Minireview

Control of Ca^{2+} Homeostasis in Neuronal Cells

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Abstract. The intracellular free Ca^{2+} concentrations show complex fluctuations in time and space in response to a variety of stimuli, and act as a pluripotent signal for many neuronal functions. Activation of cells is associated with Ca^{2+} influx from the extracellular space through voltage-dependent and/or receptor-operated Ca^{2+} channels localized on the plasma membrane, and/or by release of Ca^{2+} from intracellular stores to reach Ca^{2+} concentrations of up to micromolar levels. During cell relaxation, calcium concentration decreases to resting levels via ATP-driven Ca^{2+} transport both to the extracellular space and into the intracellular stores. Thus, Ca^{2+} homeostasis in neuronal cells is maintained by several systems differing by their mechanisms, biochemical characteristics and intracellular localization. Their biochemical properties and physiological importance as well as cellular localization are discussed in this short review.

Key words: Brain — Calcium — Ca^{2+}channels — Ca^{2+}-transport ATPases — Plasma membrane — Endoplasmic reticulum — Mitochondria — Nucleus

Abbreviations: AMPA, α-amino-3-hydroxy-5-methylisoxazolepropionate ; CaM, calmodulin; $InsP_3$, myo-D-inositol (1,4,5) trisphosphophosphate; NMDA, N-methyl-D-aspartate; PMCA, plasma membrane Ca^{2+}-ATPase; PKA, cAMP-dependent protein kinase; PKC, protein kinase C; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase

Introduction

Ionized Ca^{2+} is the most common signal transduction element in the cells ranging from bacteria to specialized neurons. The intracellular free Ca^{2+} concentrations show complex fluctuations in time and space in response to a variety of stimuli, and act as a pluripotent signal for many neuronal functions (Miller 1992; Ghosh

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and Greenberg 1995), and is therefore very tightly controlled by several systems differing by their mechanisms, biochemical characteristics and intracellular localization. Since the systems which maintain Ca^{2+} homeostasis are mostly membrane associated proteins, their classification is made on the basis of their localizations in the cell, and especially upon their location within individual cellular membranes. The following types are found in the neuronal cells:

- plasma membrane Ca^{2+} transport proteins
- endoplasmic reticulum membrane Ca^{2+} transport proteins
- mitochondrial and nuclear Ca^{2+} transport proteins.

In addition, Ca^{2+} binding proteins play an important role in the control of intracellular Ca^{2+} concentrations (for review see Carafoli 1987).

Plasma Membrane Ca^{2+} Transport Proteins

Voltage-sensitive Ca^{2+} channels

Voltage-sensitive Ca^{2+} channels are oligomeric transmembrane proteins which primarily control Ca^{2+} homeostasis in neurons. The molecular and functional diversity of voltage sensitive Ca^{2+} channels is remarkable (Tsien et al. 1991; Perez-Reyes and Schneider 1994). Different types of voltage-sensitive Ca^{2+} channels have been described in neuronal tissue, and each can be classified according to its biophysical characteristics, biochemical and pharmacological properties, molecular structure, localization and function. At present, the biophysical parameters of voltage-sensitive Ca^{2+} channels are the most common classification criteria. Table 1 illustrates the individual types of voltage-sensitive Ca^{2+} channels with their biophysical, biochemical and pharmacological properties.

The opening of voltage-sensitive Ca^{2+} channels is initiated by a change in the local membrane potential, thus two subtypes: low and high voltage-dependent Ca^{2+} channels can be recognized. Several groups of voltage-sensitive Ca^{2+} channels (high as well as low voltage-dependent channels) are expressed simultaneously in most cells of the central and peripheral nervous system. Nevertheless, sympathetic neurons express high voltage-dependent Ca^{2+} channels exclusively. In addition, the opening properties of voltage-sensitive Ca^{2+} channels are significantly modulated by several intracellular signal systems (G-proteins, phosphorylation, polyphosphatidylinositol system) (Tareilus and Breer 1995; Wickman and Clapham 1995). The distribution of different voltage-sensitive Ca^{2+} channels subtypes determined by selective binding of various radioactive labelled toxins is used to understand their contribution to various physiological processes in neurons and glial cells (Sher et al. 1991; Reuter 1996). A high density of L- and N-types of voltage-sensitive Ca^{2+} channel is expressed primarily in the grey matter and brain regions rich in synaptic connections; higher ratios of L-subtypes are seen in some specialized brain sections, e.g. lateral septal nucleus, subiculum, interpendunular and pontine

Table 1. Biophysical, biochemical and pharmacological properties of voltage-sensitive Ca^{2+} channels (VSCC) (Speddings et al. 1989; Bossu 1993; Tareilus and Breer 1995)

	Low VSCC	High VSCC		
	T	N	L	P
Conductance	8 pS	13 pS	25 pS	10 pS
Selectivity	$Ca^{2+} = Ba^{2+}$	$Ba^{2+} > Ca^{2+}$	$Ba^{2+} > Ca^{2+}$	$Ba^{2+} > Ca^{2+}$
Activation potential	−70 mV	−20 mV	−10 mV −50 mV	
Inactivation potential	−100 mV	−120 mV	−60 mV	?
Inactivation kinetics	fast	variable	slow	?
	20 ms	80–500 ms	> 500 ms	?
Effect of activators				
Atrotoxin	none	none	high	
Palmitoyl carnitine	low	?	medium	
Bay K8644	low?	none	high	
Effect of antagonists				
Metal ions	$Ni^{2+} > Cd^{2+}$	Mg^{2+}, Cd^{2+}	Mg^{2+}, Cd^{2+}	Co^{2+}
Gadolinium	none	medium	none	
ω-Conotoxin	none	high	low	none
Taicatoxin	none	none	medium	
FTX	none	none	none	low
ω-Aga IIIA	none	low	low	none
ω-Aga IVA	none	none	none	low
Phenytoin	medium	low	low	
Nifedipine/nicardipine	none	none	high	none
Verapamil/diltiazem	none	none	high	
Cinnarizine/fluspiriline	low	none?	medium	
Amiloride	low	none	medium	low

nuclei; and others, such as the facial nucleus, possess higher levels of N-subtypes. Moreover, P-subtype has the most variable distribution in the central nervous system and can be found in different parts of adult brain except of granular cells. The distribution of high-voltage channels on the cellular level is also known. While the L-subtype channels are concentrated primarily in the soma and on the basement of main dendrites of neuronal cells, N-subtype clusters are mainly present on the presynaptic part of the plasma membrane at neuromuscular junctions. P-subtype is localized predominantly on dendrites and on synaptic terminals. Unfortunately, no selective marker for T-type voltage-sensitive Ca^{2+} channels is now available, so its precise distribution in brain tissue is not yet clear.

