RESEARCH NOTE

Evidence for Functional Ryanodine Receptors in *Schistosoma mansoni* and their Putative Role in the Control of Calcium Homeostasis

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The main musculature present in adult male *Schistosoma mansoni* is mainly of the smooth muscle type (MH Silk & IM Spence 1969 *S Afr J Med Sci 34:* 11-20). As we have previously demonstrated, the contraction of *S. mansoni* presents a tonic component, dependent on extraworm calcium, and a phasic one, dependent on intracellular/intraworm calcium (S da Silva & F Noël 1995 *Parasitol Res 81:* 543-548). Since intracellular calcium channels sensitive to the alkaloid ryanodine and therefore known as ryanodine receptors (RyR) (R Coronado et al. 1994 *Am J Physiol 266:* C1485-C1504) have been demonstrated in a variety of mammalian as well as non-mammalian (chicken, frog) and non-vertebrate cells (*Caenorhabditis elegans*, lobster and drosophila) (G Meissner 1994 *Annu Rev Physiol 56:* 485-508), the main objective of this work was to investigate the presence of RyR in *S. mansoni*, as well as their subcellular distribution profile, pharmacological modulation and their putative involvement in *S. mansoni* contractility.

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About 2,000 worms were homogenized in a Dounce homogenizer at 4ºC in 0.25 M sucrose solution (5 mM Tris-HCl pH 7.4) using three sequences of ten 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 hr) (VMN Cunha et al. 1988 *FEBS Lett 241:* 65-68).

In the binding assays, 100-150 mg of proteins of subcellular fractions were incubated for 2 hr at 37ºC in a medium (0.5 ml) containing 1.5 M KCl, 10 mM Na_2ATP, 0.8 mM $CaCl_2$ (107 mM free Ca^{2+}), HEPES 10 mM, NaOH pH 7.4 and 0.3 nM [^{3}H]ryanodine (NEN, 84 Ci/mmol) in the presence or absence of 0.2-10 nM unlabelled ryanodine. Incubations were terminated by dilution of the samples with 5 ml of ice-cold buffer (150 mM KCl, 10 mM Tris, pH 7.4) followed by rapid filtration on glass fibre filters under vacuum (Whatman GF/C) in order to separate bound and free radioligand. The non-specific binding was determined in the presence 10 mM unlabelled ryanodine. Data from saturation experiments were treated by a computerised non-linear regression analysis (EBDA-LIGAND; Elsevier-Biosoft, Cambridge, UK).

For the *in vitro* studies, the worms were carefully recovered from mice portal veins, washed and placed in a glass dish containing 3 ml Tyrode's solution maintained at 37ºC. After 10 min for equilibration, 100 mM ryanodine or vehicle were added (time zero) and the grading of worm shortening was performed based on the worm length (da Silva & Noël *loc. cit.*).

The [^{3}H]ryanodine specific binding sites were mainly recovered in the heterogeneous (P_1) and microsomal (P_4) fractions of *S. mansoni* (Table I). Analysis of the data from four saturation experiments revealed an equilibrium dissociation constant (K_d) in the low nanomolar range (6.9 ± 0.6 nM), a maximal number of receptors (B_{max}) of 78 ± 18 fmol/mg protein. We tested the influence of Ca^{2+} and Mg^{2+} on the binding of [^{3}H]ryanodine to the P_1 fraction. As the free concentration of Ca^{2+} was increased from 0.3 to 107 mM there was a corresponding increase in the [^{3}H]ryanodine specific binding (Table II). On the other hand 5 mM free Mg^{2+}, in the absence of ATP, inhibited about 50% the [^{3}H]ryanodine specific binding (Table II). Praziquantel, even at a concentration 10-fold higher than its therapeutic plasma concentration, had no effect on [^{3}H]ryanodine binding (Table II). Finally ryanodine was able to contract the whole *S. mansoni* in a time-dependent manner (Fig.).

