THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 190, No. 2

Printed in U.S.A.

TRYPTAMINERGIC AND DOPAMINERGIC RESPONSES OF *SCHISTOSOMA MANSONI*[1]

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Accepted for publication March 26, 1974

ABSTRACT

TOMOSKY, THOMAS KEITH, JAMES L. BENNETT AND ERNEST BUEDING: Tryptaminergic and dopaminergic responses of *Schistosoma mansoni*. J. Pharmacol. Exp. Ther. **190:** 260–271, 1974.

Pharmacological evidence is reported indicating that 5-hydroxytryptamine is an excitatory neurotransmitter in *Schistosoma mansoni*. The stimulation of the motor activity of adult schistosomes brought about by cholinergic blockade is abolished 1) by bromlysergic acid diethylamide and other 5-hydroxytryptamine antagonists and 2) by prior 5-hydroxytryptamine depletion brought about by exposure of the parasites to chlorimipramine and reserpine. Whereas catecholamines have no effect on the motor activity of schistosomes, low concentrations of dopamine, norepinephrine and epinephrine (but not isoproterenol) produce a lengthening response of the worm which is ascribed to a relaxation of the longitudinal musculature. The same response was induced by apomorphine, considered to be a specific dopamine receptor agonist, but not by clonidine, a specific norepinephrine receptor agonist. Apomorphine and dopamine were more potent in this respect than norepinephrine and epinephrine. These effects are blocked more effectively by dopamine-blocking agents than by *alpha* and *beta* adrenergic blockers. Male worms from single-sex infections differed in their lengthening response from males paired with females.

The presence of two biogenic amines, 5-hydroxytryptamine (5-HT) and norepinephrine (NE), in *Schistosoma mansoni* and their localization within the nervous system of the parasite have been reported (Bennett *et al.*, 1969; Bennett and Bueding, 1971). Furthermore, it has been found that schistosomes have a high affinity uptake mechanism for 5-HT, providing for effective means 1) to supply the worms with a putative neurotransmitter which they may be unable to synthesize, and 2) to terminate its excitatory action (Bennett and Bueding, 1973). These observations brought up the question whether 5-HT and NE function as neurotransmitters in *S. mansoni*.

A criterion for a neurotransmitter is the response of the postsynaptic membrane, or effector

Received for publication August 3, 1973.
[1] This investigation was supported by National Institutes of Health Training Grant AI-149, National Institutes of Health Research Grants AI-03515 and AI-08022 and the Rockefeller Foundation Grant GA BMS 7225.
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cell, to chemical agents which mimic or block its effects. The effector cell should respond in a similar manner to exogenous and to neuronally released endogenous transmitters, as well as to specific blocking agents. Use of this approach indicated a role for acetylcholine as an inhibitory neuromuscular transmitter in *S. mansoni* (Barker *et al.*, 1966). For example, exposure of the worms to cholinomimetic agents or to acetylcholinesterase inhibitors resulted in a reduction or a loss of their motor activity; these effects were reversed by cholinergic blocking agents, *e.g.*, atropine or mecamylamine. Furthermore, the latter compounds, in the absence of exogenous cholinomimetic agents, produced an increase in the motor activity of the worms. Therefore, blockade of the inhibitory effects of acetylcholine unmasked the presence of an endogenous excitatory substance. The marked stimulation of the motor activity of schistosomes, induced by low concentrations of 5-HT, is consistent with the possibility that this compound is an excitatory neurotransmitter in *S. mansoni*.

In this paper pharmacological evidence is reported indicating a role for 5-HT and for NE as neuromuscular transmitters in *S. mansoni*.

Methods

Adult schistosomes were obtained as in previous studies (Bueding, 1950). Immediately after their removal from the mesenteric or portal veins of the mouse host, the worms were transferred to 75% horse serum at 37°C for 15 to 30 minutes in a covered glass dish placed on a thermostatically controlled slide warmer. Thereafter, the schistosomes were transferred into the same medium with or without added drug. Two milliliters of medium were used per worm. Unless otherwise stated, following incubation at 37°C for 15 minutes, the motor activity of the schistosomes exposed to a given drug was compared with that of worms removed from the same mouse host and incubated in the same manner, but without drugs (controls), by observation under a microscope (magnification ×10).

The following symbols were used to record the motor activity of the worms:

+ (Control activity): the frequency of contractions, affecting any portion of the worm, reflected in a displacement of the worm's body or in an undulating wave, varied between 60 and 90/min among control worms removed from hosts infected by different groups of cercariae. However, these variations were far less pronounced among worms from the same mouse. In control schistosomes the frequency and amplitude of contraction were far greater in the posterior portion of the worm than in the anterior one. At this level of motor activity, the acetabulum often adhered to the surface of the dish.

++ Moderate increase in motor activity, amounting to a contraction frequency 30 to 50% greater than that of the controls.

+++ Marked increase in motor activity with frequencies of contraction exceeding the control level by 60 to 100%. At this level, the motor activity of the anterior portion was almost as prominent as that of the posterior portion; adherence to the glass surface was observed only occasionally. There were some convulsive movements and rotations around the worm's longitudinal axis.