The different functions of the individual voltage-sensitive Ca^{2+} channel subtypes in various parts of the brain are classified according to their location on specific domains on the plasma membrane and by their various pharmacological

properties (reviewed by Bertolino and Llinás 1992). Since T-subtype channels are activated by a negative potential close to the resting potential, it seems that this type is responsible for the neuronal oscillatory activity, e.g. spontaneous fluctuations of membrane potential. This oscillatory activity is thought to play an important role in various brain functions such as regulation of breathing (Jakuš et al. 1987), motor co-ordination, wakefulness regulation, and specification of neuronal circuit during ontogenesis (Llinás 1988). From the localization of the L-subtype can be derived that Ca^{2+} which enters via these channels triggers several processes in the neuronal soma (e.g. regulation of specific enzymes, organization of cytoskeleton and gene expression). A recent study (Chavis et al. 1996) demonstrated functional coupling between L-type Ca^{2+} channels and ryanodine receptors, which are important intracellular Ca^{2+} channels (see below). N-type voltage-sensitive Ca^{2+} channels play a role in some forms of neurotransmitter release, such as release of norepinephrine by sympathetic neurons, however, it seems that most synapses do not involve these channels in the exocytosis (Jahn and Südhof 1994). The precise physiological role of the N-type channels needs further clarification. The P-type channels serve as a generator of inner cellular activity, as a modulator of neuronal integration and neurotransmitter release (Coffey et al. 1994). Recently, a new type of voltage-sensitive Ca^{2+} channel, termed Q-type, has been identified in the central nervous system (Wheeler et al. 1994). It shows sensitivity to ω-conotoxin peptide MVIIC, and so far has been found only in granular cells of the cerebellum. Its possible role in the norepinephrine release primarily in the hippocampus has also been suggested (Gaur et al. 1994).

Receptor-operated Ca^{2+} channels

Ca^{2+} channels with the opening mechanism independent of membrane potential, but dependent on the activation of specific plasma membrane receptors, have been classified as receptor-operated Ca^{2+} channels (Pietrobon et al. 1990). Excitatory amino acid glutamate is one of the most prominent neurotransmitters of excitatory synapses. It acts by binding to two main types of receptors:

i) ionotropic receptors: N-methyl-D-aspartate (NMDA) type, which are Ca^{2+} permeable (McBain and Mayer 1994), and α-amino-3-hydroxy-5-methylisoxazole-propionate (AMPA)/kainate type, which are Na^+ and K^+ permeable (Jonas 1993).

ii) metabotropic receptors, the function of which is coupled via G proteins either to specific membrane phospholipase C_β or through activation or inhibition of adenyl cyclase (Pin and Duvoisin 1995). Phospholipase C releases two second lipidic messengers from membrane inositolphospholipids: myo-D-inositol (1,4,5) trisphosphate ($InsP_3$) and diacylglycerol, and thus it significantly contributes to Ca^{2+} signalling.

NMDA receptor, the most extensively studied receptor-operated Ca^{2+} channel, is localized mainly on the post-synaptic part of the neuronal membrane, hence

an entry of Ca^{2+} via this channel triggers a cascade of processes which are controlled by intracellular Ca^{2+} concentration. As in other post-synaptic membrane systems which participate in calcium homeostasis, the NMDA receptor shows a substantial gene and isoform diversity (McBain and Mayer 1994). As far as it is currently known, phosphorylation of this channel by protein kinases is an important event for the induction of neuronal long term potentiation and memory (Bliss and Collingridge 1993). Ca^{2+} entry via this channel induces expression of immediate early genes (Vendrell et al. 1993), and as it appears from cell culture studies, NMDA receptor plays a central role in neuronal excitotoxicity, ischemia and neurodegeneration (Frandsen and Schousboe 1993). The presence of 50 μmol/l glutamate in the culture media is lethal for cells but toxicity can be relieved by removal of extracellular Ca^{2+} after 15–30 min of glutamate exposure (Manev et al. 1989). In addition, the voltage-dependent blockade of the NMDA receptor by Mg^{2+} ions and an essential requirement of glycine as co-agonist are the unique features of this Ca^{2+} channel (McBain and Mayer 1994). The biophysical, biochemical and pharmacological properties of the NMDA receptor are shown in Table 2.

Table 2. Biochemical, biophysical and pharmacological properties of N-methyl-D-aspartate receptor (McBain and Mayer 1994)

Conductance	50 pS (140 mmol/l CsCl)
Mean opening time	5 ms
Selectivity	$Ca^{2+} > Na^{+}$
Agonists	Glutamate
	N-methyl-D-aspartate
Activators	Glycine is an essential coagonist of glutamate
	Spermidine activates channel
	Arachidonic acid increases open channel probability
	PKC phosphorylation activates channel and decreases sensitivity to Mg^{2+}
Antagonists	Putrescine blocks channel
	Mg^{2+} – reversible blocks calcium entry in voltage dependent manner
	Zn^{2+} blocks entry of calcium
	Phencyclidine blocks entry of calcium

Among other types of receptor-operated Ca^{2+} channels, the ATP-dependent Ca^{2+} channel (P_{2x}-receptor) has recently been identified and described in neuronal cells. It is activated by extracellular ATP and elicits different physiological responses (Valera et al. 1994). Other members of this group are Ca^{2+} channels coupled with receptors sensitive to various stimuli and second messenger-like sub-

stances. The most important of this subclass are cyclic nucleotide-gated Ca^{2+} channels found in some sensory neurons (Matthews 1991), Ca^{2+} channels coupled to G protein receptors (Birnbaumer et al. 1990; Brown 1991), and channels sensitive to inositol 1,3,4,5 tetrakisphosphate (Pietrobon et al. 1990).

