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Plasma Membrane Ca^{2+}-Pump Functional Specialization in the Brain

Complex of Isoform Expression and Regulation by Effectors

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ABSTRACT

The plasma membrane Ca^{2+}-pump (PMCA) is a key element in the removal of intracellular Ca^{2+}. A number of PMCA pumps, encoded by a multigenic family and differing in their regulatory domains, also exist in the neuronal cells. We discuss here an idea regarding a new, higher level of specialization of PMCA protein isoforms with different sensitivities toward phospholipids and calmodulin. The idea is based on the kinetic data from PMCA stimulation by acidic phospholipids, with a combination of results describing an alternative RNA splicing at site A and C coding of regulatory domains of protein. The resulting complex modulation of the Ca^{2+}-pump underlies the specific cellular requirements for Ca^{2+} homeostasis in a tissue-selective manner and is regulated by the level and spatial distribution of enzyme isoforms as well as by the level of their regulatory factors. The possible role of PMCA protein in the neuronal injury is also discussed.

Index Entries: Plasma membrane; Ca^{2+} pump; functional specialization; calmodulin.

Abbreviations: Ca^{2+}-ATPase, Ca^{2+}-transport adenosinetriphosphatase; PMCA, Ca^{2+}-transport ATPase from plasma membrane; pNPPase, *p*-nitrophenylphosphatase; PCR, polymerase chain reaction; PC, phosphatidylcholine; PIP, phosphatidylinositol phosphate; PS, phosphatidyldserine.

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INTRODUCTION

Ca^{2+} plays a key role in a number of processes of critical importance for the neuronal system, including: neuronal excitability, neurotransmitter release, membrane conductance, axonal transport, and intracellular signaling (Miller, 1991). Precise control of the intracellular Ca^{2+} concentration is essential for these processes, yet the regulation of the different Ca^{2+} transport systems involved remains incompletely understood. This in part is a result of the interplay of many transport systems located at both intracellular sites and at the plasma membrane (Grover and Khan, 1992; Wuytack and Raeymaekers, 1992). The Ca^{2+}-pumping ATPase from the plasma membrane (PMCA) is thought to be a key element for removal of intracellular Ca^{2+} (Carafoli, 1992). The activity of the Ca^{2+} pump is modified by a large number of agents that interact either with the enzyme directly (calmodulin, acidic phospholipids) or through some integrated regulatory pathways (various kinases, proteolysis, self-association, hormones). The major effect of all of these regulators is to increase the V_{max} and the affinity of the pump for Ca^{2+} (Wang et al., 1992). Long-term control is achieved through regulation of its gene expression (Kue et al., 1991).

A number of PMCA pumps, encoded by a multigenic family and differing in their regulatory domains, also exist in the neuronal cells. This isoform multiplicity may lead both to functional complementarity or to functional redundancy (Strehler, 1991; Brandt et al., 1992; Stahl et al., 1992). The approach taken here is first to describe the distribution of different PMCA-pump isoforms in the brain and then based on known isoform-related differences for PMCA stimulation by phospholipids, to formulate a hypothesis regarding the functional specialization (plasticity) of the Ca^{2+}-transport ATPase from neuronal plasma membrane. In addition, the role of PMCA dysfunction in the pathomechanisms of neuronal injury is also discussed.

TOPOGRAPHY OF THE PMCA PUMP AND RECOGNITION OF ISOFORMS

A comparison of the primary sequences of the PMCA pumps with other P-type ATPases, including a total of some 30 different sequences, confirmed the previous presumption that they are all homologously related (Green and MacLennan, 1989). The alignment of the PMCA pump sequences with their SERCA counterparts from intracellular organelles points to two distinct domains of functionally important differences: a large, highly charged PMCA insert between membrane-spanning helices two and three (putative acidic phospholipid-binding domain) and an extended PMCA C-terminus comprising the calmodulin-binding domain and a site for kinase phosphorylation (Carafoli, 1992).

