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Review article

The role of Plasma Membrane Calcium ATPases (PMCAs) in neurodegenerative disorders

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ABSTRACT

Selective degeneration of differentiated neurons in the brain is the unifying feature of neurodegenerative disorders such as Parkinson's disease (PD) or Alzheimer's disease (AD). A broad spectrum of evidence indicates that initially subtle, but temporally early calcium dysregulation may be central to the selective neuronal vulnerability observed in these slowly progressing, chronic disorders. Moreover, it has long been evident that excitotoxicity and its major toxic effector mechanism, neuronal calcium overload, play a decisive role in the propagation of secondary neuronal death after acute brain injury from trauma or ischemia. Under physiological conditions, neuronal calcium homeostasis is maintained by a fine-tuned interplay between calcium influx and releasing mechanisms (Ca^{2+}-channels), and calcium efflux mechanisms (Ca^{2+}-pumps and -exchangers). Central functional components of the calcium efflux machinery are the Plasma Membrane Calcium ATPases (PMCAs), which represent high-affinity calcium pumps responsible for the ATP-dependent removal of calcium out of the cytosol. Beyond a growing body of experimental evidence, it is their high expression level, their independence of secondary ions or membrane potential, their profound redox regulation and autoregulation, their postsynaptic localization in close proximity to the primary mediators of pathological calcium influx, i.e. NMDA receptors, as well as evolutionary considerations which all suggest a pivotal role of the PMCAs in the etiology of neurodegeneration and make them equally challenging and alluring candidates for drug development. This review aims to summarize the recent literature on the role of PMCAs in the pathogenesis of neurodegenerative disorders.

1. Excitotoxicity and developmental neuroapoptosis set the narrow marks under which the neuronal calcium system works

Birth and death of neurons are inevitably linked to the maintenance of proper intraneuronal calcium concentrations [1]. Suppression of calcium influx through NMDA-type glutamate receptors during specific periods of development is thought to be causal to the neurotoxicity and massive neuronal apoptosis after early-life application of pharmacological NMDA antagonists [2], different anesthetic drugs [3–5], and ethanol [6]. More physiologically, neurodevelopmental deficits and apoptosis due to insufficient sensory stimulation have been similarly linked to inadequate calcium influx [7–9]. In the adult CNS, temporary loss of calcium influx does not seem to lead to any comparable cellular damage [6,10]. However, it is clear that without an adequate and regulated influx of this ion, primary functionalities of the neuron, such as learning and memory formation or neuronal repair after axonodendritic damage, cannot be sustained [7,10–12].

Conversely, it is the adult and aged CNS that is particularly affected by excitotoxicity, a form of essentially irreversible damage due to excessive stimulation of ionotropic glutamate receptors [13]. Excitotoxicity is probably most relevant as rapidly occurring phenomenon in the wake of acute brain lesions such as trauma or ischemia, and its dominating toxic effector mechanism seems to be an inordinate influx of calcium into the cytosol [13–16]. Downstream of this triggering event, a large number of damage cascades have been described, whose relative importance depends on the intensity and duration of the calcium stimulus, the subcellular site of calcium accumulation and its way of entry into the cell, and the affected neuronal cell type itself [13,16,17]. Aging seems to escalate the toxicity of various excitotoxic agents [18], potentially due to an increased baseline calcium level in aged neurons [19,20]. Hence, it is often assumed that a heightened cellular susceptibility to augmented calcium levels may be an important cause of the increased general sensitivity of the aged brain to trauma and ischemia [21]. By analogy, this conclusion might also apply to the increased incidence of chronic neurodegeneration during aging, even if the etiologic role of excitotoxicity is less firmly established in these diseases [13,22,23].

Excitotoxicity as general phenomenon of secondary damage

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propagation following various primary triggers of injury has led to widespread interest into NMDA receptors and their pharmacological inhibition, as these receptors appear to be the predominant source of toxic calcium concentrations [13,16,17]. Despite intense efforts and compelling animal data, clinical studies with NMDA antagonists as neuroprotective therapy against acute brain injury have strikingly failed [22–24]. Two major reasons have crystallized to explain these failures, which are (i) the problem of the inevitably delayed time of application, and (ii) the apparent lack of a therapeutic concentration window. Regarding the first reason, excitotoxic damage after stroke culminates within a few hours, generally too early to leave much time for calcium influx blockers such as NMDA antagonists to work [24,25]. This temporal issue is common to all neuroprotective strategies targeting acute syndroms like stroke or trauma, and continues to be of major concern also for the present standard therapy of ischemic stroke, tissue plasminogen activator (tPA) treatment [26]. The second reason is more specific, however. Clinical and preclinical data indicate that for typical NMDA antagonists, there is also no therapeutic window in terms of the drug concentrations to be applied [10,13,27]. In other words, drug concentrations needed to elicit significant neuroprotection against excitotoxicity concomitantly cause side-effects or even damage in healthy, unaffected areas of the brain. The molecular explanation for this surprising phenomenon seems to be hidden in the fact that excitotoxicity from NMDA receptor activation does not necessarily require supra-physiological concentrations of glutamate; protracted exposure times suffice. For example, NMDA receptors exhibit EC_{50} values of ~3 μM glutamate [28], which is an already toxic concentration when it is applied to cortical cell cultures for extended periods of time. Concomitantly, extracellular glutamate concentrations may well reach millimolar concentrations in the proximity of an acute cytolytic injury [13]. Hence, competitive glutamate-site antagonists are clearly disfavored as potential therapeutics as they would be overrun by the latter concentrations, unless they were employed at similarly high concentrations themselves. In that case, however, they would totally shut down physiological neurotransmission in unaffected areas of the brain.

