1044–7393/98/3303–0199 $10.50

Distribution and Isoform Diversity of the Organellar Ca^{2+} Pumps in the Brain

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Received November 5, 1996; Revised June 2, 1997; Accepted June 3, 1997

ABSTRACT

The gene family of organellar-type Ca^{2+} transport ATPases consists of three members. SERCA1 is expressed exclusively in fast skeletal muscle; SERCA2 is ubiquitously expressed, whereas SERCA3 is considered to be mainly expressed in cells of the hematopoietic lineage and in some epithelial cells. In the brain, the organellar-type Ca^{2+} transport ATPases are almost exclusively transcribed from the SERCA2 gene. Four different SERCA2 mRNAs have been described (classes 1–4). However, unlike in nonneuronal cells, which express the class 1, 2, and 3 splice variants, the main SERCA2 mRNA in the brain is the class 4 messenger. Similar to classes 2 and 3, the class 4 codes for the ubiquitously expressed SERCA2b protein. Recently, we have reported the distribution of the SERCA isoforms in the brain (Baba-Aissa et al., 1996a,b). SERCA2b was present in most neurons of all investigated brain regions. The highest levels were found in the Purkinje neurons of the cerebellum and in the pyramidal cells of the hippocampus. Interestingly, SERCA3 and SERCA2a are coexpressed along with SERCA2b in the Purkinje neurons, but are weakly expressed in the other brain regions if present at all. Since these three protein isoforms have a different affinity for Ca^{2+}, their possible roles in relation to Ca^{2+} stores in neurons are discussed.

Index Entries: SERCA; calcium; endoplasmic reticulum; brain; neuron.

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INTRODUCTION

Biological responses in nerve cells that use Ca^{2+} as a second messenger are critically dependent on the maintenance of a resting cytosolic free calcium concentration ($[Ca^{2+}]_{cyt}$) in the submicromolar range. There is now compelling evidence that a high intracellular calcium concentration for long duration leads to an impaired Ca^{2+} homeostasis in nerve cells, causing irreversible brain damage (*see*, for review, Siesjö, 1990; Heizman and Braun, 1992). On the other hand, transient elevations of $[Ca^{2+}]_{cyt}$ are crucial for transmitter release, synaptic transmission, and synaptic plasticity. To control $[Ca^{2+}]_{cyt}$ precisely, an elaborate membrane system containing channels, pumps, and exchangers is required. The Na^+/Ca^{2+} exchanger and the plasma membrane Ca^{2+} transport ATPases extrude Ca^{2+} out of the cell, whereas the Ca^{2+} transport ATPase in the internal stores sequester Ca^{2+} into the sarcoplasmic (SR) or endoplasmic reticulum (ER). The ER and its specializations are present in almost all compartments of neurons, including the soma, dendrites, and synaptic terminals. This organelle not only helps to initiate the Ca^{2+} signal by releasing Ca^{2+}, but also acts as a buffering system through the action of the Ca^{2+} transport ATPases that sequester Ca^{2+} into the lumen (*see*, for review, Simpson et al., 1995).

SERCA ISOFORM DIVERSITY

The ATPases that mediate Ca^{2+} uptake into the intracellular stores are proteins of 100–120 kDa, which belong to the family of sarco-endoplasmic reticulum Ca^{2+} transport ATPases (SERCA). Like the plasma membrane Ca^{2+} transport ATPases (which belong to the PMCA gene family), the SERCA pumps are members of the superfamily of P-type ion pumps, which are characterized by the formation of a phosphorylated intermediate as part of the catalytic cycle (Pedersen and Carafoli, 1987a,b). Three distinct genes constitute the SERCA family: SERCA1, SERCA2, and SERCA3. Alternative processing of the primary transcript of these genes generates additional SERCA isoform diversity.

SERCA1 is exclusively present in fast skeletal muscle (Brandl et al., 1986). The alternative splicing of the SERCA1 gene generates an adult (SERCA1a) and a neonatal (SERCA1b) isoform. These two isoforms differ in their carboxyl end. In SERCA1a, the last amino acid (aa) is replaced by 8 aa in SERCA1b. However, no functional differences have been detected between these two Ca^{2+} pumps (Maruyama and MacLennan, 1988).

