Biochemical Pharmacology, Vol. 56, pp. 997–1003, 1998.

ISSN 0006-2952/98/$19.00 + 0.00
PII S0006-2952(98)00219-6

Evidence for Ryanodine Receptors in *Schistosoma mansoni*

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ABSTRACT. The present study investigated the presence of ryanodine receptors in the trematode *Schistosoma mansoni*. [^{3}H]Ryanodine specific binding sites were found in the four subcellular fractions of *S. mansoni*; however, more binding sites were recovered in the heterogeneous fraction P_1 and the microsomal fraction P_4, as was thapsigargin-sensitive (Ca^{2+}-Mg^{2+})ATPase activity, marking the sarco/endoplasmic reticulum calcium ATPase (SERCA) pumps. This binding had an equilibrium dissociation constant (K_d) in the nanomolar range, an apparent maximal number of receptors (B_{max}) of about 80 fmol/mg of protein, and was modulated by ions (Ca^{2+}, Mg^{2+}) and some pharmacological tools such as caffeine. Ryanodine was able to accelerate the rate of $^{45}Ca^{2+}$ release from actively loaded vesicles, and also to induce a transient contraction of the whole worm. We conclude that ryanodine-sensitive Ca^{2+} release channels are present in *S. mansoni*, with properties very similar to the ones present in higher animals. BIOCHEM PHARMACOL **56**;8:997–1003, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. ryanodine; *Schistosoma mansoni*; calcium; praziquantel

Cell life is dependent on calcium homeostasis, and, therefore, there are several physiological systems to control the concentration of this ion within cells [1]. Classically, these systems may be present on plasma membranes or intracellularly on organelles such as the endo/sarcoplasmic reticulum, as in the case of intracellular calcium channels known as ryanodine receptors (RyR) [2, 3]. These receptors were first discovered in skeletal muscle and then later in other muscular and non-muscular tissues. They are present in a variety of mammalian as well as non-mammalian (chicken, frog) and non-vertebrate cells (*Caenorhabditis elegans*, lobster, and Drosophila) [4, 5]. In adult male *Schistosoma mansoni*, the main muscle type present resembles the smooth type present in higher animals [6]. This musculature has already been investigated pharmacologically and has been found to contract in response to stimuli such as high extracellular K^+, electrical current [7], L-glutamate [8], 5-hydroxytryptamine [9], and phorbol ester [10]. In addition, da Silva and Noël [11] showed that the tonic contraction of the whole worm induced by praziquantel, the drug of choice to treat schistosomiasis, was completely abolished by the voltage-dependent calcium channel antagonist verapamil, but not the phasic contraction, indicating that mobilization of intracellular calcium stores occurs. This hypothesis is supported by our recent characterization of a (Ca^{2+}-Mg^{2+})ATPase activity sensitive to thapsigargin and cyclopyazonic acid, two inhibitors of the SERCA† pumps, in subcellular fractions of *S. mansoni* [12].

The aim of this work was to investigate the presence of intracellular calcium channels sensitive to the alkaloid ryanodine in *S. mansoni*, as well as their subcellular distribution profile, pharmacological modulation, and putative involvement in the control of Ca^{2+} fluxes and *S. mansoni* muscular contractility.

MATERIALS AND METHODS

Infection of Mice and Harvesting of Adult Male S. mansoni

Male cercariae of *S. mansoni* (BH strain) were obtained from snails (*Biomphalaria glabrata*) previously infected with a single miracidium. Newborn mice (3–5 days) were then infected percutaneously with about 150 male cercariae and killed for collecting adult worms, as previously described [13].

Preparation of Subcellular Fractions

S. MANSONI. About 2000 worms were homogenized in a Dounce homogenizer at 4° in 0.25 M sucrose solution buffered to pH 7.4 with 5 mM Tris–HCl using 3 sequences of 10 passes of the pestle. The homogenate was centrifuged

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Received 7 January 1998; accepted 25 May 1998.

