J Comp Physiol A (2003) 189: 631–641
DOI 10.1007/s00359-003-0429-8

ORIGINAL PAPER

J. P. Boyle · J. F. Hillyer · T. P. Yoshino

Pharmacological and autoradiographical characterization of serotonin transporter-like activity in sporocysts of the human blood fluke, *Schistosoma mansoni*

Received: 7 March 2003 / Revised: 21 April 2003 / Accepted: 13 May 2003 / Published online: 12 July 2003

Abstract The present study focuses on the role of the biogenic monoamine serotonin (5-hydroxytryptamine) in the biology of sporocyst stages of the human blood fluke, *Schistosoma mansoni*, and its importance during obligate development within its snail host *Biomphalaria glabrata*. Based on previous work demonstrating that snails infected with *S. mansoni* have reduced levels of 5-hydroxytryptamine, we hypothesized that sporocysts actively transport this molecule from the host milieu. Intact sporocysts isolated in vitro take up exogenous 5-hydroxytryptamine via a high-affinity mechanism ($K_m = 1.4$ μmol l^{-1}), and this serotonin transporter-like activity is dependent upon extracellular Na^+ and Cl^- and is highly sensitive to previously characterized serotonin transporter inhibitors. Autoradiography suggests that transported [^{3}H]5-hydroxytryptamine localizes within the body of the sporocyst, and in many cases is found in apical gland cells. Moreover, serotonin transporter-like activity is absent in free-swimming miracidia, the infective stage for the snail host, and the increase in larval serotonin transporter-like activity after miracidium-to-sporocyst transformation is accompanied by a corresponding decrease in steady-state levels of transcripts for tryptophan hydroxylase, the rate-limiting enzyme in serotonin biosynthesis. Overall our data suggest that *S. mansoni* larvae express surface-exposed serotonin transporter-like molecules, and that the transition from free-living miracidium to parasitic mother sporocyst is characterized by an increased dependence upon exogenous 5-hydroxytryptamine.

Keywords Electron microscopy · Miracidia · *Schistosoma mansoni* · Serotonin transport · Sporocyst

Abbreviations 5-HT 5-hydroxytryptamine · CBSS Chernin's balanced salt solution · SERT serotonin transporter

Introduction

Schistosoma mansoni is one of four major *Schistosoma* spp. that infect over 200 million people in tropical and subtropical regions worldwide (World Health Organization 1998, 2000). Numerous control efforts have been initiated attempting to reduce parasite burdens in infected human populations using chemotherapy, and to limit the proliferation of snail intermediate hosts. In spite of these efforts, infections due to *Schistosoma* spp. continue to be an enormous public health problem in endemic areas, and there is a great need to develop new methods of transmission control. As a result, *Schistosoma* spp., and especially *S. mansoni*, have been subject to a large body of research aimed at elucidating unique aspects of their biology that could be exploited for the development of new strategies to disrupt the life cycle.

Biogenic monoamines such as serotonin (5-hydroxytryptamine; 5-HT) have been extensively investigated as potential targets for novel chemotherapies in *S. mansoni* (Thompson et al. 1996) due to the clear effects these compounds have on the parasite's behavior and physiology (reviewed in Mansour 1984; Pax et al. 1996). Bath-application of 5-HT increases muscular activity in both human- (adult) and snail-host (larval) stages of *S. mansoni* (Mansour 1984; Boyle et al. 2000), and also stimulates glucose uptake (Rahman et al. 1985), glycogen breakdown, and lactate excretion (Mansour 1984; Rahman et al. 1985) in adults. In addition, adult

J. P. Boyle · J. F. Hillyer
Department of Animal Health and Biomedical Sciences,
School of Veterinary Medicine,
University of Wisconsin-Madison,
1656 Linden Drive West, Madison, WI 53706, USA

T. P. Yoshino (✉)
Department of Pathobiological Sciences,
School of Veterinary Medicine,
University of Wisconsin-Madison,
2115 Observatory Drive, Madison, WI 53706, USA
E-mail: yoshinot@svm.vetmed.wisc.edu
Tel.: +1-608-2636002
Fax: +1-608-2658122

stages of *S. mansoni* exhibit carrier-mediated uptake of exogenous 5-HT (Catto and Ottesen 1979; Wood and Mansour 1986). While the receptors or transporters involved in these processes have not been identified molecularly, pharmacological data suggest that 5-HT can act via receptors with pharmacological similarities to those previously found in mammals and arthropods (Day et al. 1994; Boyle et al. 2000). Since *S. mansoni* is an obligate endoparasite, it has been hypothesized that responses to 5-HT in vitro indicate that parasites react to fluctuations in host 5-HT levels with changes in motility and/or metabolism which may be important for their survival within the host (Mansour 1984).

Transmission of *S. mansoni* to humans is dependent on the development of the parasite in the snail intermediate host belonging to the genus *Biomphalaria* (Basch 1976). This process is relatively complex, involving the asexual production of three phenotypically distinct life stages. Free-swimming ciliated miracidia that have hatched from eggs in the feces of an infected host actively seek out a suitable snail host (Kalbe et al. 1996), and then penetrate through the mantle using glandular secretions (Yoshino et al. 1993). Once inside the snail, miracidia transform into mother sporocysts by shedding their ciliary plates and forming a surface syncytium called the tegument (Pan 1996), which serves as an absorptive and secretory surface for the developing larvae. After ~15–20 days, daughter sporocysts that have developed from germinal cells within the mother sporocyst emerge and migrate to the host digestive gland and gonad, where they eventually give rise to cercariae. These cercariae emerge from the daughter sporocyst and exit through the snail headfoot, completing the intramolluscan phase of the life cycle. Given the species specificity of the snail/parasite interaction (Basch 1976) and the complex development and migration necessary for disease transmission, intramolluscan stages of *S. mansoni* represent a unique target for disease control.

Results from work in our laboratory suggest an important role for 5-HT during the intramolluscan development of *S. mansoni*. Snails infected with *S. mansoni* contain reduced CNS levels of 5-HT (Manger et al. 1996), and this parasite-induced depletion of host 5-HT may be partially responsible for parasitic castration, a process by which schistosome-infected snails have reduced reproductive output (Crews and Yoshino 1989; Manger et al. 1996). To follow up on these observations, we aimed to identify potential mechanisms for larval depletion of host 5-HT. The surface tegument is a single cell consisting of an outer lipid bilayer, cytoplasm, and an inner lipid bilayer supported by multiple cytons within the body of the sporocyst. In adult stages, this structure is known to contain a variety of integral membrane proteins, including solute transporters (Pappas and Read 1975; Skelly et al. 1994; Skelly and Shoemaker 1996; Skelly et al. 1999) and receptors for host factors (Camacho and Agnew 1995; Davies et al. 1998) that presumably facilitate interactions with the host environment (Yoshino et al. 2001). We hypothesized that sporocysts possess surface-exposed serotonin transporter (SERT)-like molecules that enable them to transport host 5-HT as has been described for adult stages of *S. mansoni* (Catto and Ottesen 1979; Wood and Mansour 1986), and that this activity is important for development and eventual disease transmission to humans. To address this hypothesis we first investigated the 5-HT transport capacity of *S. mansoni* sporocysts in vitro, as well as the functional pharmacology of the transporter(s) involved in 5-HT uptake. We then used autoradiography to determine the eventual location of transported 5-HT. We also compared gene expression profiles for enzymes involved in the synthesis of biogenic monoamines among free-living and intra-host stages of *S. mansoni*.