The PMCA protein is encoded by a multigenic family. In mammalian brain at least four genes have been recognized that are subject to further alternative mRNA splicing leading to additional protein variability (Strehler, 1991; Wuytack et al., 1992). Isoform diversity exclusively appears to concern the regulatory domains, none of the isoforms show variations in the active site. Alternative mRNA splicing occurs at four sites designated A–D and anticipates also some aspects of the topology of the pump (Carafoli, 1992).

Site A lies in the immediate vicinity or overlaps with the putative phospholipid-binding domain. It is used mainly in gene 2, where it gives rise to four PMCA splice variants. Site C, used in gene products 1, 3, and 4 involves the calmodulin-binding domain. RNA splicing at site C provides several isoforms with variable affinities for calmodulin and the autoinhibitory activity of the calmodulin-binding domain (Enyedi et al., 1991).

The splicing at sites B and D could in later studies not be confirmed and might thus be in all likelihood the results of cloning artifacts (Fig. 1).

DISTRIBUTION OF PMCA ISOFORMS IN BRAIN

Although most of the data have thus recently come from study of erythrocytes, the presence of several Ca^{2+}-ATPases in neuronal tissues is now extensively documented (Hakim et al., 1982; Hermoni-Levine and Rahaminoff, 1990). The Ca^{2+} pump has also been detected in CNS and PNS both by using a cytochemical method as well as by immunochemistry with antibodies specific for the PMCA (Mata et al., 1988; Borke et al., 1989; Talamoni et al., 1993).

cDNA cloning and mRNA analysis by PCR revealed unique tissue distribution the transcripts of four genes products and their different splice form in neuronal cells (Brandt et al., 1992; Keeton et al., 1993; Stauffer et al., 1993). Brain cells were shown to express all PMCA isoforms tested, however, at different levels (Table 1). Of these isoforms PMCA 1b is housekeeping for practically all tissues. In brain PMCA 1a and PMCA 1x isoforms were preferentially expressed.

PMCA 2 was found uniformly distributed in brain; all PMCA 3 isoforms as well as isoforms of PMCA 4 were present in both brain and spinal cord. In human cerebral cortex was found to contain very high relative amounts of the transcripts of each of the genes with relative quantities as follows: PMCA1–51%; PMCA2–19%; PMCA3–16% and PMCA4–24% (Stauffer et al., 1993).

The considerable diversity of Ca^{2+}-ATPase mRNAs expressed in the brain is most probably the reflection of the great cell-type diversity found there and may lead to either functional complementarity of PMCA-pump or to its functional redundancy (Wuytack et al., 1992).

