ELSEVIER

Biochemical Pharmacology 64 (2002) 1337–1344

Biochemical Pharmacology

Evidence for the presence of glutamatergic receptors in adult *Schistosoma mansoni*

Dayde Lane Mendonça-Silva, Renata Fittipaldi Pessôa, François Noël*

Departamento de Farmacologia Básica e Clínica, Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Cidade Universitária, 21941-590 Rio de Janeiro, Brazil

Received 29 November 2001; accepted 2 April 2002

Abstract

Several studies have suggested that L-glutamate is a putative neurotransmitter in helminths. The present study investigated the presence of non-*N*-methyl-D-aspartate (NMDA) ionotropic receptors for glutamate in four subcellular fractions from adult male *Schistosoma mansoni*. Low-affinity ($K_d = 221 \pm 80$ nM) binding sites for [^{3}H]kainic acid (KA) were detected in the heterogeneous (P_1) fraction, which contains pieces of unbroken worm tissues, tegument, nuclei, and some vesicles. This binding was inhibited by classical glutamatergic ligands in the following order of potency: KA > L-glutamate > α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) > quisqualate ≅ 6,7-dinitroquinoline-2,3-dione (DNQX). However, neither NMDA, a selective agonist for NMDA receptors, nor DL-*threo*-β-hydroxyaspartate (THA) and 1-*trans*-pyrollidine-2-dicarboxylic acid (PDC), inhibitors of high-affinity glutamate transporters, modified [^{3}H]KA binding to the P_1 fraction. In addition, no specific binding for 10 nM [^{3}H]AMPA was detected in any subcellular fraction from *S. mansoni*. These results suggested the presence of KA receptors in adult male worms. This is supported by the evidence that direct application of 10 μM KA to whole worms produced a corkscrew-like coiling of their bodies, modifying the motility of the worms. The KA-induced response, measured as a decrease of the body area, was time-dependent and reversible. PDC was ineffective at blocking the KA effects, indicating that KA does not depend on high-affinity glutamate transporters to reach its site of action. On the other hand, DNQX, the non-NMDA antagonist, was able to partially inhibit KA-induced responses. As a whole, the present data support the presence of a glutamatergic signaling pathway in this parasite.

Keywords: *Schistosoma mansoni*; Platyhelminths; Glutamate; Kainic acid

1. Introduction

The existence of effective and safe drugs should not discourage the search for new schistosomicidal drugs, since strains of worms resistant to oxamniquine [1] and praziquantel [2] have already been reported. Most authors suggest that the neuromuscular system of parasitic helminths is a target that is particularly amenable for anthelmintics. Thus, a better understanding of the neuromuscular physiology of *Schistosoma mansoni* could provide valuable information for the discovery of more selective drugs. In fact, we already know that differences exist between *S. mansoni* and its host regarding the nature of the putative transmitters (worm-specific FMRFamide-related peptides, e.g. [3]) and/or receptors (distinct pharmacological modulation of 5-hydroxytryptamine effects, e.g. [4]) involved in muscular contraction. With this in mind, we decided to investigate the role of glutamate, the major excitatory neurotransmitter of the vertebrate central nervous system, in adult *S. mansoni*. Although widely studied in vertebrates, the molecular targets and physiological actions of glutamate in platyhelminths have not been studied in detail. This is due, in part, to the fact that these parasitic flatworms are small, and the study of nerve or muscle systems without the interference of other tissues is rather difficult [5]. However, some experimental results support a

* Corresponding author. Tel.: +55-21-2562-6732; fax: +55-21-2562-6732.

E-mail address: fnoel@pharma.ufrj.br (F. Noël).

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid; DNQX, 6,7-dinitroquinoline-2,3-dione; 5-HT, 5-hydroxytryptamine creatine sulfate; KA, kainic acid; KSCN, potassium thiocyanate; NMDA, *N*-methyl-D-aspartate; PDC, L-*trans*-pyrollidine-2-dicarboxylic acid; PMSF, phenylmethylsulfonyl fluoride; POPOP, 1,4-bis-[2-(5-phenyloxazolyl)]-benzene; PPO, 2,5-diphenyloxazole; THA, DL-*threo*-β-hydroxyaspartate; Tris, tris(hydroxymethyl)aminomethane.

0006-2952/02/$ – see front matter
PII: S0006-2952(02)01358-8

putative role of glutamate in the neurotransmission of platyhelminths as a whole, and more specifically in *S. mansoni*. Glutamate-like immunoreactivity has been demonstrated in the central and peripheral nervous system of several species, including the cestodes *Mesocestoides corti* and *Hymenolepis diminuta* and the trematode *Fasciola hepatica* [6–8]. In *H. diminuta*, more detailed information is already available since high- and low-affinity [^{3}H]glutamate transporters are present in tissue slices [9]. Furthermore, longitudinal muscles respond to glutamate with powerful rhythmic contractions [10,11]. In *S. mansoni*, the data are apparently contradictory: direct application of KA and L-glutamate to whole worms did not alter their basal motor activity [12], whereas L-glutamate induced concentration-dependent contractions of isolated *S. mansoni* muscle fibers mediated by activation of a high-affinity glutamate transporter [13].

