ACETYLCHOLINE : A POSSIBLE NEUROTRANSMITTER IN *SETARIA CERVI*

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Summary: The total and free acetylcholine (Ach) and cholinesterase (CHE) content of adult *Setaria cervi* were estimated. The Ach was estimated by bioassay on rectus abdominis muscle of frog and the CHE by measuring the drop in pH following incubation of worm homogenate with Ach chloride. The free and total Ach contents (4.0 ± 0.57 and 6.0 ± 0.48 $\mu g/g$ wet weight of worms respectively) were as high as found in mammalian brain cortex. The cholinesterase activity was found to be 5.57 ± 0.6 units/g wet weight of worms. It is possible that there may exist a well developed system responsible for the synthesis, storage, release and destruction of Ach and that Ach may be acting as an excitatory neurohormone in *S. cervi*.

Key words: acetylcholine cholinesterase neurotransmitter *Setaria cervi*

INTRODUCTION

Rats implanted intraperitoneally with adult *Setaria cervi* have been found suitable for screening potential antifilarial agents (13, 16). The antifilarial efficacy of presently available drugs including diethylcarbamazine is largely confined to the microfilariae and the adult worms enjoy the hospitality of the host unaffected (1, 6, 8). To find a drug which could kill the adult worms as well, it is necessary to understand the factors responsible for the regulation of their motility. In our earlier studies we observed that 0.5×10^{-8} g/L acetylcholine (Ach) causes stimulation of the whole worm and the nerve-muscle preparation of *Setaria cervi* (14, 15). The effectiveness of Ach in such low concentration prompted the present investigators to explore the possible existence of a natural cholinergic system responsible for the motility in the worm.

MATERIALS AND METHODS

Adult *S. cervi* were obtained from the peritoneal cavity of the freshly slaughtered Indian water buffalo (*Bubalus bubalis* Linn.) at the slaughter house and transported to the laboratory in a vacuum flask containing "modified Ringer solution" at 37°C (17).

Estimation of acetylcholine :

Fifty worms were weighed wet (average weight 38 ± 5 *mg*/worm) and placed in ice cold modified Ringer solution containing physostigmine sulphate (1.5×10^{-5} g/*ml*) and were homogenised for the preparation of 10% homogenate which was divided in two equal portions. One part was centrifuged at 3000 x G for 10 min and the supernatant immediately thereafter assayed for free Ach. The other portion of the homogenate was acidified with 0.5 M HCl to pH 3-4 and placed for 2 min in boiling water. After neutralization with 0.5 M NaOH, the supernatant was assayed for total Ach content.

The bioassay was carried out on the rectus abdominis muscle of frog (4). Physostigmine (1×10^{-5} *g/ml*) was added to the bathing fluid to increase the sensitivity of the preparation. Three point assay was carried out to determine the concentration of Ach. At the end of each experiment d-tubocurarine (1×10^{-6} *g/ml*) was added to the bath to confirm the specificity.

Estimation of cholinesterase :

Groups of 40 adult *S. cervi* were weighed wet and incubated in 100 *ml* of "modified Ringer solution" at 37°C in a BOD incubator. After 2 hr of incubation groups of worms were transferred to 10 *ml* phosphate buffered saline (pH 7.4) in a tissue homogeniser surrounded by ice and homogenised for about 10 min. The resulting homogenate was centrifuged at 6000 x G for 30 min. The concentration of cholinesterase was determined by electrically measuring the drop in pH ($\triangle$ pH) following incubation with Ach chloride at 25°C for 1 hr by the method described by Henry (7). Bovine erythrocyte type I cholinesterase (Sigma) was used as standard.

RESULTS

The results of the present study are summarized in Table I.

Table I: Concentrations of acetylcholine and cholinesterase in *Setaria cervi*.

	Number of experiments	*Concentration/g wet weight of worms (μg or units $\pm$ S.E.)*
Acetylcholine		
Total	10	6.0 $\pm$0.48
Free	10	4.0 $\pm$0.57
Cholinesterase	8	5.57$\pm$0.6

The two homogenates prepared to estimate free and total Ach content caused contraction of frog rectus abdominis muscle which could be blocked by d-tubocurarine (1×10^{-6} *g/ml*). The amounts of free and total Ach contents were 4.0 and 6.0 $\mu g/g$ wet weight of worms.

The acetylcholinesterase activity in *S. cervi* was found to be 5.57 units/g of wet weight.

DISCUSSION

Most parasitic helminths exhibit vigorous rhythmical movements. These movements help the organism to maintain and locate itself inside the host. The first observation that Ach may be of functional significance was made by Bulbring *et al.* (3). They demonstrated the presence of Ach in motile *Trypanosoma rhodesiense* and its absence in non-motile *Plasmodium gallinaceum*. Pharmacological evidence indicates that Ach is an excitatory neuromuscular transmitter in *Ascaris lumbricoides* (5, 12) and *S. cervi* (14, 15) and possibly other nematodes.

The presence of Ach in high concentrations and of cholinesterase suggest that a system for synthesis and hydrolysis of Ach may exist in *S. cervi*. The concentration of Ach (4 and 6 $\mu g/g$ wet weight as free and total Ach content respectively) is of the same order of magnitude as that of the grey matter of mammalian brain cortex (11). The presence of Ach in both free as well as in bound form in *S. cervi* suggests that a developed system responsible for its synthesis, storage, release and destruction may exist in the worm. The Ach-cholinesterase system has been shown to exist in many other nematodes including *A. lumbricoides* (2, 10), *Litomosoides carini* (2) and *Ascaridia galli* (9). The presence of Ach-cholinesterase system tempts us to assign Ach the role of neurotransmitter in nematodes.

While the cholinergic receptors of *S. cervi* exhibit some similarity with those of mammalian myoneural junction, their responses also suggest differences with pharmacologically defined cholinoceptive mammalian receptors (14, 15). For example, in contrast to cholinergically innervated effector organs of vertebrates the motor activity of *S. cervi* is not affected by pilocarpine and the response to nicotine is different. On the nerve muscle preparation of Setaria, nicotine, at any dose level, causes only relaxation followed by irreversible paralysis (15) while on mammalian myoneural junction the effect of nicotine is biphasic in nature characterized by initial stimulation followed by paralysis. The differences occur not only among various phyla of helminths but also among various species of these organisms. The differences may be evident not only by the presence or absence of a certain physiological system but also by the functional role played by it in the parasite. To achieve the goal of a rational chemotherapy of parasitic helminths more important approach would be to find areas where the role of the neurotransmitter differs in parasite with that in the host. These differences, which are not uncommon to find, are likely to form the basis of differential chemotherapy. In the present context because of the difference in the response of cholinoceptive receptors of the host and Setaria there is, atleast, theoretically a possibility for designing cholinergic blocking agents which are selective to parasite.

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