The primary transcript of the SERCA2 gene is similarly subject to tissue-dependent differential processing, resulting in the formation of four different classes of mRNA (Eggermont et al., 1991; Plessers et al., 1991). Although the expression of the class 1 mRNA is muscle-specific,

the class 2 and 3 mRNAs are present in all nonmuscle tissues. Interestingly, the class 4 mRNA isoform is exclusively found in brain tissue (Plessers et al., 1991). The four classes of mRNA are translated all together into two distinct proteins (Fig. 1). The class 1 mRNA is translated to the muscle-specific SERCA2a protein isoform, whereas the class 2, 3, and 4 mRNAs, differing only in their 3'-untranslated region, all encode the same protein isoform SERCA2b, which is considered to be the "housekeeping" isoform. The SERCA2b protein is identical to the SERCA2a for the first 993 aa. At the C-terminal end, 4 aa are additionally present in SERCA2a. In SERCA2b this tail is replaced by a longer one of 49 (rat) or 48 aa (human and rabbit) (Lytton and MacLennan, 1988; Gunteski-Hamblin et al., 1988; Eggermont et al., 1989; Campbell et al., 1991). This extended SERCA2b tail contains a hydrophobic domain that has been suggested to form an 11th transmembrane domain (Eggermont et al., 1989; Campbell et al., 1992). SERCA2a shows a twofold lower affinity for Ca^{2+}, but a higher catalytic turnover rate than SERCA2b (Lytton et al., 1992; Verboomen et al., 1994).

The last member of the SERCA gene family, SERCA3, has been detected along with SERCA2 in platelets, lymphoid cells, mast cells, and in some endothelial cells (Bobe et al., 1994; Wuytack et al., 1994). Recent data also suggest that the primary transcript is subject to alternative processing generating at least two isoforms, the known SERCA3a isoform and a new SERCA3b isoform (Dode et al., 1996; Tokoyama et al., unpublished). In the brain, however, only the SERCA3a mRNA isoform could be detected (Dode et al., unpublished observations). As compared to the other SERCA members, SERCA3 shows the lowest Ca^{2+} affinity (Toyofuku et al., 1992). The physiological significance of the functional differences between the SERCA isoforms has not yet been elucidated.

Although the expression of these SERCA protein isoforms has been extensively studied in muscle tissues, their expression pattern in the brain remained largely unexplored for a long time. We review here our recent work on this topic (Plessers et al., 1991; Baba-Aissa et al., 1996a,b).

SERCA2b IS THE MAJOR ISOFORM EXPRESSED IN THE BRAIN

The distribution of SERCA2 mRNA in the brain has initially been studied by *in situ* hybridization using SERCA2 probes that did not discriminate between the SERCA2a- and SERCA2b-encoding mRNA classes. This work has shown that SERCA2 is widely distributed in the rat brain (Miller et al., 1991). Northern blot analysis, which is able to discriminate between the different SERCA2 mRNA classes, indicated a low level of SERCA2a mRNA (Burk et al., 1989; Wu et al., 1995), suggesting that the reported distribution of SERCA2 mRNA in the rat brain corresponded

