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STUDIES ON CHEMOTHERAPY OF PARASITIC HELMINTHS (VIII). EFFECTS OF SOME POSSIBLE NEUROTRANSMITTERS 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 some possible neurotransmitters such as GABA, adrenergic drugs, and 5-HT and their antagonists on the motility of *Angiostrongylus cantonensis* were studied. Paralysis was caused by GABA, avermectin B1a (Av-B1a), piperazine and α-adrenergic agonists such as adrenaline, noradrenaline, phenylephrine, clonidine and methoxamine, but not by β-adrenergic agonists such as isoproterenol. The paralysis by GABA or Av-B1a was antagonized by GABA antagonists such as picrotoxin and/or bicuculline with cholinergic agents such as N-methylcytisine (N-MC) or eserine. The paralysis elicited by α-adrenergic agonists was antagonized by α-adrenergic antagonists such as phentolamine and dibenamine, but not by β-adrenergic antagonists such as propranolol. 5-HT affected the motility of *A. cantonensis* paralytically or spastically. The paralysis induced by 5-HT was antagonized by α-adrenergic antagonists such as phentolamine and dibenamine, while the contraction induced by this compound was further stimulated by N-MC, but antagonized by strychnine. Other agents such as glutamine, glycine, aspartic acid, taurine, and substance P showed little effect on the motility of *A. cantonensis*. From these findings on the neuropharmacological properties of *A. cantonensis*, it is suggested that this worm is useful as an excellent nematodal model for the investigation of anthelminthics. In addition, this worm may also useful as one of screening models of drugs affecting the central nervous system in mammals.

Basical neuropharmacological studies on parasitic nematodes for the investigation of anthelminthics have been exclusively carried out on only one species, *Ascaris suum* (1–5). Recently, we have selected another species, *Angiostrongylus cantonensis*, as an excellent model of nematodes in our comparative and systematic studies on chemotherapy of parasitic helminths (6). In the previous paper, we reported effects of various cholinergic drugs on the motility of *A. cantonensis* and suggested that the excitatory cholinergic mechanism in this worm is nicotinic in nature (7). Though this mechanism in *A. cantonensis* was suggested to be basically similar to that reported in *A. suum* (1–5), *A. cantonensis* seemed to be remarkably superior to *A. sunm* regarding the sensitivity to various drugs.

In the present study, we have examined some neuropharmacological agents which are suggested to be neurotransmitters in the nervous system of both vertebrate and invertebrate animals (8, 9).

MATERIALS AND METHODS

Collecting the worms, *Angiostrongylus cantonensis*, and experiments on drug effects on the motility of this worm by the isotonic transducer method were as described in previous papers (6, 7, 10).

The following drugs were used: eserine salicylate, γ-aminobutyric acid (GABA), propranolol, bicuculline, taurine, substance P [Sigma], phenylephrine hydrochloride, papaverine hydrochloride, piperazine dihydrochloride, glycine, sodium glutamate, aspartic acid [Wako], adrenaline [Daichi], strychnine sulfate, dibenamine hydrochloride [Nakarai], serotonin creatinine sulfate (5-hydroxytryptamine, 5-HT), picrotoxin hydrochloride [Tokyokasei], avermectin Bia (Av-Bia) [Merck], phentolamine [Takeda], noradrenaline [Sankyo], isoproterenol hydrochloride [Nikenkagaku], methoxamine hydrochloride [Nihonshinyaku], clonidine hydrochloride [Tanabe], pyrantel tartrate [Pfeizer], and N-methylcytisine (N-MC), an alkaloid from *Sophora flavescens*, was kindly offered from Dr. T. Noro (Shizuoka College of Pharmacy).

RESULTS

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

Effects of GABA, Av-Bia and piperazine on the motility of *A. cantonensis*: Paralysis was caused by GABA (10^{-5}–10^{-4} M), Av-Bia (3.6×10^{-5} M) and piperazine (10^{-5}–10^{-4} M) as shown in Figs. 1–3, respectively.

