STUDIES ON CHEMOTHERAPY OF PARASITIC HELMINTHS (VII). EFFECTS OF VARIOUS CHOLINERGIC AGENTS ON THE MOTILITY OF ANGIOSTRONGYLUS CANTONENSIS

Mamoru TERADA, Akira I. ISHII, Hideto KINO and Motohito SANO
Department of Parasitology, Hamamatsu University School of Medicine, Hamamatsu 431-31, Japan

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Abstract—Effects of various cholinergic agents on the motility of Angiostrongylus cantonensis were studied to define the neuropharmacological properties of this worm. Stimulation of the motility and/or contraction were shown by eserine, ACh, carbachol, nicotine, DMPP, pyrantel, and Ba²⁺, but not by pilocarpine and McN-A-343. Contraction was similarly observed by these agents in the preparations paralyzed with praziquantel. Paralysis was caused remarkably by d-tubocurarine and slightly by succinylcholine, while the contraction induced by eserine and DMPP was little influenced by these drugs. Both the motility and the eserine-induced contraction were little influenced by hexamethonium, but stimulated remarkably by atropine. Though hemicholinium-3, morphine, and pircate showed little effect, guanidine stimulated remarkably the motility and also the eserine-induced contraction. The stimulatory action of guanidine was antagonized by strychnine. Strychnine paralyzed the motility, and the eserine-induced contraction was antagonized by the pre- and post-treatment with strychnine.

From these results, it is suggested that the excitatory cholinergic mechanism in A. cantonensis is nicotinic, and it is basically similar to that reported in Ascaris suum.

For the development of more effective and less toxic anthelminthics, it must be essential to study the biochemical, physiological, and pharmacological mechanisms of parasitic worms such as energy metabolism, ion-movements, and neural transmission (1–4). Such studies may be also interesting from other viewpoints such as comparative pharmacology and searching for screening models of drugs (5, 6).

Effects of drugs on the motility of parasitic helminths have been tested as one of the useful approaches to the investigation of anthelmintics (7–16). With some exceptions for Schistosoma mansoni (15, 16), however, these studies have been carried out exclusively on larger worms that were easily tested by kymographic techniques such as Ascaris suum and Fasciola hepatica, and in each study, only a preparation from one species was tested.

In previous studies, we have undertaken comparative studies on drug actions between various parasitic helminths and isolated host tissues using the isotonic transducer method that we recently developed (17, 18). From the practical viewpoints in experiments such as the availability of the worms, the easiness
of experiments, and the susceptibility to drugs, and also from the viewpoints of phylogenetic and pharmacological differences among three groups of helminths, *Angiostrongylus cantonensis* was selected as an excellent model for nematodes, *Dipylidium caninum* as that for cestodes, and *F. hepatica* and *Schistosoma japonicum* as those for trematodes (5, 6). Thus, comparative and systematic studies have been carried out using many preparations including these essential four parasites and isolated host smooth and skeletal muscles (5, 6, 17–21). In the present study, to define the neuropharmacological properties of *A. cantonensis*, our nematodal model, effects of various cholinergic drugs on the motility of this worm were studied.

**MATERIALS AND METHODS**

The rat lungworm, *Angiostrongylus cantonensis* is a metastrongylid nematode parasitizing in the pulmonary arteries and sometimes in the right cardiac ventricle of rats and other rodents. The worms were collected from rats (Wistar strain) experimentally infected in our laboratory. Female worms were used as whole worm preparations.

The worm preparation was suspended in Tyrode’s solution in a thermostatically controlled organ bath (7 ml capacity) at 35°C and gassed slightly with air. Responses of the preparation to drugs were recorded isotonically on a recorder (Toa, EPR-100A) with an isotonic transducer (Nihon Koden, TD-112S), producing a magnification of 15 to 30 fold and exerting a tension of 0.8 to 1.0 g.