To circumvent this and other intricacies of excitotoxicity, various refined strategies of anti-glutamatergic neuroprotection have been pursued more lately, such as uncompetitive, low-affinity open-channel blockers, which inhibit a larger fraction of the receptor response at higher agonist concentrations, a feature urgently desired for anti-excitotoxic drugs [29]. Nevertheless, the attained success was limited in most indications [22,30], or awaits further testing in others [31]. Moreover, the problem of the very narrow therapeutic concentration window has apparently not been solved yet [31]. In animal experiments, even the clinically promoted open-channel blocker memantine was unable to provide protection before it damaged the brain by itself [10,32]. Other anti-excitotoxic strategies that are currently investigated include subunit-specific NMDA antagonists [33], including drugs that may differentiate synaptic versus non-synaptic NMDA receptors [28], or the selective blockade of site-specific postsynaptic calcium actions by untethering proteins deemed particularly toxicity-prone, such as NOS, from the NMDA receptor complex, to which they are connected via the PSD-95 scaffold protein [17,34]. The latter approach has demonstrated surprisingly positive effects in a primate model of ischemic stroke [35], and significant effects in a first clinical trial in patients at risk for iatrogenic stroke after endovascular aneurysm repair [36]. A larger clinical study in stroke patients undergoing endovascular thrombectomy has been launched in early 2017 (www.clinicaltrials.gov: NCT02930018).

Surveying the numerous tested or explored strategies against neuronal calcium toxicity in a broader sense, one comes to conclude that the overwhelming majority has focused on calcium influx control, with a clear lack of interest into calcium efflux. Why could strategies to enhance or stabilize dynamic calcium efflux capacity be utterly interesting? Efflux pumps such as PMCAs are only active if added calcium is present to be exported, and they finish pumping when their setpoint calcium concentrations are reached. Hence, their effect is generally much larger under conditions of heightened calcium influx. Moreover, they respond autonomously to the situation in each cell or subcellular compartment. Thus, PMCAs seem to quite precisely fulfill the requirements of a “pathologically activated drug [target]”, i.e. an ideal anti-excitotoxic drug as sketched on the example of a hypothetical NMDA antagonist [37]. Therefore, the preventive or therapeutic transcriptional induction of the PMCAs, the prevention of their inactivation by proteolytic cleavage [38] or other modifications, and maybe their allosteric activation might all be promising strategies for neuroprotection, lacking the side-effects and inherent difficulties associated with NMDA antagonists. Finally, the physical anchoring of PMCA2 to the PSD-95 complex [39,40] suggests that a pharmacological underpinning of PMCA activity would reduce particularly those calcium components that contribute most to toxicity during calcium overload. Hence, PMCAs are not only interesting for their unquestioned physiological relevance and their established pathophysiological role in specific cases of hereditary disease (with certainly more to come regarding the chronic, age-associated neurodegenerative diseases), they might also constitute drug targets of an unusual, novel kind. Thus, the salient biochemical features of the PMCAs with respect to the nervous system are further explored in the following.

2. PMCAs are ubiquitous calcium pumps that fulfill a special role in the brain

2.1. Evolution and expression of the PMCAs

PMCAs are ATP-driven primary ion pumps that comprise the major high-affinity ($K_D \approx 100–200$ nM) calcium efflux system to remove calcium ions from the cytosol, along with SERCA, the Sarcoplasmic Reticulum Calcium ATPase [41,42]. The task of the PMCAs in the CNS is the controlled restoration of the nanomolar resting level of intracellular calcium after its temporary elevation during calcium signaling. Classic studies using red blood cell PMCA preparations have shown that the extrusion of one Ca^{2+} ion consumes one molecule of ATP, and that PMCA-mediated Ca^{2+} transport is coupled to H^+ uptake, but essentially independent of the concentrations of K^+ and Na^+, and hence, membrane potential [42–44].

PMCAs are members of the superfamily of the P-type ATPases, which is a large, diversified and ubiquitously expressed family of membrane proteins that also encompasses Sodium/Potassium ATPase and SERCA. The PMCAs are encoded by the ATP2B1-4 genes, whose protein products are termed PMCA1-4. For each PMCA isoform, a number of functional splice variants have been identified [45,46], which have a distinct and tissue-specific expression pattern. Whereas PMCA1 and PMCA4 are found ubiquitously, PMCA2 and PMCA3 expression is almost exclusively restricted to the brain. Notably, intermediate or high expression of the PMCAs in human tissues is associated throughout with low mRNA levels, confirming experimental data that PMCAs are very long-lived proteins [47]. Table 1 provides an overview of the observed expression patterns.

To explore the phylogenetic history of the PMCAs, we have mapped all four human genes against the sequences from *Drosophila melanogaster*, *Caenorhabditis elegans*, and *Saccharomyces cerevisiae* (not shown). Even if *Caenorhabditis elegans* already possesses three PMCA-like genes, the human genes most likely originate from the single *Drosophila melanogaster* sequence, which was thus chosen as reference for the analysis of vertebrate PMCA evolution (Fig. 1). PMCA1 is the human PMCA that is closest to the ancient arthropod/vertebrate PMCA precursor. PMCA4 most likely developed from a gene duplication event of PMCA1. PMCA2 and PMCA3, which are both expressed almost exclusively in neurons as mentioned (Table 1), also seem to be the youngest of the human PMCAs and probably branched off from PMCA4. During vertebrate evolution, PMCA1 is the most conserved gene, whereas the neuron-specific PMCA2 and PMCA3 genes exhibit a generally lower degree of