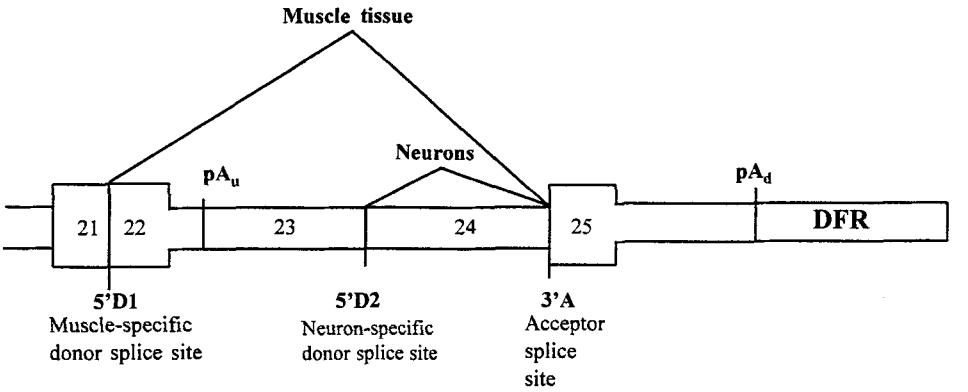

Fig. 1. Schema of the modes of alternative processing of the 3′ end of the SERCA2 gene. The exon numbers are indicated within the corresponding boxes. Regions corresponding to coding and noncoding sequences are represented by wide and narrow boxes, respectively. Exon 21 is the last constitutively spliced exon. Exons 22–25 are included or excluded in different combinations by the use or skipping of two alternative splice donor sites (5′D1 and 5′D2), as follows: Class 1: The use of 5′D1 results in splicing out of exons 22, 23, and 24; this process occurs in muscle tissue. Class 2: The use of the upstream polyadenylation site (pA_u) results in a messenger ending with exon 22. Class 3: All exons are included, resulting in polyadenylation at the downstream polyadenylation site (pA_d); both class 2 and class 3 messengers predominate in nonmuscle and nonneuronal cells. Class 4: The use of 5′D2 results in splicing out of exon 24 only; this is the brain-specific messenger.

more likely to the expression of SERCA2b than to that of SERCA2a. Using a specific SERCA2b antibody, we were able to confirm these assumptions at the protein level and to map in detail the cellular distribution of these isoforms in the rat and cat brain (Baba-Aissa et al., 1996b). It was clearly demonstrated that SERCA2b is the major isoform present in the brain and that it is exclusively found in neuronal cells. Most likely, SERCA2b is not completely absent from the glial cells, but it is present at a level below the detection limit of the immunocytochemical method. Indeed, SERCA2 mRNA and most specifically the class 3 and 4 mRNAs could be detected by the polymerase chain reaction (PCR) in rat cultured neuroglial cells (Baba-Aissa et al., unpublished observations). The low level of expression of SERCA in glial cells could be related to the divergent Ca^{2+} regulation pathways found in glial and neuronal cells. For example, Peuchen et al. (1996) have shown that in glial cells, refilling of the internal calcium store, even after repeated stimulation, is a very slow process.

Interestingly, the large neurons are usually more heavily labeled than the smaller ones when stained with the SERCA2b antisera. The processes, including axon and dendrites, were in general immunonega-

tive. Exceptions are the proximal dendrites of the pyramidal cells in the cerebral cortex and in the hippocampus, and the whole dendritic tree, including the thin branches, of the Purkinje neurons.

Although SERCA2b is widely distributed in most regions of the cat and rat brain, the SERCA2a and SERCA3 isoforms are exclusively found in the Purkinje neurons of the cerebellum. Earlier, Wu et al. (1995) found that the cerebellum was the major site of the expression of the SERCA2a and SERCA3 mRNA in the brain. Our results on the SERCA proteins are in agreement with the mRNA data. Our immunological observations, using affinity-purified antibodies that recognize specifically the SERCA2a and SERCA3 protein isoforms, have indeed shown that none of these protein isoforms could be detected in the cerebrum and brainstem, with the possible exception of the giant cells of the reticular formation, which express a very low level of SERCA2a. In contrast, the Purkinje neurons express SERCA2a and SERCA3 along with SERCA2b.