Remarkably paralyzed preparations with

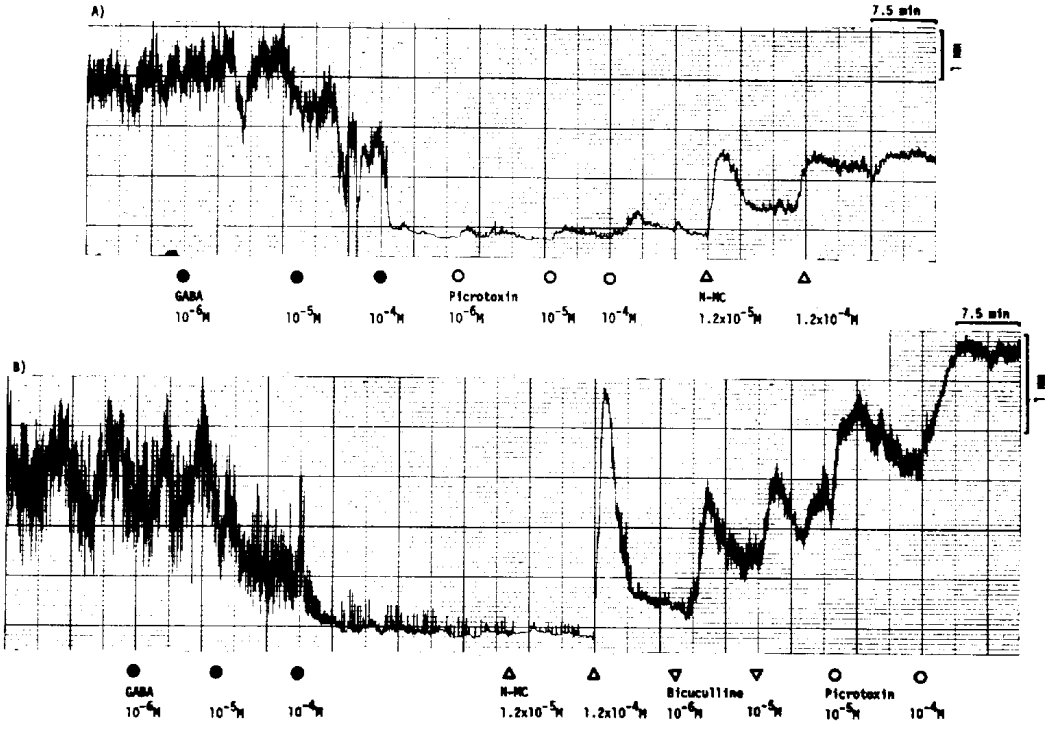

Fig. 1. Effects of GABA and its antagonists on the motility of *A. cantonensis*. Drugs were cumulatively given: and γ-aminobutyric acid (GABA, 10^{-6}–10^{-4} M), picrotoxin (10^{-6}–10^{-4} M), and N-methylcytisine (N-MC, 1.2×10^{-5}–1.2×10^{-4} M) in A) and GABA (10^{-6}–10^{-4} M), N-MC (1.2×10^{-5}–1.2×10^{-4} M), bicuculline (10^{-6}–10^{-5} M), and picrotoxin (10^{-5}–10^{-4} M) in B) were successively given.

GABA (10^{-4} M) were slightly antagonized by the single addition of picrotoxin (10^{-6}–10^{-4} M) or N-MC (1.2×10^{-5}–1.2×10^{-4} M), and they were remarkably antagonized by the combined addition of N-MC (1.2×10^{-4} M), bicuculline (10^{-6}–10^{-5} M) and picrotoxin (10^{-5}–10^{-4} M) (Fig. 1). The eserine (10^{-7}–10^{-5} M)-induced contraction was inhibited by the pretreatment with GABA (10^{-4} M), but not by picrotoxin (10^{-5} M) (Fig. 2).

The paralysis induced by Av-Bia (3.6×10^{-15} M) was not reversed by the combined addition of N-MC (1.2×10^{-5} M) with dibenamine (10^{-5}–10^{-4} M), but reversed by N-MC (1.2×10^{-4} M) with picrotoxin (10^{-5} M) and also by eserine (10^{-5} M) with picrotoxin (10^{-4} M) (Fig. 3A).