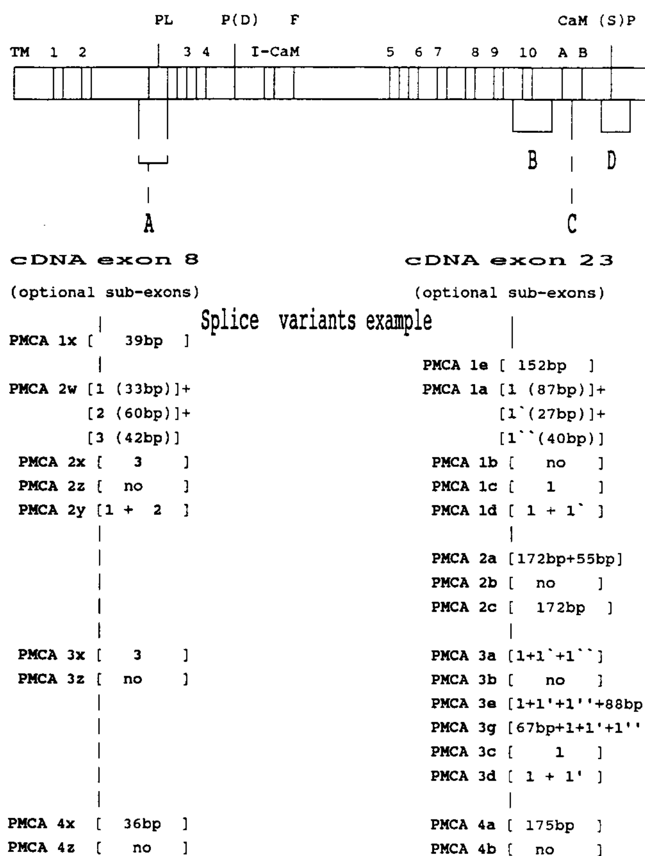

Fig. 1. Organization and mRNA processing for PMCA isoforms. Top: Scheme of a PMCA domain assignment as given by Strehler et al. (1991). Black bars above the structure give the areas where the different PMCA gene products show the highest variability. TM, putative transmembrane domains (1–10); PL, the phospholipid binding domain; P(D), aspartate residue of acylphosphate formation; I, CaM, the inhibitory region that interacts with calmodulin binding domain; F, the fluorescein isothicyanate binding site; CaM, calmodulin binding region, separated into subdomains A and B; P(S), serine residue, the cAMP-dependent protein kinase target site. Underneath the protein structure the different alternative splicing options at sites A, B, C, and D are also indicated. The putative PMCA splice variants are given for site A and C. The rat exons are numbered as given by Burk and Shull (1992). The length in basepairs is given for optional subexons, numbered 1, 2, and 3 for site A and 1, 1′, and 1″ for site C. Nomenclature of isoforms is used as given by Adamo and Penniston (1992), Burk and Shull (1992), Wuytack et al. (1992), and Carafoli and Stauffer (1993).

As can be expected from the large regional cellular diversity in the CNS, the distribution pattern of the different PMCA isoforms in brain is even more complex. By studying cellular localizations, several mRNAs that encode for different PMCA variants exhibited a distinct pattern of expression, in parallel with the reflection of a stage-dependent cellular

Table 1
Tissue-Specific Distribution of Isoforms of PMCA mRNAs Detected by Southern Blotting Probed with a Probe Specific to PCMA Isoforms[a]

mRNA	Kidney	Lung	Skeletal muscle	Liver	Heart	Brain
PMCA1a			+		+	+ +
PMCA1x	+ +	+ +	+ +	+ +	+ +	+ +
PMCA1b	+ +	+ +	+ +	+ +	+ +	+
PMCA1c			+ +		+ +	+
PMCA1d			+		+	
PMCA1e						+
PMCA2				+		+
PMCA2a					+	+
PMCA2x	+	+	+	+	+	+
PMCA2b	+	+	+	+	+	+ +
PMCA2w	+	+	+	+	+	+
PMCA2z			+		+	
PMCA3a						+
PMCA3x						+
PMCA3b			+			+
PMCA3c			+			+
PMCA3d						+
PMCA3z						+
PMCA4a	+	+	+	+	+ +	+ +
PMCA4x	+ +	+ +	+ +	+ +	+ +	+ +
PMCA4b	+ +	+ +	+ +	+ +	+ +	+ +
PMCA4z					+ +	

[a]Table shows a summary of data from Brandt et al. (1992), Keeton et al. (1993), and Stauffer et al. (1993).

+ +Represents the relative quantities of corresponding isoforms higher than 8% of the total PMCA content in human tissues (Stauffer et al., 1993).

differentiation (Brandt and Neve, 1992; Stahl et al., 1992). PMCA 1–3 isoforms appeared to be expressed primarily in neurons. PMCA was found neither in white matter nor in regions rich in astrocytes (Stahl et al., 1992). Expression of PMCA 1 mRNA was highest in CA 1 pyramidal cells of the hippocampus, PMCA 2 mRNA was most abundant in Purkinje cells and the highest level of PMCA 3 was found in the habenula and the choroid plexus (Table 2).