The aim of the present work was to investigate the presence of non-NMDA ionotropic receptors for glutamate in *S. mansoni*, as well as their pharmacological modulation and putative involvement in the control of adult worm motility.

2. Materials and methods

2.1. Preparation of subcellular fractions of S. mansoni

Adult male *S. mansoni* (BH strain) were obtained from mice infected 45 days earlier with approximately 150 male cercariae each, as previously described [14].

About 2000 worms were homogenized in a Dounce homogenizer at 4° in a 0.25 M sucrose solution buffered to pH 7.4 with 5 mM Tris-acetate using 3 sequences of 10 passes of the pestle. The homogenate was centrifuged according to the method of Smithers *et al*. [15] 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; fraction P_4 consisted mainly of vesicles or large multivesicular structures and endoplasmic reticulum membranes [16]. The pellets were resuspended in buffered sucrose solution and stored at −70° until used.

2.2. Preparation of chick cerebellar membranes

Membranes from chick cerebellum were obtained as described by Gregor *et al*. [17]. Briefly, cerebellar tissue was homogenized with a Polytron homogenizer for 2 min in 10 vol. of an ice-cold 50 mM Tris-acetate buffer, pH 7.2, containing 5 mM EDTA and 1 mM PMSF. The homogenate was centrifuged at 24,000 g_{av} for 20 min (4°). The pellet was rehomogenized, recentrifuged as above, and frozen overnight at −20°. After thawing at room temperature, the pellet was homogenized as above in water, incubated for 60 min at room temperature, washed once more by centrifugation, resuspended in 50 mM Tris-acetate buffer, pH 7.2, and stored at −70° until used. Protein concentration for all preparations was determined by the method of Bradford [18], using bovine serum albumin as the standard.

2.3. Binding assays

P_1–P_4 fractions (80–120 μg protein) were incubated for 1 hr at 4° in 0.5 mL of 50 mM Tris-acetate buffer, pH 7.2, and, unless otherwise specified, 10 nM [^{3}H]KA (58 Ci/mmol, New England Nuclear). Bound and free [^{3}H]KA were separated by rapid filtration under vacuum on glass fiber filters (Whatman GF/C) followed by two 2.5-mL washes with ice-cold 50 mM Tris–HCl buffer (pH 7.2). 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 Tri-Carb Packard liquid scintillation counter. Saturation experiments were performed by the addition of increasing concentrations of unlabelled KA (10–90 nM) to 10 nM [^{3}H]KA. This protocol, classically referred to as a competition experiment, allows a great economy of radioligand and preparation, two critical points as far as low-affinity binding to *S. mansoni* is concerned. Specific binding was calculated by subtracting non-specific binding, determined in the presence of 100 μM unlabelled KA, from total binding.

The same subcellular fractions were used to measure [^{3}H]AMPA binding. Aliquots of 150–200 μg protein were incubated in 0.5 mL of 50 mM Tris-acetate buffer (pH 7.2) containing 100 mM KSCN and 5 or 10 nM [^{3}H]AMPA (40.6 Ci/mmol, New England Nuclear) for 1 hr at 4°. After separation of bound and free ligands by rapid filtration, the glass fiber filters (Whatman GF/C) were rapidly washed twice with 3 mL of ice-cold buffer containing 100 mM KSCN. Non-specific binding was determined in the presence of 1 mM L-glutamate.

Experiments with chick cerebellar membranes were performed as previously described by Taverna and Hampson [19] with some slight modifications. About 40 μg protein was incubated for 1 hr at 4° in 0.5 mL of 50 mM Tris-acetate buffer, pH 7.2, containing 5 nM [^{3}H]KA. The binding assay was terminated by rapid filtration under vacuum. Then the filters were washed twice with 2.5 mL of ice-cold 50 mM Tris-acetate, pH 7.2. Specific binding was defined as total binding minus binding obtained in the presence of 100 μM unlabelled KA.

Binding data from saturation experiments were presented as a classical Scatchard plot. The equilibrium dissociation constant (K_d) and the apparent maximal number of receptors (B_{max}) were obtained by non-linear

regression analysis (Prism, GraphPad Software Inc.), assuming the presence of only one population of binding sites. Student's *t*-test was used for statistical comparisons in competition studies.

2.4. In vivo studies

About 12–15 adult male worms were recovered from the portal veins of mice 50 days after infection. The worms were then washed rapidly and placed in a glass dish with two wells containing 250 μL of a buffered saline solution, consisting of 82.5 mM Na^+, 4.1 mM K^+, 3.6 mM Ca^{2+}, 3.3 mM Mg^{2+}, 100.4 mM Cl^-, 79.9 mM glucose, 10 μM 5-HT, and 15 mM HEPES, pH 7.4.

In each well, four worms were preincubated for 10 min at 37°. Then, the buffered saline solution was exchanged for an identical fresh one containing different concentrations of KA. After 10 min, the buffered saline solution containing KA was exchanged for an identical fresh one containing the same concentration of KA. Putative antagonists were added during the 10-min preincubation and remained present throughout the experiment.

To quantitate the effects of the drug, body area measurements were made by analysis of images captured with a CDC camera using the Image-Pro® Plus program (Media Cybernetics, L.P.). Data represent the means (±SEM) of at least three different dishes with four worms each ($N \geq 12$). Two-way ANOVA considering drug and time factors was used for the determination of statistical significance.

