Role of Plasma Membrane Calcium ATPase Isoform 2 in Neuronal Function in the Cerebellum and Spinal Cord

MICHAEL P. KURNELLAS, AMANDA K. LEE, KAROLYNN SZCZEPANOWSKI, AND STELLA ELKABES

Department of Neurology and Neuroscience, New Jersey Medical School, University of Medicine and Dentistry of New Jersey, Newark, New Jersey 07103, USA

Neurology Service, Veterans Affairs, East Orange, New Jersey 07018, USA

ABSTRACT: The distinct role of plasma membrane calcium ATPase 2 (PMCA2) in the function of different neuronal subpopulations in the central nervous system is not well understood. We found that lack of PMCA2 leads to a reduction in the number of motor neurons in the spinal cord of PMCA2-null mice and to abnormal changes in molecular pathways in Purkinje cells. Thus, PMCA2 may have unique, nonredundant functions in spinal cord and cerebellar neurons. Our results suggest that anomalous alterations in PMCA2 activity or expression may induce pathology in some neuronal populations, a possibility that will be the focus of future investigations.

KEYWORDS: ATP2b2; glutamate receptors; axonal/neuronal pathology

Plasma membrane calcium ATPase (PMCA2) is a calcium pump, which is expressed primarily in neurons, including motor neurons of the spinal cord and Purkinje cells of the cerebellum.[1–3] Although a number of studies have implicated dysregulation of PMCA2 in pathological conditions,[4] the importance and the unique contribution of PMCA2 to the function or dysfunction of distinct neuronal subpopulations in the central nervous system is not well understood.

Earlier studies in our laboratory indicated a significant decrease in the levels of PMCA2 in the inflamed spinal cord of rats and mice affected by experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis.[5,6] Subsequently, we showed that a reduction in PMCA activity causes

Address for correspondence: Stella Elkabes, Ph.D., Department of Neurology and Neuroscience, MSB, H-506, New Jersey Medical School-UMDNJ, 185 South Orange Ave, Newark, NJ 07103. Voice: 973-676-1000; ext.: 1156 (office) or 3589 (laboratory); fax: 973-395-7233.
elkabest@umdnj.edu

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pathology of spinal cord neurons, *in vitro*.[7] Inhibition of PMCA activity delayed the clearance of depolarization-induced calcium transients. This was followed by beading of dendrites and axons, cytoskeletal abnormalities, and, finally, death of cultured spinal cord neurons. Motor neurons were most vulnerable to inhibition of PMCA activity, *in vitro*, as they were the first cells to die. In accordance with these findings, quantification of motor neurons in the lumbar spinal cord of the adult PMCA2-null mouse indicated a significant reduction in their number as compared with that in wild-type littermates.[7] Further studies are necessary to determine whether the decrease in the number of motor neurons is due to death and/or developmental abnormalities in the proliferation or differentiation of these cells in the knockout mice. Nevertheless, these findings pinpoint the importance of PMCA2 in motor neuron function.

In addition to hindlimb weakness, which may be partially attributed to motor neuron loss, the phenotype of the PMCA2-null mouse includes ataxia, movement dyscoordination, and balance deficits. Whereas some of these anomalies are due to alterations in vestibular function, as reported previously,[8] they also raise the possibility of cerebellar dysfunction. In fact, the morphometric studies of Kozel *et al.*[8] indicated an increase in the density of Purkinje neurons and a decrease in the density of granule cells. Reductions in the thickness of the molecular layer were also reported, which may be due to loss of dendritic spines or projecting axons, such as parallel and climbing fibers, which innervate

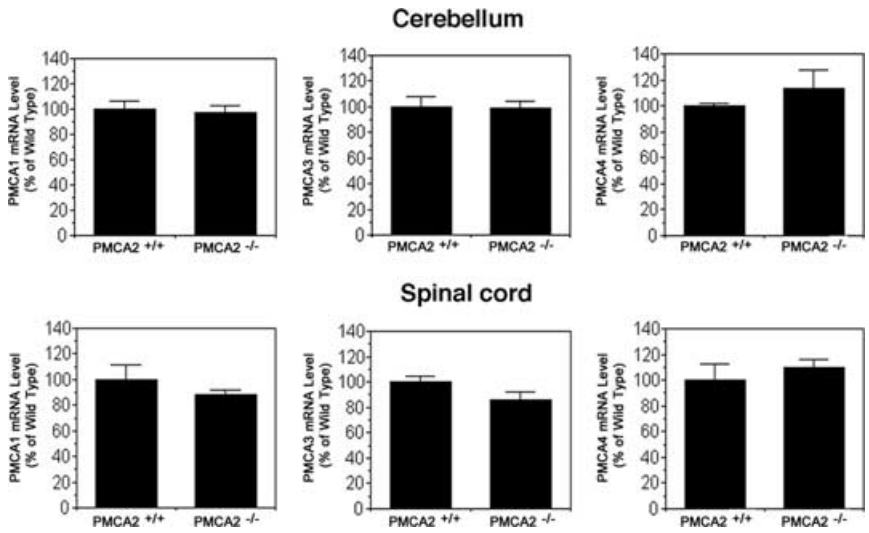

FIGURE 1. Semiquantitative analysis of PMCA1, 3, and 4 mRNA levels in the spinal cord and cerebellum of the PMCA2$^{+/+}$ and PMCA2$^{-/-}$ mice by reverse transcription-polymerase chain reaction. No significant differences were found. Graphs represent the mean ± SEM. The experiments were repeated twice and yielded similar results; n = 6.