† *Abbreviations:* cADP ribose, cyclic ADP ribose; P_i, inorganic phosphate; PMSF, phenylmethylsulfonyl fluoride; POPOP, 1,4-bis-[2-(5-phenyloxazolyl)]-benzene; PPO, 2,5-diphenyloxazole; and SERCA, sarco/endoplasmic reticulum calcium ATPase.

according to the method of Smithers *et al.* [14] to obtain four pellets (P_1, P_2, P_3, and 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). Fraction P_1 was heterogeneous, containing pieces of unbroken worm tissue, tegument with spines, nuclei, and some vesicles; fraction P_2 consisted mainly of nuclei, but had other structures such as mitochondria, dense bodies, and vesicles; fraction P_3 consisted mainly of mitochondria, with few vesicles and dense bodies also present; fraction P_4 consisted mainly of vesicles or large multivesicular structures and endoplasmic reticulum membranes [13, 14]. The pellets were resuspended in buffered 0.25 M sucrose solution and stored in liquid N_2 until use.

RAT GASTROCNEMIUS MICROSOMES. The muscle was cut into small segments and homogenized at 4° in 4 vol. (v/w) of 0.25 M sucrose buffered with 5 mM Tris–HCl, containing 0.2 mM PMSF and 2 mM dithiotreitol, using a Virtis-45 homogenizer (8 times during 30 sec). The supernatant of a centrifugation at 1000 g_{max} for 10 min was centrifuged further at 10,000 g_{max} for 20 min. After washing of the pellet, the combined supernatants were centrifuged at 110,000 g_{av} for 35 min to obtain a microsomal fraction that was resuspended and stored at −80°.

RAT HEART CRUDE PREPARATION. Heart ventricles were homogenized in a Potter homogenizer with a motor-driven teflon pestle at 4° in 2–3 vol. of 0.25 M buffered sucrose containing 0.1 mM PMSF/g organ. After filtration through gauze, the homogenates were centrifuged at 100,000 g_{av} for 1 hr, and the pellets were resuspended and stored at −80°.

Protein concentration was determined by the method of Bradford [15], using BSA as standard.

Binding Assays

The experiments were performed as previously described by Wibo and Godfraind [16]. Briefly, 100–150 μg protein of subcellular fractions was incubated for 2 hr at 37° in a medium (0.5 mL) containing 1.5 M KCl; 10 mM Na_2ATP; 0.8 mM $CaCl_2$ (107 μM free Ca^{2+}); 10 mM HEPES; adjusted to pH 7.4 with NaOH and, unless otherwise specified, 0.3 nM [^{3}H]ryanodine (NEN, 84 Ci/mmol) for equilibrium assays in the presence of 0.2 to 10 nM unlabeled 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 under vacuum on glass fiber filters (Whatman GF/C) in order to separate bound and free radioligand. The tubes were washed once again with 5 mL followed by two washings of the filters with 5 mL of the same buffer. After drying, filters were added to a scintillation mixture [POPOP (0.1 g/L) and PPO (4.0 g/L) in toluene], and the radioactivity was measured in a Packard liquid scintillation counter with 45% efficiency. The non-specific binding was determined in the presence of an excess (10 μM) of unlabeled ryanodine, and the specific binding was calculated by subtracting the value of non-specific binding from the total value. In some experiments, the concentration of free Ca^{2+} was adjusted in the micromolar range by using EGTA as a chelator. Free metal ion concentrations and their complexes with EGTA and ATP were calculated using a computer program [17].

