Control of Calcium Homeostasis in *Schistosoma mansoni*

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Calcium signalling is fundamental for muscular contractility of Schistosoma mansoni. *We have previously described the presence of transport ATPases (Na^+,K^+-ATPase and (Ca^{2+}-Mg^{2+})-ATPase) and calcium channels (ryanodine receptors - RyR) involved in control of calcium homeostasis in this worm. Here we briefly review the main technics (ATPase activity, binding with specific radioligands, fluxes of $^{45}Ca^{2+}$ and whole worm contractions) and results obtained in order to compare the distribution patterns of these proteins: thapsigargin-sensitive (Ca^{2+}-Mg^{2+})-ATPase activity and RyR co-purified in P_1 and P_4 fractions mainly, which is compatible with a sarcoplasmic reticulum localization, while basal ATPase (along with Na^+,K^+-ATPase) and thapsigargin-resistant (Ca^{2+}-Mg^{2+})-ATPase have a distinct distribution, indicative of their plasma membrane localization. Finally we attempt to integrate these contributions with data from other groups in order to propose the first synoptic model for control of calcium homeostasis in* S. mansoni.

Key words: *Schistosoma mansoni* - calcium - ATPase - ryanodine

As for mammals, the control of low cytosolic calcium concentrations appears to be very important in *Schistosoma mansoni* cell physiology as a whole, and in muscular contractility in particular. The main muscle present in adult worms presents morphologic similarities with the smooth type present in higher animals (Silk & Spence 1969). On the other hand, species differences exist in relation to the nature of the putative transmitters (worm specific FMRFamide related peptides, e.g. - Day & Maule 1999) and/or receptors (distinct pharmacological modulation of 5-hydroxytryptamine effects, e.g. - Willcokson & Hillman 1984) involved in *S. mansoni* contraction. Some years ago, we had no idea of how the worm could maintain a low cytosolic calcium after a contraction induced by such stimuli, so that we initiated studies to investigate the presence of transport ATPases and ion channels present either on plasma membrane or intracellular organelles. In the present communication we re-analyze some of our data, integrate our main contributions with data from other groups in order to propose a synoptic model for the control of calcium homeostasis in *S. mansoni*, and further add some insight to the phylogenetic importance of these proteins.

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MATERIALS AND METHODS

Preparation of subcellular fractions - About 2000 adult worms (BH strain) were homogenized in a Dounce homogenizer at 4°C in 0.25 M sucrose solution (5 mM Tris-HCl pH 7.4) using three sequences of 10 passes of the pestle. The homogenate was centrifuged to obtain four pellets (P_1, P_2, P_3, P_4) sedimenting respectively at 300 g_{av} (5 min); 1000 g_{av} (10 min); 8000 g_{av} (10 min) and 100,000 g_{av} (1 h). These fractions have been previously characterized by electronic microscopy (Cunha et al. 1988) as heterogeneous (P_1), nuclear (P_2), mitochondrial (P_3) and microsomal (P_4).

ATPase assays - The experimental conditions for measuring (Ca^{2+}-Mg^{2+})-ATPase activities through the colorimetric dosage of inorganic phosphate have been previously described in details (Cunha et al. 1996).

Binding assays - Classical radioligand binding assays with [^{3}H]ouabain and [^{3}H]ryanodine were used for labeling Na^+,K^+-ATPase and ryanodine receptors (RyR), respectively (Pardon & Noël 1994, Silva et al. 1998). In both conditions, rapid vacuum filtration on glass fibre filters (Whatman GF/C) was used in order to separate bound and free radioligands.

RESULTS AND DISCUSSION

Although a small Na^+,K^+-ATPase activity had already been reported in *S. mansoni* homogenate (Nechay et al. 1980), we initiated our studies by re-