REGULATION OF PMCA BY PHOSPHOLIPIDS

Activation of plasma membrane Ca^{2+}-ATPase by acidic phospholipids has been found to increase both the V_{max} and the affinity for Ca^{2+} (Missiaen et al., 1989). We and others have also shown that the number of

Table 2
Relative Distribution of Hybridization Probes Specific for PMCA1, PMCA2, and PMCA3 Isoforms in Different Brain Regions[a]

Isoform	Region/cell	Relative amount
PMCA 1	Hippocampus CA1	1.00 ± 0.07
	Cerebral cortex	0.46 ± 0.06
	Thalamus	0.50 ± 0.06
	Amygdaloid nuclei	0.38 ± 0.03
	Olfactory bulb (M)	0.39 ± 0.04
PMCA 2	Cerebellum-Purkinje cells	1.00 ± 0.08
	Cerebral cortex	0.24 ± 0.02
	Hippocampus-DG cells	0.20 ± 0.04
	Olfactory bulb-GLL	0.19 ± 0.02
PMCA 3	Medial habenula	0.69 ± 0.04
	Third ventricle choroid plexus	1.00 ± 0.05
	Fourth ventricle choroid plexus	0.48 ± 0.01
	Hippocampus CA3	0.41 ± 0.02
	Cerebellum-granular layer	0.33 ± 0.06

[a]Table shows the reanalyzed data originally presented in Stahl et al. (1992). Relative amount represents number of grains/100 μm^2 of each probe and highest number for each probe was taken as control. The level of radioactivity for each probe was similar, but since exposure of PMCA 3 was longer than for PMCA 1 or PMCA 2, relative comparisons of hybridization between probes is difficult.

negative charges of phospholipids involved is important for both the binding and the potency to stimulate Ca^{2+} pump activity (Wrzosek et al., 1989; Verbist et al., 1991).

To investigate the mechanism of this activation we studied the effect of acidic phospholipids on:

1. The Ca^{2+}-ATPase activity (cycling activity);
2. The steady-state phosphointermediate level (confering mainly to the level of enzyme E_1 form); and
3. On the *p*-nitrophenylphosphatase activity (pNPPase), an enzymatic reaction catalyzed by E_2 form of PMCA enzyme (Lehotsky et al., 1992b).

First, phosphatidylinositolphosphate (PIP) and phosphatidylserine (PS) affected the Ca^{2+}-ATPase activity by a common mechanism. They modified the ATP activation curve such as to increase the V_{max} of the high affinity site (results not shown). However, conformational states (E_1 and E_2) of the PMCA pump were differentially affected by the two phospholipids. The level of phosphointermediate (confering to E_1 conformer level) was not affected by PS (30% of total phospholipid, the remainder being phosphatidylcholine), but it was increased 1.6-fold by 30% of PIP (Table 3).

Table 3
Effect of Acidic Phospholipids on the Steady-State Level of Phosphointermediate of the Ca^{2+} Transport ATPase[a]

Phospholipid	Time, s			
Activity nmol^{32}P/mg protein	0	1	2	5
PC, 100%	0	0.15 ± 0.03	0.141 ± 0.02	0.151 ± 0.03
PS, 30%	0	0.142 ± 0.04	0.15 ± 0.04	0.14 ± 0.02
PIP, 30%	0	0.187 ± 0.05	0.279 ± 0.07	0.283 ± 0.06

[a]The ATPase was reactivated by adding either pure PC, or a mixture of 30% of PS and 70% of PC or 30% of PIP and 70% of PC. Table shows reanalyzed data originally presented in Lehotsky et al. (1992b).

Table 4
Effect of Phospholipids on the *p*-Nitrophenylphosphatase Activity of the Ca^{2+}-Transport ATPase[a]

			pNPPase activity nmol/mg protein/min
Phospholipid	PC	(100%)	0.32 ± 0.09
	PS	(20%)	0.52 ± 0.11
		(30%)	0.72 ± 0.13
		(50%)	0.93 ± 0.11
		(100%)	1.08 ± 0.14
	PIP	(5%)	0.43 ± 0.09
		(10%)	0.43 ± 0.10
		(20%)	0.38 ± 0.08
		(30%)	0.33 ± 0.13

[a]Phospholipids were added as described for the level of intermediate measurement. Table shows reanalyzed data originally presented in Lehotsky et al. (1992b).