2.5. Drugs

The drugs were purchased from the Sigma Chemical Co. (DNQX and 5-HT), the RBI Co. (AMPA, L-glutamate, and KA), and Tocris Cookson Ltd. (NMDA, PDC, quisqualate, and THA).

3. Results

3.1. Binding assays

Among the four subcellular fractions of *S. mansoni*, [^{3}H]KA binding sites were detected only in the heterogeneous (P_1) fraction (Table 1). Therefore, this fraction was selected for further studies of [^{3}H]KA binding to *S. mansoni*. As shown in Fig. 1, data from the saturation experiment gave rise to a linear Scatchard plot within the concentration range used, indicating a uniform population of sites with low affinity (Table 2). Note that the relatively high value of non-specific binding (50–60% of total binding) at high concentrations of [^{3}H]KA resulted in an imprecise estimation of the parameter values in the individual experiments. A better estimation of K_d and B_{max} values was obtained by averaging the parameters calculated from three different experiments (Table 2). As the cerebellum of birds contains a high density of low-affinity binding sites [17], chick cerebellar membranes were used as positive controls in our experiments. Note that under the same experimental conditions, the affinity of KA sites found in *S. mansoni* was very similar to that measured in chick cerebellum (Table 2).

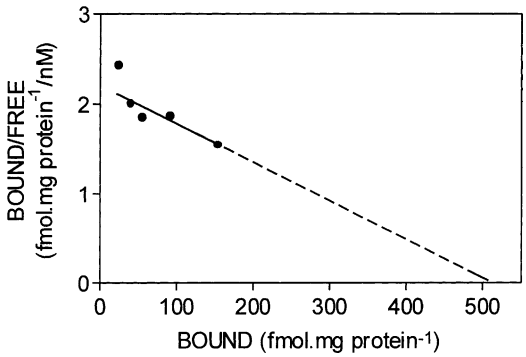

Fig. 1. Typical Scatchard plot for [^{3}H]KA binding to the P_1 fraction of *S. mansoni*. The curve was drawn using the parameters fitted by non-linear regression analysis with the model of a single class of independent binding sites. Non-specific binding accounted for 50–60% of total [^{3}H]KA binding.

As [^{3}H]KA could bind to the glutamate transporter, which has been reported in muscle fibers of *S. mansoni* [13], we tested PDC and THA, two inhibitors of high-affinity glutamate transporters [20], for their ability to inhibit [^{3}H]KA binding to the P_1 fraction. Neither PDC nor THA was effective for competing with 10 nM [^{3}H]KA in the P_1 fraction of *S. mansoni* (Fig. 2), when used at 100 μM, a concentration that effectively blocks the glutamatergic transporters in several mammalian tissues [20] and in muscle fibers of *S. mansoni* [13].

Table 1
Subcellular distribution of 5 nM [^{3}H]KA binding in subcellular fractions for *S. mansoni*

Fractions	[^{3}H]KA bound (fmol/mg protein)
P_1	9.17 ± 0.55
P_2	0.80 ± 0.45
P_3	0
P_4	0.70 ± 0.70

Values represent means ± SEM from three different preparations, each experiment being performed in quadruplicate.

Table 2
Equilibrium constants for the specific binding of [^{3}H]KA

	K_d (nM)	B_{max} (pmol/mg protein)
S. mansoni (P_1 fraction)	221 ± 80	0.724 ± 0.214
Chick cerebellar membranes	264	116

Values of parameters obtained for *S. mansoni* represent means ± SEM of three individual experiments performed in quadruplicate, while the values for chick cerebellum were obtained from a single experiment performed in quadruplicate.

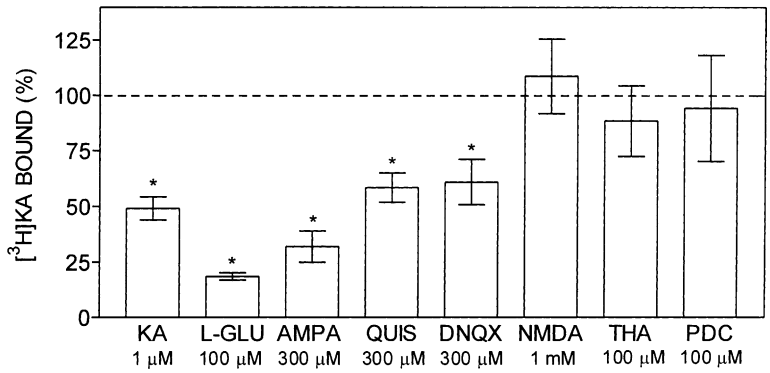

Fig. 2. Modulation of [^{3}H]KA (10 nM) binding to the P_1 fraction of *S. mansoni* by classical glutamatergic ligands. The values of specific binding expressed as percent of control (means ± SEM) were obtained from 3 to 5 experiments performed in quadruplicate. Key: (∗) significantly different from control ([^{3}H]KA binding in the absence of inhibitor), $P < 0.01$ (Student's *t*-test).

