DEFECTIVE CALCIUM PUMPS IN NEURONS IN THE AGING BRAIN AND IN PARKINSON'S DISEASE

Asma Zaidi, M.S., M. Phil., PhD
Sidrah Sheikh, B.S.
Shaneisha Williams, B.S.
Paul Ramlow, M.S.
Kansas City University of Medicine and Biosciences,
Kansas City, Missouri, USA

Abstract

The plasma membrane Ca^{2+}-ATPase (PMCA) pumps play an important role in the maintenance of precise levels of intracellular Ca^{2+}, quintessential for the optimal functioning and long term survival of neurons. In this paper, we review evidence showing alterations in the PMCAs in aging brain. Additionally, we provide evidence showing defects in these transporters in Parkinson's disease (PD). PMCA activity and protein levels in brain synaptic plasma membranes (SPMs) decline progressively with increasing age. The PMCAs undergo functional and structural changes when exposed to reactive oxygen species known to be generated in the aging brain and in neurodegenerative disorders such as PD. The major changes in the PMCAs include rapid inactivation, formation of aggregates, internalization from the plasma membrane and fragmentation by proteases. Reduction of PMCA levels as occurs in aging and under conditions of oxidative stress may play an important role in compromised neuronal function in the aging brain and in PD. Therapeutic strategies that protect the PMCAs and stabilize $[Ca^{2+}]_i$ homeostasis have the potential of serving as novel interventions in preventing and/or slowing down the degeneration of neurons in various chronic neurodegenerative disorders.

Keywords: Brain aging, Parkinson's disease, calcium, neurons, oxidative stress

Introduction

Neurons rely on calcium (Ca^{2+}) signaling to regulate a wide array of important cellular processes such as the release of neurotransmitters, signal

transduction, induction of gene expression, synaptic plasticity, and learning and memory formation (Foster & Kumar, 2002) (Foster & Norris, 1997). Following the transduction of the Ca^{2+} signal, neurons need to return the intracellular calcium $[Ca^{2+}]_i$ to baseline levels within milliseconds in order to prepare the cell for the next round of stimulation. Defects in the handling of Ca^{2+} and consequent overload is believed to result in cytotoxicity eventually leading to neuronal cell death (Miller, 1991). The various mechanisms for restoring baseline resting $[Ca^{2+}]_i$ following neuronal excitation include sequestration into the endoplasmic reticulum and mitochondria, buffering by Ca^{2+} binding proteins, and extrusion across the plasma membrane by the Na^+/Ca^{2+} exchangers (NCXs) and the plasma membrane Ca^{2+}-ATPase pumps (PMCAs) (Blaustein, 1988; Carafoli, 1992). The PMCA pumps are regulated by the Ca^{2+} sensor protein calmodulin (CaM), which induces conformational changes within the protein causing the C-terminal autoinhibitory domain to move away from the active site of the enzyme, thus relieving autoinhibition and resulting in several-fold activation (Enyedi et al., 1989; James et al., 1988). The PMCAs represent the major high affinity transport system at the plasma membrane responsible for the fine tuning of resting free $[Ca^{2+}]_i$ and counteracting transient increases that occur during Ca^{2+} signaling (Strehler & Zacharias, 2001). The PMCA proteins are coded by four different genes that produce four distinct isoforms termed PMCA 1, 2, 3 and 4. Neurons express all four isoforms of the protein and have multiple splice variants formed by alternative splicing of mRNA, attesting to the very complex nature of Ca^{2+} dynamics in nerve cells.