Calcium release activated-calcium influx

The view that Ca^{2+} entry through the plasma membrane can also be controlled by depletion of the intracellular Ca^{2+} store (principally endoplasmic reticulum) is more than a decade old (Casteels and Droogmans 1981), and has been derived from indirect findings in different cellular models. Moreover, the discovery of thapsigargin, a selective inhibitor of sarco/endoplasmic Ca^{2+}-ATPase, has given experimental support to this hypothesis (Berridge 1995). Blocking of active Ca^{2+} transport by thapsigargin causes depletion of intracellular Ca^{2+} stores and stimulates the release of yet unidentified second messenger "calcium influx factor – CIF" or "GTP-sensitive element" (Parekh et al. 1993; Randriamampita and Tsien 1993). This factor is able to initiate Ca^{2+} entry from the extracellular space through a putative Ca^{2+} channel located on the plasma membrane. It seems that Ca^{2+} influx via this route does not require binding to receptor; is not sensitive to any classical voltage-sensitive Ca^{2+} channel inhibitors, and can be initiated by various stimuli having the common ability to deplete Ca^{2+} store. Although this "capacitative" Ca^{2+} influx mechanism has not yet been in detail described in neurons, it is thought that this mechanism is a common feature of mammalian cells including neuronal cells (Fasolato et al. 1994).

Plasma membrane Ca^{2+}-ATPase

The principal mechanisms for calcium extrusion out of the cell are active calcium transport from the cytoplasm to extracellular space mediated by Ca^{2+} pump and/or by Na^+/Ca^{2+} exchanger coupled to the action of Na^+/K^+ ATPase. It is suggested that as in other tissues, in neuronal cells mainly plasma membrane Ca^{2+}-ATPase (PMCA) participates in fine tuning of the Ca^{2+} concentration. Based on molecular biology experiments, it has been shown that PMCA protein is encoded by at least four genes with different distributions within brain structures (Stahl et al. 1992):

- PMCA 1 gene encodes isoforms expressed in practically all tissues; it is expressed predominantly in hippocampal CA1 neurons
- PMCA 2 protein is expressed mainly in the brain (mainly Purkinje cells of the cerebellum), and to a lesser extent in the heart, liver and kidney
- PMCA 3 is found in the central nervous system (choroid plexus) and skeletal muscle
- PMCA 4 is expressed in various organs but at relatively low levels

PMCA 1,3 and 4 differ mainly in the calmodulin-binding domain, which is located in the C terminal part of the protein, whereas PMCA 2 isoforms differ mostly in the domain which is adjacent to phosholipid binding region (Wuytack et al. 1992). Transcripts of individual PMCA genes are subject of further alternative RNA splicing, which depends on the tissue and on the developmental stage. In all isoforms, alternative splicing concerns two principal splicing sites located in the regions responsible for both calmodulin binding and binding of acidic phospholipids (Carafoli and Guerini 1993). Translational products of matured mRNA are isoforms which differ both in the primary structure and the biochemical properties. Neuronal tissue in particular expresses quite a number of isoforms, which all are splice variants of different genes (Wuytack and Raeymaekers 1992; Carafoli 1995). It has been suggested that the genetic, isoform and structural diversity of PMCA in neuronal tissue plays an important role to meet a special cellular requirements for calcium homeostasis in individual cell types of the central nervous system (Lehotský 1995). PMCA protein belongs to the P-type cation transporting ATPases (Pedersen and Carafoli 1987). During the reaction cycle, PMCA protein binds Ca^{2+} and is phosphorylated on the aspartyl residue of the polypeptide chain. The phosphorylated intermediate alternates in two conformational stages which mediate cation transfer through the membrane and is coupled with subsequent phosphate hydrolysis and release of the cation from its binding site. A more detailed description of the biochemical properties are shown in Table 3 and also in recent reviews (Carafoli 1992; Wang et al. 1992; Lehotský 1993).

Na^+/Ca^{2+} exchanger

In all cells, the Na^+/Ca^{2+} exchanger participates in transport of Ca^{2+} out of the cells to the extracellular space when cytoplasmic Ca^{2+} concentration increases over the resting level (Missiaen et al. 1991). Since its capacity to pump out calcium is more than ten times greater compared to PMCA, it was for a long time considered to be the principal mechanism responsible for Ca^{2+} extrusion. An analysis of the kinetic parameters showed that the protein of the Na^+/Ca^{2+} exchanger has a very low Ca^{2+} affinity ($K_{0.5} = 10-15$ μmol/l). Although apparently affinity increases in the presence of ATP ($K_{0.5} = 1-3$ μmol/l), e.g. to the levels reached at excitation state of the cell, it does not seem very likely that the exchanger can decrease Ca^{2+} to resting levels (50–200 nmol/l) (Alberts et al. 1994). However, the high density of Na^+/Ca^{2+}-exchanger in the presynaptic terminals suggests the functional relevance of the exchanger in the control of intracellular Ca^{2+} and of synaptic vesicle recycling (Reuter and Porzig 1995). In many brain areas the exchanger may also be co-localized with underlying endoplasmic reticulum structures (Juhaszova et al. 1996). This localization indicates that one role of the Na^+/Ca^{2+}-exchanger is indirect regulation of intracellular Ca^{2+} stores. Due to its voltage dependency, a reverse sequence of its transport cycle during cell stimulation has also been suggested

Table 3. Biochemical and pharmacological properties of PMCA and SERCA (Carafoli 1992; Carafoli and Chiesi 1992; Wang et al. 1992)

	PMCA	SERCA
Charge balance	H^+/Ca^{2+} exchange. Stoichiometry undetermined (0.5 or 1).	H^+/Ca^{2+} exchange. pH-dependent stoichiometry (in optimum 1:1).
ATP affinity	High affinity site $K_{0.5}$ = 1–2.5 μmol/l Low affinity site $K_{0.5}$ = 145–180 μmol/l	Low affinity site $K_{0.5} \sim 100$ μmol/l High affinity site $K_{0.5} \sim 10$ μmol/l
Ca^{2+} affinity	Resting state $K_{0.5} > 10$ μmol/l Optimally activated $K_{0.5} < 0.5$ μmol/l	Activatory site $K_{0.5}$ = 0.1–0.4 μmol/l Inhibitory site $K_{0.5}$ = 100–400 μmol/l
Inhibitors	Vanadate $K_{0.5} \sim 3$ μmol/l La^{3+} $K_{0.5} \sim 1$ μmol/l	Vanadate $K_{0.5}$ = 10–100 μmol/l Thapsigargin; La^{3+} 2.5-di-(t-butyl)-1,4-hydrochinone
Activators	CaM ($K_d \sim 1$ nmol/l) increases V_{max}, decreases $K_{0.5}(Ca^{2+})$ to 0.4–0.7 μmol/l Acidic phospholipids increase V_{max}, decrease $K_{0.5}(Ca^{2+})$ to 0.2 μmol/l, relieve sensitivity to CaM Calpain proteolysis increases V_{max}, decreases $K_{0.5}(Ca^{2+})$ to 0.4–0.7 μmol/l, relieves sensitivity to CaM PKA phosphorylation increases V_{max}, decreases $K_{0.5}(Ca^{2+})$ to 2 μmol/l PKC phosphorylation increases V_{max}, does not affect $K_{0.5}(Ca^{2+})$ Oligomerization increases V_{max}, decreases $K_{0.5}(Ca^{2+})$, relieves sensitivity to CaM	

