RESEARCH NOTE

$(Ca^{2+}$-$Mg^{2+})$ATPase in *Schistosoma mansoni*: Evidence for Heterogeneity and Resistance to Praziquantel

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In mammals, Ca^{2+} pumps use the chemical energy derived from the hydrolysis of ATP to remove Ca^{2+} from the cytoplasm of a variety of cell types (L De Meis & G Inesi 1982, p. 141-169. In E Carafoli, *Membrane Transport of Calcium*, Academic Press, New York, AF Rega 1986, p. 13-20. In AF Rega, *The Ca^{2+} Pump of Plasma Membrane*, CRC Press, Boca Raton). The pumps of plasma membranes (PMCA family) and those of endoplasmic or sarcoplasmic reticulum (SERCA family) (NM Green 1992 *Ann NY Acad Sci 671*: 104-169) have the same physiological functions, despite differences in their structure, subcellular localization and modulatory mechanisms. Thapsigargin, a plant-derived sesquiterpene lactone, inhibits the activity of all of the SERCA isoenzymes ($SERCA_1$, $SERCA_2$ and $SERCA_3$) with equal potency, but has no effect on the ATPase or transport activities of PMCA pumps. In previous reports we demonstrated a $(Ca^{2+}$-$Mg^{2+})$ATPase activity coupled to the active transport of Ca^{2+} in subcellular fractions from *Schistosoma mansoni* (VMN Cunha et al. 1988 *FEBS Lett 241*: 65-68, 1992 *Mol Biochem Parasitol 52*: 167-174). The aim of the present work was, first, to investigate the subcellular localization of the $(Ca^{2+}$-$Mg^{2+})$ATPase activities present in P_1 and P_4 fractions, using thapsigargin, cyclopiazonic acid (CPA) and tamoxifen as inhibitors of Ca^{2+} pump. Second, to investigate if an ATPase responsible for the control of Ca^{2+} homeostasis, could be involved in the molecular mechanism of praziquantel-induced contraction in *S. mansoni*.

Male cercariae of *S. mansoni* (BH strain) were obtained from snails (*Biomphalaria glabrata)* previously infected with a single miracidium (Cunha 1988 *loc. cit.*). The subcellular fractions were obtained by homogenizing and centrifuging about 2000 worms to obtain four pellets (P_1, P_2, P_3, P_4) sedimenting respectively at 300 x g_{av} (5 min); 1000 x g_{av} (10 min); 8000 x g_{av} (10 min) and 100,000 x g_{av} (1hr). About 25 mg of protein from P_1 or P_4 fractions were incubated for 1 hr at 37°C in 0.5 ml of a mixture containing (unless otherwise stated) 5 mM Na_2ATP, 0.3 mM EGTA, 10 mM NaN_3, 4 mM $MgCl_2$, 60 mM KCl, 5 mM A_{23187} (calcimycin, a calcium ionophore), 50 mM HEPES-Tris buffer (pH 7.4) and 0 or 370 mM $CaCl_2$ (10 mM free Ca^{2+}), for the determination of ATPase activity. The $(Ca^{2+}$-$Mg^{2+})$ATPase was calculated by subtracting the basal ATPase activity measured in the absence of calcium from the total activity measured in the presence of calcium.

The effect of thapsigargin on $(Ca^{2+}$-$Mg^{2+})$ATPase activity in the fractions P_1 and P_4 from *S. mansoni* is shown in Fig. 1. In fraction P_4, the $(Ca^{2+}$-$Mg^{2+})$ATPase activity was inhibited by the drug with a half-maximal effect (I_{50}) at 250 nM. At a saturating concentration of thapsigargin (3 mM), the activity was completely blocked ($I_{max} = 10^3 \pm 5.1\%$, $n = 3$). Under the same conditions, about 20% of the $(Ca^{2+}$-$Mg^{2+})$ATPase activity present in P_1 was resistant to thapsigargin (I_{max}=78.3 ± 4.9%, n = 3; P<0.005, Student's t - test). There was no significant difference between fractions P_1 and P_4 in their sensitivity to the drug. However, a lower sensitivity of *S. mansoni* ATPase to thapsigargin in relation to the mammalian enzymes was observed. The same pattern of inhibition was observed using CPA, with the fraction P_4 exhibiting greater inhibition at a saturating concentration (I_{max} = 84.8 ± 2.2%, n = 3) than P_1 (I_{max} = 70.9 ± 2.8%, n = 3; P< 0.005, Student's t - test) whereas there was no significant difference in the I_{50} values. Thus, 22% to 29% of the $(Ca^{2+}$-$Mg^{2+})$ATPase activity of fraction P_1 is resistant to thapsigargin and CPA in these preparations. To explore the subcellular origin of the $(Ca^{2+}$-$Mg^{2+})$ATPase activity that is resistant to thapsigargin and CPA, tamoxifen was employed as a non-selective inhibitor of $(Ca^{2+}$-$Mg^{2+})$ATPase. This drug completely inhibits the $(Ca^{2+}$-

