Cholinergic Responses of *Schistosoma mansoni*

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In higher animals, the role of acetylcholine as a transmitter of nerve impulses is well established. However, this substance makes its appearance already at an early stage in phylogeny. Bülbring et al. (1949) demonstrated the presence of acetylcholine in the motile protozoan parasite *Trypanosoma rhodesiense* and its absence from the non-motile erythrocytic forms of malarial parasites; this suggested a role of acetylcholine concerned with the motility of protozoa. Subsequently, it has been found that acetylcholine may be of considerable physiological significance to the regulation of the muscular activity of the metazoan parasite *Schistosoma mansoni*. The habitats of the adult forms of this trematode are the mesenteric-portal venous system and the liver sinuses. According to conservative estimates, 200 million human beings are infected with this or two other species of schistosomes (*S. hematobium, S. japonicum*). Accordingly, information about mechanisms essential for the functional integrity of these parasites may be pertinent to the rational development of chemotherapeutic agents effective in the treatment of schistosomiasis.

The presence of acetylcholine, and of the enzymes catalyzing its hydrolysis and its synthesis, acetylcholinesterase and choline acetylase, have been demonstrated in *S. mansoni* (Bueding, 1952; Barker et al., 1966). The concentration of acetylcholine and the activities of acetylcholinesterase and of choline acetylase in these worms are of a high order and equal those found in the gray matter of mammalian brain cortex. By the use of histochemical methods, it has been found that acetylcholinesterase is localized primarily in the nervous system of *S. mansoni* (Bueding et al., 1963). These observations raise the question about the physiological role of acetylcholine in *S. mansoni*. This problem has been studied by determining the effect of cholinomimetic and of cholinergic blocking agents on the motor activity of schistosomes.

The choline ester carbaminoylcholine (carbachol) has the same physiological actions as acetylcholine, but it is not hydrolyzed by acetylcholinesterase. This choline ester markedly depresses the muscular activity of schistosomes. Cholinesterase inhibitors, e.g., physostigmine, prostigmine, and di-isopropylfluorophosphate, have the same effect. Therefore, inhibition of cholinesterase appears to result in an accumulation of endogenous acetyl-

TABLE 1
Effects of Cholinomimetic Agents on Muscular Activity of *S. mansoni*

Compound	Minimal molar concentration reducing muscular activity of *S. mansoni*	Lack of effect on motor activity of *S. mansoni* at molar concentration range
Carbachol	2×10^{-5}	
Arecoline	2×10^{-7}	
Pilocarpine		1×10^{-7} to 1×10^{-2}
Muscarine		1×10^{-7} to 1×10^{-2}
Physostigmine	2×10^{-6}	
Prostigmine	5×10^{-5}	
Diisopropylfluoro-phosphate (DFP)	1×10^{-4}	

choline; this, in turn, produces decreased muscular activity and paralysis of the worm in the same manner as exogenous carbachol.

The three alkaloids, muscarine, arecoline, and pilocarpine, have the same actions on parasympathetic effector organs as acetylcholine. By contrast, only arecoline is a potent depressant of the muscular activity of *S. mansoni,* while muscarine and pilocarpine are inactive in this respect.

Reduction of the motor activity and paralysis of schistosomes produced by carbachol, cholinesterase inhibitors and arecoline, are abolished by atropine and two non-quaternary ganglion blocking agents, mecamylamine and pempi-

TABLE 2
Effects of Cholinergic Blocking Agents on *S. mansoni*

Blocking Agent	Site of blockade in mammalian host	Minimal molar concentration required to reverse cholinergic paralysis of *S. mansoni*	Lack of cholinergic blockade in *S. mansoni* at molar concentration range
Atropine	Parasympathetic effector organ at low concentrations (approx. 1×10^{-7} molar) and autonomic ganglia at higher concentrations	5×10^{-5}	
Mecamylamine		5×10^{-5}	
Pempidine		2×10^{-4}	
Hexamethonium	Autonomic ganglia		5×10^{-5} to 2×10^{-2}
Pentolinium			2×10^{-4} to 2×10^{-2}
Chlorisondamine			1×10^{-4} to 1×10^{-2}
Nicotine			1×10^{-7} to 1×10^{-3}
d-Tubocurarine	Neuromuscular junction		5×10^{-5} to 1×10^{-2}
Decamethonium			5×10^{-5} to 1×10^{-2}
Succinylcholine			1×10^{-4} to 1×10^{-2}

dine. Exposure of schistosomes to these compounds alone (i.e., in the absence of cholinergic agents) results in a marked motor hyperactivity of the parasite. This stimulatory effect can be accounted for by a block of an interaction of the worm's cholinergic receptors with endogenous acetylcholine, resulting in the failure of this humoral transmitter to exert its inhibitory effect on motor activity. In contrast to these secondary and tertiary amine blocking agents, even high concentrations of nicotine, of quaternary ganglion blocking agents, such as hexamethonium, chlorisondamine, and pentolinium, or of neuromuscular blocking agents (e.g., tubocurarine, decamethonium, and succinylcholine, are completely devoid of cholinergic blocking activity in schistosomes.

These observations, summarized in tables 1 and 2, indicate that cholinergic receptors of schistosomes have some similarities with cholinergic receptors of their mammalian hosts, but nevertheless are distinguishable from those of vertebrate autonomic cholinergic effectors organs and of autonomic ganglia because the muscular activity of the worms is affected neither by muscarine or pilocarpine, on the one hand, nor by quaternary ammonium ganglion blocking agents or nicotine, on the other.

One of the first effects of the administration of the antischistosomal drug, p-rosaniline (tris (p-aminophenyl) carbonium) to mice infected with S. mansoni (Elslager et al., 1961; Thompson et al., 1962) is a localized paralysis of two muscular organs of the worm, the oral sucker and the acetabulum, with which the parasite attaches itself to the mesenteric veins. In vitro, this paralysis is reversed within less than two minutes by atropine or by mecamylamine. This suggests that the paralysis is due to an accumulation of acetylcholine, whose action in depressing muscular activity of schistosomes is abolished by cholinergic blocking agents.

This interpretation is confirmed by a histochemically demonstrable inhibition of acetylcholinesterase activity of the two muscular organs and of the central nervous system of the parasite following the administration of p-rosaniline (Bueding et al., 1963).

It is concluded that the use of pharmacological agents can provide opportunities to recognize and define similarities and differences in the mechanisms of transmission of nerve impulses in the parasite and the mammalian host. Such studies contribute to better understanding of comparative physiology and of the mode of action of antiparasitic drugs.

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