Effects of adrenergic agents on the motility of *A. cantonensis*: Paralysis was caused by adrenaline (2.7×10^{-5}–2.7×10^{-4} M), noradrenaline (3×10^{-6}–6×10^{-5} M), phenylephrine (10^{-6}–3×10^{-5} M), methoxamine (4×10^{-5}–1.2×10^{-4} M), and clonidine (2.8×10^{-6}–1.4×10^{-5} M), but not by isoproterenol (4.7×10^{-7}–4.7×10^{-5} M) (Figs. 4–7).

Dibenamine (10^{-5}–10^{-4} M) stimulated tonically and phasically the motility and also the preparations paralyzed by phenylephrine (3×10^{-5} M), methoxamine (8×10^{-5} M), and clonidine (7×10^{-6} M) as respectively shown in Figs. 4C, 5B, 6. Phentolamine (3.6×10^{-6}–10^{-5} M) antagonized the preparation paralyzed by phenylephrine (3×10^{-5} M) (Fig. 5A). On the other hand, propranolol (10^{-7}–10^{-5} M) showed little effect on the motility and also on the paralysis induced by adrenaline (2.7×10^{-4} M) and phenylephrine (3×10^{-5} M) (Figs. 4A, B, 5A).

Paralysis elicited by noradrenaline (6×10^{-5}

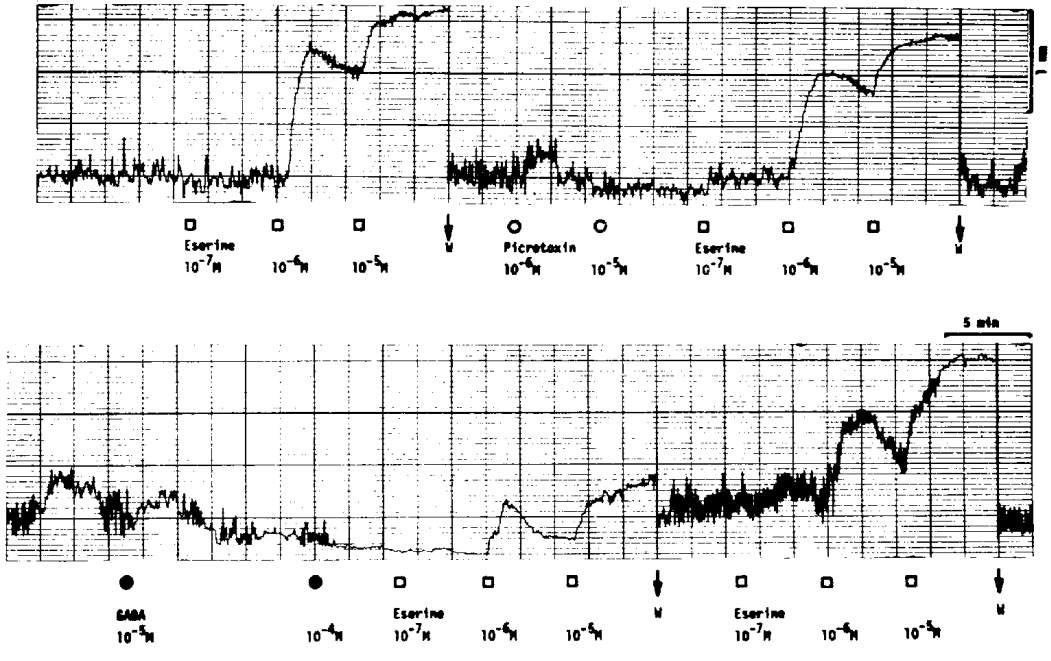

Fig. 2. Effects of picrotoxin and GABA on the eserine-induced contraction in *A. cantonensis*. Upper and lower traces are continuous. Drugs were cumulatively given, and eserine (10^{-7}–10^{-5} M) was given with or without treatment by picrotoxin (10^{-6}–10^{-5} M) or γ-aminobutyric acid (GABA, 10^{-5}–10^{-4} M). At the point W, the preparation was washed by Tyrode's solution.