ATPase Activity

The preparations (20–25 μg of protein) were incubated for 1 hr at 37° 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 μM calcium ionophore A23187; 50 mM HEPES–Tris buffer (pH 7.4); and 0 or 370 μM $CaCl_2$ (10 μM free Ca^{2+}) [17] in the absence or in the presence of 1 μM thapsigargin. The reaction was started by the addition of protein, and the ATPase activity was determined colorimetrically by measuring the P_i liberated. The (Ca^{2+}-Mg^{2+})ATPase activity was calculated by subtracting the basal ATPase activity, measured in the absence of calcium, from the total ATPase activity measured in the presence of this ion. The (Ca^{2+}-Mg^{2+})ATPase activity is the sum of thapsigargin-resistant (measured in the presence of 1 μM thapsigargin) and -sensitive activities. The total (Ca^{2+}-Mg^{2+})ATPase activity varied from 3.0 to 17.0 μmol $P_i \cdot$ mg protein$^{-1} \cdot$ hr^{-1} depending on the preparation and subcellular fraction. The basic ATPase activity varied from 1.6 to 10.0 μmol $P_i \cdot$ mg protein$^{-1} \cdot$ hr^{-1}.

$^{45}Ca^{2+}$ Uptake Measurements

Vesicles (50 μg of protein) were incubated for 30 min at 37° in 0.5 mL of a mixture containing 5 mM Na_2ATP; 0.3 mM EGTA; 10 mM NaN_3; 4 mM $MgCl_2$; 60 mM KCl; 40 mM P_i; 50 mM HEPES–Tris (pH 7.4); and 348 μM $CaCl_2$ (5 μM free Ca^{2+}) [17] supplemented with $^{45}Ca^{2+}$. Afterwards, $^{45}Ca^{2+}$ loaded vesicles were exposed to 5 mM EGTA (controls) or to 5 mM EGTA plus 10 μM ryanodine. The reaction was stopped at different times by rapid filtration using 0.45 μm pore size filters (Millipore HAWP 29325). Filters were washed and counted in a liquid scintillation counter as previously described [18]. The $^{45}Ca^{2+}$ content actively transported was calculated by subtracting the $^{45}Ca^{2+}$ retained by the vesicles in the absence of ATP from that retained in the presence of ATP.

In Vitro *Studies*

The protocol used was described previously by da Silva and Noël [11]. Briefly, worms carefully recovered from portal veins of mice were washed and placed in a glass dish containing 3 mL of Tyrode's solution maintained at 37°. After 10 min for equilibration, the worm length was monitored. Either 10 or 100 μM ryanodine or vehicle was added at time zero. Grading of the worm shortening induced by ryanodine was based on the length of the worms. It was visually evaluated, at different intervals

(0–15 min), considering the population as a whole as follows: 0, no shortening; 1, small reduction in worm length; 2, reduction to three-fifths to one-half of their original length; and 3, maximal contraction.

Statistics

Binding data from equilibrium experiments were shown in a classical Scatchard plot; however, the untransformed data from these experiments were treated by a computerized non-linear regression analysis (EBDA-LIGAND; Elsevier–Biosoft), assuming the presence of only one population of binding sites, in order to calculate the equilibrium dissociation constant (K_d), the apparent maximal number of receptors (B_{max}), and the Hill coefficient. $^{45}Ca^{2+}$ release half-lives were calculated by non-linear regression assuming a mono-exponential decay (Prism, GraphPad Softerware Inc.). Student's *t*-test, paired *t*-test or the Mann–Whitney's nonparametric *U*-test was used for statistical significance determinations.

Drugs

Caffeine, cADP ribose, dantrolene, and ATP were purchased from the Sigma Chemical Co., and ryanodine was obtained from Agrisystems International. Praziquantel was provided by Dr. R. Martins from Merck®. All drugs were prepared daily just before the experiments. Dantrolene was first dissolved in absolute ethanol and subsequently in deionized water. For all experiments, there were controls of nonspecific binding.