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TABLE I

Subcellular distribution of ryanodine binding

Fraction	[^{3}H]ryanodine bound (%)
P_1	42.3 ± 6.1
P_2	5.2 ± 0.8
P_3	7.5 ± 1.6
P_4	45.0 ± 5.0

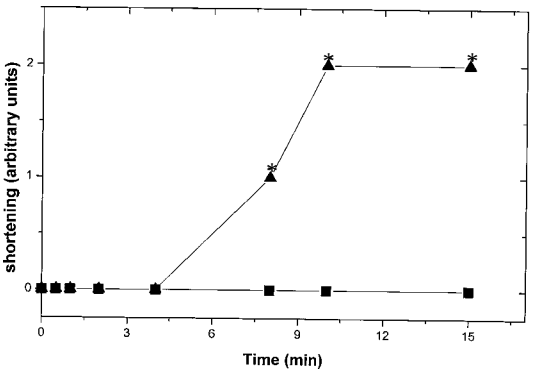
The values are expressed as % of recovery calculated as follows: 100 x binding (specific binding of 0.5 nM [^{3}H]ryanodine x protein content) divided by the sum of all four *Schistosoma mansoni* fractions ($P_1 - P_4$); $n = 6$.

TABLE II

Pharmacological modulation of ryanodine binding to the subcellular P_1 fraction from *Schistosoma mansoni*

	[^{3}H]ryanodine bound (%)
Ca^{2+} modulation	
Ca^{2+} 107 mM, ATP 10 mM (control)	100
Ca^{2+} 10 mM, ATP 10 mM	55.4 ± 1.3[a]
Ca^{2+} 0.3 mM, ATP 10 mM	15.1 ± 4.6[a]
Mg^{2+} modulation	
Ca^{2+} 107 mM (control)	100
Ca^{2+} 107 mM, Mg^{2+} 5 mM	53.6 ± 7.1[a]
Praziquantel (10 mM)	96.1 ± 5.2

The values represent means ± SEM of three individual experiments performed with different preparations in quadruplicate expressed as percentage of control. The Ca^{2+} and Mg^{2+} concentrations shown represent the free ion concentration. [^{3}H]ryanodine concentration was 0.5 nM; *a: $p < 0.05$,* paired *t*-test.



Time-course of ryanodine-induced contractions of whole *Schistosoma mansoni*. Grading of shortening was set according to the worm length (see Methods). Each point represents the median value for 8-14 experiments. (■) control and (▲)100 mM ryanodine. * $p < 0.05$ versus control using Mann-Whitney's non-parametric *U*-test.

binding sites have a similar pattern of subcellular distribution as the (Ca^{2+}-Mg^{2+})ATPase sensitive to thapsigargin, that corresponds to a SERCA ATPase (VMN Cunha et al. 1996, *Comp Biochem Physiol 114B*: 199-205). The saturation experiment at equilibrium revealed the labelling of only one homogeneous population of receptors in the range of concentrations used, and a dissociation constant (K_d) in the nanomolar range, as observed in higher animals (Coronado *loc. cit*.) or even in the nematode *C. elegans* (YK Kim et al. 1992 *Biophys J 63:* 1379-1384). [^{3}H]ryanodine binding correlates with the functional state of the ionic channel (Coronado *loc. cit.*) so that conditions increasing the open channel probability usually favour the [^{3}H]ryanodine binding whereas conditions that close the channel decrease this binding, as for Ca^{2+} and Mg^{2+} respectively. With respect to the ryanodine receptors present in *S. mansoni* they possess some characteristics qualitatively very similar to those present in higher animals regarding the affinity for ryanodine and the modulatory effect of ions (Ca^{2+} and Mg^{2+}). Recently, CA Redman et al. (1996 *Parasitol Today 12:* 14-20) suggested that the mechanism of action of praziquantel could be related to mobilisation of intracellular Ca^{2+} stores sensitive to IP_3 or Ca^{2+}. Here we observed that praziquantel was not able to alter the [^{3}H]ryanodine binding to the subcellular P_1 fraction. Other possibilities have just been discharged such as inhibition of (Ca^{2+}- Mg^{2+}) ATPase and (Na^+/ K^+) ATPase (VMN Cunha & F Noël 1997 *Life Sci 60:* PL289-294), thus the exact mechanism of action of praziquantel-induced contraction still remains to be elucidated. Finally, a high concentration of ryanodine (100 mM) was able to induce contractions of the whole adult worms, indicating that these receptors may have a role in the control of calcium homeostasis within the worm musculature.

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