Materials and methods

Parasite collection

All parasites used were the NMRI strain of *S. mansoni* (Lewis et al. 1986). Free-swimming miracidia were hatched from eggs harvested from the livers of 48-day *S. mansoni* infected mice and axenically transformed into mother sporocysts as described previously (Boyle et al. 2000). Briefly, dissected livers containing *S. mansoni* eggs were blender-homogenized in 0.4 mol l^{-1} NaCl containing antibiotics (penicillin 60 μg ml^{-1} and streptomycin 50 μg ml^{-1}; NaCl-PS), washed three times by centrifugation in NaCl-PS, and the homogenate was resuspended in artificial pond water (Nolan and Carriker 1946) to induce hatching. Phototactic miracidia were collected from the top of a 1-l volumetric flask and washed twice in Chernin's balanced salt solution (CBSS, pH 7.2; containing in mmol l^{-1}: 47.9 NaCl, 2.0 KCl, 0.5 Na_2HPO_4, 1.8 $MgSO_4$, 3.6 $CaCl_2$, and 0.6 $NaHCO_3$; Chernin 1963) supplemented with 1 g l^{-1} each of glucose and trehalose and the same antibiotics as in NaCl-PS ($CBSS^+$). After resuspension in $CBSS^+$, they were either used immediately in experiments (for miracidia 5-HT uptake determinations, see below), or plated in a volume of 1 ml in 24-well plates (Costar, Cambridge, Mass., USA) at a density of approximately 5,000 miracidia/well. Under these conditions, 90% of the miracidia shed their ciliary plates and form the tegumental syncytium within 48 h, becoming primary, or mother, sporocysts. Prior to all experiments, sporocysts were transferred to clean 1.5-ml microcentrifuge tubes, pelleted by brief (~3 s at 500×*g*) centrifugation, and washed with CBSS to remove sugars and antibiotics. It should be noted that sporocysts cultivated in this fashion, while not identical to sporocysts generated in vivo, are capable of producing progeny daughter sporocysts when cultured in vitro (Yoshino and Laursen 1995; Ivanchenko et al. 1999).

Serotonin uptake assay

All assays were carried out in 1.5-ml microcentrifuge tubes in a final volume of 20–100 μl CBSS, and *S. mansoni* miracidia or sporocysts were added last to tubes containing [^{3}H]5-HT and drugs or salines of interest. Uptake was allowed to proceed for either 10 or 15 min at 22°C, a time during which uptake was consistently linear (data not shown). Assays were terminated by washing (3×1.5 ml) in ice-cold CBSS using brief centrifugations (~3 s at ~500×*g*) between washes to pellet the larvae. Initiation and termination of individual uptake assays were staggered to ensure equal incubation times for each treatment. Following the final wash, larvae were resuspended in 100 μl CBSS, and 70–90 μl of the of the suspension was added to 3.5 ml scintillation fluid (Scintiverse, Fisher) and subjected to scintillation counting in a TriCarb 2200CA liquid scintillation

analyzer (Packard Instrument Company, Downers Grove, Ill., USA). The number of worms in an aliquot (5–20 µl) of the suspension was determined in order to normalize the data to uptake per 1,000 larvae. Non-specific uptake was determined by carrying out identical assays in the presence of 200 µmol l^{-1} unlabeled 5-HT at 4°C. The mean non-specific uptake was subtracted from each data point prior to analysis, and all assays were performed in duplicate or triplicate. For K_m determinations sporocysts were incubated in 10, 5, 1, 0.5, and 0.05 µmol l^{-1} [^{3}H]5-HT for 15 min. Parallel assays were carried out in the presence of the serotonin transport blocker fluoxetine (10 µmol l^{-1}) to differentiate SERT-like (e.g., fluoxetine-sensitive) uptake from non-specific uptake.

To determine the effects of Na^+ and Cl^- ions on [^{3}H]5-HT uptake, sporocysts were divided into five pools and washed three times with 1.5 ml normal CBSS (49.0 mmol l^{-1} Na^+, 57.1 mmol l^{-1} Cl^-), low-Na^+ CBSS (0.6 mmol l^{-1} Na^+), low-Cl^- CBSS (0 mmol l^{-1} Cl^-), or low-Na^+ or low-Cl^- CBSS mixed with an equal part of normal CBSS (24.8 mmol l^{-1} Na^+ or 28.6 mmol l^{-1} Cl^-, respectively). For low-Na^+ saline, choline chloride isotonically replaced NaCl, and K_2HPO_4 replaced Na_2HPO_4. For low-Cl^- saline, sodium and potassium gluconate salts replaced NaCl and KCl, respectively, and $Ca(NO_3)_2$ replaced $CaCl_2$. Sporocysts were then incubated for 10 min in the presence of 1 µmol l^{-1} [^{3}H]5-HT in the proper saline, washed and subjected to liquid scintillation counting as described above.

For K_i determinations, sporocysts were treated with 500 nmol l^{-1} [^{3}H]5-HT containing different drug concentrations ranging from 0.001 to 10 µmol l^{-1} for 10–15 min, and processed for scintillation counting as above. At least two separate experiments were carried out for each drug.

Autoradiographic analysis and electron microscopy

To determine the fate of transported 5-HT, sporocysts were incubated for 15 min with 1 µmol l^{-1} [^{3}H]5-HT as described above in the presence or absence of 10 µmol l^{-1} fluoxetine, washed extensively (4×1.5 ml) with CBSS, and fixed for 2 h in 4% paraformaldehyde and 2% glutaraldehyde in snail PBS (sPBS, pH 7.2; Crews and Yoshino 1991). After a 2-h wash in sPBS and brief centrifugation, the sporocyst pellet was overlaid with one drop of 2% low melting point agarose (Invitrogen, Carlsbad, Calif., USA) in sPBS and incubated at 42°C for 15 min to ensure mixing of the agarose with the remaining CBSS. After hardening, the agarose plug was dehydrated through a graded ethanol series with gentle agitation at 22°C, and all subsequent manipulations were carried out at this temperature. Plugs were infiltrated for 1 h in a 1:1 mixture of EtOH and embedding resin, consisting of a 1:1 mixture of EMBed 812 and Spurr's low viscosity embedding media (Electron Microscopy Sciences, Fort Washington, Pa., USA). After an overnight incubation in 100% resin and three more resin changes the following day, samples were polymerized for 24 h at 60°C. Sections of 1–2 µm thickness were cut with a diamond knife and fixed to glass microscope slides by heating. For autoradiography, slides were dipped in photographic emulsion NTB-2 (Kodak, Rochester, N.Y., USA) in a darkroom equipped with Safelight Filter 2 (Kodak) and stored in the dark at 4°C in the presence of desiccant for 20–25 days. Samples were then allowed to warm to room temperature, developed for 4 min in 50% strength D-19 developer (Kodak), rinsed in distilled water, and fixed for 8 min in 50% strength all-purpose fixer (Kodak). A subset of the slides were not exposed to emulsion, but rather were stained with 1% toluidine blue O (in 1% sodium borate) and rinsed with deionized water. Slides were dried, mounted in Polymount (Polysciences, Warrington, Pa., USA), and visualized by light and differential interference contrast microscopy (DIC) on a Provis AX70 light microscope (Olympus, Melville, N.Y., USA) connected to a Spot RT Slider digital camera (Diagnostic Instruments, Sterling Heights, Mich., USA). Digital images were collected, brightness and contrast were adjusted using Spot software, and then subjected to HiGauss filtering using Image-Pro Plus software (MediaCybernetics, Carlsbad, Calif., USA).

To further clarify which sporocyst tissues took up exogenous [^{3}H]5-HT, the embedded sporocysts used above were also analyzed by transmission electron microscopy (TEM). Blocks were trimmed and ultrathin sections (70–100 nm) were cut with a diamond knife on a Reichert Om U3 ultramicrotome. Sections were transferred to 200-mesh nickel EM grids (Polysciences), stained with uranyl acetate and lead citrate, and viewed on a Philips CM120 TEM operating at 80-kV acceleration voltage under conditions described previously (Hillyer and Albrecht 1998). Photographic negatives were scanned and processed manually to optimize brightness and contrast. It should be noted that sections were processed for TEM for the purpose of examining identified sporocyst tissues at higher magnification, and were *not* exposed to photographic emulsion.