RESULTS

The specific binding of 0.3 nM [^{3}H]ryanodine to subcellular fractions of *S. mansoni* increased as a function of time, reaching steady state within 1 hr and remaining stable for 3 hr (data not shown); hence, equilibrium assays were performed after incubation for 2 hr. The [^{3}H]ryanodine specific binding sites were mainly recovered in the heterogeneous (P_1) and microsomal (P_4) fractions of *S. mansoni*. These fractions were also enriched in the (Ca^{2+}-Mg^{2+})ATPase activity sensitive to thapsigargin, a classical inhibitor of the (Ca^{2+}-Mg^{2+})ATPase present in the endo/sarcoplasmic reticulum (Table 1). The concentration-dependence of ryanodine binding at equilibrium was studied using the microsomal fraction P_4. As shown in Fig. 1, for a typical experiment the Scatchard plot was linear in the concentration range used, indicating the labeling of a single homogeneous population of receptors. The analysis of these data revealed an equilibrium dissociation constant (K_d) in the low nanomolar range (6.9 ± 0.6 nM, N = 4), an apparent maximal number of receptors (B_{max}) of 78 ± 18 fmnol/mg protein (N = 4) and a Hill coefficient equal to 0.98 ± 0.01 (N = 4).

As the binding of [^{3}H]ryanodine correlates with the functional state of the ionic channel, any condition that favors the open state of the channel increases this binding. In view of this, we first 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 μM, there was also an increase in [^{3}H]ryanodine specific binding (Table 2). On the other hand, 5 mM free Mg^{2+}, in the absence of ATP, inhibited about 50% of the [^{3}H]ryanodine specific binding to the P_1 fraction (Table 2). Among the drugs known to modulate the functional release of Ca^{2+} through the mammalian ryanodine receptors, we decided to test caffeine, dantrolene, and cADP ribose. A summary is given in Table 3 of the effects of these drugs on [^{3}H]ryanodine specific binding to *S. mansoni*, as well as to rat gastrocnemius or heart ventricles, used as controls. Caffeine (6 mM), a classical modulator of ryanodine receptors when used in the presence of low free Ca^{2+} concentrations (here, 0.3 μM), increased the binding of [^{3}H]ryanodine to both the P_1 fraction and gastrocnemius micro-

TABLE 1. Subcellular distribution of 0.5 nM [^{3}H]ryanodine binding and thapsigargin-sensitive (Ca^{2+}-Mg^{2+})ATPase activity

Fraction	[^{3}H]Ryanodine bound (%)	ATPase activity (%)
P_1	42.3 ± 6.1	40.0 ± 7.5
P_2	5.2 ± 0.8	3.4 ± 0.7
P_3	7.5 ± 1.6	4.4 ± 0.9
P_4	45.0 ± 5.0	51.8 ± 6.7
	N = 6	N = 7

Values (means ± SEM) are expressed as percent of recovery calculated as follows: 100 × binding or activity (specific binding or activity × protein content) divided by the sum of the binding (50.2 ± 11.6 fmol/1000 worms) or activity (111.3 ± 22.6 μmol P_i/1000 worms) 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 quadruplicate. Values of [^{3}H]ryanodine binding and ATPase activity were not significantly different ($P > 0.1$, Student's *t*-test).

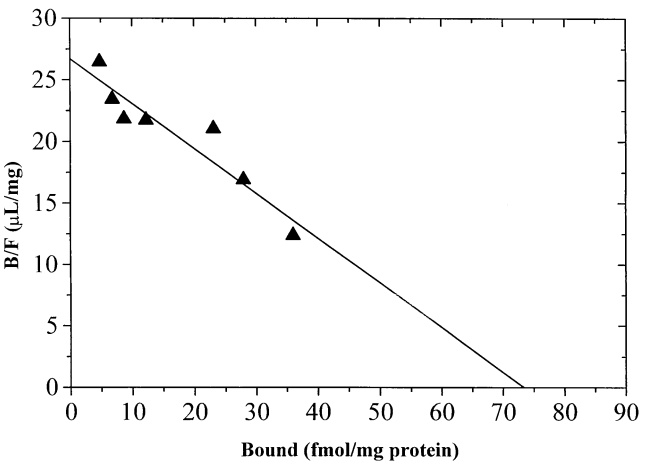

FIG. 1. Typical Scatchard plot for [^{3}H]ryanodine binding to microsomes from *S. mansoni*. The curve was drawn using the parameters fitted by non-linear regression analysis with the model of a single class of independent binding sites (B = ryanodine specifically bound; F = concentration of free ryanodine). Non-specific binding accounts for 40–50% of total [^{3}H]ryanodine binding to the P_4 fraction.