(Miller 1991). Since it pumps out only one Ca^{2+} per 3 Na^+, the exchange process is clearly electrogenic. The protein of the brain Na^+/Ca^{2+}-exchanger has been purified only partially (Michaelis et al. 1992), but the brain exchanger was cloned and sequenced (Furman et al. 1993), and the presence of at least three different brain isoforms has been proposed (Kofuji et al. 1994). Na^+/Ca^{2+}-exchanger mRNA with a fragment sharing 91.4% sequence identity with the human cardiac Na^+/Ca^{2+}-exchanger has been detected in the rat brain (Marlier et al. 1993). The kinetic properties of Na^+/Ca^{2+}-exchanger in the rat brain synaptosomes have been studied thoroughly (Fontana et al. 1995). The recent data suggest that phosphorylation of brain Na^+/Ca^{2+}-exchanger by protein kinase C can increase the maximum velocity by nearly 50% (Blaustein et al. 1996). The heart muscle Na^+/Ca^{2+}-exchanger is regulated by inositolphospholipids (Pierce and Panagia 1989), by composition of phospholipid fatty acids chains (Vemuri and Philipson 1990) as well as by cholesterol (Kutryuk and Pierce 1988). Since the brain Na^+/Ca^{2+}-exchanger appears to

be similar to the cardiac exchanger in molecular structure and some kinetic properties, we can expect that also brain Na^+/Ca^{2+}-exchanger could be regulated by the composition of the plasmalemma.

Calcium Transport Proteins of the Endoplasmic Reticulum Membrane

Intracellular calcium channels (ryanodine- and $InsP_3$-receptors)

The intracellular non-mitochondrial Ca^{2+} store, which is thought to be the endoplasmic reticulum, can release Ca^{2+} through Ca^{2+} channel proteins located on the endoplasmic reticulum membrane. Two receptor proteins have been identified on the membrane of neuronal intracellular Ca^{2+} store which can act as a receptor-operated Ca^{2+} channel:

– receptor sensitive to plant alkaloid ryanodine (ryanodine receptor). It is regulated both by intracellular Ca^{2+} concentration (Furuichi et al. 1994a), and in nonmuscle cells, including neuronal, by cyclic ADP-ribose (Mészáros et al. 1993)

– receptor controlled by binding of $InsP_3$ and/or by intra- and extra-luminal Ca^{2+} ($InsP_3$ receptor) (Furuichi et al. 1994a).

The ryanodine receptor was identified for the first time in skeletal and later on in cardiac muscle, where it plays an essential role in the control of the excitation/contraction cycle (McPherson and Campbell 1993). Via immunochemical and molecular biology techniques, this type of receptor has also been identified in endoplasmic reticulum of both neuronal and glial cells.

Ryanodine receptor protein is encoded by three different genes which give rise to three different isoforms of the receptor each having different properties and distribution in various tissues (Furuichi et al. 1994b; Sorrentino and Volpe 1994):

– RyR1 – skeletal muscle type, expressed in skeletal muscle and the cerebellum

– RyR2 – cardiac type, expressed in various tissues, such as the heart and endothelial cells. In the central nervous system, it has been localized in different regions and seems to be the dominant brain form.

– RyR3 – expressed in epithelial cells, smooth muscle and the brain, however at much lower levels. Likely due to extreme differences among the neuronal cells, the central nervous system expresses all types of the ryanodine receptor each having very similar basic properties, however, with particular differences in their primary structures and pharmacological characteristics (Ehrlich et al. 1994; McPherson and Campbell 1993). The biochemical and pharmacological properties of the ryanodine receptor are shown in Table 4.

Myo-D-inositol (1,4,5) trisphosphate ($InsP_3$), as a second messenger molecule has been discovered only recently, however in recent years, the study of this compound has achieved substantial progress. In parallel, an important role of other inositol polyphosphates in signal transduction in the neuronal tissue has been suggested (Fisher et al. 1992). Since the ability of $InsP_3$ to release Ca^{2+} from intracel-

lular store after binding to its own receptor has been unambiguously proved, $InsP_3$ appears to play a dominant role in Ca^{2+} signal transduction in the neuronal cells (Furuichi and Mikoshiba 1995). $InsP_3$ is produced in the cell in response to various extracellular stimuli by two signal cascades (Berridge 1993).

Binding of several neurotransmitters (glutamate, acetylcholine, serotonine and others) and/or hormones (vasopressin, oxytocin and others) to the plasma membrane receptors activates, through a particular G protein, phospholipase C_β. Activation of a second type of plasma membrane receptors by several neurotrophic factors and hormones mediates autophosphorylation of the receptor, and consequently stimulation of γ-isoform of phospholipase C (Fisher 1995). Hydrolysis of membrane polyinositolphospholipids by phospholipases C generates two second intracellular messengers derived from lipids, diacylglycerols and $InsP_3$, which trigger and regulate several physiological processes dependent on calcium in all eukaryotic cells including neuronal cells (Berridge 1993). Three different genes have been identified and described which encode this type of the Ca^{2+} channel (Furuichi et al. 1994a):

– $InsP_3R1$ type – is the principal type of $InsP_3$ receptor found in the brain; it is expressed in virtually all brain structures with predominant distribution in the cerebellum and the hippocampus

– $InsP_3R2$ type – is expressed mainly in the brain and the spinal cord, however in less quantities and with 68% homology in primary structure compared to type 1 receptor

– $InsP_3R3$ – type was shown to be expressed in granular cells of the cerebellum and in some parts of the hippocampus. It shows a 60% homology in primary protein structure to both of the previous receptor types. As it has been already proved, similarly to other proteins expressed in neuronal cells, $InsP_3$ receptor diversity can be expected to underlie specific cellular requirements for Ca^{2+} homeostasis in the neuronal cells. Differences shown in primary structures and in biochemical and pharmacological properties, as well as differences shown in the distribution in the brain strongly support this view (Marshall and Taylor 1993; Miyazaki 1995). A list of basic biochemical and pharmacological properties is given in Table 4.