Dyshomeostasis of Calcium in the Aging Brain: Role of the Calcium Pumps

There is an extensive body of literature showing that defects in Ca^{2+} homeostasis underlie neuronal dysfunction. If left unchecked, elevated levels of $[Ca^{2+}]_i$ lead to cytotoxicity and eventual cell death. Evidence from our laboratory and work of others has shown alterations in Ca^{2+} regulating systems in brain neurons with increasing age (*reviewed in* (Foster & Kumar, 2002; Gibson & Peterson, 1987; Squier & Bigelow, 2000; Thibault, Gant, & Landfield, 2007; Wojda, Salinska, & Kuznicki, 2008; A. Zaidi, 2010)). Aged neurons have elevated levels of voltage-gated Ca^{2+} channels, the major system involved in the influx of extracellular Ca^{2+}. To make matters worse, aged neurons have decreased activity of the NCXs, the sarco-endoplasmic reticulum Ca^{2+}-ATPases (SERCAs) (Gomez-Villafuertes, Mellstrom, & Naranjo, 2007; Martinez, Vitorica, & Satrustegui, 1988; Thibault, Hadley, & Landfield, 2001; Verkhratsky & Toescu, 1998) (Michaelis, 1989; Michaelis et al., 1996; Michaelis, Foster, & Jayawickreme, 1992; Michaelis, Johe, & Kitos, 1984; Yao et al., 1996) and the PMCAs (A. Zaidi, Gao, Squier, &

Michaelis, 1998), (Lipman, Chrisp, Hazzard, & Bronson, 1996). The resultant effect of age-associated changes in the proteins that regulate Ca^{2+} is an elevation in $[Ca^{2+}]_i$ which is consequently detrimental to the health of neurons.

Our laboratory has been interested in the PMCAs for the last 20 years (A. Zaidi et al., 2003; A. Zaidi, Fernandes, Bean, & Michaelis, 2009; A. Zaidi et al., 1998; A. Zaidi & Michaelis, 1999). We were the first to show a progressive and significant decline in the activity (~50%) of the PMCAs in SPMs isolated from rat brain at five different ages covering the life span of the animal (Lipman et al., 1996; A. Zaidi et al., 1998). Reduction in PMCA activity is associated with a statistically significant decrease in maximum velocity (V^{max}) with no appreciable change in the affinity of the enzyme for Ca^{2+} (K_{act}) (A. Zaidi et al., 1998). An approximately 20% reduction in PMCA protein is observed at 34 months, the highest age of rats we tested, compared to the 5 month young adults (A. Zaidi et al., 1998). The disproportional loss of activity vs protein levels indicate modifications in the protein that cause inactivation without complete removal from the SPMs. A more recent study on lipid raft microdomains isolated from SPMs at different ages has shown similar results, i.e., a disproportional loss of PMCA activity vs its protein levels (Jiang et al., 2014). Age-related reduction in the PMCA in SPMs and lipid rafts may be attributed to multiple causes such as decreased synthesis, altered stability, abnormal trafficking/targeting to SPMs, structural alterations leading to enhanced removal from the synaptic terminals, or modifications leading to reduced immunoreactivity, although none of these possibilities have been experimentally confirmed and validated. Lowered PMCA activity and protein levels in SPMs as observed with increasing age is likely to contribute to the disruption of Ca^{2+} homeostasis, a hallmark of aged neurons.

Oxidative Stress and the PMCAs

Post-translational modification to proteins and lipid peroxidation are major contributors of neuronal dysfunction in normal aging and in the pathophysiology of several neurodegenerative disorders including Parkinson's disease (PD). Reactive oxygen species-mediated oxidative modification of proteins may involve altered conformation, misfolding, aggregation and oxidation of amino acid residues. To determine the vulnerability of the PMCAs to oxidative stress and to identify the pattern of oxidative modification that may appear on the protein upon exposure to oxidants with physiological relevance, we performed a series of studies using a variety of experimental approaches (A. Zaidi et al., 2003; A. Zaidi et al., 2009; A. Zaidi & Michaelis, 1999). Exposure of synaptic membranes to oxidizing agents such as H_2O_2, peroxynitrite and peroxyl radical generating

agents for a brief period of time (10 min at 37°C) caused rapid inactivation of the PMCA (A. Zaidi & Michaelis, 1999). Loss of activity was due to a significant reduction in V^{max} with no change in the affinity for Ca^{2+} or K_{act} consistent with our observations in the aging brain (A. Zaidi et al., 1998). The major structural alteration was the formation of high molecular weight aggregates of PMCA which were reversed by the addition of a reducing agent (dithiothreitol) and chaotropic agent (urea). These data suggested the contribution of the oxidation of cysteine residues to form disulfide bonds and increased hydrophobic interactions between PMCA molecules, respectively (Souza dos Santos, Saraiva, Ferraz da Costa, Scofano, & de Carvalho-Alves, 2007; A. Zaidi & Michaelis, 1999).