Conversely, the pNPPase activity that is exposed when the enzyme is in the E_2 conformation was stimulated in a dose-dependent manner by PS but, it was not affected by PIP (Table 4). This suggests that more than one reaction step of the ATPase cycle is affected by acidic phospholipids. PIP mainly affects a reaction step which leads to the accelerated formation of the phosphointermediate, whereas the action of PS would affect two steps, one upstream and one downstream of the intermediate. Both hydrolytic activities catalyzed by PMCA enzyme were also differentially affected by organic solvents and polycations with respect to the type of the phospholipids (Lehotsky et al., 1992a).

Evidence has been presented that besides the proposed phospholipid-binding domain (splice site A) (Zvaritch et al., 1990), a second domain

could also mediate the binding of activating acidic phospholipids. A synthetic peptide corresponding to the calmodulin-binding domain (splicing site C) interfered with the activation by negatively charged phospholipids (Brodin et al., 1992; Filoteo et al., 1992). Acidic phospholipids also interfere with calmodulin binding. In light of these facts, our kinetic results can most easily be explained by assuming that phospholipids interact with at least two sites rather than one site. Phospholipid species thus would differ in their selectivity for one site (proposed phospholipid-binding site) or the other site (calmodulin-binding site). Unfortunately, we have no data to suggest which site is preferred by PIP (PIP_2) and which by PS.

PMCA PUMP FUNCTIONAL SPECIALIZATION

Alternative splicing of PMCA gene transcript (Carafoli, 1992; Wuytack et al., 1992; Carafoli and Stauffer, 1993) at site A (phospholipid-binding site) is thought to modulate the sensitivity and specificity of the resulting isoenzymes toward phospholipids (Adamo and Penniston, 1992). Considering this, in parallel with the splicing at site C (calmodulin-binding site) affecting both the affinity of isoenzymes for calmodulin, calmodulin-binding domain and phospholipids (Enyedi et al., 1991) (Fig. 1) and, taking into account our data cited earlier, this points to a new, higher level of functional modulation-specialization of the PMCA protein. In vivo all of these slicing patterns allow to express very delicate fine-tuning of the pump activity with a variable sensitivity of enzyme isoform toward different phospholipids and calmodulin.

Furthermore, specialization must be the underlying factor of PMCA's ability to meet the specific cellular requirements for Ca^{2+} homeostasis in a tissue selective manner. This results in a functional modulation of the Ca^{2+}-transport ATPase(s) in different cell types achieved simultaneously by regulating (Fig. 2):

1. The level and the spatial distribution of the PMCA proteins;
2. The mode of alternative splicing of the gene transcript and expression of appropriate isoforms; and
3. The level of regulatory agents, among them calmodulin, acidic phospholipids, and cAMP-dependent kinase, is thought to be very relevant also in vivo.

In fact, the modulation of phosphoinositide concentration in response to agonist of the cell appears possible and has already been proved (Berridge and Irvine, 1989).