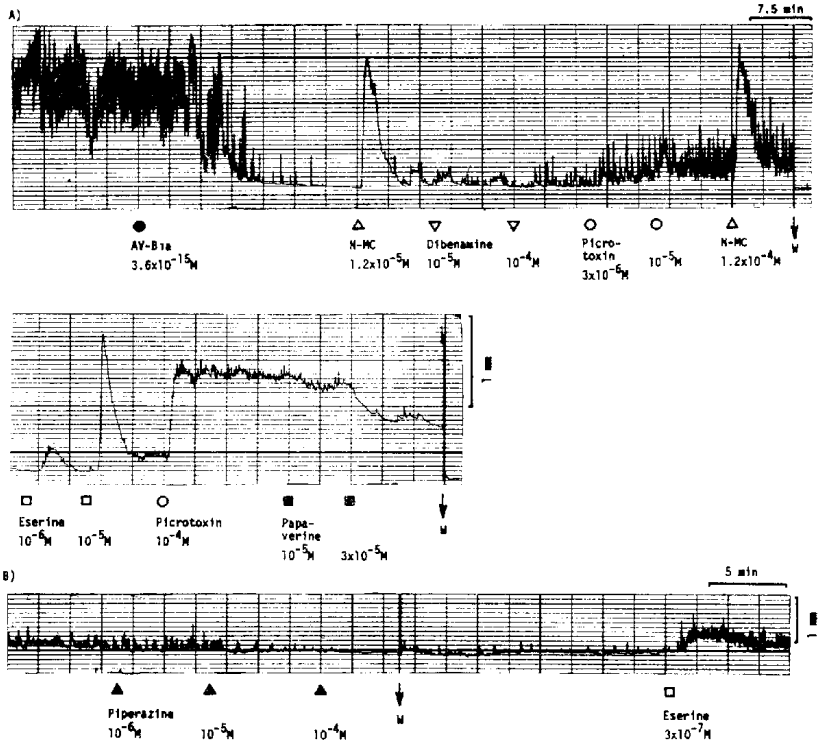

Fig. 3. Effects of avermectin B1a (Av-B1a), piperazine, and other agents on the motility of *A. cantonensis*. Some drugs were cumulatively given. A): Upper and middle traces are continuous. Av-B1a (3.6×10^{-15} M), N-methylcytisine (N-MC, 1.2×10^{-5} M), dibenamine (10^{-5}–10^{-4} M), picrotoxin (3×10^{-6}–10^{-5} M), and N-MC (1.2×10^{-4} M) in the upper trace and eserine (10^{-6}–10^{-5} M), picrotoxin (10^{-4} M), and papaverine (10^{-5}–3×10^{-5} M) in the middle trace were successively given. B): Effects of piperazine (10^{-6}–10^{-4} M) and eserine (3×10^{-7} M) were examined. At the point W, preparations were washed by Tyrode's solution.

M) or clonidine (7×10^{-6} M) was antagonized and contraction was caused by spasmogens such as N-MC (1.2×10^{-4} M) and eserine (10^{-6} M) (Fig. 7).

Effects of 5-HT on the motility of *A. cantonensis*: 5-HT (10^{-6}–10^{-4} M) caused paralysis in many preparations (70%, n=10), but caused contraction in other preparations (30%, n=10) (Figs. 8, 9A, B).

The paralysis induced by 5-HT was antagonized by the addition of dibenamine (10^{-5}–3×10^{-5} M) or phentolamine (10^{-5} M) (Figs. 8B, C). The action of eserine (10^{-7}–10^{-5} M) was inhibited in the paralyzed preparation by 5-HT (10^{-5} or 10^{-4} M), while this inhibition was reversed by the treatment with dibenamine (3×10^{-5} M) or phentolamine (10^{-5} M) (Fig. 8). On the other hand, the contraction induced by 5-HT (10^{-5} M) was further stimulated by N-MC (1.2×10^{-5}–1.2×10^{-4} M), but antagonized by strychnine (3×10^{-5} M) (Fig. 9A, B).