investigating this enzyme due to its importance for maintaining membrane potential, sodium gradient and, indirectly, intracellular calcium concentrations (Sweadner 1989). The presence of Na^+, K^+-ATPase activities with different sensitivity to ouabain in tegumental and carcass preparations (Noël & Soares de Moura 1986) and the characterization of two classes of [^{3}H]ouabain binding sites in the homogenate (Pardon & Noël 1994) let us propose the existence of more than one isoenzyme in this worm, just as in mammals. Although very similar to the mammalian enzyme with respect to (physiological) activators (Noël & Soares de Moura 1986), the worm enzyme exhibited some pharmacological difference being much more resistant to vanadate, the classical inhibitor of P-ATPases (Noël & Pardon 1989). *S. mansoni* Na^+, K^+-ATPase has not been cloned yet, but a PCR product (SMIV) exhibiting 84% homology with mammalian Na^+, K^+-ATPase has been described (de Mendonça et al. 1995). Later on, we characterized the presence of a calcium pump activity (Cunha et al. 1992) that was mainly related to a Sarco/Endoplasmic Reticulum (Ca^{2+}-Mg^{2+})-ATPase (SERCA). Here too, although (physiological) activation of the enzyme occurred at the same submicromolar concentrations of calcium as for mammalian SERCA, a difference in the pharmacological modulation by thapsigargin, a specific inhibitor of SERCA, was observed (Cunha et al. 1996). In fact when re-expressed in nmol thapsigargin/enzymatic unit (EU) in order to correct for different quantities of enzyme molecules in the assay (Sagara & Inesi 1991), the worm enzyme (IC_{50} = 50 nmol/EU) was about 140-250 fold more resistant to thapsigargin than mammalian enzymes (IC_{50} = 0.2-0.35 nmol/EU; Lytton et al. 1991, Mason et al. 1993). A physiological role for this ATPase has recently been confirmed when the full-length SMA2 cDNA clone from *S. mansoni* was expressed in (Ca^{2+}-Mg^{2+})-ATPase-deficient yeast (Talla et al. 1998). A second (Ca^{2+}-Mg^{2+})-ATPase activity, resistant to thapsigargin and cyclopiazonic acid (two selective inhibitors of SERCA) was also reported, that could be located in the plasma membrane (Cunha et al. 1996).

Just as in mammals or invertebrates, stored calcium is supposed to be mobilized from intracellular stores through intracellular calcium channels. We were recently able to characterize the presence of specific binding sites for [^{3}H]ryanodine with an affinity similar to the mammalian RyR (Silva et al. 1998). This binding was modulated by Mg^{2+} and Ca^{2+} ions as well as by caffeine. Furthermore, the addition of ryanodine induced the contraction of whole worms, in vitro. These data were corroborated by Day et al. (2000) which have showed that caffeine, an agonist of RyR, was able to contract isolated muscle fibres. In this way we may propose that the presence of sarcoplasmic reticulum calcium stores, with both SERCA and RyR, is essencial to support worm contraction. In Table we compare the distribution patterns of both (Ca^{2+}- Mg^{2+})-ATPases (sensitive and resistant to thapsigargin), RyR and basal ATPase. Basal ATPase activity, mea-

TABLE

Distribution patterns of basal ATPase, (Ca^{2+}- Mg^{2+})-ATPases and ryanodine receptors in subcellular fractions from *Schistosoma mansoni* homogenate

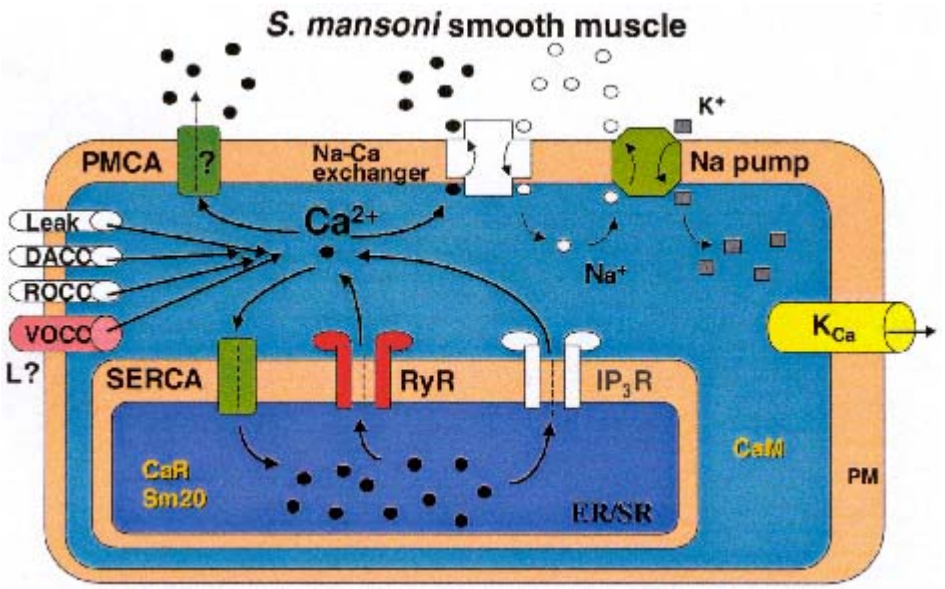
Subcellular Fractions	Basal ATPase (%)	(Ca^{2+}- Mg^{2+})-ATPase Tg-resistant (%)	(Ca^{2+}- Mg^{2+})-ATPase Tg-sensitive (%)	[^{3}H]ryanodine bound (%)
P_1	26.3 ± 5.7	*50.8 ± 8.3	*40.0 ± 7.5	*42.3 ± 6.1
P_2	*15.7 ± 1.9	11.0 ± 4.1	3.4 ± 0.7	5.2 ± 0.8
P_3	23.3 ± 4.7	11.1 ± 3.9	4.4 ± 0.9	7.5 ± 1.6
P_4	34.5 ± 3.0	26.7 ± 6.4	*51.8 ± 6.7	*45.0 ± 5.0
	n = 7	*n* = 7	*n* = 7	*n* = 6