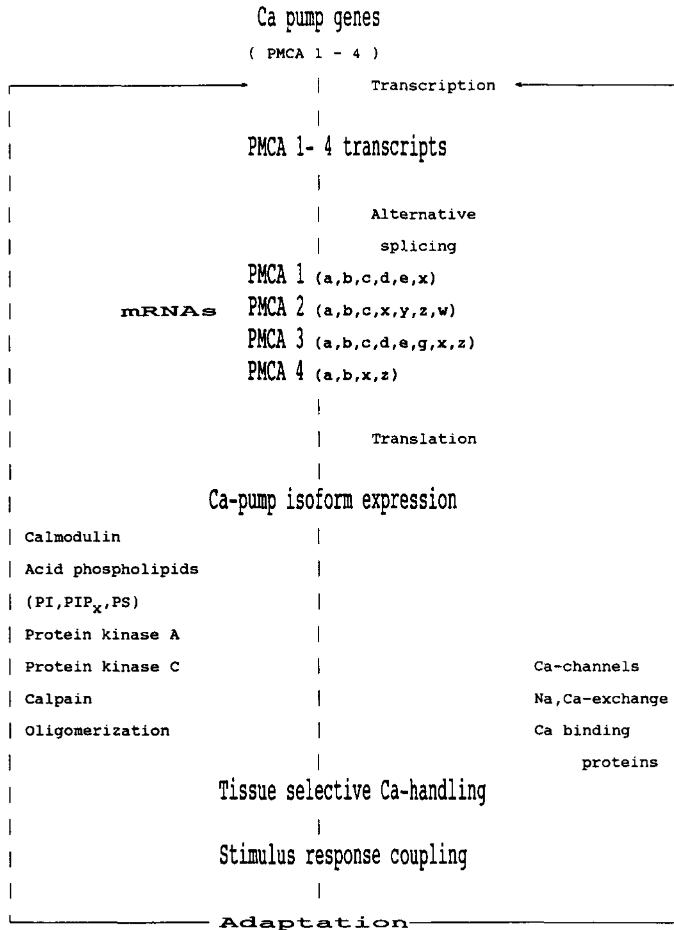

Fig. 2. Functional specialization of the Ca^{2+} transport ATPase protein from plasma membrane in a different tissue.

THE ROLE OF PMCA IN NEURONAL INJURY

Only very little is known about the possible role of PMCA in the pathomechanism of neuronal disorders. Cerebral energy failure, loss of ion- (Na^+, K^+ and Ca^{2+}) and acid-base homeostasis and generation of free radicals are important events associated with brain ischemia, stroke, and other neurological disorders. In a number of studies, emphasis has been on the influx of Ca^{2+} into the cells as a critical prelude to cell death (Siesjö, 1992). If we presume that the ability of the cell to recover from the elevated ionized Ca^{2+} is dependent not only on the activity of Na^+, Ca^{2+} exchanger but on the Ca^{2+}-ATPase, then the pump can be considered as the another potential site for altered ionic regulation.

ATPase-dependent Ca^{2+} transport by synaptic vesicles is reduced in aged animals (Michaelis, 1989). Senescence and Alzheimer disease have been associated with the reduction in Ca^{2+} pumps and other Ca^{2+} movements in the brain (Roth, 1990). Altered Ca^{2+} movements have been linked with neuronal growth and diabetes (Murrain et al., 1990; Levy et al., 1994). Ca^{2+}-ATPase might also be inhibited directly by lower pH observed during ischemia owing to altered Ca^{2+} affinity (Dixon and Haynes, 1990). Acidic lipids, especially PIP and PIP_2, are the primary polyamine binding sites in the membrane. They are able to neutralize the negative charges and therefore to counterpart the stimulation of Ca^{2+}-ATPase (Missiaen et al., 1992). Polyamine synthesis is markedly activated in different pathological states of the brain (Paschen, 1989). Polyamines could therefore be an endogenous inhibitor for the lipid stimulation of the Ca^{2+} pump with augmented effect during cellular stress.

Ischemia and subsequent reperfusion favor the formation of free radicals. They can disrupt membrane integrity by reacting with proteins and unsaturated lipid in the plasma membrane. Oxidative damage caused by free radicals inhibits the activity of the Ca^{2+} pump in the erythrocyte as well as microsomal Ca^{2+} transport in the brain (Rohn et al., 1993; Račay et al., 1994).

An increase of intracellular Ca^{2+} following insult is sufficient to alter protein phosphorylation, to activate hydrolytic enzymes and to release fatty acids from membrane phospholipids (Farooqui et al., 1992). Changes in the activities of second messenger systems and several metabolic pathways associated with membrane phospholipids are altered because of ischemia as well; this pertains especially to a rapid hydrolysis of polyphosphoinositides and the accumulation of free fatty acids. Interestingly, the activation of muscarinic acetylcholine receptors inhibits Ca^{2+}-ATPase in synaptosomes (Ross et al., 1985); inositol phosphates are able to regulate the Na^{+}, Ca^{2+}-exchanger as well as Ca^{2+}-ATPase in the brain (Frasier and Sarnacki, 1992), and the Ca^{2+} pump is regulated at different levels via phosphorylation of lipids and protein itself (Wuytack and Raeymaekers, 1992). In vivo, all of these influences may considerably affect the Ca^{2+} pump activity and may lead to an uncontrolled sustained rise in intracellular Ca^{2+} and consequently to neuronal injury.

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