Effects of papaverine on the motility of *A. cantonensis*: Papaverine (10^{-5}–3×10^{-5} M) paralyzed the motility of *A. cantonensis*, and it also blocked the eserine (10^{-7}–10^{-6} M)-induced contraction (Fig. 9C). This alkaloid (10^{-5}–3×10^{-5} M) also paralyzed the reversed preparation by the combined addition of eserine (10^{-5} M) with picrotoxin

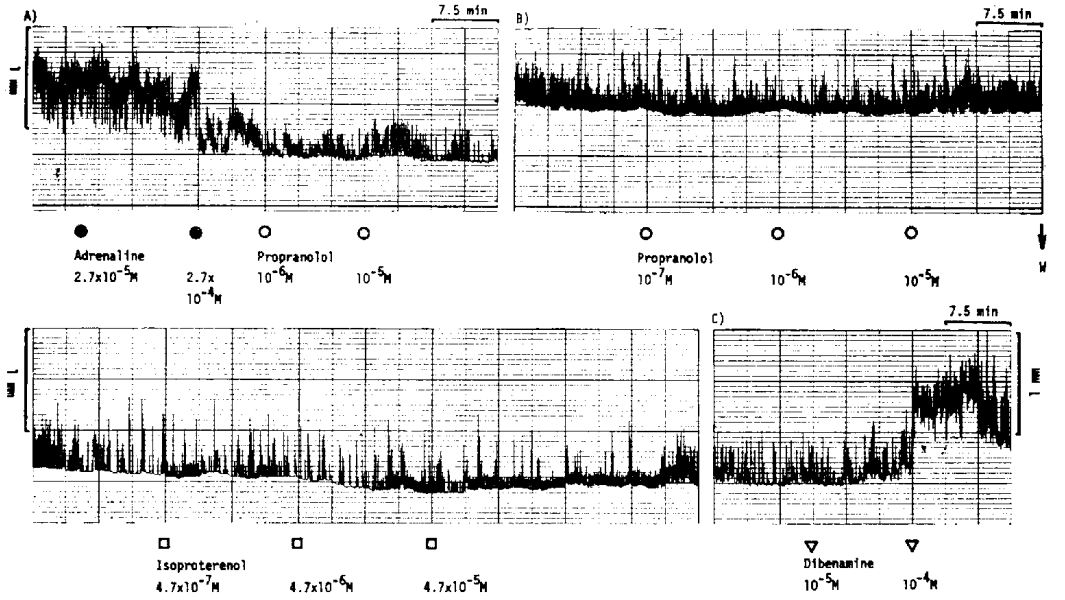

Fig. 4. Effects of various adrenergic agonists and antagonists on the motility of *A. cantonensis*. Upper and middle traces of B) are continuous. Drugs were cumulatively given. Effects of adrenaline (2.7×10^{-5}–2.7×10^{-4} M), propranolol (10^{-7}–10^{-5} M), isoproterenol (4.7×10^{-7}–4.7×10^{-5} M), and dibenamine (10^{-5}–10^{-4} M) were examined. Adrenaline and propranolol in A) were successively given. At the point W, the preparation was washed by Tyrode's solution.

(10^{-4} M) in *A. cantonensis* which had been treated with Av-Bia (3.6×10^{-15} M) (Fig. 3A).

Effects of other possible neurotransmitters on the motility of *A. cantonensis*: Glutamate (10^{-5}–10^{-4} M), glycine (10^{-5}–10^{-4} M), aspartic acid (10^{-5}–10^{-4} M), taurine (10^{-5}–10^{-4} M), and substance P (10^{-5}–5×10^{-5} g/ml) showed little effect on the motility of *A. cantonensis*.

DISCUSSION

In contrast to *A. suum*, a traditional experimental model of parasitic nematodes, there has been no report regarding the neuropharmacology of *A. cantonensis*, a new model introduced by us for pharmacological studies. Up to this time, no data have been reported concerning neuropharmacological responses, the presence of neurotransmitters and enzymes responsible for synthesizing or inactivating these transmitters in this worm.

In the present study, inhibitory mechanisms including the GABA mechanism which is similar to that reported in *A. suum* (2, 4, 5, 11) and the α-adrenergic mechanism were suggested to be in *A. cantonensis*.