(Ca^{2+}-Mg^{2+})-ATPase activity corresponds to the Mg^{2+}-dependent ATPase that is stimulated by micromolar concentrations of free calcium and that has been shown to be responsible for active calcium pumping (Cunha et al. 1988). The thapsigargin-sensitive (TgS) activity was calculated subtracting the thapsigargin-resistant (TgR) activity (measured in the presence of 1 µM thapsigargin) from the total (Ca^{2+}-Mg^{2+})-ATPase activity (measured in the absence of thapsigargin). Basal ATPase was measured in the absence of Ca^{2+}, Na^+ and K^+ ions (Cunha et al. 1996). The results are expressed as percent of recovery calculated as follows: 100 X activity or binding (specific activity or binding X protein content) divided by the sum of the activity or binding of all four *S. mansoni* fractions (P_1-P_4). Values are means ± SEM; *n* = the number of different preparations used in experiments performed in triplicate or quadruplicate. Note that the distribution patterns of TgS (Ca^{2+}-Mg^{2+})-ATPase, TgR (Ca^{2+}-Mg^{2+})-ATPase and basal ATPase activities are not the same (lines and * show the fractions which present a significant difference in the percent of recovery for each fraction, $P < 0.05$ - ANOVA). On the contrary, distribution patterns for [^{3}H]ryanodine binding and thapsigargin-sensitive ATPase activity are not different (the two activities are not significantly different when compared in each fraction, $P > 0.1$ - ANOVA).

sured in the absence of Na^+, K^+ and Ca^{2+} ions, has been shown to share the same pattern of distribution as that of high-affinity binding sites for [^{3}H]ouabain, a marker of Na^+,K^+-ATPase, when assayed simultaneously in a single preparation (Cunha et al. 1996). In fact, since all our data have been obtained using the same procedures we may compare the recovery of these activities/binding sites along the four subcellular fractions prepared from male adult worm homogenate. Table indicates that thapsigargin-sensitive (Ca^{2+}-Mg^{2+})-ATPase activity and RyR co-purified in P_1 and P_4 fractions mainly, while basal ATPase (along with Na^+,K^+-ATPase) and thapsigargin-resistant (Ca^{2+}-Mg^{2+})-ATPase have a distinct distribution. All these data are compatible with the nature/localization proposed above for these proteins.

The similarity with mammalian systems for controlling calcium concentrations in muscles goes further since we have good evidences for the presence of calcium channels (of the L-type ?) (Fetterer et al. 1980, da Silva & Noël 1995) and Ca^{2+}-activated K^+-channels (Blair et al. 1991) in the plasma membrane that could interact with RyR in order to control muscular tone (Jaggar et al. 1998). Other important participants in calcium signalling are also present in *S. mansoni* since different calcium-binding proteins (CaBP) have been reported: an EF-hand CaBP like calmodulin, the ubiquitous partner of calcium for diverse enzymatic pathways (Thompson et al. 1986) and Sm20, possibly acting as a reservoir for calcium in SR muscle (Stewart et al. 1992). Calreticulin, a non-EF-hand CaBP normally resident in the endoplasmic reticulum, has also been described but seems to be mainly expressed in the digestive gut and genital organs of the worm (Khalife et al. 1994). An overview of all the proteins participating in the control of calcium homeostasis is presented in a cartoon representing a hypothetical smooth muscle cell of *S. mansoni* (Figure).

Based on the phylogenetic importance of platyhelminths that are the first metazoan group to possess a centralized nervous system, being considered as the link between lower and higher invertebrates (Day & Maule 1999), we may propose that current systems controlling calcium homeostasis were selected very early in the evolution process and that the presence of different isoforms to perform the same work has to be considered a phylogenetic advantage.



Synoptic view of proteins controlling calcium homeostasis in a putative smooth muscle cell of *Schistosoma mansoni*. Colored proteins have already been described (see text for explanation) whereas putative proteins not yet described are represented in white. ATPases: PMCA (plasma membrane (Ca^{2+}-Mg^{2+})-ATPase), SERCA (sarco-endoplasmic reticulum (Ca^{2+}-Mg^{2+})-ATPase) and Na^+ pump (Na^+, K^+-ATPase). Plasma membrane ion channels: Leak, DOCC (depletion-operated calcium channel), ROCC (receptor-operated calcium channel) and VOCC (voltage-operated calcium channel) for entry of calcium, and K_{ca} for extrusion of potassium ions. Intracellular calcium channels: RyR (ryanodine receptors) and IP_3R (inositol 1,4,5-triphosphate receptors). Calcium Binding Proteins: CaR (calreticulin), SM20 (20-kilodalton calcium-binding protein of *S. mansoni*) and CaM (calmodulin). PM: plasma membrane. SR/ER: Sarco-Endoplasmic Reticulum

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