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Mg^{2+})ATPase activity in both fractions whereas only the inhibitory effect on (Ca^{2+}-Mg^{2+})ATPase activity from fraction P_1 could be partially antagonized by the addition of 90 mg calmodulin to the incubation mixture (VMN Cunha et al. 1996 *Comp Biochem Parasitol 114B*: 199-205). Several lines of evidence suggest that praziquantel interacts specifically with a molecule involved in Ca^{2+} regulation within the worm to promote contraction. Fig. 2 shows that neither the (Ca^{2+}-Mg^{2+})ATPase activities of P_1 nor of P_4 fractions were inhibited by increased concentrations of praziquantel. As a whole our data show that the majority of the (Ca^{2+}-Mg^{2+})ATPases from *S. mansoni* should be a SERCA isoform and that therapeutic concentrations of praziquantel have no direct action on the (Ca^{2+}-Mg^{2+})ATPases described in *S. mansoni.*

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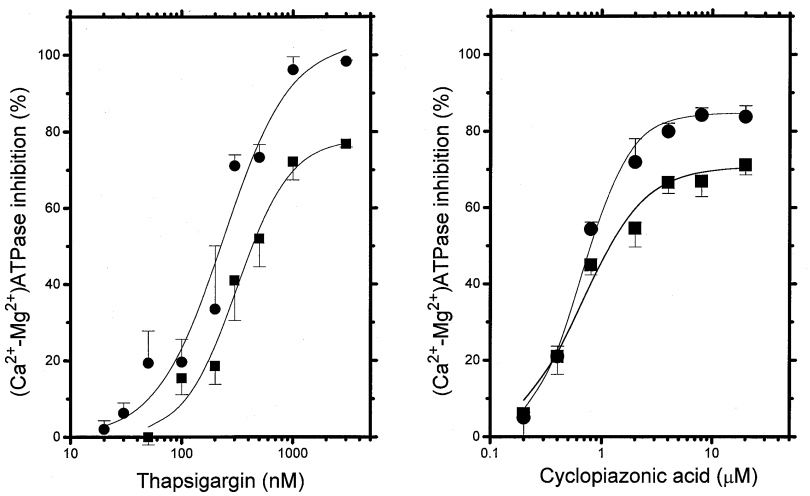

Fig. 1: thapsigargin and cyclopiazonic acid inhibition of (Ca^{2+}-Mg^{2+})ATPase activity in fractions P_4 (●) and P_1 (■) from S*chistosoma mansoni*. Fractions (16-30 mg protein) were incubated for 1 hr at 37ºC. Each point is the mean of three assays performed in triplicate in different enzymatic preparations, with bars denoting SEM. The curves were drawn using the parameters obtained by non-linear regression analysis according to the Hill equation.

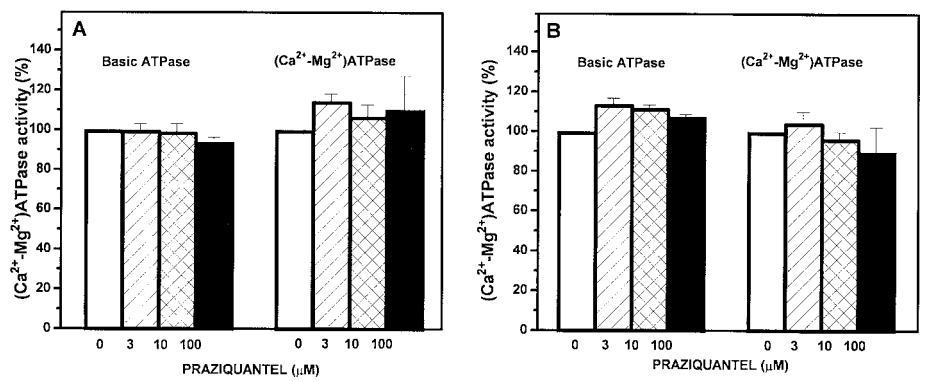

Fig. 2: effect of praziquantel on basal ATPase and (Ca^{2+}-Mg^{2+})ATPase activities of heterogenous (P_1) and microsomal (P_4) fractions from *Schistosoma mansoni*. About 55 mg protein of P_1 (A) and P_4 (B) were incubated for 1 hr at 37º C. Results are means ± SEM of four assays performed in triplicate and expressed as percent of the control values (n=4). The specific basal ATPase and (Ca^{2+}-Mg^{2+})ATPase activities (expressed as mmol P_i. mg^{-1} protein. hr^{-1}) were 3.3 ± 0.34 and 2.7 ± 0.89, respectively, for fraction P_1; 3.4 ± 0.59 and 3.7 ± 0.61, respectively, for fraction P_4.