Similar to the results on *Ascaris* muscle preparations (2, 4, 5, 11), the motility of a whole worm preparation of *A. cantonensis* was paralyzed by GABA, piperazine, and Av-Bia. The sensitivity of *A. cantonensis* to Av-Bia was remarkably better than that of the *Ascaris* muscle preparations, while the sensitivity to GABA and piperazine was not different (2, 4, 5, 11, 12). The paralytic action of GABA was antagonized by GABA antagonists such as bicuculline and picrotoxin (13) with N-MC, a stimulant of the release of ACh in *A. cantonensis* (14). The paralytic action of Av-Bia was also antagonized by GABA antagonists with N-MC or eserine,

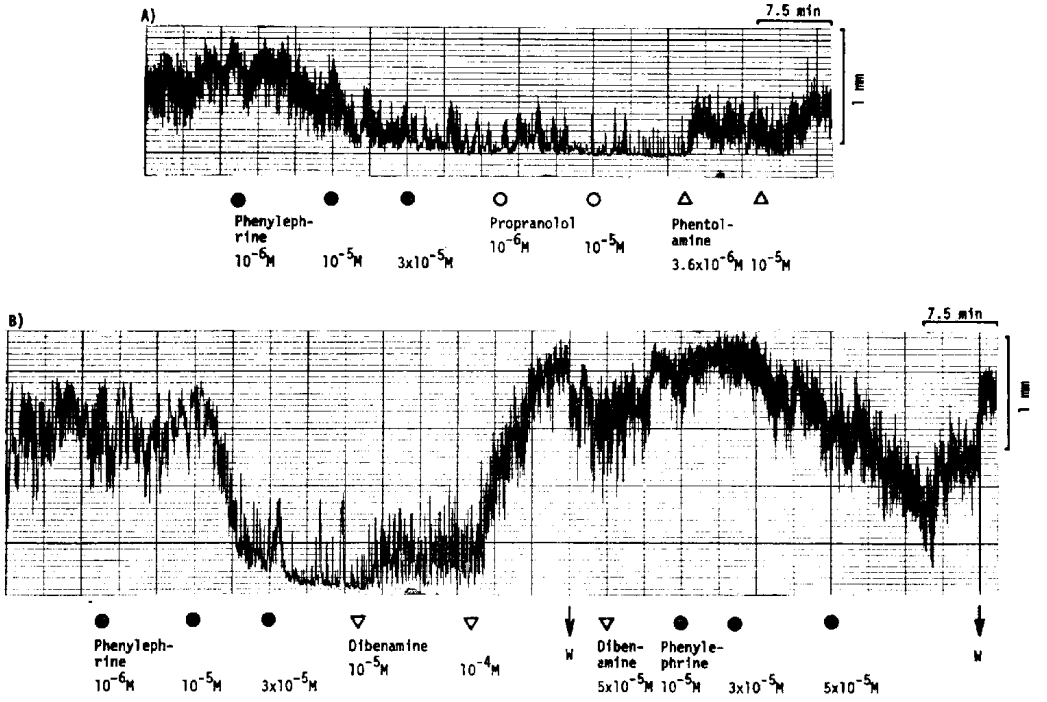

Fig. 5. Effects of phenylephrine and its antagonists on the motility of *A. cantonensis*. Drugs were given cumulatively, and agonists and antagonists were successively given. A): Effects of propranolol (10^{-6}–10^{-5} M) and phentolamine (3.6×10^{-6}–10^{-5} M) on the preparation paralyzed with phenylephrine (10^{-6}–3×10^{-5} M) were examined. B): Dibenamine (10^{-5}–10^{-4} or 5×10^{-5} M) was given before or after the treatment with phenylephrine (10^{-6}–3×10^{-5} or 10^{-5}–5×10^{-5} M). At the point W, the preparation was washed by Tyrode's solution.

but not by dibenamine with N-MC. Thus, it is suggested that GABA has a role as an inhibitory neurotransmitter in *A. cantonensis* as well as in *A. suum* (2, 4, 5, 11).

It was reported that adrenergic agents such as adrenaline showed little effect on *Ascaris* muscle preparations (1). On the other hand, the motility of *A. cantonensis* was paralyzed by α-adrenergic agonists such as adrenaline, noradrenaline, phenylephrine, methoxamine, and clonidine, but not by β-adrenergic agonists such as isoproterenol. The paralysis induced by α-adrenergic agonists was blocked by α-adrenergic antagonists such as dibenamine and phentolamine, but not by β-adrenergic antagonists such as propranolol. In addition, α-adrenergic agonists as well as GABA were affected antagonistically with excitatory cholinergic agents such as eserine and N-MC. These results suggest a possible involvement of an α-adrenergic mechanism as an inhibitory one in *A. cantonensis*. Since dibenamine stimulated remarkably the motility with or without the treatment by α-adrenergic agonists, the inhibitory role of the α-adrenergic mechanism appears to be rather significant. However, there are probably other reasons for the stimulating effect of dibenamine. For example, this effect appears to be reasonably supported through postulating that dibenamine also has a stimulant action on the excitatory cholinergic mechanism in this worm.

