in $[Ca^{2+}]_i$, such as those evoked by injury, stimulate PMCA function priming the pump for the next Ca^{2+} challenge [84], and possibly protecting the cell from accumulating toxic levels of Ca^{2+}. This Ca^{2+} memory phenomenon may result from a slow dissociation of calmodulin from the PMCA [23, 24]. Overall, the acute response to injury appears to be an increase in PMCA-mediated Ca^{2+} clearance that increases excitability and improves tolerance to potentially toxic Ca^{2+} loads.

However, the sustained response to injury is less clear. PMCA2 gene expression is down regulated by more the 50% following contusive spinal cord injury [100]. Axotomy decreases expression of PMCA isoforms 1–3 in large DRG neurons but increases isoform 4 in small sensory neurons [74]. DRG neurons of different size convey information from different sensory modalities, suggesting that changes in PMCA expression may contribute to injury–induced changes in sensory perception. Finally, PMCAs may affect sensory perception in a sex-specific manner: in a recent study, the 50% reduction of PMCA2 in the dorsal horn neurons of Atp2b2$^{+/-}$ heterozygote mice was shown to affect the mechanical and heat-induced pain sensitivity of female, but not male, mice in a modality-specific manner [61]. Remarkably, this was accompanied by significant changes in the expression of several glutamate and GABA receptor subunits and astroglial glutamate transporters in the dorsal horn of the female (but not the male) mice, although PMCA2 was similarly reduced in male and female Atp2b2 heterozygotes. How the reduction in PMCA2 and consequent change of Ca^{2+} signaling affects the expression of the excitatory toolkit in a sex-specific manner is unknown. However, PMCA2 is an integral component of signaling complexes that include glutamate receptors, and the composition, abundance and function of these complexes may be regulated in an estrogen-dependent manner resulting in sex-specific differences in nociception.

PMCAs in cerebellar function: Synaptic function and role in ataxias

PMCA isoforms 2 and 3 are abundant in cerebellum. Their heterogeneous distribution underlies isoform-specific roles in synaptic transmission that when disturbed produce motor phenotypes. PMCA2 is highly expressed in Purkinje neuron soma, dendrites and spines and PMCA3 highly expressed in parallel fiber, but not climbing fiber, terminals [18]. PMCA2 is also expressed in parallel fiber terminals and both the 2 and 3 isoforms are expressed in the basket and stellate cells of the molecular layer [16, 31].

PMCA2 knockout mice exhibit overt cerebellar ataxia resulting from impaired Purkinje neuron Ca^{2+} homeostasis, as well as altered excitatory and inhibitory input to Purkinje neurons (Figure 2). In these mice, Purkinje neuron Ca^{2+} recovery kinetics are slowed and basal $[Ca^{2+}]_i$ is elevated, resulting in a reduced firing rate due to increased activity of Ca^{2+}-activated K^+ channels [32] (Figure 2, bottom insets). Inhibition of PMCA2 results in pruning of the elaborate Purkinje neuron dendritic tree, suggesting that PMCA2-mediated $[Ca^{2+}]_i$ homeostasis is required for maintenance of dendritic structure [92] (Figure 2, upper right). This dramatic simplification of Purkinje neuron structure alters dendritic integration [33]. The parallel fiber to Purkinje neuron synapse displays marked paired-pulse facilitation, a form of short-term synaptic plasticity that results from enhanced neurotransmitter release due to the accumulation of residual Ca^{2+} in the presynaptic terminal [2]. PMCA2 plays a major role in regulating resting $[Ca^{2+}]_i$ and in returning presynaptic $[Ca^{2+}]_i$ to basal levels following weak stimulation of parallel fibers [87]. Thus, in PMCA2 knockout animals the kinetics of Ca^{2+} removal from parallel fiber nerve terminals is slowed resulting in enhanced paired-pulse facilitation [33] (Figure 2, upper left). Altered short-term plasticity likely contributes to the motor impairment in these animals. Finally, PMCA2$^{-/-}$ mice exhibit increased synaptic inhibition onto Purkinje neurons, likely resulting from an adaptive response that includes an increased number of stellate and basket cells and increased firing

rate of these molecular layer interneurons [34] (Figure 2, middle insets). The loss of the "fast" PMCA2a splice variant expressed in basket cell terminals may also contribute to enhanced GABA release onto Purkinje neurons [19]. Thus, several factors contribute to changes in Purkinje neuron spike timing that are responsible for ataxia in PMCA2$^{-/-}$ mice. These include direct effects on Purkinje neuron function and structure as well as altered plasticity of the parallel fiber input and increased inhibitory input.