The pharmacological modulation of binding is best studied by comparing IC_{50} values calculated from whole competition curves performed with different putative ligands, as was done with the chick cerebellar membranes (Table 3). Unfortunately, such a protocol was not possible with *S. mansoni* due to the aforementioned limitations of our worm preparation. As a surrogate, we tested a single concentration of each putative ligand, generally a concentration equal to or near the IC_{50} value measured in the chick cerebellum (Fig. 2). [^{3}H]KA binding to *S. mansoni* was inhibited by KA, L-glutamate, and quisqualate, classical glutamatergic agonists, with intensities compatible with their potency in chick cerebellum (Table 3). On the other hand, 300 μM AMPA inhibited [^{3}H]KA binding to *S. mansoni* by more than 50% (Fig. 2), whereas it was nearly without effect (10% inhibition) on [^{3}H]KA binding to chick cerebellum (Table 3). Another difference observed between KA binding in chick cerebellum and *S. mansoni* was the effect of DNQX, a non-NMDA antagonist of glutamatergic ionotropic receptors. DNQX (300 μM) reduced KA binding to *S. mansoni* by only 40% (Fig. 2), whereas total inhibition of [^{3}H]KA binding occurred with chick cerebellum (data not shown). NMDA was also less effective in *S. mansoni* than in chick cerebellum, since it had no effect on [^{3}H]KA binding to *S. mansoni* (Fig. 2) when used at the IC_{50} concentration for chick cerebellum (Table 3).

Table 3
Inhibition of [^{3}H]KA binding to chick cerebellar membranes

Compound	IC_{50} (μM)
KA	1
L-Glutamate	60
Quisqualate	200
NMDA	1000
AMPA	⩾300*

The IC_{50} values (concentration required to inhibit 50% of the specific binding) were obtained from single competition experiments performed as described in "Materials and Methods."

* AMPA at 300 μM only inhibited 10% of [^{3}H]KA binding to chick cerebellar membranes.

As KA could bind with low affinity to the other subclass of non-NMDA ionotropic glutamate receptors, we investigated the presence of AMPA receptors in the four subcellular fractions of *S. mansoni*. None of these exhibited specific binding for [^{3}H]AMPA, at either 5 nM (N = 1, data not shown) or at 10 nM (N = 2, data not shown), a relatively high concentration considering the affinity of this ligand at mammalian AMPA receptors.

3.2. In vivo studies

Adult male worms in saline solution display a variety of body movements, including small and fast generalized shortening and lengthening of the body, and undulatory body waves propagated along the anterior/posterior axis, similar to peristaltic waves; however, the rate at which movements occurred varied for each worm. KA at 10 μM abolished the fast shortening and lengthening of the body, as well as the waveforms of the worms. Although the treated worms were immobile (they still exhibited infrequent movements in the head region), their locomotory behavior was completely different when compared to the worms in saline solution. As shown in Fig. 3, 10 μM KA produced a corkscrew-like coiling of the body of the worm, and this posture remained throughout exposure to KA. To quantitate the effects of KA, we measured the body area of the worms before and after addition of the drug. We observed that 10 μM KA produced a time-dependent reduction of the body area and that this effect was reversible (Fig. 4). Increasing the concentration of KA to 100 μM did not alter its maximal effect, but did accelerate the kinetics of its action (the maximal effect was attained more rapidly; data not shown).

Fig. 5 shows that 1 mM PDC, a competitive antagonist of the amino acid transporter present in *S. mansoni* muscle fibers, did not alter the effect of KA on whole worms. Since the binding assays suggested the presence of low-affinity KA receptors in *S. mansoni*, we next investigated the pharmacology of the KA effect on whole worm motility. To characterize the putative antagonism exerted by DNQX,