++++ Very marked increase in motor activity with frequencies of contraction exceeding control levels by more than 100%. The convulsions and rotations were more pronounced than in +++; no adhesions of the acetabulum to the glass surface were observed, and the contractile waves in both anterior and posterior portions were not always synchronous.

± decrease in the frequency of contractions to between 10 and 70% of control levels.

− frequency of contraction less than 10% of control levels or complete paralysis.

The length of the worms was determined as follows. Paired worms were gently separated before use. The term "paired male" refers to a male found *in copula* with the female before use. "Single male" refers to a male from a single sex infection, established as described by Standen (1953). Analogous terms apply to female schistosomes. Since it has been suggested that paired male schistosomes furnish some nutritive substances to the females (Holden, 1967; Lennox and Schiller, 1972), physiological differences may exist between paired and single males. In some experiments worms were decapitated below the acetabulum by using a sharp razor blade. The worm with its incubation medium was aspirated gently into a polyethylene tube (Adams Intramedic) connected to a needle which in turn was attached to the outer part of a 0.5-ml syringe. Tubing of 0.38 mm (inside diameter) (PE 20) and a 26-gauge needle were used for male length determinations and tubing of 0.28 mm inside diameter tubing (PE 10) and a 30-gauge needle for the females. The tubing was then placed on a slide warmer next to a ruler and the length of the worm was measured at a 10-fold magnification. Worms

were observed for approximately 1.5 minutes and returned to the medium for 2 minutes. This procedure was repeated until a consistent base-line length was obtained (usually two or three measurements were required). Subsequently the worms were placed into the test medium, maintained at 37°C, and the length was measured in the same manner at predetermined intervals. The range of base-line lengths varied between 7 and 11 mm among males and between 10 and 14 mm among females. The length of individual control worms incubated for up to 30 minutes in 75% horse serum did not vary by more than ±4%.

5-HT-depleted worms were obtained by incubating schistosomes for 3 hours at 37°C in 75% horse serum containing both reserpine (1×10^{-3} M) and chlorimipramine (1×10^{-4} M). At the end of the incubation period, the 5-HT concentrations had been reduced to less than 10% of their control levels (table 1).

5-HT and NE levels, the fluorescence characteristics of 5-HT derivatives (at concentrations of 1 μg/ml) and the distribution of these amines in the nervous system of the worms were determined as described previously (Bennett and Bueding, 1971, 1973). P values were calculated by Student's *t* test.

Each drug was dissolved in an appropriate solvent [75% horse serum, water, ethanol or dimethylsulfoxide (DMSO)] and then mixed with 75% horse serum. In experiments with catecholamines or apomorphine, 25 mg of ascorbic acid per 100 ml of 75% horse serum was used in control and experimental solutions. Drug solutions were prepared immediately before use. Three types of controls for the length measurements were employed: 1) worms in 75% horse serum; 2) worms in 75% horse serum containing the same concentrations of ethanol or DMSO as that used for a given drug; the final concentrations of these two organic solvents in the incubation medium (2% or less) affected neither the length nor the motor activity of the worms; 3) appropriate corrections were made for the effects on the length of the worms by receptor blocking agents in the absence of agonist.

Results

Tryptaminergic responses. Brom-lysergic acid diethylamide (BOL) has been used extensively as a specific blocking agent of 5-HT receptors (Gaddum and Picarelli, 1957; Asher, 1971; Maddrell *et al.*, 1971; Crowder and Shankland, 1972). This compound was found to be a potent inhibitor of the excitatory effects of 5-HT on *S. mansoni* (table 2). Qualitatively, similar blocking effects were observed with another known 5-HT antagonist, 8-β-carbobenzyoxy-aminomethyl-1,6-dimethyl-10-α-ergoline (MCE) (table 2), which has been reported to block the effects of 5-HT in *Fasciola hepatica* (Beretta and Locatelli, 1968). In addition, dihydroergotamine proved to be a weak 5-HT blocking agent; similarly, it blocked the excitatory effects of cholin-

TABLE 1

Effect of amine-depleting agents on 5-HT and NE levels of schistosomes

The worms were incubated with each compound in 75% horse serum at 37°C for 3 hours.

Drug	Conc.	5-HT			NE		
		No. of Expts.	Amount[a]	% change	No. of Expts.	Amount[a]	% change
	M		*μg/g (wet wt.)*			*μg/g (wet wt.)*	
Control		13	2.79 (±0.77)		5	0.52 (±0.20)	
Reserpine	1×10^{-3}	7	1.58 (±0.29)	−42[b]	2	0.20 (±0.2)	−60[c]
Chlorimipramine	1×10^{-4}	7	0.84 (±0.31)	−70[b]	2	0.29 (±0.1)	−44[c]
Reserpine + chlorimipramine	1×10^{-3} + 1×10^{-4}	9	0.26 (±0.04)	−91[b]	4	0.10 (±0.1)	−80[b]
Protryptyline	1×10^{-4}	7	0.91 (±0.36)	−67[b]			
d-Amphetamine	1×10^{-4}	5	1.01 (±0.30)	−64[b]			
4-α-Dimethyl-*m*-tyramine	3×10^{-5}	4	1.25 (±0.40)	−55[b]			
Tryptamine	1×10^{-5}	2	1.80 (±0.4)	−35[c]			
Reserpine *in vivo*[d]		2	2.10 (±0.20)	−25[c]			

[a] Mean (±S.D.).