Analysis of tryptophan and tyrosine hydroxylase transcript levels during development by real-time quantitative PCR

Real-time quantitative PCR (qPCR) with SYBR green detection (Morrison et al. 1998) was used to determine developmental changes in steady-state mRNA levels of tryptophan hydroxylase (TPH; GenBank Acc. No. AF031034) and tyrosine hydroxylase (TH; GenBank Acc. No. AF030336), the rate limiting enzymes in serotonin and catecholamine biosynthesis, respectively. Adult worms were obtained by perfusion (Day et al. 1994) or dissection from the mesenteric veins of 48-day-infected mice, and miracidia and sporocysts were obtained as described above. Total RNA was harvested from worms either directly (miracidia and sporocysts) or after pulverizing in liquid N_2 (adults), using RNAzol. cDNA was synthesized from 2 µg of total RNA in a 25-µl reaction using 200 U of MMLV-reverse transcriptase (Promega, Madison, Wis., USA) and oligo-$dT_{(18)}$ and random hexamer primers, and control reactions were carried out for each sample in the absence of MMLV-RT to account for the presence of any contaminating genomic DNA. For qPCR, cDNA samples were diluted 1:5 with sterile water for the analysis of poly-adenylated transcripts, or 1:500 for determination of 18 s ribosomal transcript levels. qPCR reactions contained the following: 2.5 µl diluted cDNA, 240 nmol l^{-1} of each primer, 3 mmol l^{-1} $MgCl_2$, 200 µmol l^{-1} each of dATP, dCTP and dGTP, 400 µmol l^{-1} dUTP, 0.1×SYBR green (Molecular Probes, Eugene, Ore., USA), 225 µmol l^{-1} ROX-I dye labeled 6-mer (Synthegen, Houston, Tex., USA), and 0.5 U of HotStar Taq polymerase (Qiagen, Valencia, Calif., USA). Reactions were subjected to 95°C for 15 min, followed by 40 cycles of 15 s at 95°C and 60 s at 60°C on a GeneAmp 5700 Sequence Detection system (Applied Biosystems, Foster City, Calif., USA). Dissociation curves were carried out at the end of each PCR run to determine product purity, and data were analyzed with GeneAmp 5700 SDS software (Applied Biosystems). To determine the cycle threshold (Ct) value (Livak and Schmittgen 2001; Morrison et al. 1998), the amplification threshold was set at 0.2, and the background fluorescence was determined during cycles 6–10. The metabolic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH; GenBank Acc. No. M92359; Charrier-Ferrara et al. 1992) also was measured to serve as a control mRNA unrelated to biogenic monoamine synthetic pathways.

Quantitative PCR primers were designed using Primer Express Software (Applied Biosystems, Foster City, Calif., USA) and were selected if they would produce a product ranging from 50–150 bp, had melting temperatures between 58 and 60°C, and had 2 or fewer G-C pairs in the final 5 bp of the 3' end. Primer sequences were as follows (Target gene: Forward, Reverse, [region amplified]): TPH: ttggtttccaagacacatttctga, cgacgttttctgtattccttatctttg [526–642]; TH: tgactttatccaatgccaatcg, aactgttgccccgtaattcttc [274–382]; GAPDH: tcgttgagtctactggagtctttacg, aatatgagcctgagctttatcaatgg [284–336]; Sm18 s: gtgtcggcgacggatctt, ccggaatcgaaccctgatt, [299–420].

Data analysis

K_m and IC_{50} values for 5-HT uptake data were determined using nonlinear regression analysis in GraphPad Prism software (Version 3.00 for Windows, GraphPad Software, San Diego, Calif., USA).

For IC_{50} determinations, all data were converted to percent of control prior to analysis, and values output from multiple experiments were averaged to generate the final values. The empirically determined K_m (see Results) was used to convert IC_{50} values of transport inhibitors to K_i values using the method of Cheng and Prusoff (1973). When necessary, standard curves of known molar amounts of [^{3}H]5-HT were subjected to scintillation counting in parallel to convert uptake data to picomoles [^{3}H]5-HT min^{-1}/1,000 sporocysts. For qPCR data, statistical comparisons were made by conducting a one-way ANOVA on the delta Ct values (target gene Ct minus 18s ribosomal Ct; Livak and Schmittgen 2001) determined for each developmental stage. For graphical representation only, relative gene levels were calculated using the $2^{(-\Delta\Delta Ct)}$ formula (Livak and Schmittgen 2001) and standard deviations were estimated. All non-repeated measures ANOVAs were carried out using the General Linear Models (GLM) procedure in Statistical Analysis Software (SAS; SAS Institute, Cary, N.C., USA) and Fischer's Protected Procedure (Zolman 1993), and when appropriate we performed multiple comparisons of treatment groups using either the LSMeans procedure or Tukey's Honest Significant difference test in SAS. We performed only preplanned comparisons to minimize Type I error (Zolman 1993), and all differences were deemed significant at $P < 0.05$.

Results

5-HT transport is saturable, dependent upon extracellular Na^+ and Cl^-, and sensitive to antidepressants

In vitro transformed sporocysts exhibited specific 5-HT uptake. A 15 min incubation in 500 nM [^{3}H]5-HT resulted in significant [^{3}H]5-HT accumulation in 5-HT-treated sporocysts (16,210 ± 1,900 cpm/1,000 sporocysts; Fig. 1A). The presence of 10 μmol l^{-1} fluoxetine in the assay medium significantly reduced serotonin uptake by over 80% to 3,090.8 ± 790 cpm/1,000 sporocysts ($P < 0.0001$). Similar results were observed when the assay was conducted at 4°C in the presence or absence of 200 μmol l^{-1} unlabeled 5-HT ($P < 0.0001$ for all treatments when compared to control).

K_m determinations were carried out on both the total uptake (total [^{3}H]5-HT accumulation) as well as on the fluoxetine-sensitive component of uptake (total uptake minus uptake in the presence of fluoxetine). K_m for total uptake was 2.4 ± 0.38 μmol l^{-1}, with a V_{max} of 0.21 ± 0.011 pmol min^{-1}/1,000 sporocysts (Fig. 1B). The fluoxetine-sensitive component had a K_m of 1.4 ± 0.33 μmol l^{-1}, and a corresponding V_{max} of 0.13 ± 0.0088 pmol min^{-1}/1,000 sporocysts. At [^{3}H]5-HT concentrations lower than or equal to 1.0 μmol l^{-1}, total uptake and fluoxetine-sensitive uptake were nearly identical (Fig. 1B). However, at higher concentrations of [^{3}H]5-HT (5.0 and 10 μmol l^{-1}), substantial binding and/or uptake was observed above the fluoxetine-sensitive component. Therefore, to look specifically at the fluoxetine-sensitive, and therefore SERT-like, component of 5-HT transport, concentrations of 0.5–1 μmol l^{-1} [^{3}H]5-HT were used in all subsequent uptake assays.

To examine the developmental regulation of *S. mansoni* SERT-like activity, 5-HT uptake capacity of freshly hatched miracidia (0 h in culture) was compared to that of in vitro transformed mother sporocysts (20 h, 3 days, and 6 days in culture). We detected very little 5-HT transport activity in miracidia (Fig. 1C). However, when members of this same batch of parasites were cultured in CBSS$^+$ and induced to transform into sporocysts, SERT-like activity became apparent after 20 h in culture (Fig. 1C). In 20-h sporocysts specific 5-HT uptake increased by over 25-fold to 6,916 ± 650 cpm/1,000 larvae when compared to miracidia (260.5 ± 240 cpm/1,000 larvae). Observations of larvae at this time point showed that 74.9 ± 4.9% were fully transformed into sporocysts, as revealed by the lack of attached ciliary plates and formation of the tegumental surface. This uptake was highly sensitive to 10 μmol l^{-1} fluoxetine, and persisted for up to 6 days in culture (Fig. 1C).