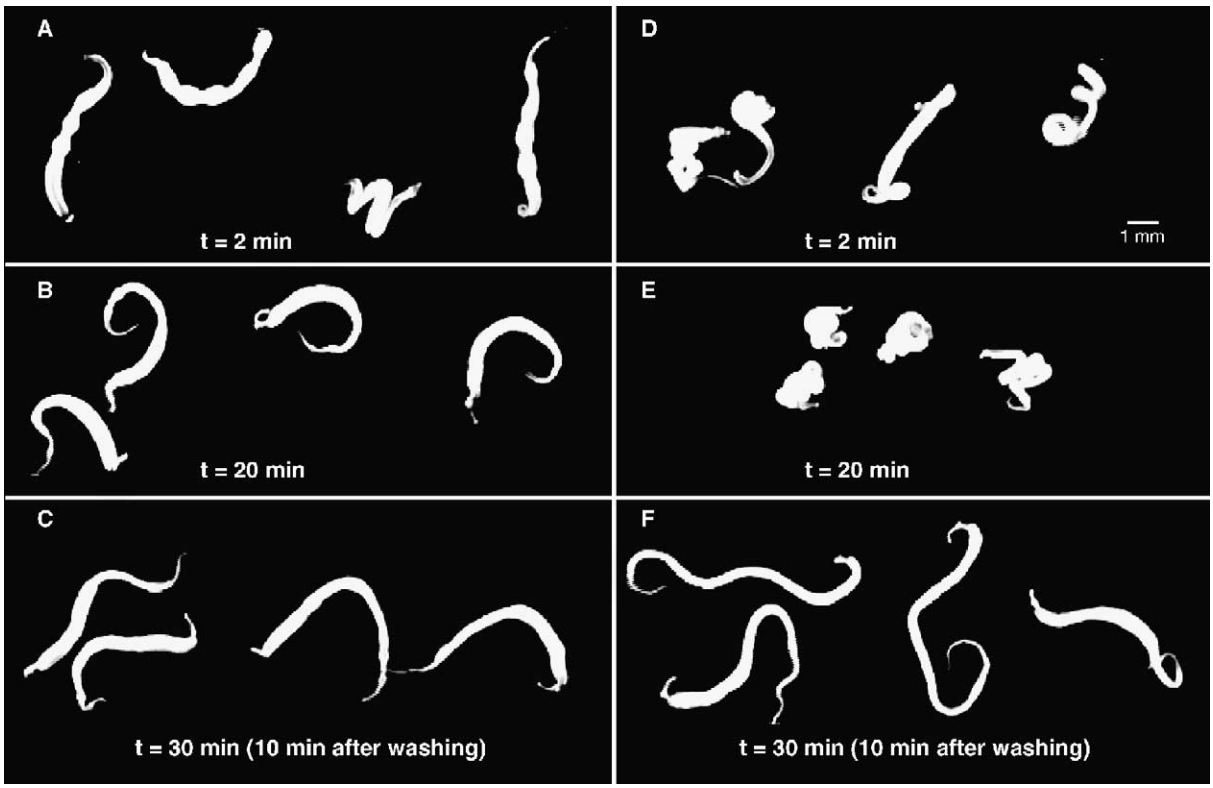

Fig. 3. Digitalized images showing the body postures of individual adult male *S. mansoni* in the absence (control, A–C) and presence of 10 μM KA (D–F). Control worms presented a variety of postures, including body waves (A). Note that 10 μM KA produced corkscrew-like coilings of parasite bodies, modifying the motor activity in a time-dependent manner (D, E). The KA-induced response was reversed completely after washing (F).

a non-specific antagonist at AMPA/kainate receptors, we performed experiments in parallel comparing the effect of KA in the absence and presence of DNQX. Fig. 6 shows that 1 mM DNQX was able to partially block the effect of KA on *S. mansoni* body area. Finally, 1 mM L-glutamate, the endogenous agonist of the mammalian KA receptor, did not modify the motor activity of whole worms (data not shown).

4. Discussion

Although the presence of glutamate-like immunoreactivity has been reported in the central and peripheral nervous systems of *S. mansoni* cercariae and adult *F. hepatica* [8,21], there is no direct evidence for the presence of a glutamatergic neurotransmission system in trematodes. As the identification of glutamate within neuronal-like

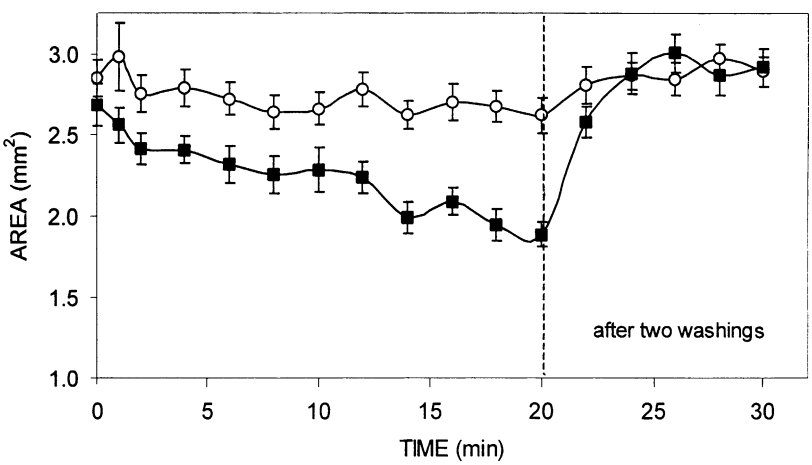

Fig. 4. Time course of the effect of KA on the body area of *S. mansoni* adult males. Twenty minutes after the addition of 10 μM KA (■) or control saline (○), the worms were washed twice with saline solution. Each point represents the mean ± SEM of three experiments with four worms each (N = 12). The effect of KA (measured during the 20-min incubation) was statistically significant ($P < 0.0001$), as determined by two-way ANOVA (for drug and time factors).

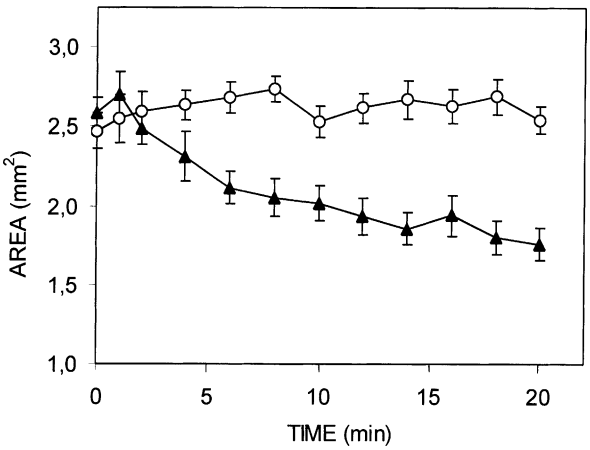

Fig. 5. Effect of KA on the body area of *S. mansoni* adult males in the presence of PDC. The body area of whole adult worms was measured in the presence of 10 μM KA and 1 mM PDC (▲) or control saline solution (○). PDC was added during preincubation and remained present throughout the experiment. Each point represents the mean ± SEM from three experiments, each performed with four worms (N = 12). The effect of KA (in the presence of PDC) was statistically significant ($P < 0.0001$), as determined by two-way ANOVA (for drug and time factors).

structures is not sufficient to classify it as a putative neurotransmitter, we provide here pharmacological evidence for the presence of ionotropic glutamatergic receptors in adult *S. mansoni*, using a classical radioligand binding approach, as well as direct measurements of the effect of KA on whole worm motility.