[b] $P < .01$.

[c] Not significant.

[d] Twenty hours after the i.p. administration of 3 mg/kg to the mouse host.

TABLE 2

Effect of 5-HT-blocking agents on motor stimulation of S. mansoni *induced by 5-HT, atropine and mecamylamine*

Prior to the addition of the 5-HT-blocking agents the worms were incubated for 15 minutes at 37°C (control period) with the stimulatory compounds. At least three worms were used to determine each motor response.

Stimulatory Compound	Conc.	Control Period	BOL			MCE	LSD		Dihydroergotamine	
			1×10^{-6} M	1×10^{-5} M	1×10^{-4} M	1×10^{-4} M	1×10^{-5} M	1×10^{-4} M	1×10^{-5} M	5×10^{-5} M
	M									
Control		+	±	−	−	−	+	+	++	+
5-HT	1×10^{-6}	+++	+	+	+	+	++	++	+	+
	1×10^{-5}	++++	++	+	+	+	++++	++++	++	++
	1×10^{-4}	++++	+++	+	+	+	++++	++++	++++	+++
Atropine	1×10^{-4}	+++	+	±	−	±	+++	+++	++	+
Mecamylamine	1×10^{-4}	+++	+	±	−	±	+++	+++	++	+

ergic blockade (table 2). In contrast to BOL, lysergic acid diethylamide (LSD) had no 5-HT blocking activity (table 2).

Stimulation of the motor activity of schistosomes by two cholinergic blocking agents, mecamylamine and atropine, was blocked by BOL, MCE and dihydroergotamine (table 2). Furthermore, mecamylamine or atropine failed to increase the motor activity of worms whose 5-HT stores had been depleted by prior exposure to chlorimipramine and reserpine (table 3). These 5-HT-depleted worms responded to exogenous 5-HT in the same manner as nondepleted worms (table 3).

Incubation of the worms with a number of compounds known to inhibit the reuptake or to induce the release of biogenic amines by mammalian nerve terminals produced a reduction in the 5-HT levels of the worms (table 1). This was associated with an increase in their motor activity (table 3). The latter effect was blocked by BOL and MCE and was not observed in 5-HT-depleted worms (table 3). Therefore, it appears that these compounds produce motor stimulation indirectly, *i.e.*, by a displacement of 5-HT from its presynaptic stores, resulting in an increased concentration of the amine at the postsynaptic receptor site.

Nimmo-Smith and Raison (1968) have reported that tryptamine stimulates motor activity of schistosomes. Tryptamine increased the motor activity even in 5-HT-depleted worms (table 3). The stimulatory properties of tryptamine and some of its analogs on *S. mansoni*, together with their fluorescence characteristics, are recorded in table 4. Some structure-activity relationships derived from these observations are as follows. 1) 5-Hydroxylation of the benzene ring is not required for activity, because tryptamine is as potent as 5-HT. 2) Hydroxylation in positions 4 and 6 reduces, but does not abolish, activity. 3) Hydroxylation in both positions 5 and

TABLE 3

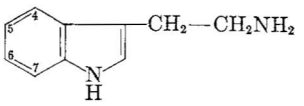
Effect of 5-HT depletion on motor responses of schistosomes

BOL or MCE was added to nondepleted worms after a preincubation (control) period of 15 minutes (37°C) with the drug recorded in the first horizontal column. At least three worms were used to determine each response. There was no variability in the response to each drug or a combination thereof.

Drug	Conc.	Nondepleted Worms			5-HT-Depleted Worms
		Control	BOL, 1×10^{-5} M	MCE, 1×10^{-4} M	
	M				
Atropine	1×10^{-4}	+++	±	±	±
Mecamylamine	1×10^{-4}	+++	±	±	±
Reserpine	1×10^{-3}	+++	+	+	±
Chlorimipramine	1×10^{-4}	++++	+	+	±
Reserpine + chlorimipramine	1×10^{-3} 1×10^{-4}	++++	+	+	±
Protryptyline	1×10^{-4}	+++	+	+	±
d-Amphetamine	1×10^{-4}	+++	+	+	±
4-α-Dimethyl-*m*-tyramine	3×10^{-5}	+++	+	+	±
5-HT	1×10^{-5}	++++	+	+	++++
Tryptamine	1×10^{-5}	++++	+	+	++++
N-methyl-5-HT	1×10^{-5}	++	+	+	++
N,*N*-dimethyl-5-HT	1×10^{-5}	++	+	+	++

TABLE 4

Effect on motor activity of S. mansoni *by, and fluorescence characteristics of, tryptamine derivatives*
At least three worms were used to determine each motor response.