Purkinje cells or interneurons. PMCA2 levels are severalfold higher in Purkinje cells as compared to other neuronal populations in the brain[1] and the lack of PMCA2 does not appear to induce compensatory increases in the mRNA expression of other PMCA isoforms either in the spinal cord or in the cerebellum of the PMCA2-null mouse (FIG. 1). Although it is not yet known whether the protein levels or the activity of other PMCAs are modified in the absence of PMCA2, it is possible to speculate that the lack of a major calcium pump in these neurons together with the absence of compensatory changes in other PMCAs may lead to calcium dyshomeostasis, a trigger that can affect Purkinje cell signaling. Therefore, we initiated studies to investigate the role of PMCA2 in Purkinje neurons. Proteomic analysis using either the isobaric tags for relative and absolute quantitation (iTRAQ) or two-dimensional gel electrophoresis followed by mass spectroscopy identified a number of differentially expressed proteins in the cerebellum of the PMCA2-null mouse, including inositol-3-phosphate receptor 1 (IP3R1)[9] and Homer 3, a scaffold protein that links IP3Rs to metabotropic glutamate receptors (mGluRs). IP3R1 is a downstream effector of mGluR1, which has been implicated in plasticity at the Purkinje cell-parallel fiber synapse, cerebellum-dependent associative learning, such as classical eyeblink conditioning and movement coordination.[10–12] As

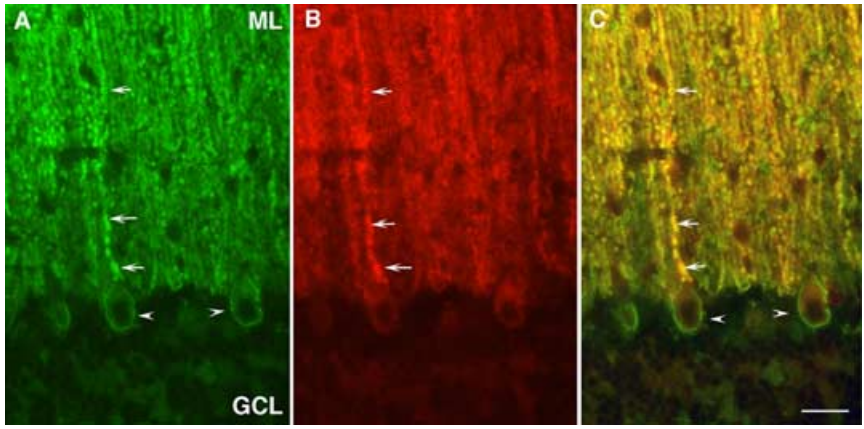

FIGURE 2. Colocalization of PMCA2 with mGluR1 in the mouse cerebellum by immunocytochemistry. (**A**) A coronal section through the cerebellum showing distribution of PMCA2 in somata (arrowheads) and dendrites (arrows) of Purkinje neurons. A FITC-tagged secondary antibody was used for visualization (green). (**B**) The same section was labeled with an antibody against mGluR1, which stained dendrites (arrows) but not cell bodies of Purkinje neurons. A Texas Red tagged secondary antibody was used for visualization (red). (**C**) Merged picture showing colocalization of mGluR1 with PMCA2 in dendrites (yellow; arrows). The arrowhead points to a Purkinje somata, which is labeled only with the PMCA2 antibody (green). ML: molecular layer, GCL: granule cell layer. Bar represents 75 μM. Color picture available online.

mGluR1-Homer-IP3R form a complex in the cerebellum,[13,14] we hypothesized that PMCA2 may play a role in mGluR1 signaling by associating with the receptor and its signaling complex. This would implicate colocalization of mGluR1 with PMCA2. To investigate this possibility, we performed immunocytochemical studies on mouse cerebellar sections. PMCA2 immunoreactivity was localized to dendrites and plasma membranes of Purkinje somata (FIG. 2 A). mGluR1 staining was at background level in cell bodies but strong in dendrites of Purkinje cells (FIG. 2 B). The distribution of PMCA2 and mGluR1 immunostaining was similar, and the merged picture indicated colocalization in dendrites of Purkinje neurons (FIG. 2 C). In agreement with these results, preliminary studies have shown that PMCA2 coimmunoprecipitates with mGluR1, Homer 3, and IP3R1[15] suggesting that PMCA2 is a component of mGluR1 signaling complex.

In conclusion, PMCA2 appears to play an important function in the integrity of spinal cord neurons and might contribute to mGluR signaling in the cerebellum. Our results, taken together, suggest that PMCA2 may have unique and nonredundant functions in spinal cord neurons and Purkinje cells. Future studies will define the exact role of PMCA2 in mGluR1 signaling and will identify the mechanisms and pathways that are affected in the spinal cord and cerebellum when PMCA2 activity is dysregulated.

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