The following drugs were used: acetylcholine chloride (ACh), carbamylcholine chloride (Carbachol), eserine salicylate [Sigma], hexamethonium chloride, sodium picrate, guanidine hydrochloride [Wako], strychnine sulfate [Nakarai], pilocarpine hydrochloride, nicotine tartrate, picrotoxin hydrochloride [Tokyokasei], atropine sulfate [Merck], d-tubocurarine chloride [Takeda], morphine hydrochloride [Sankyo], 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP), hemicholinium-3 [Aldrich], 4-(m-chlorophenylcarbamoyloxy)-2-butylnyltrimethylammonium chloride (McN-A-343) [McNeil labs], barium chloride [Katayama], succinylcholine chloride [Yamanouchi], pyrantel tartrate [Pfeizer], praziquantel (PQ) [Bayer]. Drugs were dissolved in a 0.9% NaCl solution, and the concentrations refer to the weights of the salts.

**RESULTS**

Each figure shows the representative of 3 to 5 similar tracings.

**Effects of various cholinergic agonists and Ba²⁺ on the motility of *A. cantonensis***: Remarkable and dose-related contraction was caused by nicotine (10⁻⁷–3 x 10⁻⁶ M), eserine (3 x 10⁻⁷–10⁻⁶ M), DMPP (10⁻⁶–10⁻⁴ M), and pyrantel (10⁻⁸–10⁻⁷ M), and spastic paralysis was observed in higher concentrations of these agonists (Figs. 1, 2). On the other hand, slight contraction was caused by ACh (10⁻⁵–10⁻⁴ M) and carbachol (10⁻⁴ M), but little effect was observed by pilocarpine (10⁻⁶–10⁻⁴ M) and MCN-A-343 (1.6 x 10⁻⁵–1.6 x 10⁻⁴ M) (Figs. 1, 2).

By the addition of Ba²⁺ (10⁻³ M), the motility was stimulated tonically as well as phasically (Fig. 2B).

**Effects of various cholinergic agonists and Ba²⁺ on the paralyzed preparations with praziquantel in *A. cantonensis***: Though praziquantel (PQ), a newly developed anthelmintic for cestodes and trematodes, paralyzed the motility of *A. cantonensis*, PQ-induced paralysis was antagonized and contraction was caused remarkably by nicotine (10⁻⁸–3 x 10⁻⁷ M), DMPP (10⁻⁷–3 x 10⁻⁶ M), pyrantel (10⁻¹⁰–10⁻⁸ M), eserine (10⁻⁷–3 x 10⁻⁶ M), Ba²⁺ (10⁻³ M), carbachol (10⁻⁶–
Fig. 1. Effects of various cholinergic agonists on the motility of *A. cantonensis*. Effects of nicotine ($10^{-9}$-$3 \times 10^{-6}$ M), pilocarpine ($10^{-6}$-$10^{-4}$ M), eserine ($10^{-6}$ M), 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP, $10^{-6}$-$10^{-4}$ M), and pyrantel ($10^{-11}$-$10^{-7}$ M) were examined. Drugs were cumulatively given, and pilocarpine and eserine in A) were successively given. At the point W, preparations were washed by Tyrode's solution.

Fig. 2. Effects of various cholinergic agonists and Ba$^{2+}$ on the motility of *A. cantonensis*. Effects of acetylcholine (ACh, $10^{-5}$-$10^{-4}$ M), carbachol ($10^{-5}$-$10^{-4}$ M), eserine ($10^{-7}$-$10^{-6}$ M), 4-(m-chlorophenylcarbamoyloxy)-2-butynyl-trimethylammonium chloride (McN-A-343, $1.6 \times 10^{-5}$-$1.6 \times 10^{-4}$ M), and Ba$^{2+}$ ($10^{-3}$ M) were examined. Drugs were cumulatively given, and McN-A-343 and Ba$^{2+}$ in C) were successively given. At the point W, preparations were washed by Tyrode's solution.
10^{-4} M), and ACh (10^{-6}-10^{-5} M), but very slightly by pilocarpine (10^{-5}-10^{-4} M) and McN-A-343 (1.6 \times 10^{-5}-1.6 \times 10^{-4} M) (Figs. 3, 4).