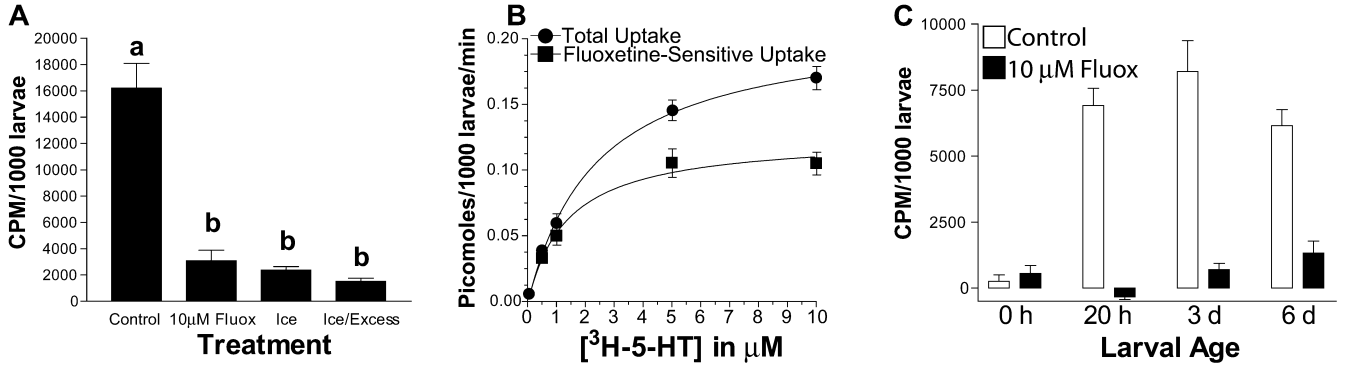

Fig. 1A–C Specificity, saturability and developmental regulation of *S. mansoni* serotonin transporter (SERT) activity. **A** Total [^{3}H]5-HT uptake in sporocysts (cpm/1,000 sporocysts) during a 15-min incubation under drug-free conditions (*Control*), in the presence of 10 μmol l^{-1} fluoxetine (*fluox*), at 4°C (*ice*), or at 4°C in the presence of 200 μmol l^{-1} unlabled 5-HT (*ice/excess*). Total uptake was significantly inhibited compared to control under all three conditions ($P < 0.0001$ for all treatments). $n = 5$/treatment. **B** Total (*solid circles*) and fluoxetine-sensitive (*solid squares*) uptake in the presence of different [^{3}H]5-HT concentrations. K_m for total uptake was 2.4 ± 0.38 μmol l^{-1}, with a V_{max} of 0.21 ± 0.011 pmol min^{-1}/1,000 sporocysts. The K_m for the fluoxetine-sensitive, and therefore SERT-like, component of uptake was 1.4 ± 0.33 μmol l^{-1}, with a V_{max} of 0.13 ± 0.0088 pmol min^{-1}/1,000 sporocysts. $n = 4$ per treatment. **C** Specific 5-HT uptake in miracidia (0 h) and sporocysts cultured in vitro (20 h, 3 days, 6 days). Fluoxetine-sensitive SERT-like activity was highly upregulated in sporocysts compared to miracidia, and persisted for up to 6 days in culture. $n = 3$/treatment. All error bars shown represent the standard error of the mean (SEM)

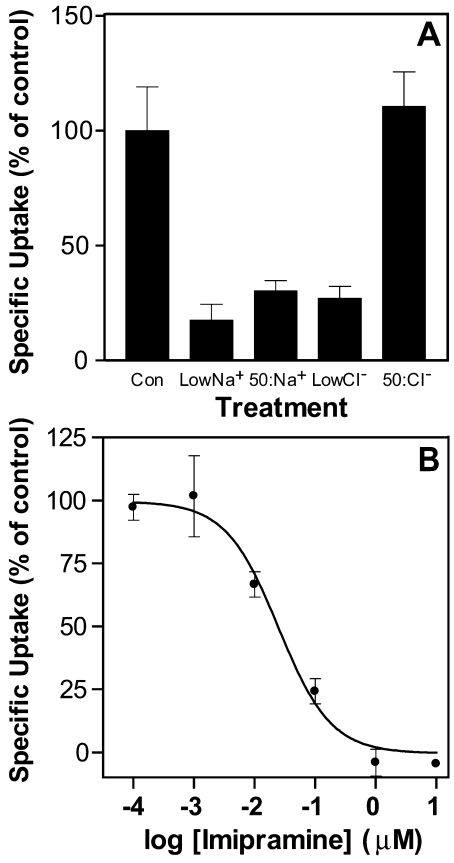

Fig. 2A, B Sporocyst SERT-like activity is dependent on extracellular Na^+ and Cl^-, and is sensitive to imipramine. **A** In CBSS containing 0.6 mmol l^{-1} Na^+ (*LowNa*$^+$), 24.8 mmol l^{-1} Na^+ (*50:Na*$^+$), or 0 mmol l^{-1} Cl^- (*LowCl*$^-$), sporocyst 5-HT uptake was significantly reduced when compared to controls ($P \leq 0.01$ for each). In contrast, CBSS with 28.6 mmol l^{-1} Cl^- (*50:Cl*$^-$) had no effect on 5-HT uptake ($P = 0.68$ when compared to control). $n = 3$/treatment. **B** Sporocysts were incubated in 500 nmol l^{-1} [^{3}H]5-HT in the presence of different concentrations of imipramine (10 μmol l^{-1} to 0.1 nmol l^{-1}), washed, and subjected to liquid scintillation counting. Imipramine had a K_i of 30.5 ± 8.8 nmol l^{-1}, and K_i values determined for other compounds are listed in Table 1. All error bars shown represent the standard error of the mean (SEM)

These data suggest that SERT-like proteins are functional only after the miracidium-to-sporocyst transition and the resultant formation of the tegumental syncytium.

To elucidate the similarities and differences between SERT proteins studied to date and sporocyst SERT-like activity, we determined functional properties of *S. mansoni* 5-HT transport in situ. Sporocyst 5-HT uptake is dependent upon extracellular sodium and chloride (Fig. 2A). In low-Na^+ saline, uptake was reduced to $15.0 \pm 3.3\%$ of control ($P = 0.0003$), and was $28.8 \pm 2.5\%$ of control ($P = 0.0010$) in intermediate (24.8 mmol l^{-1}) Na^+ saline. Chloride ion-free CBSS reduced uptake to $25.3 \pm 2.9\%$ of control ($P = 0.0007$), while intermediate chloride saline (28.6 mmol l^{-1}) had no inhibitory effect on 5-HT uptake ($P = 0.6802$ when compared to control).

Table 1 Inhibitory constants (K_i) for serotonin transport (SERT) blockers in *Schistosoma mansoni*, *Drosophila melanogaster*, and *Caenorhabditis elegans*

Drug	K_i (nmol l^{-1})		
	S. mansoni	dSERT[a]	MOD-5[b]
6-Nitroquipazine	4.61	ND	ND
Clomipramine	229	2670	ND
Desipramine	1,680	580	334
Chlorpromazine	280	ND	ND
Imipramine	30.9	1450	89
Fluoxetine	182	73	133
Methiothepin	1,610	ND	ND
5-HT	782	490	ND
GBR 12,909[c]	10,800	> 10,000	ND
Dopamine	> 100,000	> 10,000	> 10,000
Norepinephrine	> 100,000	> 10,000	ND

[a]Data from Demchyshyn et al. (1994), except clomipramine from Corey et al. (1994)
[b]Data from Ranganathan et al. (2001)
[c]Dopamine transport inhibitor
5-HT 5-hydroxytryptamine, *ND* not determined

Sporocyst SERT-like activity is sensitive to a wide range of antidepressant and psychotropic drugs. The tricyclic antidepressant imipramine inhibited 5-HT uptake with a K_i of 30.9 ± 8.84 nmol l^{-1}, and at high concentrations (1 and 10 μmol l^{-1}) uptake was completely blocked (Fig. 2B). Table 1 lists the calculated K_i values for the compounds tested. The compound 6-nitroquipazine was the most potent inhibitor of sporocyst 5-HT uptake, with a K_i of 4.61 ± 2.7 nmol l^{-1}. Overall, there is agreement between the K_i values calculated for sporocyst SERT-like activity in situ and those determined for SERT genes cloned and characterized from *Drosophila melanogaster* (dSERT; Corey et al. 1994; Demchyshyn et al. 1994) and *Caenorhabditis elegans* (MOD-5; Ranganathan et al. 2001). Furthermore, we also found that the general 5-HT receptor antagonist methiothepin was effective at inhibiting [^{3}H]5-HT uptake with a K_i of 1.6 μmol l^{-1}. In contrast, GBR 12,909, a dopamine transport inhibitor, as well as dopamine and norepinephrine, did not significantly inhibit 5-HT uptake in sporocysts at the concentrations tested (10 μmol l^{-1}), confirming the specificity of the sporocyst transport system for 5-HT alone.