Tryptamine

Compound	Conc. and Motor Activity			Fluorescence Intensity	
	1×10^{-3} M	1×10^{-4} M	1×10^{-5} M	295/545 mμ 3 N HCl	385/495 mμ ninhydrin
Tryptamine	++++	++++	++++	0	0
5-HT	++++	++++	++++	100	100
4-Hydroxytryptamine	+++	++	+	0	0
5-Methoxytryptamine	++++	+++	++	100	0
6-Hydroxytryptamine	++	+	+	0	1
6-Methoxytryptamine	+++	++	+	0	0
5,6-Dihydroxytryptamine	+	+	+	0	0
5-Hydroxy-6-methoxytryptamine	+++	+++	++	0	0
5-Methoxy-6-hydroxytryptamine	+++	++	+	0	0
7-Hydroxytryptamine	+++	++	+	0	0
7-Benzoxytryptamine	+++	++	+	0	0
N-Methyl-5-hydroxytryptamine	++++	+++	++	25	100
N-Dimethyl-5-hydroxytryptamine	++++	+++	++	65	0
5-Hydroxyindoleacetic acid	++	+	−	0	0

6 abolishes activity but the latter is partially restored if one of the two substituents is a methoxy, rather than a hydroxyl, group. 4) N-methylation or N-dimethylation results only in a slight reduction in potency. The product of the action of amine oxidase on 5-HT, 5-hydroxyindoleacetic acid, is inactive. This enzyme has been demonstrated in *S. mansoni* by Nimmo-Smith and Raison (1968). The stimulatory properties of some of these tryptamine analogs on *S. mansoni* together with their fluorescence characteristics recorded in table 4 are of potential usefulness for the identification of small quantities of these compounds isolated from biological materials. For example, only 5-methoxytryptamine, N-methyl-5-HT and dimethyl-5-HT (bufotenine) share with 5-HT the fluorescence spectrum induced in 3 N HCl and only 5-HT and N-methyl-5-HT yield a highly fluorescent compound when reacted with ninhydrin. The fluorescence intensity of the reaction product of 6-hydroxytryptamine with ninhydrin is only 1% of that of 5-HT. Confirming and extending observations of Snyder *et al.* (1965), it was found that, with the exception of N-methyl-5-HT, no fluorescence was detectable when the other compounds listed in table 4 were treated with ninhydrin.

Dopaminergic responses. Incubation of paired males with dopamine (DA) and NE resulted in a significant increase in the lengths of the worms (figs. 1 and 2). The maximum increase occurred after exposure for 3 minutes. Incubation with apomorphine (AP) (fig. 3) and epinephrine (E) produced the same type of response. The length of the worm was not affected by clonidine (1×10^{-7} to 1×10^{-4} M), isoproterenol (1×10^{-8} to 1×10^{-3} M) or octopamine (3×10^{-7} to 2×10^{-4} M), nor was it increased by dibutyryl cyclic 3′,5′-adenosine monophosphate (1×10^{-5} to 1×10^{-3} M). AP at a low concentration (1×10^{-6} M) was more effective than was NE in producing a lengthening response ($P < .025$); in fact, at 1×10^{-6} M this response was already more than half-maximal with AP and DA, whereas NE at the same concentration had virtually no effect. The maximal response to all three compounds was observed at concentrations varying between 1×10^{-5} and 1×10^{-4} M. When the concentrations were increased further, the lengthening response was reduced with AP

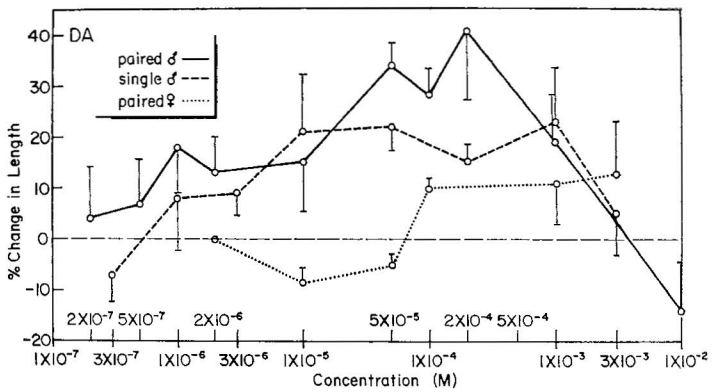


FIG. 1. Lengthening response of paired and single male and of paired female schistosomes to DA. The differences in the response between paired and single males were significant at concentrations of 5×10^{-5} M ($P < .01$) and of 2×10^{-4} M ($P < .01$). An average number of six worms was used for each point in figures 1 to 3. Vertical bars represent S.E.M.

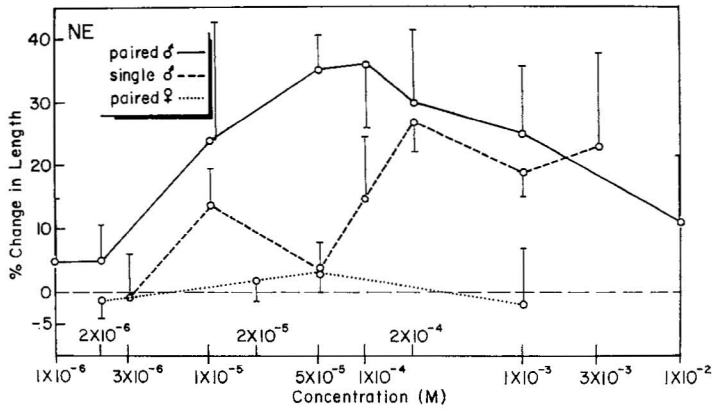


FIG. 2. Lengthening response of paired and single male and of paired female schistosomes to NE. The differences in the response between paired and single males were significant at concentrations of 5×10^{-5} M ($P < .001$) and of 1×10^{-4} M ($P < .01$). At 3×10^{-6} M the response of single male schistosomes to NE was significantly lower than to AP ($P < .025$) and to DA ($P < .05$). Vertical bars represent S.E.M.