To determine whether the effects of oxidants on the PMCA are a direct oxidation of the protein or mediated indirectly via lipid peroxidation, we purified the PMCA protein from its membrane environment. Red blood cell membranes rich in one of the isoforms (PMCA 4) were utilized for these studies. The purified PMCA protein was reconstituted into mixed micelles made from the phospholipid phosphatidylcholine and exposed to increasing doses of H_2O_2 for 10 minutes at 37ºC (A. Zaidi et al., 2003). As in the case of synaptic membranes, the PMCA in red blood cell membranes was rapidly inactivated and formed high molecular weight aggregates suggesting a direct effect of the oxidant on the protein.

In the next series of studies, we used primary neurons to assess the effects of oxidants on the PMCA (A. Zaidi et al., 2009). The logic was to investigate the effects of oxidative stress on the PMCAs in a system closer to the physiological one, with antioxidant defense mechanisms in place. Primary cultured neurons (cortical) were exposed to increasing concentrations of the superoxide free radical generating agent paraquat. (Smith & Heath, 1976). A 24 hour exposure to paraquat (5 µM – 100 µM) resulted in significant changes in PMCA activity which exhibited a biphasic response (A. Zaidi et al., 2009). While low concentrations of paraquat (5-25 µM) activated PMCA basal activity by ~two-fold and abolished its sensitivity to CaM, higher concentrations (50-100 µM) inhibited both basal and CaM-stimulated PMCA activity. Paraquat treatment also led to aggregate formation and calpain-mediated proteolysis of the PMCA protein (A. Zaidi et al., 2009). Our results are consistent with several studies investigating the effects of excitotoxic agents and neurotoxins on the PMCAs present in neurons (Hajimohammadreza et al., 1997; Kip & Strehler, 2007; Pottorf et al., 2006; Wang, Roufogalis, & Villalobo, 1989; Wang, Villalobo, & Roufogalis, 1988; A. Zaidi et al., 2009), and in non-neuronal cells (Bruce & Elliott, 2007; Marian, Mukhopadhyay, Borchman, Tang, & Paterson, 2008; Xiao et al.).

Increase in oxidative stress and elevations in $[Ca^{2+}]_i$ have also been linked to the activation of caspases, enzymes that mediate apoptosis, a form of programmed cell death (Berridge, 1998). The link between the PMCAs and apoptosis was established when PMCA 4b was shown to be degraded by caspase 3 (Paszty et al., 2005; Paszty et al., 2002; Schwab et al., 2002). Cleavage by caspase forms a 120 kDa truncated form of the PMCA minus its CaM-binding autoinhibitory domain. (James et al., 1989; Papp et al., 1989; Wang et al., 1988), As expected this form is fully active even in the absence of CaM (Paszty et al., 2005; Paszty et al., 2002), a condition that would help the cell to counteract the increased Ca^{2+} load and protect it against death (Schwab et al., 2002). However, under severe and more chronic conditions, the PMCAs may be down-regulated further impairing Ca^{2+} homeostasis and promoting cell death.

The PMCAs and Neurodegeneration

While the contribution of overall neuronal Ca^{2+} dysregulation in age-associated neurodegenerative disorders has been experimentally validated (*reviewed in* (Bezprozvanny & Mattson, 2008; Green & LaFerla, 2008; Mattson, 2007; Wojda et al., 2008)), the specific contribution of the PMCAs is just beginning to be elucidated. Conceptually, there are two major ways by which the PMCAs may either contribute to the initiation and progression of neurodegenerative diseases or be impacted by these chronic conditions. Any change in PMCA function is likely to disrupt neuronal Ca^{2+} homeostasis and elevate $[Ca^{2+}]_i$ which may promote the generation of abnormal forms of peptides/proteins such as the amyloid beta peptide in Alzheimer's disease and alpha synuclein in PD. Conversely, events downstream from the accumulation of the pathological forms of these peptides/proteins may disrupt energy homeostasis, increase membrane excitability, promote the formation of free radicals, elevate membrane-associated oxidative stress, and activate proteolytic enzymes all of which may consequently have an inhibitory effect on PMCA function. Convincing experimental support for either one or both of these possibilities is still lacking in the literature.