In situ localization of transported 5-HT

When sporocysts were incubated for 15 min in 1 μmol l^{-1} [^{3}H]5-HT, sectioned, and processed for autoradiography, we observed an increased density of exposed silver grains directly associated with sporocysts in comparison to background silver grain levels (Fig. 3A). While in certain cases the grains were evenly distributed throughout the interior of sporocysts (denoted by asterisks in Fig. 3E), in most cases the signal was localized primarily in discrete areas within the larvae (see arrows, Fig. 3C). In all sections examined, [^{3}H]5-HT-derived silver grains were never

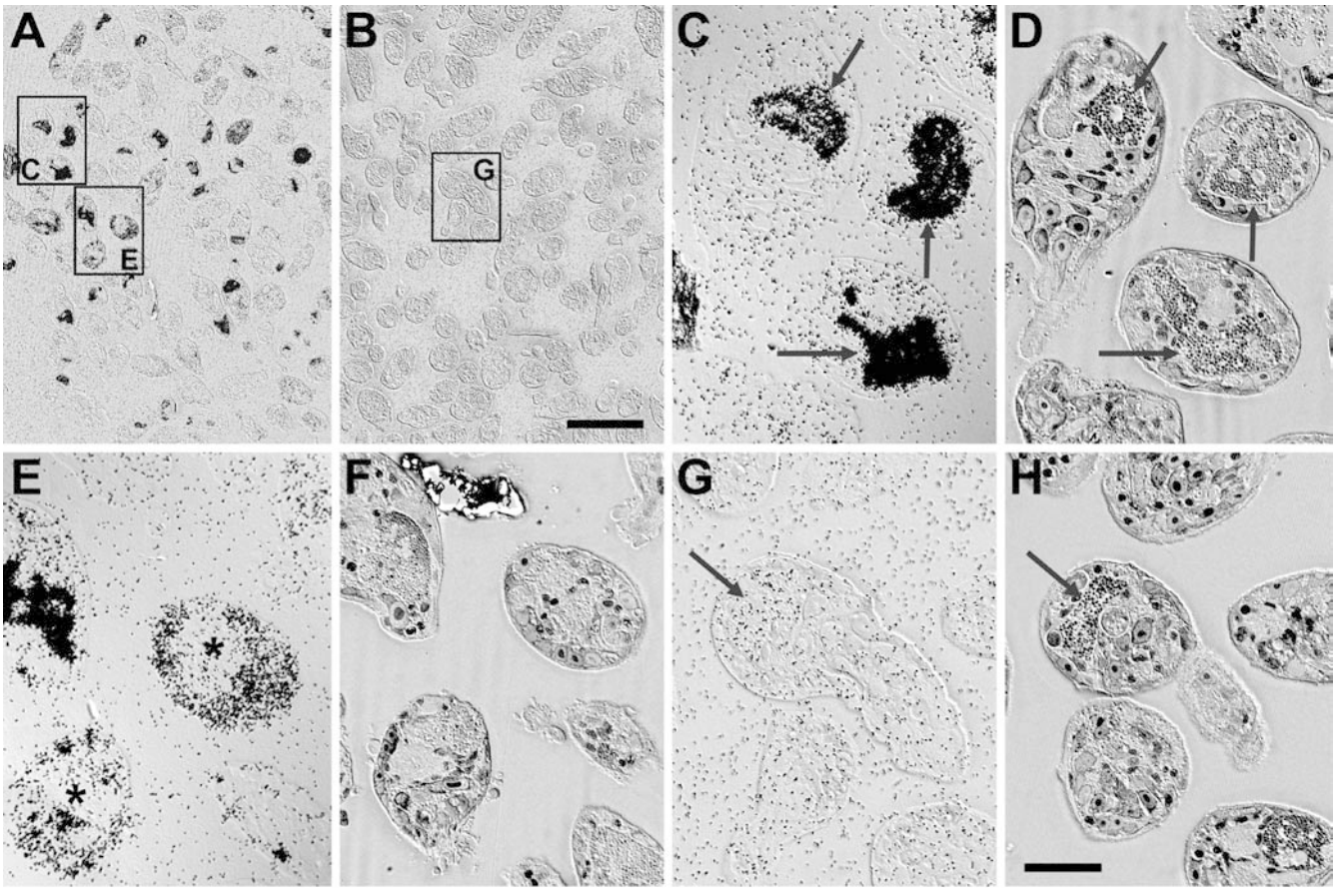

Fig. 3 Localization of transported [^{3}H]5-HT in *S. mansoni* sporocysts using autoradiography. *Panel A* Low magnification differential interference contrast (DIC) light micrograph of sporocysts treated with [^{3}H]5-HT alone. The emulsion above sporocysts contains a high concentration of exposed silver grains in comparison to background. The areas inside the squares are magnified on *panels C and E*. *Panel B* Low magnification DIC image of sporocysts treated with [^{3}H]5-HT in the presence of 10 μmol l^{-1} fluoxetine. No signal above background is observed. The area inside the square is magnified in *panel G*. *Panels C and D* DIC images of sporocysts treated with [^{3}H]5-HT from serial sections exposed to emulsion (*panel C*) or stained with toluidine blue (*panel D*). In most sporocysts signal was restricted to the apical gland (*arrows*). *Panels E and F* DIC images of sporocysts treated with [^{3}H]5-HT from serial sections exposed to emulsion (*panel E*) or stained with toluidine blue (*panel F*). On occasion signal could be found evenly dispersed throughout the sporocysts (*asterisk*). *Panels G and H* DIC images of sporocysts treated with [^{3}H]5-HT in the presence of 10 μmol l^{-1} fluoxetine from serial sections exposed to emulsion (*panel G*) or stained with toluidine blue (*panel H*). No labeling above background is observed, including in the apical gland (*arrow*), confirming that the majority of the radioactive signal in sporocysts treated with [^{3}H]5-HT was due to SERT-like activity. Scale bars: *panels A, B* 100 μm; *panels C–H* 20 μM

observed in the tegument or tegumental-associated structures. Serial histological sections counterstained with toluidine blue O and visualized with DIC optics (Fig. 3D, F, H) revealed that the major source of the highly localized radioactivity was a sporocyst secretory cell found in close proximity, and anterior, to the neural mass, and filled with large secretory vesicles (Figs. 3D and 4). Based on detailed descriptions of gland cells by Pan (Pan 1980, 1996) and our electron microscopic observations (Fig. 4), we have concluded that apical gland cells serve to concentrate exogenously acquired 5-HT. This staining was specific and the result of SERT-like activity, since sporocysts treated with [^{3}H]5-HT in the presence of 10 μmol l^{-1} fluoxetine exhibited very little staining above background in the apical gland cell and other parts of the sporocyst (see arrow, Fig. 3B, G and H).

Tryptophan hydroxylase transcripts are down-regulated after miracidium-to-sporocyst transformation

Using real-time quantitative PCR, we examined transcript levels for enzymes involved in monoamine biosynthesis in freshly hatched miracidia, 3–4-day-old in vitro-derived sporocysts, and adult stages of *S. mansoni*. As shown in Fig. 5, sporocysts had significantly lower steady-state levels of tryptophan hydroxylase (TPH) transcript when compared to freshly-hatched miracidia ($8.6 \pm 8.5\%$ of miracidia level; $P = 0.0072$), while levels of transcript for the glycolytic enzyme GAPDH were similar between miracidia and sporocysts ($P = 0.92$; Fig. 5). Adults also had reduced levels of TPH transcript, but this reduction was not statistically significant ($P = 0.060$ when compared to miracidia TPH levels). Tyrosine hydroxylase (TH) levels were significantly reduced in adults ($11.1 \pm 3.4\%$ of miracidia level; $P = 0.016$), and also

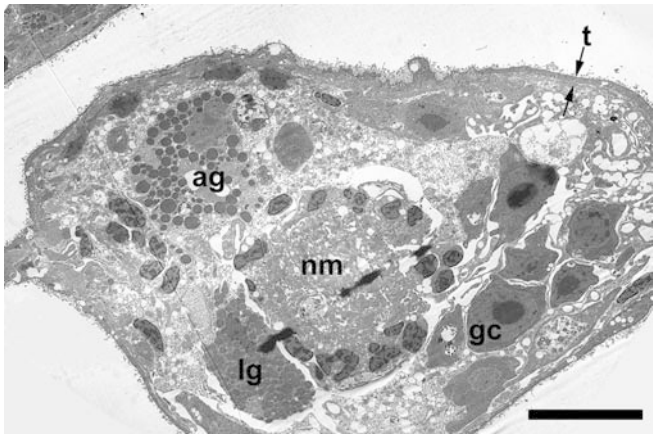

Fig. 4 Electron micrograph of an *S. mansoni* sporocyst showing the apical (*ag*) and lateral (*lg*) gland cells. Based on the size and distribution of the vesicles, we were able to determine that in cases where the [^{3}H]5-HT was highly localized (Fig. 3), it was present in the apical gland (*ag*). Other structures are denoted as follows: *t* tegument; *gc* germ cells; *nm* neural mass. Scale bar: 10 μm