Subtle changes in $[Ca^{2+}]_i$ regulation observed in PMCA2 heterozygous mice also result in impaired function and reduced survival of Purkinje neurons. Purkinje neurons from PMCA2$^{+/-}$ mice exhibit slowed Ca^{2+} recovery kinetics and reduced frequency of action potential firing, disrupting the accuracy of cerebellar processing and motor coordination [35]. A number of adaptive changes contribute to altered cerebellar function in PMCA2$^{+/-}$ mice. The expression of AMPA receptors and metabotropic glutamate receptors is decreased and Ca^{2+} influx via voltage-gated Ca^{2+} channels increased possibly underlying an age-dependent loss of Purkinje neurons [37]. In contrast, in mice homozygous for the PMCA2 point mutant *wriggle mouse sagami*, Purkinje neurons also have slow Ca^{2+} recovery kinetics, but exhibit reduced Ca^{2+} influx presumably due to down-regulation of voltage-gated Ca^{2+} channels [103]. Thus, the nature of the compensatory/consequential changes that contribute to loss of motor control appear unique to the specific PMCA2 defect.

Defects in PMCA3 are also causally linked to cerebellar ataxia as first demonstrated in an X-exome sequencing study in a family suffering from X-linked congenital cerebellar ataxia [116]. In affected individuals the ATP2B3 gene (located on the human X-chromosome) carries a point mutation resulting in a G1107D amino acid change in the region encoding the calmodulin-binding domain of the PMCA3 [116]. This mutation impairs pump activation and autoinhibition reducing Ca^{2+} clearance rate [20]. More recently, a different PMCA3 missense mutation (R482H) was identified in another patient afflicted by cerebellar ataxia. This mutation impairs Ca^{2+} clearance rate and elevates basal $[Ca^{2+}]_i$ but, is innocuous alone. However, when combined with missense mutations in the 1α subunit of laminin it results in developmental delay, generalized hypotonia and cerebellar ataxia [21]. This appears to be another case of PMCA mutations acting as genetic modifiers, similar to PMCA2 mutants contributing to hearing loss discussed above. Even PMCA mutations that do not result in an overt functional deficit when analyzed *in vitro* may contribute to ataxia, as illustrated by the R35C missense mutation in PMCA3 that co-segregates with the X-linked tremor-ataxia phenotype in the *shaker* rat [40]. PMCAs are integrated in signaling complexes *in vivo*, and even subtle differences in their protein interactions or membrane targeting may suffice to result in changes in Ca^{2+} regulation with pronounced physiological consequences. The R35C mutant of PMCA3 may exemplify this concept although proof of its causative involvement in the shaker rat phenotype is still elusive.

The specialized and non-redundant roles for PMCA gene products in cerebellum and the dramatic phenotypes that result from impaired cerebellar synaptic transmission highlight the diverse roles played by PMCAs in the CNS. Ca^{2+} pumps regulate synaptic plasticity, dendritic structure, and cellular excitability. In the absence of the fine control of $[Ca^{2+}]_i$ provided by PMCAs compensatory changes in inhibitory neurons, expression of

neurotransmitter receptors and Ca^{2+} channel function are important consequences that contribute to impaired motor function.

PMCAs in schizophrenia and autism spectrum disorder

Altered and aberrant communication between neurons is a hallmark of complex neurological diseases such as schizophrenia. Because of the essential role of Ca^{2+} in synaptic plasticity and signal transmission it is not surprising that the PMCA is among the proteins showing highly significant differences in abundance in postmortem brain tissue (from the anterior temporal lobe) in normal vs schizophrenic patients [68]. Unexpectedly, however, the affected isoform is PMCA4, and it is upregulated in the schizophrenic brain compared to the normal controls. Unfortunately, information on the specific role of PMCA4 in neuronal function and survival is limited, hampering interpretation of these data. Since the above proteomic study was performed on whole tissue, it is also likely that cells other than neurons (e.g. glia) contributed to the observed protein changes. PMCA2 may also play a role in schizophrenia: in a pharmacogenomics study, single nucleotide polymorphism (SNP) markers in the ATP2B2 (PMCA2) gene were highly predictive of treatment response to the schizophrenia drug risperidone [54]. In mice, PMCA2 gene expression in the prefrontal cortex is altered with risperidone exposure [54]. However, in the absence of functional data on PMCA2-mediated Ca^{2+} regulation in the schizophrenic vs normal brain the above data remain strictly correlative.