The PMCAs have been found to be significantly down-regulated in experimental models of global ischemia-reperfusion injury and seizures (Paszty et al., 2005). In preliminary studies, we found that exposure to hypoxic conditions led to a significant decline in PMCA activity with no change in its protein levels (*unpublished observations*). Evidence suggests that the suppression of PMCA activity is not simply due to the disruption of ion gradients and altered energy homeostasis due to lowered ATP levels, but rather due to a direct oxidative modification and degradation of the PMCA protein (Lehotsky et al., 2002). The relationship between the PMCAs and Alzheimer's disease, the most common neurodegenerative disorder, was first

demonstarted by functional inativation of the PMCA by the amyloid beta peptide (Mark, Hensley, Butterfield, & Mattson, 1995; Mark, Lovell, Markesbery, Uchida, & Mattson, 1997; Mattson, Mark, Furukawa, & Bruce, 1997). More recent studies have further validated the involvement of the PMCA in Alzheimer's disease (Berrocal et al., 2009). PMCA activity in human brain tissue from Alzheimer's disease patients shows altered affinity to Ca^{2+} as compared to age-matched controls (Berrocal et al., 2009) suggesting structural/conformational changes in the Ca^{2+} binding sites in the protein. More interestingly, addition of exogenous amyloid beta peptide to control brain simulated many of the effects observed in Alzheimer's disease brain (Berrocal et al., 2009).

In our laboratory, we are studying the PMCAs in PD, the most common movement neurodegenerative disorder. In a cell model of PD, we exposed the dopaminergic cell line SH-SY5Y neuroblastoma cells to the Parkinsonian mimetics methyl phenyl pyridinium (Zaidi A, 2009) and 6-hydroxydopamine (A. R. Zaidi, A; Clark, S; Elliot, K and Ramlow, P 2012). We observed a significant loss of activity and protein levels upon exposure to these neurotoxins. Preliminary studies on human brain tissue from PD patients are consistent with the cell model showing a very significant reduction in PMCA activity. Overall, the findings presented here suggest that the loss of the PMCAs are a common theme across several neurodegenerative diseases. Further studies are needed to elucidate the underlying mechanisms and determine whether the observed changes in the PMCA pumps are a cause or consequence of the disease. Therapeutic approaches that can protect the PMCAs and stabilize $[Ca^{2+}]_i$ homeostasis may be capable of slowing or even preventing neuronal degeneration. The PMCAs are therefore presenting themselves as novel drug targets for therapeutic interventions in various chronic degenerative disorders.

Conclusion:

The plasma membrane Ca^{2+}-ATPase (PMCA) pumps are critical to the maintenance of precise levels of intracellular Ca^{2+}, quintessential to the functioning of nerve cells. The PMCAs in the SPMs and lipid raft microdomains diminish progressively with increasing age. PMCA loss also occurs in Alzheimer's disease and Parkinson's disease, the two most common neurodegenerative disorders. This may be due to elevated levels of oxidative stress present in brain neurons in these chronic disorders. Downregulation of the PMCAs may further elevate the levels of intracellular Ca^{2+} leading to Ca^{2+} overload, subsequent cytotoxicity and eventual cell death. Strategies that protect the PMCA from oxidative damage and/or elevate its expression in neurons represent novel future therapeutic interventions.

References:

Berridge, M. J. (1998). Neuronal calcium signaling. Neuron, 21(1), 13-26.

Berrocal, M., Marcos, D., Sepulveda, M. R., Perez, M., Avila, J., & Mata, A. M. (2009). Altered Ca2+ dependence of synaptosomal plasma membrane Ca2+-ATPase in human brain affected by Alzheimer's disease. Faseb J, 23(6), 1826-1834.