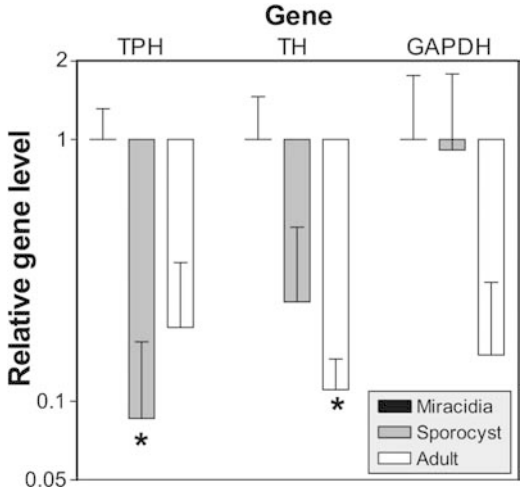

Fig. 5 Transcript levels for tryptophan hydroxylase (*TPH*), tyrosine hydroxylase (*TH*) and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) in miracidia, sporocysts, and adults of *S. mansoni*. TPH levels, but not GAPDH levels, were significantly lower in sporocysts when compared to miracidia ($P = 0.0072$), while TH levels were significantly reduced in adults ($P = 0.016$). While not statistically significant, TH levels were also reduced in sporocysts when compared to miracidia ($P = 0.07$), and GAPDH transcript levels were reduced in adults ($P = 0.08$). $n = 5$ for miracidia, 4 for sporocysts, and 3 for adults. $^*P < 0.05$ as determined by ANOVA and multiple means comparisons on the ΔCt values. Error bars shown represent the standard error of the mean (SEM)

reduced in sporocysts, although this difference was not statistically significant ($24.0 \pm 22\%$ of miracidia level; $P = 0.067$).

Discussion

To date, cDNAs corresponding to SERTs have been cloned and characterized from several mammalian species, as well as *Drosophila melanogaster* (Corey et al. 1994; Demchyshyn et al. 1994) and *Caenorhabditis elegans* (Ranganathan et al. 2001), suggesting that these proteins have been conserved throughout evolution. Importantly, these "divergent" SERT paralogues share functional characteristics with those transporters described in mammals, in that they utilize ion gradients to generate energy for substrate translocation (Demchyshyn et al. 1994; Ranganathan et al. 2001), and are blocked by the same pharmacological agents. They are typically expressed in neuronal tissues (Demchyshyn et al. 1994; Ranganathan et al. 2001), where they function to terminate serotonergic neurotransmission by reuptake of synaptically released 5-HT. When we assayed specific SERT-like activity in larval stages of *S. mansoni* in vitro, it was clear that *S. mansoni* sporocysts are capable of high-affinity 5-HT transport. The properties of the high-affinity component of the uptake system described here are similar to that reported for schistosomules (Catto and Ottesen 1979) and adult worms (Wood and Mansour 1986) of *S. mansoni*, although the work here provides a much more detailed functional and autoradiographical analysis of *S. mansoni* SERT-like activity.

Functional and pharmacological characteristics of *S. mansoni* SERT-like activity in situ are consistent with SERTs characterized from other organisms

Importantly, sporocyst SERT activity showed remarkable similarity to SERT proteins from other organisms studied in heterologous expression systems. Uptake was highly dependent upon extracellular Na^+ in the assay medium, as demonstrated by a significant inhibition of uptake even when the Na^+ concentration was only reduced to half that of control (24.8 mmol l^{-1}). In contrast, uptake was not as sensitive to the presence of Cl^-. While saline completely lacking in Cl^- significantly inhibited 5-HT uptake, Cl^- at half of the normal concentration had no significant inhibitory effect. This is similar to the SERT cloned from *D. melanogaster* (dSERT), which appeared to be Cl^- facilitated rather than Cl^- dependent (Demchyshyn et al. 1994). Sporocyst 5-HT uptake was also sensitive to a number of SERT inhibitors, and in certain cases the drugs tested had affinities similar to those reported for transporters from other organisms (see Table 1). For example, *S. mansoni* K_i values for fluoxetine and desipramine were within an order of magnitude of those determined for *Drosophila* (dSERT) and *C. elegans* (MOD-5). Moreover, dopamine and the dopamine uptake inhibitor GBR 12,909 were ineffective at inhibiting sporocyst 5-HT uptake, suggesting a substrate specificity consistent with other SERTs characterized to date. Such high similarity between K_i values is somewhat remarkable given that our experiments with *S. mansoni* were carried out on whole organisms, while those for both *Drosophila* and *C. elegans* were carried out in

heterologous expression systems. However, in certain cases sporocyst 5-HT uptake activity demonstrated possibly novel pharmacological properties. The tricyclic antidepressants clomipramine and imipramine were both at least ten times more effective at inhibiting sporocyst 5-HT uptake in comparison to dSERT (Table 1). In addition, the non-selective 5-HT receptor antagonist methiothepin blocked sporocyst 5-HT uptake with a K_i of 1.6 μmol l^{-1}, even though it is not considered to be a SERT inhibitor (Granas and Larhammar 1999), and this sensitivity to methiothepin may be a unique property of *S. mansoni* 5-HT transport. Methiothepin induces 5-HT release from mammalian neurons (Jacoby et al. 1975; Pettibone and Pflueger 1984), and this is thought to occur via inhibition of serotonin autoreceptors (Pettibone and Pflueger 1984). While we have not encountered reports in the literature in which the effect of methiothepin on mammalian SERTs has been determined, it is possible that the SERT-blocking activity of methiothepin may be at least partially responsible for increased extracellular levels of 5-HT in the mammalian CNS after methiothepin administration (Jacoby et al. 1975; Pettibone and Pflueger 1984). However, experiments examining the direct effect of methiothepin on 5-HT uptake in other organisms would be necessary to confirm this.

S. mansoni serotonin transporters are likely to be surface exposed

An important difference between sporocyst SERT-like activity and that described in other organisms is its likely site of expression. Sporocysts are multicellular acoelomates lacking a functional gut, and their sole interface with the host environment is the tegument, a syncytial structure unique to the parasitic platyhelminths. In sporocysts, this organ is bounded by a single lipid bilayer that covers the entire surface of the worm, and functional proteins are known to be expressed in this membrane (Zhong et al. 1995). Several lines of evidence presented here suggest that a likely location for sporocyst SERT-like molecules is this tegumental surface: (1) SERT-like activity is not detected in *S. mansoni* miracidia, a free-swimming life cycle stage that has yet to form its tegument; (2) SERT-like activity can be measured directly in sporocysts without prior manipulation; and (3) according to both scintillation counting and autoradiography, the presence of fluoxetine during incubation with [^{3}H]5-HT results in complete abrogation of sporocyst 5-HT uptake, suggesting that the fluoxetine targets are on the outer surface of the sporocyst. It is known from previous work that proteins destined for the sporocyst tegumental surface can be preformed and associated with vesicles within the miracidium, and become surface-exposed only after these vesicles fuse with the nascent tegumental membrane during the transformation process (Dunn and Yoshino 1988). It is therefore likely that the "activation" of serotonin uptake activity in sporocysts does not require the synthesis of new serotonin transporter proteins, but is rather due to the exposure of functional polypeptides to the external environment post-transformation. Regardless, the presence of a neurotransmitter transporter on the surface of a metazoan organism is an excellent example of co-evolution between host and pathogen, and suggests that intra-host stages of *S. mansoni* may depend on exogenous 5-HT for their successful development, and therefore transmission. Interestingly, when whole worms of the rat tapeworm *Hymenolepis diminuta* were exposed to to [^{3}H]5-HT in vitro, a tegument-associated 5-HT active transport system like that described here for *S. mansoni* was not detected (El-Razek and Webb 1997). It is possible that, among the parasitic flatworms, tegumental transport of host 5-HT may be unique to members of the class Trematoda (like *S. mansoni*), a fact that would have important implications in the evolutionary biology of this important group of parasites. Following exposure to exogenous [^{3}H]5-HT, radioactivity derived from the transported compound was often observed within the body of the sporocyst, and these data are consistent with the hypothesis that exogenous 5-HT is able to traverse both the outer and inner tegumental membranes of the sporocyst. Although it might be expected that we would occasionally observe the radiolabeled compound "in transit" in the tegumental cytoplasm, in all slides examined, exposed silver grains were never observed within the tegument. However, following incubation in [^{3}H]5-HT, a series of washes were performed prior to fixation, and during this time (approximately 5 min) the radiolabel may have had ample time to traverse the tegument and enter the body of the sporocyst. Moreover, any radiolabel traversing the tegument at the time of fixation would be at a much lower concentration than that observed in the body of the worm, and may not be detectable under the exposure conditions applied. Regardless, the transport of 5-HT through both tegumental membranes implies that there may be transport molecules present on the inner membrane of the tegument that mediate the flux of 5-HT into the worm's interior. Such a mechanism has been proposed for the transport of exogenous (e.g., host-derived) glucose by adult stages of *S. mansoni*, where two facilitated diffusion glucose transporters were found to be differentially expressed on the apical (outer) and basal (inner) tegumental membranes (Skelly et al. 1994; Zhong et al. 1995; Skelly and Shoemaker 1996). A similar model could be proposed to explain the transport of exogenous 5-HT through the apical and basal tegumental membranes to the inside of the sporocyst. This problem raises interesting questions as to how proteins are trafficked to the different tegumental membranes in schistosomes and other parasitic flatworms, and how these proteins are oriented or function in vivo to allow for selective and "directional" permeability to various compounds.