For the first time, our binding assays revealed the presence of low-affinity binding sites for KA in *S. mansoni*. These were detected only in the heterogeneous (P_1) fraction obtained from differential centrifugation of adult worm homogenates. To propose that a specific binding site is, in fact, a receptor, some features must be present, such as proper affinity and pharmacological modulation [22]. Likewise, other alternatives must be ruled out, such as binding to catabolic enzymes or transporters. The affinity of worm binding sites for KA is low, but similar to that of the low-affinity binding sites present in great amounts in chick cerebellum (Table 2, [17]). Note that the K_d obtained with our preparation of chick cerebellar membranes was very similar to that reported in the literature for this preparation [17]. Therefore, we did not repeat this control. Furthermore, [^{3}H]KA binding to the P_1 fraction was modulated by classical glutamatergic ligands in a similar manner to the experiments with chick cerebellum: the order of potency for [^{3}H]KA inhibition was KA > L-glutamate > AMPA > quisqualate. NMDA, even at a high concentration, did not compete with KA in *S. mansoni*. This pharmacological profile was qualitatively consistent with that reported for the low-affinity KA binding sites present in vertebrates [17,23]. However, this similarity (but not identity) between the sites detected in *S. mansoni* and chick cerebellum does not indicate whether they are species homologues (the same gene in different species) or different isoforms (subtypes represented by different genes) [24]. Because the binding sites present in *S. mansoni* have a low affinity for KA, we had to consider the possibility of a binding to AMPA receptors, since KA is known to bind and activate this ionotropic glutamate receptor, in mammals [25]. However, the order of potency of the glutamatergic ligands used to compete with AMPA was very different from that observed in *S. mansoni*. In addition, our attempt to directly label binding sites with [^{3}H]AMPA was unsuccessful in all subcellular fractions of *S. mansoni*, so that we ruled out the hypothesis of [^{3}H]KA binding to a putative AMPA receptor in our preparation. Interestingly, when compared to chick cerebellum and mammalian brain, the KA binding sites detected in *S. mansoni* displayed some quantitative differences regarding the potency of some glutamatergic ligands [17,23]. For example, AMPA was more potent, whereas DNQX and NMDA were less potent, in *S. mansoni* than in chick cerebellum (Table 3, Fig. 2). These differences suggest that the design of kainate analogs may allow the distinction between host and parasite glutamatergic receptors. This would be an important aspect in the search for new specific schistosomicidal drugs.

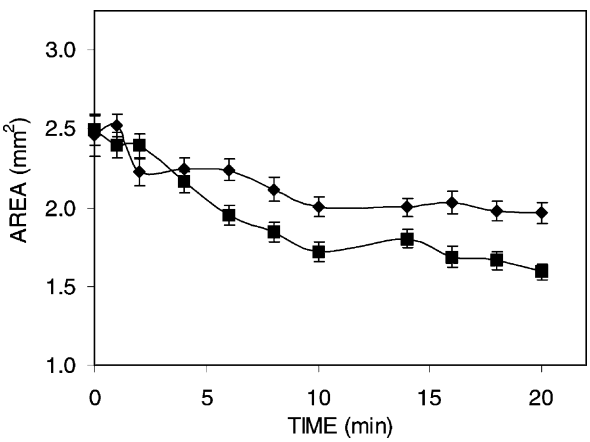

Fig. 6. DNQX inhibition of the *S. mansoni* response to KA. The body area of whole adult worms was measured in the presence of 10 μM KA in the absence (■) and presence (◆) of 1 mM DNQX. DNQX was added during preincubation and remained present throughout the experiment. Each point represents the mean ± SEM from five experiments, each performed with four worms (N = 20). The effect of DNQX (comparing the effect of KA in the presence of DNQX to the effect of KA alone, as a control) was statistically significant ($P < 0.0001$), as determined by two-way ANOVA (for drug and time factors).

Although the glutamate transporter detected in a particular schistosoma muscle fiber was not blocked by KA [13], we could not rule out, *a priori*, the possibility that KA was binding to another glutamate transporter similar to the mammalian $EAAT_2$, which is competitively inhibited by KA [20]. This hypothesis is improbable since the glutamate transporter inhibitors PDC and THA were not able to inhibit [^{3}H]KA binding to the P_1 fraction.