Calcium pump of the endoplasmic reticulum membrane

The intracellular Ca^{2+} stores in the cells of higher organisms, including neuronal cells, act as a calcium sink for excessive cytoplasmic Ca^{2+} and as a source of Ca^{2+} to be released in response to various stimuli (Henzi and MacDermott 1992; Kostyuk and Verkhratsky 1994). Active transport of Ca^{2+} against its electrochemical gradient between the cytoplasm and the lumen of the endoplasmic reticulum is accomplished by a protein of Ca^{2+}-transporting ATPase localized in the endoplasmic reticulum membrane. In comparison to the sarcoplasmic reticulum of skeletal as well as cardiac muscle, endoplasmic reticulum membranes of the brain

Table 4. Biochemical and pharmacological properties of $InsP_3$ and ryanodine receptors (Henzi and MacDermott 1992; Ehrlich et al. 1994)

	$InsP_3$ receptor	Ryanodine receptor
Molecular weight	4 × 313 kDa	4 × 565 kDa
Physiological activators	$InsP_3$: $EC_{50} \sim 100$ nmol/l other inositol polyphosphates: $EC_{50} \sim \mu$mol/l	cytoplasmic Ca^{2+} cyclic ADP-ribose
Modulation by Ca^{2+}	< 0.3 μmol/l Ca^{2+} increases activity > 0.3 μmol/l Ca^{2+} decreases activity	< 100 μmol/l Ca^{2+} increases activity > 1 mmol/l Ca^{2+} decreases activity > 1 mmol/l of luminal Ca^{2+} decreases activity
Modulation by ATP	< 2 mmol/l ATP increases activity > 4 mmol/l ATP decreases activity	ATP increases activity $EC_{50} \sim$ mmol/l
Activators	none discovered	Caffeine $EC_{50} \sim$ mmol/l Ryanodine < 10 μmol/l Heparin 2 μg/ml Doxorubicin $EC_{50} \sim \mu$mol/l $InsP_3$
Inhibitors	Heparin: competitive inhibition of $InsP_3$ binding Mg^{2+}: noncompetitive inhibition of $InsP_3$ binding H^+: noncompetitive inhibition of $InsP_3$ binding PKA phosphorylation decreases sensitivity of receptor to $InsP_3$	Ryanodine > 10 μmol/l Ruthenium red: $EC_{50} \sim \mu$mol/l H^+: cytoplasmic pH < 7.4 luminal pH < 7.4 Dantrolene: $EC_{50} \sim \mu$mol/l Mg^{2+}: $EC_{50} \sim$ mmol/l

contain only minute Ca^{2+}-transporting ATPase activity (Michelangeli 1991; Račay et al. 1994). This fact does not, however, significantly diminish the physiological importance of active Ca^{2+} transport across the endoplasmic reticulum membrane in neurons (Simpson et al. 1995; Taylor 1995). Likewise, PMCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) also belongs to the P type of ATPases, however its homology to PMCA is very low and its biochemical properties differ markedly as well (Table 3). Only the tertiary structure and the topology in the membrane seem to be similar. The gene and isoform diversity of SERCA is smaller than that of PMCA. Three genes encoding SERCA protein in a variety of tissues have been identified (Wuytack et al. 1996):

– SERCA1 gene is expressed exclusively in fast-twitch skeletal muscle, and its splicing is developmentally regulated which give rise to SERCA 1a - adult isoform and SERCA 1b – neonatal isoform

– SERCA 2 gene encodes protein isoforms which are expressed in slow-twitch

skeletal muscle, cardiac muscle, smooth muscle, in the liver, the kidney and the brain. Two isoforms are products of alternative splicing: SERCA 2a – muscle form with a high affinity to Ca^{2+} ($K_{0.5}$ = 0.17 μmol/l) and SERCA 2b – non-muscle form with lower affinity to Ca^{2+} ($K_{0.5}$ = 0.31 μmol/l).

– SERCA 3 gene is expressed in various tissues, especially in hematopoietic and epithelial tissues, and as has been recently shown also in the cerebellum (Baba-Aissa et al. 1996).

In general, it has been documented that the biochemical properties of SERCA 1 and SERCA 2a isoforms are very similar, however, there are differences between SERCA 2b and other isoforms with regard to lower Ca^{2+} affinity, lower turnover both for ATPase activity and Ca^{2+} uptake, and phospholambane sensitivity. SERCA 3 seems to be exceptional, it shows a reduced affinity to Ca^{2+}, an altered pH optimum, and an almost 10 fold higher apparent affinity for vanadate inhibition (Lytton et al. 1992). SERCA 2b is the dominant isoform expressed in neuronal cells whereas SERCA 3 isoform is expressed in significantly smaller amounts (Burk et al. 1989; Wu et al. 1995). The general properties of this Ca^{2+}-ATPase are given in Table 3 and in recent reviews (Carafoli and Chiesi 1992; Lehotský and Kaplán 1994).

Calcium Transport Through Mitochondrial Membranes

Mitochondria have long been considered as important organelles in the regulation and buffering of intracellular calcium due to their Ca^{2+} uptake ability. This view seems to have lost favour, since the Ca^{2+} affinity of the energetically coupled Ca^{2+} uptake system is very low (K_m = 10 μmol/l) (Carafoli 1987). In addition, Ca^{2+} accumulation rate is about an order of magnitude smaller than the rate of Ca^{2+} accumulation by cardiac endoplasmic reticulum. In the brain, however, this difference is not so marked. A reasonable physiological significance of calcium accumulation by the mitochondria was clarified, when it was discovered that several ATP-producing enzymes, all located in the mitochondrial matrix, are dependent on mitochondrial Ca^{2+} in the range of 1 μmol/l (Hansford 1994). Two transport systems for Ca^{2+} have been identified on the inner mitochondrial membrane (reviewed by Gunter et al. 1994). An electrophoretic uniporter, a membrane protein driven by oxidative phosphorylation, transports Ca^{2+} into the mitochodrial matrix. In the brain, this uniporter is activated by cytoplasmic Ca^{2+} and polyamines, and is inhibited by ions of Mg^{2+} and ruthenium red. Backward flux of Ca^{2+} requires another transport system which exchanges ions of Na^+ and Ca^{2+} along the inner mitochondrial membrane. Generally, lower interest is given to Ca^{2+} transport systems on mitochondrial membranes in comparison to systems from the plasma membrane or the intracellular Ca^{2+} store.