Effects of cholinergic antagonists on the motility and on the eserine-induced contraction in A. cantonensis: Though paralysis was caused remarkably by d-tubocurarine (1.9 \times 10^{-5}-5.7 \times 10^{-5} M) and slightly by succinylcholine (2.5 \times 10^{-5}-7.5 \times 10^{-5} M), the contraction induced by DMPP (10^{-4} M) and eserine (10^{-6} M) was little influenced by these drugs (Fig. 5). Hexamethonium (10^{-5}-10^{-4} M) showed little effect on the motility and on the eserine (10^{-7}-10^{-6} M)-induced contraction (Fig. 6). On the other hand, atropine (10^{-5} M) stimulated the motility and caused contraction and it also stimulated the eserine (10^{-7}-3 \times 10^{-7} M)-induced contraction (Fig. 6).

Effects of morphine, picrate, guanidine, hemicholinium-3, and strychnine on the motility and on the eserine-induced contraction in A. cantonensis: Though morphine (10^{-6}-10^{-5} M), picrate (10^{-4} M), and hemicholinium-3 (10^{-5}-10^{-4} M) showed little effect, guanidine (10^{-3}-2.5 \times 10^{-3} M) stimulated remarkably the motility and also the eserine (10^{-7}-3 \times 10^{-7} M)-induced contraction (Figs. 7, 8A). The stimulant effect of guanidine was characterized by an increase of tone and amplitude (Fig. 7). The action of

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**Fig. 3.** Effects of various cholinergic agonists on the preparations of A. cantonensis paralyzed with praziquantel. Effects of nicotine (10^{-9}-3 \times 10^{-7} M), N,N-diethyl-4-phenylpiperazinium iodide (DMPP, 10^{-8}-3 \times 10^{-6} M), pilocarpine (10^{-5}-10^{-4} M), 4-(m-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride (McN-A-343, 1.6 \times 10^{-5}-1.6 \times 10^{-4} M), pyrantel (10^{-11}-10^{-9} M), and eserine (10^{-8}-3 \times 10^{-9} M) were examined. Drugs were cumulatively given, and pilocarpine and McN-A-343 in A) were successively given. At points W & PO, preparations were washed by Tyrode's solution and treated with praziquantel (2 \times 10^{-4} M).
Effects of Ba\(^{2+}\), carbachol, and acetylcholine on the preparations of *A. cantonensis* paralyzed with praziquantel. Effects of Ba\(^{2+}\) \((10^{-3} \text{ M})\), carbachol \((10^{-6}-10^{-4} \text{ M})\), and acetylcholine (ACh, \(10^{-6}-10^{-5} \text{ M}\)) were examined. Drugs were cumulatively given. At the point W, preparations were washed by Tyrode's solution, and at points W & PQ \((3 \times 10^{-4} \text{ M})\), the preparation was washed by Tyrode's solution and treated with praziquantel.

Strychnine \((3 \times 10^{-6} \text{ M})\) paralyzed the motility, and the eserine \((10^{-6} \text{ M})\)-induced contraction was antagonized by the pre- and post-treatment with strychnine \((3 \times 10^{-6} \text{ M})\) (Fig. 8B).

**DISCUSSION**

As to the neuropharmacology of parasitic nematodes, studies on one species, *Ascaris suum*, have been exclusively carried out using kymographic methods (7-9, 11-14) or electrophysiological techniques (22). From these physiological and pharmacological studies, it is suggested that the neuromuscular junctions in *A. suum* consist of two different types: (a) depolarizing or excitatory ones which are cholinergic in nature, and (b) hyperpolarizing or inhibitory ones in which the transmitter is likely to be \(\gamma\)-aminobutyric acid (GABA) or a related compound (22).

It is also suggested that the cholinceptor in *Ascaris* muscle preparation is pharmacologically similar to that of the mammalian autonomic ganglion (13) or skeletal muscle (9, 14).

In the present study, an excitatory cholinergic mechanism, which is similar to that reported in *A. suum* (9, 12-14, 22), was suggested to be in *A. cantonensis*.