Bezprozvanny, I., & Mattson, M. P. (2008). Neuronal calcium mishandling and the pathogenesis of Alzheimer's disease. Trends Neurosci, 31(9), 454-463.

Blaustein, M. P. (1988). Calcium transport and buffering in neurons. Trends Neurosci, 11(10), 438-443.

Bruce, J. I., & Elliott, A. C. (2007). Oxidant-impaired intracellular Ca2+ signaling in pancreatic acinar cells: role of the plasma membrane Ca2+-ATPase. Am J Physiol Cell Physiol, 293(3), C938-950.

Carafoli, E. (1992). The plasma membrane calcium pump. Structure, function, regulation. Biochim Biophys Acta, 1101(2), 266-267.

Enyedi, A., Vorherr, T., James, P., McCormick, D. J., Filoteo, A. G., Carafoli, E., & Penniston, J. T. (1989). The calmodulin binding domain of the plasma membrane Ca2+ pump interacts both with calmodulin and with another part of the pump. J Biol Chem, 264(21), 12313-12321.

Foster, T. C., & Kumar, A. (2002). Calcium dysregulation in the aging brain. Neuroscientist, 8(4), 297-301.

Foster, T. C., & Norris, C. M. (1997). Age-associated changes in Ca(2+)-dependent processes: relation to hippocampal synaptic plasticity. Hippocampus, 7(6), 602-612.

Gibson, G. E., & Peterson, C. (1987). Calcium and the aging nervous system. Neurobiol Aging, 8(4), 329-343.

Gomez-Villafuertes, R., Mellstrom, B., & Naranjo, J. R. (2007). Searching for a role of NCX/NCKX exchangers in neurodegeneration. Mol Neurobiol, 35(2), 195-202.

Green, K. N., & LaFerla, F. M. (2008). Linking calcium to Abeta and Alzheimer's disease. Neuron, 59(2), 190-194.

Hajimohammadreza, I., Raser, K. J., Nath, R., Nadimpalli, R., Scott, M., & Wang, K. K. (1997). Neuronal nitric oxide synthase and calmodulin-dependent protein kinase IIalpha undergo neurotoxin-induced proteolysis. J. Neurochem., 69(3), 1006-1013.

James, P., Maeda, M., Fischer, R., Verma, A. K., Krebs, J., Penniston, J. T., & Carafoli, E. (1988). Identification and primary structure of a calmodulin binding domain of the Ca2+ pump of human erythrocytes. J Biol Chem, 263(6), 2905-2910.

James, P., Vorherr, T., Krebs, J., Morelli, A., Castello, G., McCormick, D. J., . . . Carafoli, E. (1989). Modulation of erythrocyte Ca2+-ATPase by

selective calpain cleavage of the calmodulin-binding domain. J Biol Chem, 264(14), 8289-8296.

Jiang, L., Bechtel, M. D., Bean, J. L., Winefield, R., Williams, T. D., Zaidi, A., . . . Michaelis, M. L. (2014). Effects of gangliosides on the activity of the plasma membrane Ca2+-ATPase. [Research Support, N.I.H., Extramural]. Biochim Biophys Acta, 1838(5), 1255-1265. doi: 10.1016/j.bbamem.2014.01.003

Kip, S. N., & Strehler, E. E. (2007). Rapid downregulation of NCX and PMCA in hippocampal neurons following H2O2 oxidative stress. Ann N Y Acad Sci, 1099, 436-439.

Lehotsky, J., Kaplan, P., Matejovicova, M., Murin, R., Racay, P., & Raeymaekers, L. (2002). Ion transport systems as targets of free radicals during ischemia reperfusion injury. Gen Physiol Biophys, 21(1), 31-37.

Lipman, R. D., Chrisp, C. E., Hazzard, D. G., & Bronson, R. T. (1996). Pathologic characterization of brown Norway, brown Norway x Fischer 344, and Fischer 344 x brown Norway rats with relation to age. J Gerontol A Biol Sci Med Sci, 51(1), B54-59.