Ca^{2+} Transport Proteins of the Nuclear Membrane

Transient increases of nuclear Ca^{2+} concentration control gene transcription, DNA synthesis and repair, and other nuclear functions (Bachs et al. 1992). The mechanism of Ca^{2+} signalling and transport in the nucleus is not fully known (Csermely et al. 1995), but an inositol signalling system has been identified in the inner membrane of the nuclear envelope (Payrastre et al. 1992) and $InsP_3$-gated Ca^{2+} release from isolated nuclei has been observed (Matter et al. 1993). The earlier suggestion that nuclear and cytoplasmic Ca^{2+} concentrations are regulated independently, has been recently shown to be contradictory. It has been demonstrated that in rat basophilic leukaemia cells nuclear Ca^{2+} increases follow cytoplasmic Ca^{2+} increases and thus that those processes are not regulated independently (Allbritton et al. 1994). The limited amount of information on this topic does not allow to make final conclusions and the problem of nuclear Ca^{2+} transport systems in neurons has to be clarified by further experiments.

References

Albers R. W., Siegel G. J., Stahl W. L. (1994): Membrane transport. In: Basic Neurochemistry: Molecular, Cellular and Medical Aspects (Eds. G. J. Siegel, B. W. Agranoff, R. W. Albers and P. B. Molinoff), pp. 49 73, Raven Press, New York

Allbritton N. L., Oancea E., Kuhn M. A., Meyer T. (1994): Source of nuclear calcium signals. Proc. Natl. Acad. Sci. USA **91,** 12458–12462

Baba-Aissa F., Raeymaekers L., Wuytack F., Callewaert G., Dode L., Missiaen L., Casteels R. (1996): Purkinje neurons express the SERCA3 isoform of the organellar type Ca^{2+} transport ATPase. Mol. Brain Res. **41,** 169 174

Bachs O., Agell N., Carafoli E. (1992): Calcium and calmodulin function in the cell nucleus. Biochim. Biophys. Acta **1113,** 259–270

Berridge M. J. (1993): Inositol trisphosphate and calcium signalling. Nature **361,** 315 325

Berridge M. J. (1995): Capacitative calcium entry. Biochem. J. **312,** 1–11

Bertolino M., Llinás R. R. (1992): The central role of voltage-activated and receptor-operated calcium channels in neuronal cells. Annu. Rev. Pharmacol. Toxicol. **32,** 399–421

Birnbaumer L., Abramowitz J., Yatani A., Okabe K., Mattera R., Graf R., Sanford J., Codina J., Brown A. M. (1990): Roles of G proteins in coupling of receptors to ionic channels and other effector systems. Crit. Rev. Biochem. Molec. Biol. **25,** 225–244

Blaustein M. P., Fontana G., Rogowski R. S. (1996): The Na^+-Ca^{2+} exchanger in rat brain synaptosomes. Kinetics and regulation. Ann. N. Y. Acad. Sci. **779,** 300–317

Bliss T. V. P., Collongridge G. L. (1993): A synaptic model of memory: long-term potentiation in the hippocampus. Nature **361,** 31–39

Bossu J. L. (1993): Canaux calciques du systeme nerveux central. Pathol. Biol. **41,** 137–142

Brown A. M. (1991): A cellular logic for G protein-coupled ion channel pathways. FASEB J. **5,** 2175–2179

Burk S. E., Lytton J., MacLennan D. H., Shull G. E. (1989): cDNA cloning, functional expression, and mRNA tissue distribution of a third organellar Ca^{2+} pump. J. Biol. Chem. **264,** 18561–18568

Carafoli E. (1987): Intracellular calcium homeostasis. Annu. Rev. Biochem. **56,** 395–433

Carafoli E. (1992): The Ca^{2+} pump of the plasma membrane. J. Biol. Chem. **267,** 2115 2118

Carafoli E. (1995): Biogenesis: Plasma membrane calcium pump ATPase: 15 years of work on the purified enzyme. FASEB J. **8,** 993–1002

Carafoli E., Chiesi M. (1992): Calcium pumps in the plasma and intracellular membranes. Curr. Topics Cell. Regul. **32,** 209–241

Carafoli E., Guerini D. (1993): Molecular and cellular biology of plasma membrane calcium ATPase. Trends Cardiovasc. Med. **3,** 177–184

Casteels R., Droogmans G. (1981): Exchange characteristic of the noradrenaline-sensitive calcium store in vascular smooth muscle cells of rabbit ear artery. J. Physiol. (London) **317,** 263–279

Chavis P., Fagni L., Lansman J. B., Bockaert J. (1996): Functional coupling between ryanodine receptors and L-type calcium channels in neurones. Nature **382,** 719-722

Coffey E. T., Sihra T. S., Nicholls D. G., Pocock J. M. (1994): Phosphorylation of synapsin I and MARCKS in nerve terminals is mediated by Ca^{2+} entry via an Aga-GI sensitive Ca^{2+} channel which is coupled to glutamate exocytosis. FEBS Lett. **353,** 264–268

Csermely P., Schnaider T., Szántó I. (1995): Signalling and transport through the nuclear membrane. Biochim. Biophys. Acta **1241,** 425–452

Ehrlich B. E., Kaftan E., Bezprozvannaya S., Bezprozvanny I. (1994): The pharmacology of intracellular Ca^{2+}-release channels. Trends Pharmacol. Sci. **15,** 145–149

Fasolato C., Innocenti B., Pozzan T. (1994): Receptor-activated Ca^{2+} influx: how many mechanisms for how many channels? Trends Pharmacol. Sci. **15,** 77-83

Fisher S. K. (1995): Homologous and heterologous regulation of receptor-stimulated phosphoinosite hydrolysis. Eur. J. Pharmacol. **288,** 231–250

Fisher S. K., Heacock A. M., Agranoff B. W. (1992): Inositol lipids and signal transduction in the nervous system: An update. J. Neurochem. **58,** 18–38

Fontana G., Rogowski R. S., Blaustein M. P. (1995): Kinetic properties of the sodium-calcium exchanger in rat brain synaptosomes. J. Physiol. (London) **485,** 349 364

Frandsen A., Schousboe A. (1993): Excitatory amino acid-mediated cytotoxicity and calcium homeostasis in cultured neurons. J. Neurochem. **60,** 1202-1211

Furman I., Cook O., Kasir J., Rahamimoff H. (1993): Cloning of two isoforms of rat brain Na^{+}-Ca^{2+}calmodulin-dependent-cGMP-phosphodiesterase exchanger gene and their functional expression in HeLa cells. FEBS Lett. **319,** 105 109