DISTRIBUTION PATTERN OF SERCA2b IN THE CEREBRUM AND BRAINSTEM

Although all neurons of the rat and cat brain express the SERCA2b isoform, the expression level is not the same in all brain regions. The highest density is found in the cerebellum, in the hippocampus, followed by the cerebral cortex, and the inferior olivary nucleus, whereas the different thalamic nuclei in the cerebrum and most nuclei of the brainstem express a moderate to low level of SERCA2b (for details, *see* Baba-Aissa et al., 1996b). Within these regions, further slight variations of the signal intensity are seen. For example, CA3 and CA1 pyramidal cells of the hippocampus express the highest level of SERCA2b protein as compared to the other hippocampal subfields and to the dentate gyrus. Similarly, the pyramidal cells of the layers III and V of the cerebral cortex exhibit a more intense SERCA2b labeling than the other cortical layers. Some regions show a very low expression level of SERCA2b (substantia nigra and the hypothalamus nuclei), whereas a few are completely immunonegative (pyramid and spinal trigeminal tract).

It is interesting to mention the high expression level of SERCA2b in neurons, probably mossy cells, within the hilus of the hippocampus. Until now, labeling for any of the Ca^{2+}-binding proteins, like paravalbumin or calbindin, has not been found in these cells (Sloviter and Nivaler, 1987; Sloviter, 1989). Mossy cells are vulnerable to seizure-induced damage (Sloviter, 1987; Strowbridge et al., 1992). During such events, oxygen radicals are formed, and Ca^{2+} pumps have been shown to be very susceptible to these compounds (Pereira et al., 1996).

In spite of some discrepancies, the distribution pattern of SERCA generally agrees with the reported distribution of $^{45}Ca^{2+}$ uptake in the rat

brain (Miller et al., 1991). Moreover, Kostyuk et al. (1989) have shown that, in snail neurons, the rate of $[Ca^{2+}]_{cyt}$ recovery following Ca^{2+} influx is mainly determined by the Ca^{2+} pump of the ER. These results suggest that, in neurons, SERCA2b could play a crucial role for a fast refilling of the Ca^{2+} stores, thereby assisting Ca^{2+}-binding proteins in lowering $[Ca^{2+}]_i$ down to the range found in resting cells.

PURKINJE NEURONS OF THE CEREBELLUM ARE THE ONLY CELL TYPE KNOWN TO EXPRESS THREE DIFFERENT SERCA ISOFORMS

Purkinje neurons of the cerebellum are remarkable in that many types of proteins involved in the regulation of Ca^{2+} metabolism are expressed in these cells and in that these proteins are expressed at an exceptionally high level. These proteins include the Ca^{2+}-release channels of the ER (inositol 1,4,5-trisphosphate receptors and ryanodine receptors) and the cytosolic Ca^{2+}-binding proteins (calbindin and parvalbumin). For example, the number of binding sites for inositol 1,4,5-trisphosphate, the intracellular messenger that opens the corresponding Ca^{2+}-release channels in the endoplasmic reticulum, is in the molecular layer of the cerebellum more than threefold higher than in the CA1 layer of the hippocampus, and more than 10-fold higher than in the cerebral cortex (Worley et al., 1987). It may not be surprising, therefore, that Purkinje neurons are unusual also with respect to SERCA expression. First, Purkinje neurons express the ubiquitous SERCA2b at a significantly higher concentration than other neurons. Second, Purkinje neurons in addition coexpress the SERCA2a and SERCA3 isoform. The expression of SERCA2a is restricted to the cell body of the Purkinje neurons, whereas that of SERCA2b and SERCA3 can be demonstrated by immunocytochemistry both in the cell body and in the dendritic processes of these neurons (Fig. 2). We have established by single-cell PCR that, in one single Purkinje neuron, the level of SERCA2 mRNA is threefold higher than that of SERCA3 (Baba-Aissa et al., 1996a). At the light-microscopical level, we could not see any evidence for a differential distribution of SERCA2b and SERCA3 in the Purkinje neurons. It would be interesting to find out whether or not SERCA2b and SERCA3 are colocalized also at the electron microscopical level. Since SERCA3 has a lower calcium affinity than SERCA2b, SERCA3 may be better adapted to cytosolic compartments in which Ca^{2+} reaches a high level. Such unusually high Ca^{2+} transients have been demonstrated by Petrozzino et al. (1995) in the spines of the CA1 pyramidal in cell culture. Using a low-affinity Ca^{2+} indicator (fura-5), these authors have demonstrated that following synaptic stimulation, the local $[Ca^{2+}]_{cyt}$ in the spines increases up to 20–40 μM, which is a value higher than any other one thus far reported. However, although exten-