Aberrant neuronal calcium homeostasis is also implicated in autism-spectrum disorders. These complex disorders show a high degree of heritability, with multiple genetic loci contributing to disease susceptibility. Among these, the ATP2B2 gene showed linkage with autism susceptibility in a family-based association study [22]. Association of specific ATP2B2 alleles with autism was further reported in studies on Italian and Chinese populations [85, 108]. The functional consequences of these alleles on the PMCA2 are not yet known. However, because most of the allele-specific single-nucleotide polymorphisms reside in introns of the ATP2B2 gene, they most likely affect PMCA2 mRNA transcription or splicing, resulting in an altered level of PMCA2 or a change in PMCA2 localization.

PMCAs in neuroinflammation, the aging brain and in Alzheimer's disease

An inflammatory response often accompanies neurodegeneration. In multiple sclerosis and its animal model experimental autoimmune encephalomyelitis (EAE), an autoimmune reaction impairs axonal function. PMCA2 expression is markedly depressed in spinal neurons early in the course of EAE and it recovers during remission [73]. Inhibition of PMCA function or knockout of PMCA2 expression in spinal cord neurons *in vitro* mimics much of the neuropathology observed in EAE [65]. Furthermore, PMCA2 heterozygous mice display an age-dependent loss of motor neurons, consistent with a critical role for PMCA2 in the survival of these cells [93]. The mechanism by which this demyelinating disease alters PMCA expression is unclear. The inflammatory cytokine inerleukin-1β modulates PMCA function via a tyrosine kinase pathway [45]; perhaps cytokines also modulate PMCA gene expression.

PMCA function in brain synaptic membranes decreases significantly with aging, and most of this decrease is due to loss of activity rather than to decreased PMCA abundance [114]. A likely reason for the decrease in PMCA function during aging is oxidative stress and the ensuing damage resulting in structural modification and conformational changes of the PMCA as well as its surrounding membrane lipids [112]. The PMCA specific activity is higher in membrane lipid rafts than in non-raft domains, and the raft PMCA appears to be particularly vulnerable to age-dependent decrease in activity [57]. Cholesterol, by increasing lipid order, may partially protect PMCA activity in aging membranes but cannot overcome the decline in PMCA function with aging. The decrease of PMCA in the aging brain could also be due to chronic stress, since high doses of the stress hormone corticosterone reduce PMCA1 expression in the rat hippocampus [9].

Impairment of Ca^{2+} handling is exacerbated in the Alzheimer's disease (AD) compared to the normally aging brain. The Ca^{2+} sensitivity and activity of the PMCA are reduced in the cortex of AD compared to age-matched control brains, and biochemical studies have shown that the amyloid β-peptide characteristic for AD inhibits the activity of the PMCA [6]. The strongest inhibitory effect of Aβ was seen with PMCA4 [6], which is abundantly expressed in the dentate gyrus and CA2 of the human hippocampus [110]. Recent studies have shown that PMCA inhibition by Aβ is effectively antagonized by CaM either by competing for access to the PMCA [7] or by directly binding to Aβ [28]. CaM levels are reduced by over 50% in the frontal and temporal cortex in AD compared to normal brains [71]. This decrease of CaM in the AD brain thus further compounds the "normal" age-dependent decline of PMCA function because CaM not only acts as major activator of the PMCA but may also shield the pump from Aβ inhibition as well as proteolytic and oxidative damage [113].