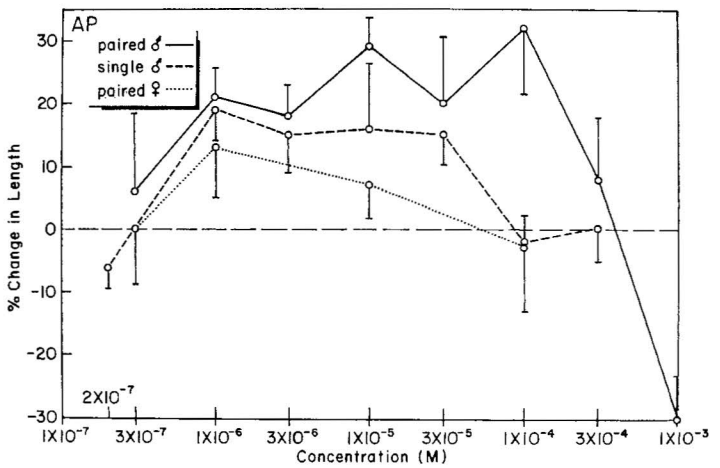


FIG. 3. Lengthening response of paired and single male and of paired female schistosomes to AP. The differences in the response between paired and single males were significant at concentrations of 1×10^{-5} M ($P < .005$) and of 1×10^{-4} M ($P < .005$). Vertical bars represent S.E.M.

and DA and a shortening rather than a lengthening response occurred at 1×10^{-3} and 1×10^{-2} M, respectively. The dose-response relationship to E was very similar to that to NE.

The lengthening responses of single males differed in several respects from those of paired males. With AP (fig. 3) the maximal response was obtained at 1×10^{-6} M and, in contrast to paired males, failed to increase at higher concentrations. The maximal response to DA was observed at 1×10^{-5} M (fig. 1). There was a biphasic response of the single males to NE (fig. 2) because the lengthening response was inhibited almost completely at 5×10^{-5} M, but then began to rise again at twice this concentration, reaching a maximum at 2×10^{-4} M. As was the case with paired males, isoproterenol (1×10^{-5} to 1×10^{-3} M) had no effect on single males. Decapitated paired and single males responded in the same manner to DA and NE as intact worms indicating that these effects are not centrally mediated.

No differences could be found between paired and single males in the distribution and fluorescence intensity of catecholamines in the neuronal structures as determined histochemically.

Because of differences between paired and single males in response to catecholamines and AP, the susceptibility of these two groups of worms to cholinergic agents and 5-HT was determined. The minimal concentrations of some of these compounds required to produce a motor response of paired males were lower than for single males (table 5).

Generally, the lengthening response of paired females was far less than that of paired and single males. A response to DA (fig. 1) was observed only at high concentrations (1×10^{-4} M and above). Conversely, the response to AP (fig. 3) was limited to lower concentrations, with a maximum of 13% lengthening at a concentration of 1×10^{-6} M. With NE (fig. 2) no significant effect was observed with any concentrations of NE used in these experiments (2×10^{-6} to 1×10^{-3} M). In contrast to paired and single males, the motor activity of paired females was markedly depressed by NE concentrations of 5×10^{-5} M and above, and by DA at concentrations varying between 1×10^{-5} and 1×10^{-4} M. There was no change in motor activity at DA concentrations of 1×10^{-3} and 3×10^{-3} M. AP did not affect the motor activity of paired females at concentrations between 3×10^{-7} and 1×10^{-4} M.

TABLE 5

Minimal effective concentrations of cholinergic agents and 5-HT for paired and single male schistosomes

At least three worms were used for each determination. There was no variability in the minimal response to each drug.

Agent	Paired Males	Single Males
Metrifonate[a]	1×10^{-4}	1×10^{-4}
Dichlorvos[b]	3×10^{-5}	3×10^{-5}
Neostigmine[a]	5×10^{-5}	1×10^{-3}
Arecoline[b]	1×10^{-6}	1×10^{-6}
Carbachol[b]	1×10^{-5}	1×10^{-3}
5-HT[c]	1×10^{-6}	1.5×10^{-5}

[a] Minimal concentration producing paralysis in 20 minutes.

[b] Minimal concentration producing paralysis in 10 minutes.

[c] Minimal concentration producing +++ activity in 2 minutes.