TABLE 2. Ionic modulation of [^{3}H]ryanodine binding to the subcellular P_1 fraction from *S. mansoni*

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

Values represent means ± SEM of three individual experiments performed with different preparations in quadruplicate, expressed as a percentage of control (6.25 ± 0.89 fmol/mg of protein for Ca^{2+} modulation and 2.82 ± 0.4 fmol/mg of protein for Mg^{2+} modulation). The Ca^{2+} and Mg^{2+} concentrations shown represent the free ion concentration (see Materials and Methods). [^{3}H]Ryanodine concentration was 0.5 nM.

*$P < 0.05$, paired *t*-test.

somes. On the other hand, dantrolene had no effect on [^{3}H]ryanodine binding to either the P_1 fraction or to microsomes of skeletal muscle. Finally, the metabolite cADP ribose [19], which was postulated initially as another possible endogenous modulator of ryanodine receptors, had no action on [^{3}H]ryanodine binding to P_1 fraction and heart membranes. Praziquantel, even at a concentration 10-fold higher than its therapeutic plasma concentration, had no effect on [^{3}H]ryanodine binding.

The ryanodine receptors present in *S. mansoni* were investigated further, looking for a ryanodine effect on $^{45}Ca^{2+}$ release from actively loaded microsomal vesicles. Figure 2 shows that the $^{45}Ca^{2+}$ release observed in the presence of EGTA followed a mono-exponential decay and that 10 μM ryanodine decreased significantly the half-life of the process (insert). Note the presence of about 27% of residual $^{45}Ca^{2+}$, of unknown nature, which was released rapidly and completely by the ionophore A23187, as we reported earlier [18].

Finally, ryanodine was able to contract the whole *S. mansoni* in a concentration- and time-dependent manner (Fig. 3). Note that ryanodine induced rhythmic contractions that were qualitatively different from the intense and sustained contractions induced by 1 μM praziquantel [11].

TABLE 3. Pharmacological modulation of [^{3}H]ryanodine binding to P_1 fraction from *S. mansoni*, rat cardiac ventricles, or skeletal muscle microsomes

	S. mansoni P_1	Rat cardiac muscle	Rat skeletal muscle
Caffeine, 6 mM	564 ± 144*	ND†	1426 ± 216*
Dantrolene, 10 μM	91.0 ± 5.7	98.3‡	99.2‡
cADP ribose, 2 μM	100‡	82.3‡	ND
Praziquantel, 10 μM	96.1 ± 5.2	ND	ND

Data are expressed as a percent of control for each condition. Control values for *S. mansoni* P_1: caffeine, 3.63 fmol/mg of protein; dantrolene and praziquantel (standard conditions), 6.25 fmol/mg of protein; and cADP ribose, 0.23 fmol/mg of protein. Control values for rat cardiac muscle: dantrolene, 223.8 fmol/mg of protein; and cADP ribose, 16.4 fmol/mg of protein. Control values for rat skeletal muscle: caffeine, 16.3 fmol/mg of protein; and dantrolene, 791 fmol/mg of protein. See Materials and Methods for incubation conditions, except for caffeine and cADP ribose where the free Ca^{2+} concentration was lower (0.3 and 0.1 μM, respectively). Values are the means ± SEM of three individual experiments performed in quadruplicate. The [^{3}H]ryanodine concentration was 0.5 nM.

*$P < 0.05$ vs control (paired *t*-test).