Furuichi T., Mikoshiba K. (1995): Inositol 1,4,5-trisphosphate receptor-mediated Ca^{2+} signaling in the brain. J. Neurochem. **64,** 953 960

Furuichi T., Kohda K., Miyawaki A., Mikoshiba K. (1994a): Intracellular channels. Curr. Opin. Neurobiol. **4,** 294–303

Furuichi T., Furutama D., Hakamata Y., Nakai J., Takeshima H., Mikoshiba K. (1994b): Multiple types of ryanodine receptor/Ca^{2+} release channels are differentially expressed in rabbit brain. J. Neurosci. **14,** 1791 1805

Gaur S., Newcomb R., Rivnay B., Bell J. R., Yamashiro D., Ramachandran J., Miljanich G. P. (1994): Calcium channel antagonist peptides define several components of

transmitter release in the hippocampus. Neuropharmacology **33,** 1211–1219

Ghosh A., Greenberg M. E. (1995): Calcium signaling in neurons: Molecular mechanisms and cellular consequences. Science **268,** 239–247

Gunter T. E., Gunter K. K., Sheu S.-S., Gavin C. E. (1994): Mitochondrial calcium transport: physiological and pathological relevance. Amer. J. Physiol. **267,** 313–339

Hansford R. G. (1994): Physiological role of mitochondrial Ca^{2+} transport. J. Bioenerg. Biomembr. **26,** 495–508

Henzi V., MacDermott A. B. (1992): Characteristics and function of Ca^{2+}- and inositol 1,4,5-triphosphate-releasable stores of Ca^{2+} in neurons. Neuroscience **46,** 251–273

Jahn R., Südhof T. C. (1994): Synaptic vesicles and exocytosis. Annu. Rev. Neurosci. **17,** 219–246

Jakuš J., Tomori Z., Stránsky A., Bošeľová L. (1987): Bulbar respiratory activity during defensive airway reflexes in cats. Acta Physiol. Hung. **70,** 245–254

Jonas P. (1993): AMPA-type glutamate receptors – nonselective cation channels mediating fast excitatory transmission in the CNS. In: Nonselective Cation Channels: Pharmacology, Physiology and Biophysics (Eds. D. Siemen and J. Hescheler), pp. 61–76, Birkhäuser Verlag, Basel

Juhaszova M., Shimizu H., Borin M. L., Yip R. K., Santiago E. M., Lindenmayer G. E., Blaustein M. P. (1996): Localization of the Na^{+}-Ca^{2+} exchanger in vascular smooth muscle, and in neurons and astrocytes. Ann. N. Y. Acad. Sci. **779,** 318–335

Kofuji P., Lederer W. J., Schulze D. H. (1994): Mutually exclusive and cassette exons underlie alternatively spliced isoforms of the Na/Ca exchanger. J. Biol. Chem. **269,** 5145–5149

Kostyuk P., Verkhratsky A. (1994): Calcium stores in neurons and glia. Neuroscience **63,** 381–404

Kutryuk M. J. B., Pierce G. N. (1988): Stimulation of sodium-calcium exchange by cholesterol incorporation into isolated cardiac sarcolemmal vesicles. J. Biol. Chem. **263,** 13167–13172

Lehotský J. (1993): Regulation of Ca^{2+}-transporting ATPase from the plasma membrane. Biológia (Bratislava) **48,** 295–300

Lehotský J. (1995): Plasma membrane Ca^{2+}-pump functional specialization in the brain. Complex of isoform expression and regulation by effectors. Mol. Chem. Neuropathol. **25,** 175–187

Lehotský J., Kaplán P. (1994): Calcium transport in the smooth muscle endoplasmic reticulum. Biológia (Bratislava) **49,** 377–384

Llinás R. R. (1988): The intrinsic electrophysiological properties of mammalian neurons: insight into central nervous system function. Science **242,** 1654–1664

Lytton J., Westlin M., Burk S. E., Shull G., MacLennan D. H. (1992): Functional comparisons between isoforms of the sarcoplasmic or endoplasmic reticulum family of calcium pumps. J. Biol. Chem. **267,** 14483–14489

Manev H., Favaron M., Guidotti A., Costa E. (1989): Delayed increase of Ca^{2+} influx elicited by glutamate: role in neuronal death. Mol. Pharmacol. **36,** 106–112

Marlier L. N. J.-L., Zheng T., Tang J., Grayson D. R. (1993): Regional distribution in the rat central nervous system of a mRNA encoding a portion of the cardiac sodium/calcium exchanger isolated from cerebellar granule neurons. Mol. Brain Res. **20,** 21–39

Marshall I. C. B., Taylor C. W. (1993): Regulation of inositol 1,4,5-trisphosphate receptors. J. Exp. Biol. **184,** 161–182

Matter N., Ritz M., Freyermuth S., Rogue P., Malviya A. N. (1993): Stimulation of nuclear protein kinase C leads to phosphorylation of nuclear inositol 1,4,5-trisphosphate receptor and accelerated calcium release by inositol 1,4,5-trisphosphate from isolated rat liver nuclei. J. Biol. Chem. **268,** 732–736

Matthews G. (1991): Ionic channels that are directly activated by cyclic nucleotides. Trends Pharmacol. Sci. **12,** 245–247

McBain C. J., Mayer M. L. (1994): N-methyl-D-aspartic acid receptor structure and function. Physiol. Rev. **74,** 723–760

McPherson P. S., Campbell K. P. (1993): The ryanodine receptor/Ca^{2+} release channel. J. Biol. Chem. **268,** 13765–13768

Mészáros L. G., Bak J., Chu A. (1993): Cyclic ADP-ribose as an endogenous regulator of the non-skeletal type ryanodine receptor. Nature **364,** 76–79

Michaelis M. L., Nunley E. W., Jayawickreme C., Hurlbert M., Schueler S., Guilly C. (1992): Purification of a synaptic membrane Na^+/Ca^{2+} antiporter and immunoextraction with antibodies to a 36-kDa protein. J. Neurochem. **58,** 147–157

Michelangeli F., Di Virgilio F., Villa A., Podini P., Meldolesi J., Pozzan T. (1991): Identification, kinetic properties and intracellular localization of the (Ca^{2+}-Mg^{2+})-ATPase from the intracellular stores of chicken cerebellum. Biochem. J. **275,** 555–561

Miller R. J. (1991): The control of neuronal Ca^{2+} homeostasis. Progr. Neurobiol. **37,** 255–285