Once the presence of specific binding sites for KA in adult *S. mansoni* is demonstrated, the question of their physiological role arises. Present data provide preliminary

insights to this important question by showing that the addition of KA to the culture medium induced a major and reversible alteration of the worm motility pattern. Indeed, macroscopic observation, as well as rough, but quantitative, measurement of body area, showed inhibition of waveform peristaltism and corkscrew-like coiling of the body of the worm. The lack of effect by KA on motor activity, as reported by Mellin *et al*. [12], can be explained by the different experimental conditions used. On the other hand, the present results do not contradict the observation that KA had no observable effects when applied to isolated worm muscle fibers [13], as only one morphological muscle type is targeted in these studies. It is known that in trematodes, pharmacological differences among distinct muscle types exist [26]. In addition, whole-animal studies showed that KA can also act on nonmuscular receptors in the central or peripheral nervous system. Even a surface localization in the tegument is possible, since KA binding sites were found in the heterogeneous fraction, which contains fragments of tegument among other structures. Such a nonneuronal localization cannot be discarded since glutamate signaling has been reported to be functional in tissues such as bone, pancreas, and skin [27]. It can be postulated that KA receptors located at the cell surface could mediate the effect of KA without transporting KA across the plasma membrane. The inability of PDC to block the responses elicited by KA further suggests that KA does not depend on a high-affinity amino acid transporter for reaching its site of action. Unfortunately, the above hypothesis is only speculative. The exact localization of KA binding sites is still unknown. The lack of effect of L-glutamate on whole worm motility could be due to its low affinity for the KA binding sites and to the efficient uptake through different amino acid transporters present in the tegument [28]. The uptake of amino acids is important particularly in stressful conditions, e.g. when the worms maintained in saline solution depend on an aerobic metabolic pathway [29,30]. Thus, we can hypothesize that glutamate is not only taken up from the incubation medium, but is also inside the worm tegument (or in deeper structures) out of the extracellular space in contact with the KA receptors involved in the control of worm motility. Alternatively, glutamate taken up from the external medium could be in some way sequestered in the tegument and unavailable for binding to the KA receptors (for which its affinity is very low since 100 μM inhibited KA binding by only 70%).

Phylogenetically, platyhelminths are important as the first metazoan group to possess a centralized nervous system [31] and so are considered as a link between lower and higher invertebrates. Therefore, it is relevant to imagine that glutamate is present not only in mammalian brains, but also in lower invertebrates, such as nematodes. Although helminths, nematodes are phylogenetically distant from platyhelminths and are characterized by nervous systems that are anatomically less complex and better characterized than those found in platyhelminths. Recently, electrophysiological and pharmacological experiments have suggested the presence of an electrogenic glutamate transporter in the hypodermis and DE2 motoneurons of the parasitic *Ascaris suum* [32]. These DE2 neurons also harbor a glutamate receptor pharmacologically related to the KA receptor, which participates in the depolarizing response elicited by L-glutamate [33]. Finally, glutamate or KA injections into whole *A. suum* produce a paralyzed quasi-static body posture, suggesting that this glutamatergic pathway may play a role in nematode locomotory behavior.

In conclusion, we report the presence of specific binding sites for KA in adult *S. mansoni* as well as its effects on whole worm motility raising the possibility of a glutamatergic signaling pathway in this parasite, putatively useful for the development of new selective schistosomicidal drugs.

Acknowledgments

The authors wish to thank Dr. Lygia dos Reis Corrêa (Instituto Oswaldo Cruz, Rio de Janeiro) for providing the infected mice. We thank Dr. Tim A. Day (Department of Biomedical Sciences, Iowa State University, USA) and Franklin D. Rumjanek (Departamento de Bioquímica Médica, UFRJ, Brazil) for critically reading the manuscript. Financial support was provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), PRONEX 0888/96 and Fundação Carlos Chagas Filho de Amparo a Pesquisa do Estado do Rio de Janeiro (FAPERJ E-26/152.018/2000).

References

[1] Cioli D, Pica-Mattoccia L, Moroni R. *Schistosoma mansoni*: hycanthone/oxamniquine resistance is controlled by a single autosomal recessive gene. Exp Parasitol 1992;75:425–32.
[2] Ismail M, Botros S, Metwally A, William S, Farghally A, Tao L, Day TA, Bennett JL. Resistance to praziquantel: direct evidence from *Schistosoma mansoni* isolated from Egyptian villagers. Am J Trop Med Hyg 1999;60:932–5.
[3] Day TA, Maule AG. Parasitic peptides! The structure and function of neuropeptides in parasitic worms. Peptides 1999;20:999–1019.
[4] Willcockson WS, Hillman GR. Drug effects on the 5-HT response of *Schistosoma mansoni*. Comp Biochem Physiol C 1984;77:199–203.
[5] Pax RA, Bennett JL. Neurobiology of parasitic platyhelminths: possible solutions to the problems of correlating structure with function. Parasitology 1991;102:531–9.
[6] Webb RA, Eklove H. Demonstration of intense glutamate-like immunoreactivity in the longitudinal nerve cords of the cestode *Hymenolepis diminuta*. Parasitol Res 1989;75:545–8.
[7] Eklove H, Webb RA. Glutamate-like immunoreactivity in the cestode *Hymenolepis diminuta*. Can J Zool 1990;68:2417–23.
[8] Brownlee DJA, Fairweather I. Immunocytochemical localization of glutamate-like immunoreactivity within the nervous system of the cestode *Mesocestoides corti* and the trematode *Fasciola hepatica*. Parasitol Res 1996;82:423–7.