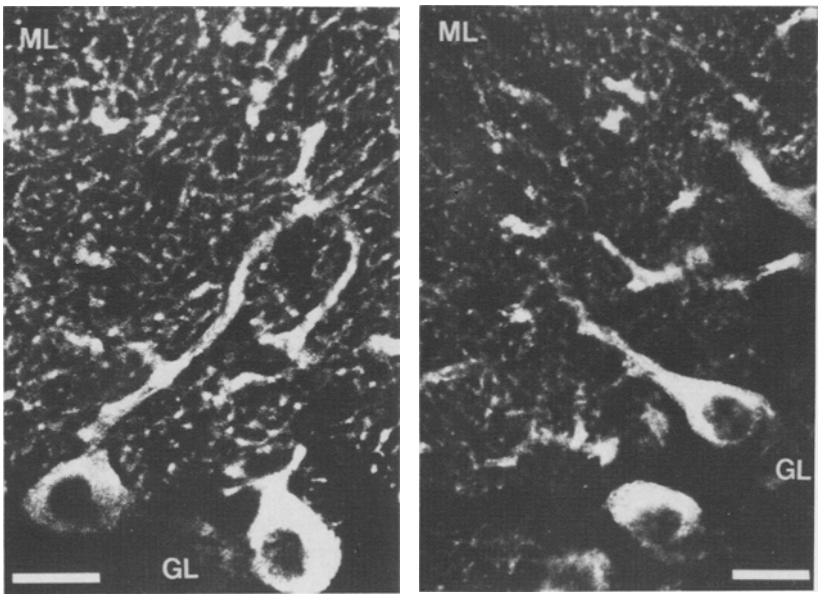

Fig. 2. Confocal immunofluorescence image of SERCA3(A) and SERCA2b(B) Ca^{2+} pumps in rat cerebellum. Both Ca^{2+} pump isoforms are localized in the cell body and in the proximal and distal dendrites. ML, molecular layer; GL, granular layer. Scale bars = 10 μm.

sive studies have been done on the properties of Ca^{2+} transients in Purkinje neurons, experimental data using fura-5 are lacking for this cell type.

By subfractionation of a crude microsomal fraction from the cerebellum on a sucrose gradient, Villa et al. (1992) have shown that SERCA protein was concentrated in particular in the very light subfractions, but some was also recovered in the heaviest subfraction. However, the antibody they used could bind the SERCA2a as well as the SERCA2b and SERCA3 protein isoforms. Therefore, further analysis of subcellular membrane fractions using isoform-specific antibodies is required.

Purkinje neurons are presently the only cell type known to coexpress three different SERCA isoforms. These isoforms differ in their Ca^{2+} affinity. However, the functional meaning of the coexpression within one cell type of three functionally different Ca^{2+} pumps is not yet understood.

NEURONS PREFERENTIALLY EXPRESS THE CLASS 4 mRNA

If at the protein level the SERCA2b is the major isoform expressed in the brain, at the mRNA level, the situation is more complex. As mentioned above, SERCA2b protein is encoded by three different mRNAs: classes 2, 3, and 4. One of them, the class 4 mRNA, is predominately

expressed in neuronal tissue (Plessers et al., 1991). Although the protein isoform generated by the class 4 is the same as that encoded by the class 2 and 3 mRNAs, we do not yet understand why this transcript is preferentially expressed in the brain. The class 2 and 3 mRNAs are also expressed in the brain, but at a much lower level than the class 4 mRNA (Plessers et al., 1991). As mentioned above, not only in neurons, but also in glial cells (at least in rat C6 cell line), the class 4 mRNA variant is present. The meaning of the expression of three different transcripts (expressed or not in the same cell type?) and the factors regulating the processing in brain tissue of the SERCA2 gene to the neuron-specific class 4 mRNA is presently not understood.

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