Miller R. J. (1992): Neuronal Ca^{2+}: getting it up and keeping it up. Trends Neurosci. **15,** 317–319

Missiaen L., Wuytack F., Raeymaekers L., DeSmedt H., Droogmans G., Declerck I., Casteels R. (1991): Ca^{2+} extrusion across plasma membrane and Ca^{2+} uptake by intracellular stores. Pharmac. Ther. **50,** 191–232

Miyazaki S. (1995): Inositol trisphosphate receptor mediated spatiotemporal calcium signalling. Curr. Opin. Cell Biol. **7,** 190–196

Parekh A. B., Terlau H., Stühmer W. (1993): Depletion of $InsP_3$ stores activates a Ca^{2+} and K^+ current by means of a phosphatase and a diffusible messenger. Nature **364,** 814–818

Payastre B., Nievers M., Boonstra J., Breton M., Verkleij A. J., VanBergen en Henegouwen P. M. (1992): A differential location of phosphoinositide kinases, diacylglycerol kinase, and phospholipase C in the nuclear matrix. J. Biol. Chem. **267,** 5078–5084

Pedersen P. L., Carafoli E. (1987): Ion motive ATPases. II. Energy coupling and work output. Trends Biochem. Sci. **12,** 186–189

Perez-Reyes E., Schneider T. (1994): Calcium channels: structure, function and classification. Drug Develop. Res. **33,** 295–318

Pierce G. N., Panagia V. (1989): Role of phosphatidylinositol in cardiac sarcolemmal membrane sodium-calcium exchange. J. Biol. Chem. **264,** 15344–15350

Pietrobon D., Virgilio F. D., Pozzan T. (1990): Structural and functional aspects of calcium homeostasis in eukaryotic cells. Eur. J. Biochem. **193,** 599–622

Pin J.-P., Duvoisin R. (1995): Neurotransmitter receptors I. The metabotropic glutamate receptors: structure and functions. Neuropharmacology **34,** 1–26

Račay P., Bezáková G., Kaplán P., Lehotský J. (1994): Intracellular Ca^{2+} store from rabbit brain: Kinetic aspects of Ca^{2+} stimulated ATPase. Biochem. Mol. Biol. Int. **33,** 981–990

Randriamampita C., Tsien R. Y. (1993): Emptying of intracellular Ca^{2+} stores releases a novel small messenger that stimulates Ca^{2+} influx. Nature **364,** 809–814

Reuter H. (1996): Diversity and function of presynaptic calcium channels in the brain. Curr. Opin. Neurobiol. **6,** 331–337

Reuter H., Porzig H. (1995): Localization and functional significance of the Na^{+}/Ca^{2+} exchanger in presynaptic boutons of hippocampal cells in culture. Neuron **15,** 1077–1084

Sher E., Biancardi E., Passafaro M., Clementi F. (1991): Physiopathology of neuronal voltage-operated calcium channels. FASEB J. **5,** 2677–2683

Simpson P. B., Challis J. R. A., Nahorski S. R. (1995): Neuronal Ca^{2+} stores: activation and function. Trends Neurosci. **18,** 299–306

Sorrentino V., Volpe P. (1994): Ryanodine receptors: how many, where and why? Trends Pharmacol. Sci. **14,** 98–103

Speddings M., Kilpatrick A. T., Alps B. J. (1989): Activators and inactivators of calcium channels: effects in the central nervous system. Fundam. Clin. Pharmacol. **3,** 3s–29s

Stahl W. L., Eakin T. J., Owens J. W. M. Jr., Breininger J. F., Filuk P. E., Anderson W. R. (1992): Plasma membrane Ca^{2+}-ATPase isoforms: distribution of mRNAs in the rat brain by in situ hybridization. Mol. Brain Res. **16,** 223–231

Tareilus E., Breer H. (1995): Presynaptic calcium channels: pharmacology and regulation. Neurochem. Int. **26,** 539–558

Taylor C. W. (1995): Why do hormones stimulate Ca^{2+} mobilization? Biochem. Soc. Trans. **23,** 637–642

Tsien R. W., Ellinor P., Horne W. A. (1991): Molecular diversity of voltage-dependent Ca^{2+} channels. Trends Pharmacol. Sci. **12,** 349–354

Valera S., Hussy N., Evans R. J., Adami N., North R. A., Surprenant A., Buell B. (1994): A new class of ligand-gated ion channel defined by P_{2X} receptor for extracellular ATP. Nature **371,** 516–519

Vemuri R., Philipson K. D. (1990): Influence of phospholipid fatty acyl composition on sarcolemal and sarcoplasmic reticular cation transporters. Biochem. Biophys. Res. Commun. **168,** 917–922

Vendrell M., Curran T., Morgan J. I. (1993): Glutamate, immediate-early genes, and cell death in the nervous system. Ann. N. Y. Acad. Sci. **679,** 132–141

Wang K. K. W., Villalobo A., Roufogalis B. D. (1992): The plasma membrane calcium pump: a multiregulated transporter. Trends Cell Biol. **2,** 46–52

Wheeler D. B., Randall A., Tsien R. W. (1994): Roles of N-type and Q-type Ca^{2+} channels in supporting synaptic transmission. Science **264,** 107–111

Wickman K., Clapham D. E. (1995): Ion channel regulation by G proteins. Physiol. Rev. **75,** 865–885

Wu K.-D., Lee W. S., Wey J., Bungard D., Lytton J. (1995): Localization nad quantification of endoplasmic reticulum Ca^{2+}-ATPase isoform transcripts. Amer. J. Physiol. **269,** c775–c784

Wuytack F., Raeymaekers L. (1992): The Ca^{2+}-transport ATPases from the plasma membrane. J. Bioenerg. Biomembr. **24,** 285–300

Wuytack F., Raeymaekers L., De Smedt H., Eggermont J. A., Missiaen L., Van Den Bosch L., De Jaegere S., Verboomen H., Plessers L., Casteels R. (1992): Ca^{2+}-transport ATPases and their regulation in muscle and brain. Ann. N. Y. Acad. Sci. **671,** 82–91

Wuytack F., Raeymaekers L., Eggermont J., VanDenBosch L., Verboomen H., Mertens L. (1996): Isoform diversity and regulation of organellar-type Ca^{2+}-transport ATPases. In: Ion Pumps. Advances in Molecular and Cell Biology (Ed. J. P. Andersen), JAI Press, Greenwich, (in press)

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