[9] Webb RA. The uptake and metabolism of L-glutamate by tissue slices of the cestode *Hymenolepis diminuta*. Comp Biochem Physiol C 1986;85:151–62.
[10] Webb RA. Release of exogenously supplied [^{3}H]glutamate and endogenous glutamate from tissue slices of the cestode *Hymenolepis diminuta*. Can J Physiol Pharmacol 1988;66:889–94.
[11] Thompson CS, Mettrick DF. The effects of 5-hydroxytryptamine and glutamate on muscle contraction in *Hymenolepis diminuta* (Cestoda). Can J Zool 1989;67:1257–62.
[12] Mellin TN, Busch RD, Wang CC, Kath G. Neuropharmacology of the parasitic trematode, *Schistosoma mansoni*. Am J Trop Med Hyg 1983;32:83–93.
[13] Miller CL, Day TA, Bennett JL, Pax RA. *Schistosoma mansoni*: L-glutamate-induced contractions in isolated muscle fibers; evidence for a glutamate transporter. Exp Parasitol 1996;84:410–9.
[14] Cunha VMN, Mever-Fernandes JR, Noël F. A (Ca^{2+}–Mg^{2+}) ATPase from *Schistosoma mansoni* is coupled to an active transport of calcium. Mol Biochem Parasitol 1992;52:167–74.
[15] Smithers SR, Roodyn DB, Wilson RJM. Biochemical and morphological characterization of subcellular fractions of male *Schistosoma mansoni*. Exp Parasitol 1965;16:195–206.
[16] Cunha VMN, De Souza W, Noël F. A Ca^{2+}-stimulated, Mg^{2+}-dependent ATPase activity in subcellular fractions from *Schistosoma mansoni*. FEBS Lett 1988;241:65–8.
[17] Gregor P, Eshhar N, Ortega A, Teichberg VI. Isolation, immunochemical characterization and localization of the kainate sub-class of glutamate receptor from chick cerebellum. EMBO J 1988;7:2673–9.
[18] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal Biochem 1976;72:248–54.
[19] Taverna FA, Hampson DR. Properties of a recombinant kainate receptor expressed in baculovirus-infected insect cells. Eur J Pharmacol 1994;266:181–6.
[20] Arriza JL, Fairman WA, Wadiche JI, Murdoch GH, Kavanaugh MP, Amara SG. Functional comparisons of three glutamate transporter subtypes cloned from human motor cortex. J Neurosci 1994;14:5559–69.
[21] Solis-Soto JM, Brink MDJ. Immunocytochemical study on biologically active neurosubstances in daughter sporocysts and cercariae of *Trichobilharzia ocellata* and *Schistosoma mansoni*. Parasitology 1994;108:301–11.
[22] Burt DR. Criteria for receptor identification. In: Yamamura HI, Enna SJ, Kuhar MI, editors. Neurotransmitter receptor binding. New York: Raven Press; 1978, p. 41–55.
[23] Johansen TJ, Drejer J, Watjen F, Nielsen EO. A novel non-NMDA receptor antagonist shows selective displacement of low-affinity [^{3}H]kainate binding. Eur J Pharmacol 1993;246:195–204.
[24] Hartig PR, Branchek TA, Weinshank RL. A subfamily of 5-HT_{1D} receptor genes. Trends Pharmacol Sci 1992;13:152–9.
[25] Monaghan DT, Bridges RJ, Cotman CW. The excitatory amino acid receptors: their classes, pharmacology and distinct properties in the function of the central nervous system. Annu Rev Pharmacol Toxicol 1989;29:365–402.
[26] Pax RA, Siefker C, Bennett JL. *Schistosoma mansoni*: differences in acetylcholine, dopamine and serotonin control of circular and longitudinal parasite muscle. Exp Parasitol 1984;58:314–24.
[27] Skerry TM, Genever PG. Glutamate signalling in non-neuronal tissues. Trends Pharmacol Sci 2001;22:174–81.
[28] Asch HL, Read CP. Membrane transport in *Schistosoma mansoni*: transport of amino acids by adult males. Exp Parasitol 1975;38:123–35.
[29] Van Oordt BEP, Van Den Heuvel JM, Tielens AGM, Van Den Bergh SC. The energy production of the adult *Schistosoma mansoni* is for a large part aerobic. Mol Biochem Parasitol 1985;16:117–26.
[30] Foster LA, Chen GZ, VandeWaa EA, Pax RA, Bennett JL. Glutamine- vs. glucose-supported motor activity in *Schistosoma mansoni*: physiological relevance of aerobic metabolism. Exp Parasitol 1989;69:44–53.
[31] Graham MK, McGeown JG, Fairweather I. Ionic mechanisms underlying spontaneous muscle contractions in the liver fluke, *Fasciola hepatica*. Am J Physiol 1999;277:R374–83.
[32] Davis RE. Action of excitatory amino acids on hypodermis and the motornervous system of *Ascaris suum*: pharmacological evidence for a glutamate transporter. Parasitology 1998;116:487–500.
[33] Davis RE. Neurophysiology of glutamatergic signalling and anthelmintic action in *Ascaris suum*: pharmacological evidence for a kainate receptor. Parasitology 1998;116:471–86.