From the results on cholinergic agonists, the cholinergic mechanism in *A. cantonensis* is suggested to be nicotinic because the motility of this worm was stimulated by nicotinic agonists with or without muscarinic properties, but not by muscarinic agonists such as pilocarpine and McN-A-343. A similar relationship was observed when these agonists were given to the preparations paralyzed with praziquantel, a newly developed anthelminthic for cestodes and...
trematodes (16). That is, a remarkable contraction was caused by nicotinic agonists, but not by muscarinic ones. From the results on cholinergic antagonists, the mechanism is also suggested to be nicotinic because paralysis was caused by d-tubocurarine and succinylcholine, but not by hexamethonium and atropine. However, the eserine-induced contraction was little influenced by d-tubocurarine and succinylcholine. We have obtained no reasonable explanation for this discrepancy as yet.

From the results on agents which influence the release of neurotransmitters (23, 24), the muscle of *A. cantonensis* is suggested to be similar to skeletal muscle rather than ileal smooth muscle because the motility of this worm was affected by strychnine and guanidine, but not by morphine and picrate. Though all of these results on cholinergic agents suggested a similarity of the muscle of *A. cantonensis* to skeletal muscle, the muscle of this worm is also suggested to have other properties because drugs such as GABA, α-adrenergic agonists, and papaverine paralyzed the motility (25).

Though these results on drugs including cholinergic agents and GABA in our experiments basically agreed well with those reported in *A. suum* (9, 12–14, 22), there seemed to be remarkable differences regarding the sensitivity to drugs between these two worms. In general, the sensitivity to various drugs in *A. cantonensis* was greater than that in *A. suum*. For example, eserine stimulated remarkably the motility of *A. cantonensis* at concentrations of $3 \times 10^{-7}$ M or more, while this drug caused only a spontaneous move-
Fig. 6. Effects of hexamethonium and atropine on the motility and on the eserine-induced contraction in *A. cantonensis*. Upper and lower traces are continuous. Drugs were cumulatively given, and eserine (10^{-7} or 10^{-6} M) was successively given after the treatment with hexamethonium (10^{-5} M, the upper trace) or atropine (10^{-6} M, the lower trace). At the point W, preparations were washed by Tyrode’s solution.

The habitat of *A. cantonensis*, the pulmonary arteries, is highly homeostatic compared to that of *A. suum*, the intestinal tract. Therefore, these differences in sensitivity to drugs seems to be intrinsically attributed to structural and functional differences such as those in the cuticular layers and in permeability of drugs, besides those in the susceptibility of receptor sites. Since an intact worm or anterior piece of *A. suum* showed less susceptibility to almost neuropharmacological agents, investigators have used this worm as muscle strips with a longitudinal cut along the lateral line or eviscerated preparations. Therefore, in comparison to *A. cantonensis*, which was used as...
Fig. 7. Effects of morphine, picrate, and guanidine on the motility and on the eserine-induced contraction in A. cantonensis. Drugs were cumulatively given, and morphine (10^{-6}-10^{-5} M), picrate (10^{-4} M), guanidine (10^{-3}-2.5 \times 10^{-3} M), and strychnine (10^{-5} M) in A) and guanidine (10^{-3}-2.5 \times 10^{-3} M) and eserine (10^{-2}-3 \times 10^{-2} M) in B) were successively given. At the point W, preparations were washed by Tyrode's solution.

Fig. 8. Effects of hemicholinium-3 and strychnine on the motility and on the eserine-induced contraction in A. cantonensis. A) Hemicholinium-3 (10^{-8}-10^{-4} M) and eserine (3 \times 10^{-7}-10^{-6} M) were successively given. B) Strychnine (3 \times 10^{-6} M) was given before and after the treatment with eserine (3 \times 10^{-2}-10^{-6} M). At the point W, preparations were washed by Tyrode's solution.
a whole worm preparation, less response to drugs in Ascaris muscle preparations may be partially due to lack of nervous system integrity.

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