†ND = not determined.

‡Values from a typical experiment. The same pattern was observed in another experiment.

DISCUSSION

This paper is the first evidence for the presence of ryanodine receptors in the trematode *S. mansoni*. Because their characterization in skeletal and cardiac muscle, these receptors were identified in several other mammalian tissues including brain, vascular and intestinal smooth muscle, and fibroblasts, and also in non-mammalian tissues from chicken, frog, lobster, and the nematode *C. elegans* [4]. In fact, there are three isoforms of these receptors, namely RyR_1, RyR_2, and RyR_3, found preferentially in skeletal muscle, cardiac muscle, and brain, respectively, although they may be co-expressed in the same tissue. [^{3}H]Ryanodine specific binding sites are recovered mainly in the heterogeneous (P_1) and microsomal (P_4) fractions of *S. mansoni*. As expected, these binding sites have a pattern of subcellular distribution similar to that of the (Ca^{2+}-Mg^{2+})ATPase sensitive to thapsigargin, which corresponds to a SERCA ATPase [12] and, hence, is used as a marker of sarco/endoplasmic reticulum. On the other hand, [^{3}H]ryanodine binding sites have a different subcellular distribution from the [^{3}H]ouabain binding sites, a classical marker of the plasma membrane [12].

The equilibrium experiment revealed the presence of only one homogeneous population of receptors in the range of concentrations used, and a dissociation constant (K_d) in the nanomolar range, just as observed in higher animals [4] or even in the nematode *C. elegans* [20]. However, the existence of low affinity binding sites cannot be ruled out since we were not able to use the high concentrations of ryanodine necessary to saturate the binding sites because of the relative high specific/total ratio. The apparent maximal number of receptors (B_{max}), which has a big intertissue and interspecies variation, was about 80 fmol/mg of protein. This low value of binding was expected first because the smooth muscle tissue has a low content of ryanodine receptors [21], and second because we used here the whole animal, and not only the muscle tissue as starting material for homogenization. As an illustration, Kim *et al.* [20] found a specific binding of 10 fmol/mg of protein in the whole homogenate from *C. elegans* and 110 fmol/mg of protein in a CHAPS-solubilized 18% sucrose peak.

As already known from the literature [4], the [^{3}H]ryanodine binding correlates with the functional state of the ionic channel. Actually, conditions that are associated with