The immediate response to AP, NE, DA or E was blocked either only partially or not at all by most *alpha* and all *beta* adrenergic blocking agents (table 6). On prolonged incubation (30 minutes) with equimolar concentrations of NE and phenoxybenzamine, complete blockade was observed. On the other hand, phenoxybenzamine failed to produce complete blockade when DA instead of NE was the agonist. The blocking activity of a combination of an *alpha* and a *beta* blocker, phenoxybenzamine and propranolol, was less effective than that of each compound alone. Similar observations were reported using rabbit aortic muscle (Kohli and Ling, 1967). Preincubation for periods of 3 to 40 minutes with either single *alpha* or *beta* adrenergic blocking agents or both phenoxybenzamine and propranolol failed to produce complete blockade.

Four other types of blocking agents inhibited more effectively the lengthening response to the three catecholamines and to AP (table 7). 1) In concentrations equal to those of NE, dihydroergotamine produced complete blockade within 3 minutes, while dihydroergocristine produced 71% inhibition within 3 minutes, increasing to 96% within 15 minutes. 2) Spiroperidol, which

TABLE 6

Effect of alpha and beta adrenergic blocking agents on the NE- and DA-induced lengthening response of paired male schistosomes

Agonist and Conc.		Blocking Agent and Conc.		% Blockage ± S.E.M.		No. of Worms
				3 min	30 min	
	M		*M*			
NE	1×10^{-4}	Phenoxybenzamine	1×10^{-4}	23 ± 33[a]	131 ± 54[d]	6
	5×10^{-5}	Phenoxybenzamine	1×10^{-4}	39 ± 16[a]	104 ± 12[e]	20
	5×10^{-5}	Butoxamine	5×10^{-5}	53 ± 28[c]		6
	5×10^{-5}	Dibenamine	1×10^{-4}	0 ± 23[a]		6
	5×10^{-5}	Phentolamine	5×10^{-5}	53 ± 6[d]		6
	5×10^{-5}	Yohimbine	1×10^{-5}	−35 ± 58[a]		6
	5×10^{-5}	Propranolol	5×10^{-5}	−26 ± 31[c]	−33 ± 6[c]	8
	5×10^{-5}	Propranolol	1×10^{-4}	49 ± 34[e]	63 ± 34[b]	18
	5×10^{-5}	Pronethalol	5×10^{-5}	49 ± 5[f]		6
	5×10^{-5}	Practolol	1×10^{-4}	0 ± 57[a]		6
	5×10^{-5}	Dichloroisopro-terenol	5×10^{-5}	48 ± 14[c]		6
DA	5×10^{-5}	Phenoxybenzamine	5×10^{-5}	14 ± 16[b]		10
	5×10^{-5}	Phenoxybenzamine	1×10^{-4}	29 ± 4[d]	21 ± 27[a]	6
	5×10^{-5}	Propranolol	5×10^{-5}	38 ± 14[d]		10
	5×10^{-5}	Sandoz LB-46[g]	5×10^{-5}	−9 ± 13[a]		10

[a] $P > .5$.
[b] $P < .5$.
[c] $P < .25$.
[d] $P < .1$.
[e] $P < .05$.
[f] $P < .005$.
[g] 4-(2-Hydroxy-3-isopropylamino)-propoxyindole.

predominantly blocks mammalian DA receptors (Andén *et al.*, 1970a), was highly effective. At concentrations 10 times lower than of NE or E, or 5 times lower than that of DA, this compound produced complete blockade within 3 minutes. Haloperidol, which qualitatively has similar effects on mammalian DA receptors (Andén *et al.*, 1970a; Yeh *et al.*, 1969), produced 63% inhibition within 3 minutes at a concentration equal to that of NE. Spiroperidol also blocked completely the effect of AP and was effective in blocking the NE-induced lengthening of decapitated worms; it was less effective in blocking the responses of the separated anterior portion to NE. 3) Pimozide, a highly specific blocking agent of mammalian DA receptors (Andén *et al.*, 1970a), produced within 3 minutes complete inhibition of the effect of NE or of DA at 4 times the concentration of the agonist. 4) Ergonovine reported to be a DA blocker in *Helix aspersa* (Walker *et al.*, 1968) was almost as effective as spiroperidol.

Discussion

Previous studies have indicated that acetylcholine is an inhibitory neuromuscular transmitter in *S. mansoni* (Barker *et al.*, 1966). Furthermore, cholinergic blockade resulted in motor stimulation of the worms, revealing the presence in this organism of an excitatory transmitter whose effects were abolished 1) by two specific 5-HT receptor blocking agents, BOL and MCE, and 2) by prior depletion of the 5-HT stores of the parasite. These observations indicate that the excitatory transmitter is either closely related to, or identical with, 5-HT. The presence of high concentrations of 5-HT in the nervous system of *S. mansoni* (Bennett and Bueding, 1971) and of a high affinity uptake mechanism for 5-HT (Bennett and Bueding, 1973) provides additional

TABLE 7

Effect of DA blocking agents on the NE-, E-, DA- and AP- induced lengthening response of paired male schistosomes