The sporocyst apical gland concentrates transported 5-HT

In many cases exogenously supplied radiolabel localized to the apical gland, an organ involved in producing secretions necessary for the successful penetration of miracidia into snail tissue (Yoshino et al. 1993). However, both the apical and lateral gland cells are known to persist for a number of days after miracidium-to-sporocyst transformation in vitro (Basch and DiConza 1974; Wippersteg et al. 2002) and in vivo (Pan 1980, 1996), and sporocysts are known to secrete polypeptides and other products into culture medium in vitro (Lodes and Yoshino 1989). Based on this information, it has been proposed that these gland cells may play additional roles during the intramolluscan development of *S. mansoni* sporocysts. Firstly, the possibility that the [^{3}H]5-HT has been enzymatically altered before or after its arrival in the apical gland cannot be ruled out. At least one degradative enzyme (a cysteine protease) is known to be expressed in the sporocyst lateral gland (Wippersteg et al. 2002), and enzymes capable of degrading 5-HT could be present in the apical gland. However, preliminary studies using HPLC with electrochemical detection to examine the fate of transported 5-HT (data not shown) demonstrate that there is a significant increase in sporocyst 5-HT content after a 15-min 5-HT exposure, and we detect no signs that it is enzymatically altered during this time frame (i.e., we do not detect any extra peaks in our chromatograms that could represent a 5-HT metabolite), but it is still possible that some 5-HT degradation may occur. We believe that if a certain portion of the transported 5-HT is not degraded, this compound could possibly be important for the manipulation of the snail host microenvironment and/or the regulation of the physiology of developing sporocysts. Snails infected with trematodes often exhibit aberrant metabolism and reproductive behavior (Crews and Yoshino 1991; Hoek et al. 1997; Jong-Brink et al. 1999), and the role of 5-HT in modulating the motility of *S. mansoni* sporocysts has been well-established (Bayne et al. 1994; Boyle et al. 2000). However, further experiments examining the fate of 5-HT transported by sporocysts, as well as its importance in larval physiological processes, will be necessary to determine its exact role in the intramolluscan development of *S. mansoni*.

Down-regulation of transcripts for a serotonin biosynthetic enzyme correlate with upregulation of *S. mansoni* serotonin transporter activity

Further support for the importance of exogenous 5-HT in larval physiology is provided by our observation that the expression of the rate-limiting enzyme for serotonin biosynthesis, tryptophan hydroxylase, is significantly down-regulated in sporocysts compared to free-swimming miracidia. The gene for this enzyme was cloned from adult stages of *S. mansoni* (Hamdan and Ribeiro 1999), and it was shown that its expression was down-regulated during the transition from free-living cercariae to sexually mature adult (Hamdan and Ribeiro 1999). Coupled with our similar observations that free-living intramolluscan stages (miracidia) express significantly higher levels of transcripts for this enzyme than do parasitic (sporocyst) stages, it is possible that the relative necessity for the production of endogenous 5-HT changes throughout *S. mansoni* development. This is consistent with the hypothesis that *S. mansoni* larvae switch between different metabolic programs depending on the compounds available in their environment, and also that 5-HT itself may play an important role in the intramolluscan development, and therefore transmission, of this medically important parasite.

Conclusion

Overall these data provide strong evidence for the presence of SERT-like molecules on the tegumental surface of *S. mansoni* sporocysts, and raise a number of important questions regarding the mechanisms used by this parasite to acquire and utilize host compounds, including the neurotransmitter serotonin. Clearly, addressing these questions will require the cloning and characterization of genes encoding transporter proteins, and their subsequent functional characterization (i.e., substrate specificity) and localization. To this end, using homology-based PCR we have recently cloned and sequenced an *S. mansoni* cDNA (Accession No. AY090636) that encodes a predicted polypeptide with high homology to other members of the Na^+/Cl^--dependent superfamily of neurotransmitter transporters, although neither its site of expression or substrate specificity are known. In addition, expressed sequence tag (EST) databases for *Schistosoma* spp. are growing at a relatively rapid rate, and can be routinely screened using BLAST to detect the presence of other members of this superfamily. These data will hopefully facilitate the characterization of transporter proteins from *S. mansoni*, a step which will greatly improve our understanding of how helminth parasites acquire and utilize host nutrients and signaling molecules.

Acknowledgements The authors wish to thank Laura Johnston and Kate Zachman for technical assistance, and Dr. Fred Lewis (Biomedical Research Institute, Rockville, MD, USA) and Dr. Jim Tracy (University of Wisconsin-Madison) for providing infected mice. Dr. John Svaren (University of Wisconsin-Madison) and members of his laboratory were extremely helpful in the design and analysis of qPCR experiments. This research was supported by Individual NIH predoctoral NRSA No. MH12992 to J.P.B., NIH Grant Nos. AI38263 and AI15503 to T.P.Y. and NIAID schistosome supply contract No. AI55270 to Dr. Lewis. All experimental protocols involving animals complied with the *Principles of animal care*, revised 1985 of the National Institutes of Health publication no. 86-23.

References

Basch PF (1976) Intermediate host specificity in *Schistosoma mansoni*. Exp Parasitol 39:150–169

Basch PF, DiConza JJ (1974) The miracidium-sporocyst transition in *Schistosoma mansoni*: surface changes in vitro with ultrastructural correlation. J Parasitol 60:935–941

Bayne CJ, Menino JS, Hobbs DJ, Barnes DW (1994) In vitro cultivation of cells from larval *Schistosoma mansoni*. J Parasitol 80:29–35

Boyle JP, Zaide JV, Yoshino TP (2000) *Schistosoma mansoni*: effects of serotonin and serotonin receptor antagonists on motility and length of primary sporocysts in vitro. Exp Parasitol 94:217–226

Camacho M, Agnew A (1995) *Schistosoma*: rate of glucose import is altered by acetylcholine interaction with tegumental acetylcholine receptors and acetylcholinesterase. Exp Parasitol 81:584–591

Catto BA, Ottesen EA (1979) Serotonin uptake in schistosomules of *Schistosoma mansoni*. Comp Biochem Physiol C 63C:235–242

Charrier-Ferrara S, Caillol D, Goudot-Crozel V (1992) Complete sequence of the *Schistosoma mansoni* glyceraldehyde-3-phosphate dehydrogenase gene encoding a major surface antigen. Mol Biochem Parasitol 56:339–343

Cheng Y, Prusoff WH (1973) Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent reduction (IC_{50}) of an enzymatic reaction. Biochem Pharmacol 22:3099–3108

Chernin E (1963) Observations on hearts explanted in vitro from the snail *Australorbis glabratus*. J Parasitol 49:353–364

Corey JL, Quick MW, Davidson N, Lester HA, Guastella J (1994) A cocaine-sensitive *Drosophila* serotonin transporter: cloning, expression, and electrophysiological characterization. Proc Natl Acad Sci USA 91:1188–1192

Crews AE, Yoshino TP (1989) *Schistosoma mansoni*: effect of infection on reproduction and gonadal growth in *Biomphalaria glabrata*. Exp Parasitol 68:326–334

Crews AE, Yoshino TP (1991) *Schistosoma mansoni*: influence of infection on levels of translatable mRNA and on polypeptide synthesis in the ovotestis and albumen gland of *Biomphalaria glabrata*. Exp Parasitol 72:368–380