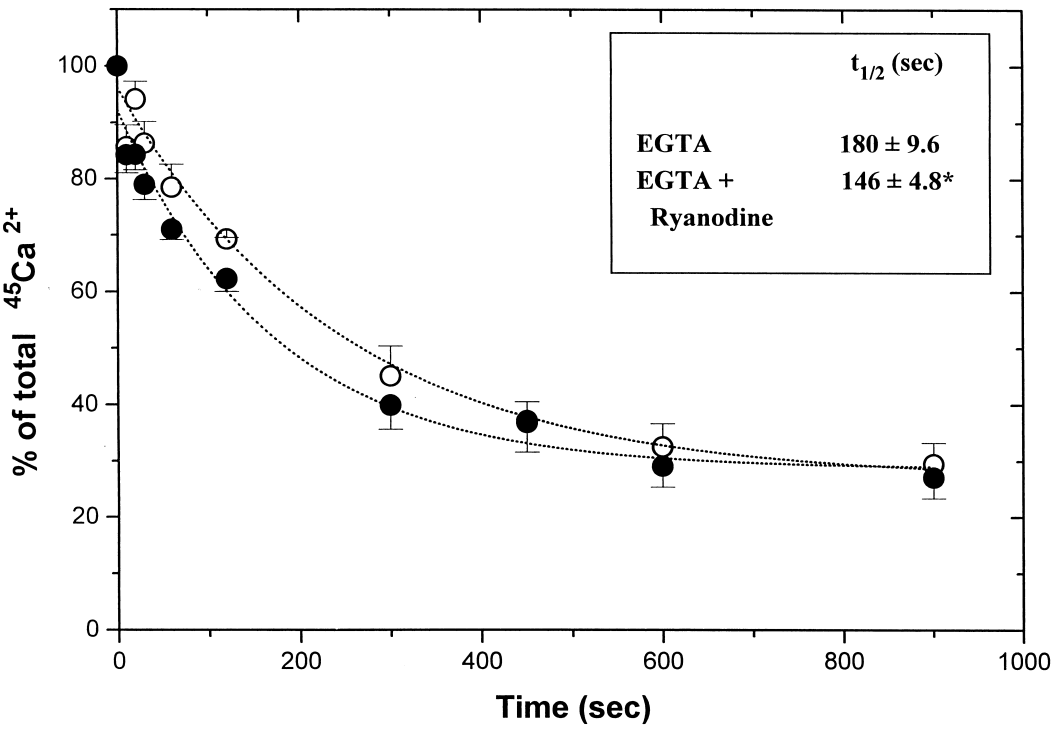

FIG. 2. Time course of $^{45}Ca^{2+}$ release from microsomal vesicles of *S. mansoni*. Vesicles were actively loaded with 5 μM free $^{45}Ca^{2+}$ (80,000 dpm/mL) for 30 min before the addition of 5 mM EGTA (○) or 5 mM EGTA plus 10 μM ryanodine (●). $t_{1/2}$ values (insert) correspond to the half-lives of the mono-exponential decays calculated by non-linear regression analysis. Values represent the means ± SEM of three individual experiments performed in triplicate. *$P = 0.03$, paired *t*-test.

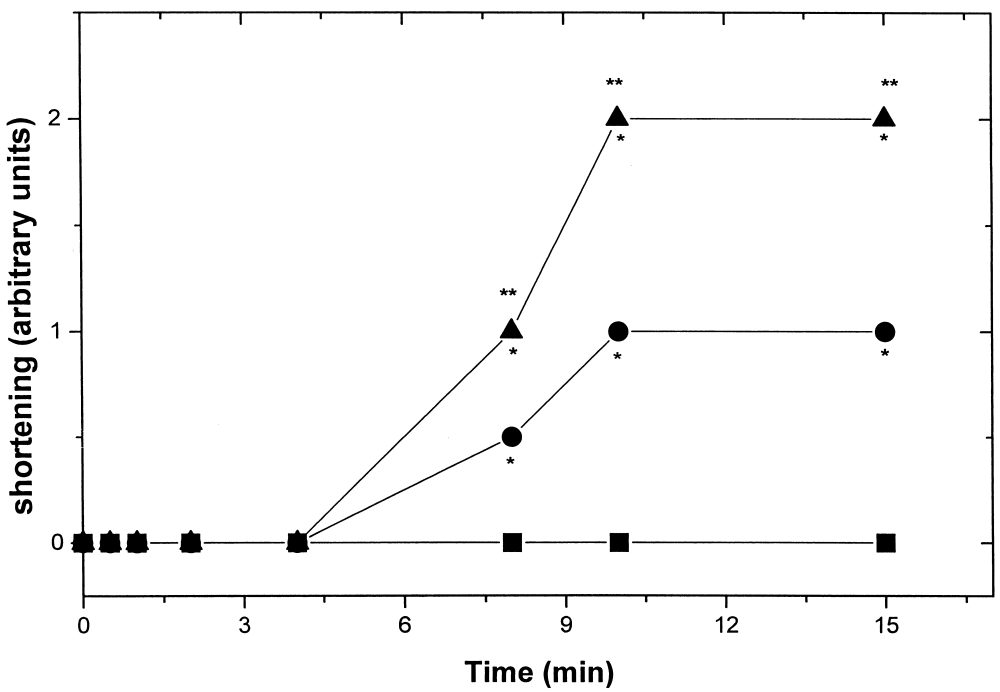

FIG. 3. Time course of ryanodine-induced contractions of whole *S. mansoni*. Grading of shortening was set according to the worm length (see Materials and Methods). Each point represents the median value for 8–14 experiments. (■) control, (●) 10 μM ryanodine, and (▲) 100 μM ryanodine. *$P < 0.05$ vs control and **$P < 0.05$ 10 μM ryanodine vs 100 μM ryanodine, using Mann–Whitney's nonparametric *U*-test.