Conclusion

PMCAs are causally implicated in an ever-growing number of neurodegenerative disorders and complex syndromes, emphasizing the specialized roles that PMCA isoforms and splice variants fulfill in the regulation and fine-tuning of neuronal Ca^{2+} signaling and communication. Altered expression and function of specific PMCA isoforms due to mutation, targeting defects, changing binding partners, or oxidative damage are a characteristic feature of many neuronal diseases. Reduced PMCA function results in altered Ca^{2+} homeostasis and may lead to a sustained increase in $[Ca^{2+}]_i$, which can become toxic to the cell. Future work will continue to show the tight integration of different PMCAs in dynamic processes of neuronal adaptation, function, and repair. Concomitantly, our understanding of the role of PMCA isoforms in diverse neurodegenerative disorders will be enhanced. Ultimately, this may help in the identification of potential strategies for selective PMCA-centered therapeutic interventions.

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Abbreviations

AD Alzheimer's disease

CaM	calmodulin
CNS	central nervous system
PMCA	plasma membrane calcium pump

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Highlights

- PMCA dysfunction impairs precise Ca^{2+} signaling in neurons producing neurotoxicity
- Loss of PMCA function accompanies excitotoxicity and contributes to ischemia and spinal cord pathology
- PMCA2 and PMCA3 mutations are linked to cerebellar ataxias and sensory neuron diseases
- PMCA mutations are often pathogenic when combined with modifier gene mutations
- Decreases in PMCA function during aging and in AD may accelerate neurodegeneration

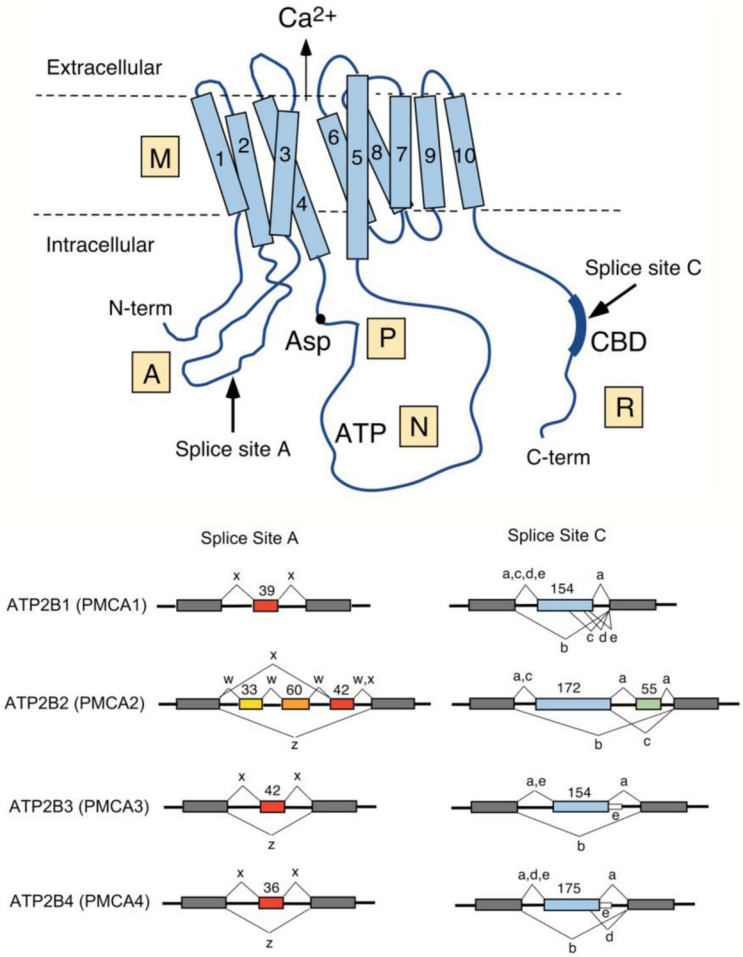

Figure 1. Scheme of the PMCA and major alternative splice pattern of human ATP2B genes

Top: Schematic representation of the PMCA with major domains indicated M, membrane; A, actuator, P, phosphorylation; N, nucleotide-binding; and R, regulatory domain. The ten membrane-spanning regions are numbered, the N- and C-terminal ends are indicated, and the conserved aspartate (Asp) residue phosphorylated during the reaction cycle, the catalytic ATP-binding site (ATP), and the CaM-binding region (CBD) are labeled. The direction of Ca^{2+} transport is indicated by an arrow. Arrows also mark the positions where alternative splicing leads to isoform diversity at sites A and C. *Bottom:* Splicing options of human PMCA genes ATP2B1-ATP2B4. The exon structure in the region of alternative splicing at sites A and C is shown for each ATB2B (PMCA) gene. Constitutively spliced exons are represented by gray boxes, alternatively spliced exons are colored and their size is shown in nucleotides. Splice options are indicated by connecting lines, and the resulting splice variants are labeled by their lowercase symbol. Note that only splice option "x" has been found in PMCA1 at site A, and that variant "e" in PMCA3 and PMCA4 results from a read-through of the last alternatively spliced exon into the following intron (shown as thin white box).