Marian, M. J., Mukhopadhyay, P., Borchman, D., Tang, D., & Paterson, C. A. (2008). The effect of hydrogen peroxide on sarco/endoplasmic and plasma membrane calcium ATPase gene expression in cultured human lens epithelial cells. Open Ophthalmol J, 2, 123-129.

Mark, R. J., Hensley, K., Butterfield, D. A., & Mattson, M. P. (1995). Amyloid beta-peptide impairs ion-motive ATPase activities: evidence for a role in loss of neuronal Ca2+ homeostasis and cell death. J Neurosci, 15(9), 6239-6249.

Mark, R. J., Lovell, M. A., Markesbery, W. R., Uchida, K., & Mattson, M. P. (1997). A role for 4-hydroxynonenal, an aldehydic product of lipid peroxidation, in disruption of ion homeostasis and neuronal death induced by amyloid beta-peptide. J Neurochem, 68(1), 255-264.

Martinez, A., Vitorica, J., & Satrustegui, J. (1988). Cytosolic free calcium levels increase with age in rat brain synaptosomes. Neurosci Lett, 88(3), 336-342.

Mattson, M. P. (2007). Calcium and neurodegeneration. Aging Cell, 6(3), 337-350.

Mattson, M. P., Mark, R. J., Furukawa, K., & Bruce, A. J. (1997). Disruption of brain cell ion homeostasis in Alzheimer's disease by oxy radicals, and signaling pathways that protect therefrom. Chem Res Toxicol, 10(5), 507-517.

Michaelis, M. L. (1989). Ca2+ handling systems and neuronal aging. Ann N Y Acad Sci, 568, 89-94.

Michaelis, M. L., Bigelow, D. J., Schoneich, C., Williams, T. D., Ramonda, L., Yin, D., . . . Squier, T. C. (1996). Decreased plasma membrane calcium transport activity in aging brain. Life Sci, 59(5-6), 405-412.

Michaelis, M. L., Foster, C. T., & Jayawickreme, C. (1992). Regulation of calcium levels in brain tissue from adult and aged rats. Mech Ageing Dev, 62(3), 291-306.

Michaelis, M. L., Johe, K., & Kitos, T. E. (1984). Age-dependent alterations in synaptic membrane systems for Ca2+ regulation. Mech Ageing Dev, 25(1-2), 215-225.

Miller, R. J. (1991). The control of neuronal Ca2+ homeostasis. Prog Neurobiol, 37(3), 255-285.

Papp, B., Sarkadi, B., Enyedi, A., Caride, A. J., Penniston, J. T., & Gardos, G. (1989). Functional domains of the in situ red cell membrane calcium pump revealed by proteolysis and monoclonal antibodies. Possible sites for regulation by calpain and acidic lipids. J. Biol. Chem., 264(8), 4577-4582.

Paszty, K., Antalffy, G., Penheiter, A. R., Homolya, L., Padanyi, R., Ilias, A., . . . Enyedi, A. (2005). The caspase-3 cleavage product of the plasma membrane Ca2+-ATPase 4b is activated and appropriately targeted. Biochem J, 391(Pt 3), 687-692.

Paszty, K., Verma, A. K., Padanyi, R., Filoteo, A. G., Penniston, J. T., & Enyedi, A. (2002). Plasma membrane Ca2+ATPase isoform 4b is cleaved and activated by caspase-3 during the early phase of apoptosis. J Biol Chem, 277(9), 6822-6829.

Pottorf, W. J., 2nd, Johanns, T. M., Derrington, S. M., Strehler, E. E., Enyedi, A., & Thayer, S. A. (2006). Glutamate-induced protease-mediated loss of plasma membrane Ca2+ pump activity in rat hippocampal neurons. J Neurochem, 98(5), 1646-1656.

Schwab, B. L., Guerini, D., Didszun, C., Bano, D., Ferrando-May, E., Fava, E., . . . Nicotera, P. (2002). Cleavage of plasma membrane calcium pumps by caspases: a link between apoptosis and necrosis. Cell Death Differ., 9(8), 818-831.

Smith, P., & Heath, D. (1976). Paraquat. CRC Crit Rev Toxicol, 4(4), 411-445.