Agonist and Conc.		Blocking Agent and Conc.		% Blockage ± S.E.M.		No. of Worms
				3 min	15–30 min	
	M		*M*			
NE	5×10^{-5}	Dihydroergotamine	5×10^{-5}	103 ± 22[f]		6
	5×10^{-5}	Dihydroergocristine	5×10^{-5}	71 ± 15[f]	96 ± 4[h]	6
	5×10^{-5}	Haloperidol	1×10^{-5}	53 ± 44[e]		14
	5×10^{-5}	Haloperidol	5×10^{-5}	63 ± 20[g]		10
	5×10^{-5}	Pimozide	5×10^{-6}	15 ± 57[a]		6
	5×10^{-5}	Pimozide	1×10^{-5}	39 ± 42[b]		6
	5×10^{-5}	Pimozide	5×10^{-5}	46 ± 30[c]		6
	5×10^{-5}	Pimozide	1×10^{-4}	85 ± 25[e]		6
	5×10^{-5}	Pimozide	2×10^{-4}	97 ± 13[f]		6
	5×10^{-5}	Spiroperidol	1×10^{-6}	0 ± 4[a]		6
	5×10^{-5}	Spiroperidol	2×10^{-6}	15 ± 22[a]		6
	5×10^{-5}	Spiroperidol	5×10^{-6}	119 ± 19[c]		6
	5×10^{-5}	Ergonovine	5×10^{-6}	−24 ± 2[a]	11 ± 6[a]	6
	5×10^{-5}	Ergonovine	1×10^{-5}	108 ± 3[d]	78 ± 18[c]	6
E	5×10^{-5}	Spiroperidol	2×10^{-6}	63 ± 0[c]		6
	5×10^{-5}	Spiroperidol	5×10^{-6}	91 ± 28[d]		6
DA	5×10^{-5}	Pimozide	5×10^{-5}	49 ± 12[c]		6
	5×10^{-5}	Pimozide	2×10^{-4}	90 ± 3[e]	124 ± 36[c]	6
	5×10^{-5}	Spiroperidol	2×10^{-6}	35 ± 53[b]		8
	5×10^{-5}	Spiroperidol	5×10^{-6}	49 ± 37[b]		8
	5×10^{-5}	Spiroperidol	1×10^{-5}	112 ± 7[f]	97 ± 30[b]	8
	5×10^{-5}	Ergonovine	5×10^{-6}	44 ± 20[f]	72 ± 20[d]	6
	5×10^{-5}	Ergonovine	1×10^{-5}	109 ± 8[c]	95 ± 20[c]	6
AP	1×10^{-5}	Spiroperidol	1×10^{-5}	79 ± 0[e]		6
	1×10^{-5}	Ergonovine	1×10^{-5}	67 ± 5[d]	107 ± 6[e]	6
	1×10^{-5}	Ergonovine	5×10^{-5}	105 ± 11[d]	138 ± 9[e]	6

[a] $P > .5$.
[b] $P < .5$.
[c] $P < .25$.
[d] $P < .1$.
[e] $P < .05$.
[f] $P < .025$.
[g] $P < .01$.
[h] $P < .005$.

support for a role of this substance as a neurotransmitter in this trematode.

The motor stimulation of *S. mansoni* produced by reserpine and by other 5-HT-depleting agents, chlorimipramine, protryptyline, *d*-amphetamine, and 4-α-dimethyl-*m*-tyramine, can be explained by an accumulation of released 5-HT at postsynaptic sites, rather than by a direct interaction of the depleting agent with the receptor, because these stimulatory effects could not be induced in 5-HT-depleted worms.

It appears that the 5-HT receptors of schistosomes lack a high degree of specificity because the motor activity of the worms is stimulated also by tryptamine (Nimmo-Smith and Raison, 1968) and by a number of tryptamine analogs other than 5-HT. Therefore, it would be appropriate to characterize this type of response as tryptaminergic. In one respect the tryptaminergic receptors of *S. mansoni* are similar to those present in the malpighian tubules of two insect species, *Rhodnius prolixus* and *Carausius morosus* (Maddrell *et al.*, 1971) because the 5-HT-induced stimulation of fluid secretion by these

tubules, on one hand, and of the motor activity of *S. mansoni*, on the other, are blocked by BOL, but not by LSD.

Hillman and Senft (1973) have proposed the use of an instrument for the study of the motor activity of schistosomes. The minimal concentrations of 5-HT (5×10^{-5} M) which produces a change in motor activity reported by the use of this device is at least 50 times higher than that which brings about motor stimulation observable directly under a dissecting microscope. Furthermore, this instrument failed to record any effect of catecholamines on the worms. Therefore, the design and use of this type of instrumentation must take into consideration such limitations, *i.e.*, very low sensitivity and the inability to record certain changes in muscular activity detectable by direct observation.

The increase in the length of schistosomes produced by catecholamines could be explained by an inhibitory effect on the longitudinal musculature of the parasite. Possibly this is brought about by a stabilization of the membrane, as in the case of mammalian intestinal smooth muscle (Bülbring, 1957; Kuriyama, 1970). On the other hand, the motor stimulation or inhibition of schistosomes produced by 5-HT or acetylcholine, respectively, could be accounted for by effects on the circular musculature of the worms. The markedly reduced lengthening response of female schistosomes to DA and AP might be explained by the far lower concentration of longitudinal musculature in the females in comparison with male worms.