increased open channel probability usually favor [^{3}H]ryanodine binding, whereas conditions that close the channels decrease this binding, as for Ca^{2+} or caffeine and Mg^{2+}, respectively. With respect to the ryanodine receptors present in subcellular fractions of *S. mansoni*, they present some characteristics qualitatively very similar to the ones present in higher animals regarding the affinity and the modulatory effect of ions (Ca^{2+} and Mg^{2+}) and caffeine. In our hands, cADP ribose lacked any effect on [^{3}H]ryanodine binding to P_1 fraction or heart ventricle membranes. This latter is in agreement with the data from Fruen *et al.* [22] and Lahouratate *et al.* [23]. In our experimental conditions, dantrolene had no effect on [^{3}H]ryanodine specific binding to P_1 fraction or to a rat skeletal muscle preparation. Although this drug is able to inhibit the Ca^{2+} release from sarcoplasmic reticulum, its putative effect on [^{3}H]ryanodine binding is still a matter of controversy [24, 25]. Recently, Redman and colleagues [26] suggested that one possible explanation for the mechanism of action of praziquantel, the drug of choice for the treatment of schistosomiasis, could be related to mobilization of intracellular Ca^{2+} stores sensitive to IP_3 or Ca^{2+}. The present work enabled us to test this hypothesis and to report that praziquantel, at these experimental conditions, was not able to alter the specific binding of [^{3}H]ryanodine to the subcellular P_1 fraction. Other possibilities suggested by these authors, such as inhibition of ($Ca^{2+}-Mg^{2+}$)ATPase and (Na^+/K^+)ATPase, have just been ruled out [27], so that the exact mechanism of action of praziquantel-induced contraction still remains to be elucidated.

Ryanodine was able to accelerate the release of $^{45}Ca^{2+}$ from actively loaded microsomal vesicles. This relatively small effect, as demonstrated by the 20% decrease in the decay half-life, has already been shown in other tissues such as longitudinal intestinal smooth muscle, aortic smooth muscle cells, and skeletal sarcoplasmic reticulum vesicles [28–30], and may be explained by the fact that not all the vesicles that were able to take up $^{45}Ca^{2+}$ possess the ryanodine receptor needed for $^{45}Ca^{2+}$ release, since we are working with vesicles from different tissues and/or organelles. Besides, free concentrations of Ca^{2+} and Mg^{2+} in the micromolar and millimolar range, respectively, necessary for a satisfactory active $^{45}Ca^{2+}$ uptake [18], are not as adequate for the optimal binding of ryanodine so that the ryanodine effect may be underestimated in this experiment. Finally, high concentrations of ryanodine (10 and 100 μM) applied to the incubation medium were able to induce contractions of the whole adult worms observed using an experimental model previously described in detail [11].

In conclusion, the existence of ryanodine receptors in *S. mansoni* supports our idea that current systems controlling the Ca^{2+} homeostasis in mammals, such as ($Ca^{2+}-Mg^{2+}$) ATPase, Na^+/K^+(ATPase), and now the ryanodine receptors, were selected very early in the evolutionary process [31].

We are grateful to Dr. Dr. Lígia Paraense (FIOCRUZ, Rio de Janeiro, Brazil), who provided the infected mice. We thanks Eliana Freitas and José Ferreira Oliveira for their skillful technical assistance. This work was supported by PRONEX No. 41.96.0888.00, FAPERJ, CNPq. Cláudia Lúcia Martins Silva is a fellow of CAPES.

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