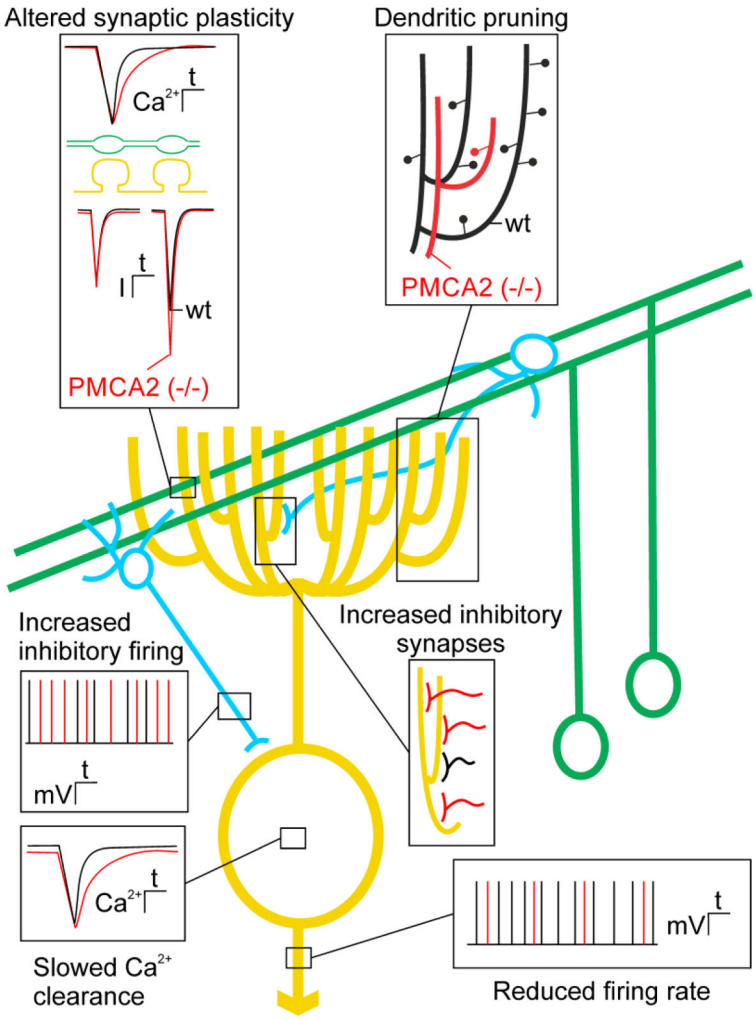

Figure 2. Impaired Purkinje neuron function in PMCA2 knockout mice highlights the role of PMCAs in synaptic transmission

Scheme shows major neuronal connections in a simplified cerebellar circuit showing Purkinje neuron (yellow), parallel fibers (green) and molecular layer interneurons (blue). Insets show pathophysiological changes in PMCA2$^{-/-}$ mice (red) overlaid on wild type responses (wt; black). Loss of PMCA2 alters short-term synaptic plasticity at parallel fiber Purkinje neuron synapses. Slowed Ca^{2+} clearance from presynaptic terminals results in enhanced paired-pulse facilitation of excitatory postsynaptic currents (inset upper left). Loss of PMCA2 results in dendritic pruning. Dendritic purning produces a dramatic simplification of the Purkinje neuron dentritic tree (inset upper right). PMCA2$^{-/-}$ animals exhibit maladaptive changes in inhibitory basket and stellate cells (blue). There are increases in the number of inhibitory synaptic inputs and the rate of inhibitory firing (insets in middle right and left). Ca^{2+} transient recovery in the Purkinje neuron cell body is prolonged and spontaneous action potential frequency is slowed in PMCA2$^{-/-}$ mice (insets on lower left and lower right).