Souza dos Santos, P., Saraiva, D. F., Ferraz da Costa, D. C., Scofano, H. M., & de Carvalho-Alves, P. C. (2007). Trifluoperazine protects brain plasma membrane Ca(2+)-ATPase from oxidative damaging. Exp Brain Res, 177(3), 347-357.

Squier, T. C., & Bigelow, D. J. (2000). Protein oxidation and age-dependent alterations in calcium homeostasis. Front Biosci, 5, D504-526.

Strehler, E. E., & Zacharias, D. A. (2001). Role of alternative splicing in generating isoform diversity among plasma membrane calcium pumps. Physiol Rev, 81(1), 21-50.

Thibault, O., Gant, J. C., & Landfield, P. W. (2007). Expansion of the calcium hypothesis of brain aging and Alzheimer's disease: minding the store. Aging Cell, 6(3), 307-317.

Thibault, O., Hadley, R., & Landfield, P. W. (2001). Elevated postsynaptic [Ca2+]i and L-type calcium channel activity in aged hippocampal neurons: relationship to impaired synaptic plasticity. J Neurosci, 21(24), 9744-9756.

Verkhratsky, A., & Toescu, E. C. (1998). Calcium and neuronal ageing. Trends Neurosci, 21(1), 2-7.

Wang, K. K., Roufogalis, B. D., & Villalobo, A. (1989). Characterization of the fragmented forms of calcineurin produced by calpain I. Biochem. Cell Biol., 67(10), 703-711.

Wang, K. K., Villalobo, A., & Roufogalis, B. D. (1988). Activation of the Ca2+-ATPase of human erythrocyte membrane by an endogenous Ca2+-dependent neutral protease. Arch. Biochem. Biophys., 260(2), 696-704.

Wojda, U., Salinska, E., & Kuznicki, J. (2008). Calcium ions in neuronal degeneration. IUBMB Life, 60(9), 575-590.

Xiao, Y., Cui, J., Shi, Y. H., Sun, J., Wang, Z. P., & Le, G. W. Effects of duodenal redox status on calcium absorption and related genes expression in high-fat diet-fed mice. Nutrition.

Yao, Y., Yin, D., Jas, G. S., Kuczer, K., Williams, T. D., Schoneich, C., & Squier, T. C. (1996). Oxidative modification of a carboxyl-terminal vicinal methionine in calmodulin by hydrogen peroxide inhibits calmodulin-dependent activation of the plasma membrane Ca-ATPase. Biochemistry, 35(8), 2767-2787.

Zaidi, A. (2010). Plasma membrane Ca2+-ATPases: Targets of oxidative stress in brain aging and neurodegeneration. World Journal of Biological Chemistry, 1(9), 271-280.

Zaidi A, A. S., and Ramlow, P. (2009). The plasma membrane Ca2+-ATPase and Parkinson's disease. [Abstract]. Free Radical Biology and Medicine, 47 (supplement 1).

Zaidi, A., Barron, L., Sharov, V. S., Schoneich, C., Michaelis, E. K., & Michaelis, M. L. (2003). Oxidative inactivation of purified plasma membrane Ca2+-ATPase by hydrogen peroxide and protection by calmodulin. Biochemistry, 42(41), 12001-12010.

Zaidi, A., Fernandes, D., Bean, J. L., & Michaelis, M. L. (2009). Effects of paraquat-induced oxidative stress on the neuronal plasma membrane Ca(2+)-ATPase. Free Radic Biol Med, 47(10), 1507-1514.

Zaidi, A., Gao, J., Squier, T. C., & Michaelis, M. L. (1998). Age-related decrease in brain synaptic membrane Ca2+-ATPase in F344/BNF1 rats. Neurobiol Aging, 19(5), 487-495.

Zaidi, A., & Michaelis, M. L. (1999). Effects of reactive oxygen species on brain synaptic plasma membrane Ca(2+)-ATPase. Free Radic Biol Med, 27(7-8), 810-821.
Zaidi, A. R., A; Clark, S; Elliot, K and Ramlow, P (2012). [Abstract]. Free Radical Biology and Medicine, 53(S2).