Davies SJ, Shoemaker CB, Pearce EJ (1998) A divergent member of the transforming growth factor beta receptor family from *Schistosoma mansoni* is expressed on the parasite surface membrane. J Biol Chem 273:11234–11240

Day TA, Bennett JL, Pax RA (1994) Serotonin and its requirement for maintenance of contractility in muscle fibres isolated from *Schistosoma mansoni*. Parasitology 108:425–432

Demchyshyn LL, Pristupa ZB, Sugamori KS, Barker EL, Blakely RD, Wolfgang WJ, Forte MA, Niznik HB (1994) Cloning, expression, and localization of a chloride-facilitated, cocaine-sensitive serotonin transporter from *Drosophila melanogaster*. Proc Natl Acad Sci USA 91:5158–5162

Dunn TS, Yoshino TP (1988) *Schistosoma mansoni*: origin and expression of a tegumental surface antigen on the miracidium and primary sporocyst. Exp Parasitol 67:167–181

El-Razek MA, Webb RA (1997) Transport of exogenous 5-hydroxytryptamine across the outer plasma membrane of the syncytial tegument of *Hymenolepis diminuta* is by simple diffusion. Can J Zool 75:1605–1617

Granas C, Larhammar D (1999) Identification of an amino acid residue important for binding of methiothepin and sumatriptan to the human 5-HT(1B) receptor. Eur J Pharmacol 380:171–181

Hamdan FF, Ribeiro P (1999) Characterization of a stable form of tryptophan hydroxylase from the human parasite *Schistosoma mansoni*. J Biol Chem 274:21746–21754

Hillyer JF, Albrecht RM (1998) Correlative instrumental neutron activation analysis, light microscopy, transmission electron microscopy, and X-ray microanalysis for qualitative and quantitative detection of colloidal gold spheres in biological specimens. Microsc Microanal 4:481–490

Hoek RM, Kesteren RE van, Smit AB, Jong-Brink M, Geraerts WP (1997) Altered gene expression in the host brain caused by a trematode parasite: neuropeptide genes are preferentially affected during parasitosis. Proc Natl Acad Sci USA 94:14072–14076

Ivanchenko MG, Lerner JP, McCormick RS, Toumadje A, Allen B, Fischer K, Hedstrom O, Helmrich A, Barnes DW, Bayne CJ (1999) Continuous in vitro propagation and differentiation of cultures of the intramolluscan stages of the human parasite *Schistosoma mansoni*. Proc Natl Acad Sci USA 96:4965–4970

Jacoby JH, Shabshelowitz H, Fernstrom JD, Wurtman RJ (1975) The mechanism by which methiothepin, a putative serotonin receptor antagonist, increases brain 5-hydroxyindole levels. J Pharmacol Exp Ther 195:257–264

Jong-Brink M, Reid CN, Tensen CP, Ter Maat A (1999) Parasites flicking the NPY gene on the host's switchboard: why NPY? FASEB J 13:1972–1984

Kalbe M, Haberl B, Haas W (1996) *Schistosoma mansoni* miracidial host-finding: species specificity of an Egyptian strain. Parasitol Res 82:8–13

Lewis FA, Stirewalt MA, Souza CP, Gazzinelli G (1986) Large-scale laboratory maintenance of *Schistosoma mansoni*, with observations on three schistosome/snail host combinations. J Parasitol 72:813–829

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{(-\Delta\Delta Ct)}$ method. Methods 25:402–408

Lodes MJ, Yoshino TP (1989) Characterization of excretory-secretory proteins synthesized in vitro by *Schistosoma mansoni* primary sporocysts. J Parasitol 75:853–862

Manger P, Li J, Christensen BM, Yoshino TP (1996) Biogenic monoamines in the freshwater snail, *Biomphalaria glabrata*: influence of infection by the human blood fluke, *Schistosoma mansoni*. Comp Biochem Physiol A 114:227–234

Mansour TE (1984) Serotonin receptors in parasitic worms. Adv Parasitol 23:1–36

Morrison TB, Weis JJ, Wittwer CT (1998) Quantification of low-copy transcripts by continuous SYBR Green I monitoring during amplification. Biotechniques 24:954–958, 960, 962

Nolan LE, Carriker JP (1946) Observations on the biology of the snail *Lymnaea stagnalis appressa* during twenty years of laboratory culture. Am Midl Nat 36:467–493

Pan SC (1980) The fine structure of the miracidium of *Schistosoma mansoni*. J Invertebr Pathol 36:307–372

Pan SC (1996) *Schistosoma mansoni*: the ultrastructure of larval morphogenesis in *Biomphalaria glabrata* and of associated host-parasite interactions. Jpn J Med Sci Biol 49:129–149

Pappas PW, Read CP (1975) Membrane transport in helminth parasites: a review. Exp Parasitol 37:469–530

Pax RA, Day TA, Miller CL, Bennett JL (1996) Neuromuscular physiology and pharmacology of parasitic flatworms. Parasitology 113 [Suppl] S83–S96

Pettibone DJ, Pflueger AB (1984) Effects of methiothepin and lysergic acid diethylamide on serotonin release in vitro and serotonin synthesis in vivo: possible relation to serotonin autoreceptor function. J Neurochem 43:83–90

Rahman MS, Mettrick DF, Podesta RB (1985) *Schistosoma mansoni*: effects of in vitro serotonin (5-HT) on aerobic and anaerobic carbohydrate metabolism. Exp Parasitol 60:10–17

Ranganathan R, Sawin ER, Trent C, Horvitz HR (2001) Mutations in the *Caenorhabditis elegans* serotonin reuptake transporter MOD-5 reveal serotonin-dependent and -independent activities of fluoxetine. J Neurosci 21:5871–5884

Skelly PJ, Shoemaker CB (1996) Rapid appearance and asymmetric distribution of glucose transporter SGTP4 at the apical surface of intramammalian-stage *Schistosoma mansoni*. Proc Natl Acad Sci USA 93:3642–3646

Skelly PJ, Kim JW, Cunningham J, Shoemaker CB (1994) Cloning, characterization, and functional expression of cDNAs encoding glucose transporter proteins from the human parasite *Schistosoma mansoni*. J Biol Chem 269:4247–4253

Skelly PJ, Pfeiffer R, Verrey F, Shoemaker CB (1999) SPRM1lc, a heterodimeric amino acid permease light chain of the human parasitic platyhelminth, *Schistosoma mansoni*. Parasitology 119:569–576

Thompson DP, Klein RD, Geary TG (1996) Prospects for rational approaches to anthelmintic discovery. Parasitology 113 [Suppl] S217–S238

Wippersteg V, Kapp K, Kunz W, Grevelding C (2002) Characterisation of the cysteine protease ER60 in transgenic *Schistosoma mansoni* larvae. Int J Parasitol 32:1219

Wood PJ, Mansour TE (1986) *Schistosoma mansoni*: serotonin uptake and its drug inhibition. Exp Parasitol 62:114–119

World Health Organization (1998) Report of the WHO informal consultation on schistosomiasis control. WHO, Geneva, pp 1–45

World Health Organization (2000) Report of the WHO informal consultation on schistosomiasis in low transmission areas: control strategies and criteria for elimination. WHO, Geneva, pp 1–51

Yoshino TP, Laursen JR (1995) Production of *Schistosoma mansoni* daughter sporocysts from mother sporocysts maintained in synxenic culture with *Biomphalaria glabrata* embryonic (Bge) cells. J Parasitol 81:714–722

Yoshino TP, Lodes MJ, Rege AA, Chappell CL (1993) Proteinase activity in miracidia, transformation excretory-secretory products, and primary sporocysts of *Schistosoma mansoni*. J Parasitol 79:23–31

Yoshino TP, Boyle JP, Humphries JE (2001) Receptor-ligand interactions and cellular signalling at the host-parasite interface. Parasitology 123 [Suppl] S143–S157

Zhong C, Skelly PJ, Leaffer D, Cohn RG, Caulfield JP, Shoemaker CB (1995) Immunolocalization of a *Schistosoma mansoni* facilitated diffusion glucose transporter to the basal, but not the apical, membranes of the surface syncytium. Parasitology 110:383–394

Zolman JF (1993) Biostatistics: experimental design and statistical inference. Oxford University Press, New York