Among invertebrates the most widely distributed and quantitatively most important catecholamine is DA (Cottrell and Laverack, 1968; Kerkut, 1973). In the intermediate host of *S. mansoni*, *Biomphalaria glabrata*, the presence of DA has been demonstrated, whereas NE could not be detected (P. Chang, J. B. Bourgeois and E. Bueding, unpublished observations). Evidence has been reported that DA functions as a neurotransmitter in molluscs (Kerkut and Walker, 1961; Gerschenfeld and Chiarandini, 1965; Walker *et al.*, 1971a,b). The lengthening response of *S. mansoni* is not specific for DA; it is induced also by NE and by E, but not by clonidine, a specific *alpha* adrenergic agonist (Andén *et al.*, 1970b), isoproterenol, a *beta* adrenergic agonist, nor by octopamine, an amine which has been proposed as a neurotransmitter in certain invertebrates (Barker *et al.*, 1972; Walker *et al.*, 1972). The effects of the former three catecholamines on *S. mansoni* are abolished by dopamine blocking agents but, with the exception of dihydroergotamine, only incompletely or not at all by *alpha* adrenergic blockers. Furthermore, AP mimics the effects of catecholamines on *S. mansoni*. This compound is known to interact specifically with mammalian DA receptors (Andén *et al.*, 1967; Ungerstedt *et al.*, 1969) and to mimic the effect of DA in two invertebrates—earthworms (Bieger and Hornykiewicz, 1972) and bivalve molluscs (Malanga, 1973), but not in *Helix aspersa* (Woodruff and Walker, 1969). Moreover, both single and paired males were more responsive to low concentrations of DA and AP than to NE. The presence of DA, rather than of NE, receptors in *S. mansoni* is indicated also by the lengthening response, albeit weak, of female worms to DA and AP and their lack of response to NE. Therefore, the characteristics of these responses are consistent with the presence of DA receptors in the longitudinal musculature of *S. mansoni*. Such receptors may have been developed in, and carried over from, the previous molluscan stages in the life cycle of the parasite. Yet, the catecholamine stored in the neuronal structures extending into the musculature of *S. mansoni* has been identified as NE; in addition, no DA was detectable in the worms (Bennett and Bueding, 1971; Chou *et al.*, 1972). Therefore, it would appear that in *S. mansoni*, NE can act as a neurotransmitter which, on its release, combines with, and activates, DA receptors located in the longitudinal muscles of the worm. The generally weaker lengthening response of single males as compared with that of paired males may suggest differences either in the structure or conformation of the receptors or in other factors determining the response to receptor activation. It is less likely that such differences can be ascribed to a lower permeability of single male worms to the agonists because at some concentrations the lengthening response of the single males was equal to (*e.g.*, 1×10^{-6} M AP), if not greater than, that of the paired males (*e.g.*, 1×10^{-5} M DA). Possibly the musculature of paired males is better developed because of the use of their musculature to keep the females in their gynecophoric canals.

In a previous study, no evidence could be found for the presence of tyrosine or tryptophan

hydroxylases in schistosomes (Bennett and Bueding, 1973). Thus the worm may depend on the host for its supply of biogenic amines. Schistosomes have the ability to take up 5-HT and NE against a concentration gradient by means of active transport mechanisms. The NE concentration of host plasma, although low, is considerably higher than that of DA (Callingham, 1967). By means of a high affinity uptake mechanism, schistosomes would be capable of obtaining enough NE to account for the concentration of 0.2 μg/g (wet weight) (Bennett and Bueding, 1971) stored in the worm. Moreover, since the receptors of the parasite respond to both DA and to NE, the latter can serve as an inhibitory neurotransmitter in the longitudinal musculature of *S. mansoni*.

Drugs that interact specifically with mammalian DA receptors, such as AP and spiroperidol, are most effective in mimicking or blocking the response of schistosomes to DA. On the other hand, the specific mammalian *alpha* adrenergic agonist, clonidine, had no effect at all on the length of the worms. Therefore, *S. mansoni* may be useful for assaying compounds for potential DA agonist or antagonist activity.

Acknowledgments. We wish to express our indebtedness to Dr. John W. Daly, National Institutes of Health, Bethesda, Md., for the tryptamine and 5-HT analogs (listed in table 4); to Dr. Paul Janssen, Beerse, Belgium, for spiroperidol and pimozide; to Dr. A. H. Glässer, of Farmitalia, Milan, Italy, for MCE; to Dr. Lawrence Peters, McNeil Laboratories, Fort Washington, Pa., for haloperidol; to Dr. Joseph Trapold of Sandoz Pharmaceuticals, Hanover, N.J., for LB-46, phentolamine, yohimbine, propranolol and pronethalol; to Dr. Robert A. Maxwell, Wellcome Laboratories, Research Triangle Park, N.C., for butoxamine; to Dr. Henry LeMein, Jr., Ayerst Laboratories, New York, N.Y., for practolol; and to Dr. Irvin H. Slater, Eli Lilly Laboratories, Indianapolis, Ind., for ergonovine.

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