From the results on cholinergic agents (7),

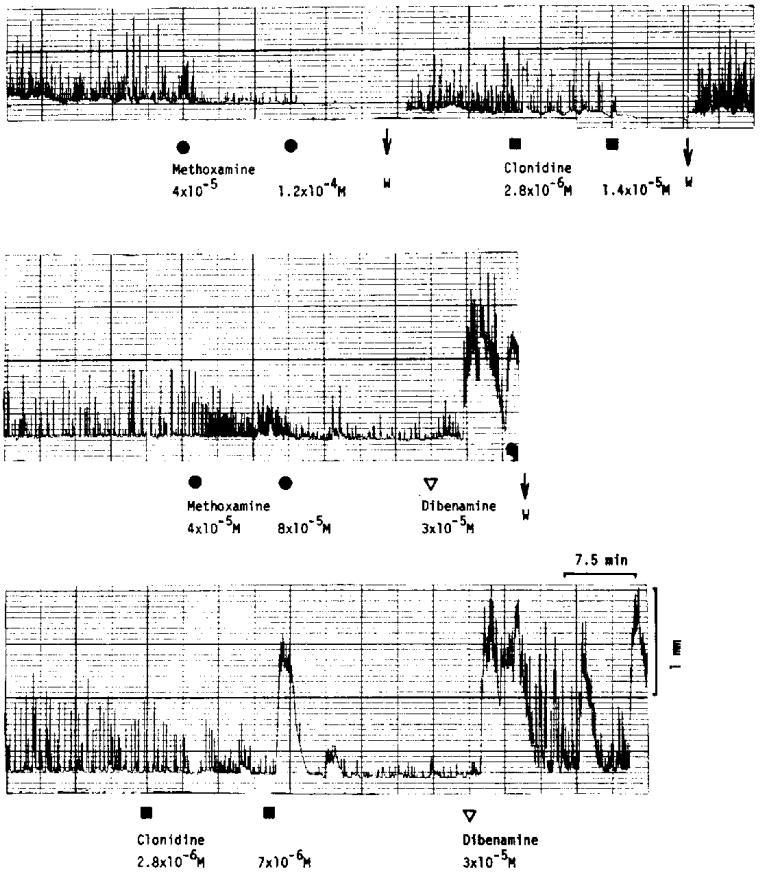

Fig. 6. Effects of methoxamine and clonidine and their antagonist, dibenamine, on the motility of *A. cantonensis*. All traces are continuous. Upper trace: Effects of methoxamine (4×10^{-5}–1.2×10^{-4} M) and clonidine (2.8×10^{-6}–1.4×10^{-5} M) were examined. Middle and lower traces: Dibenamine (3×10^{-5} M) was given after the treatment with methoxamine (4×10^{-5}–8×10^{-5} M) or clonidine (2.8×10^{-6}–7×10^{-6} M). At the point W, the preparation was washed by Tyrode's solution.

the muscle of *A. cantonensis* is suggested to be similar to the skeletal muscle rather than the ileal smooth muscle. However, the muscle is suggested to have some properties similar to the smooth muscle from the results on α-adrenergic agents and papaverine. It has been reported that 5-HT acts spastically or paralytically on the motility of the intestinal smooth muscle in mammals (15). This compound also acted spastically or paralytically on the motility of *A. cantonensis*. From the relationship between 5-HT and other agents such as α-adrenergic antagonists, eserine and strychnine, it is suggested that 5-HT probably acts through increasing the release of neurotransmitters in this worm.

The motility of *A. cantonensis* was affected by many important neuropharmacological agents such as nicotinic cholinergic agonists, GABA, α-adrenergic agonists and 5-HT, and their antagonists. In addition, atropine stimulated the motility of this worm (7). These results suggest that the responses to drugs in *A. cantonensis* are partially similar to those in the brain of mammals. Since the larvae of this worm migrate to the central nervous system

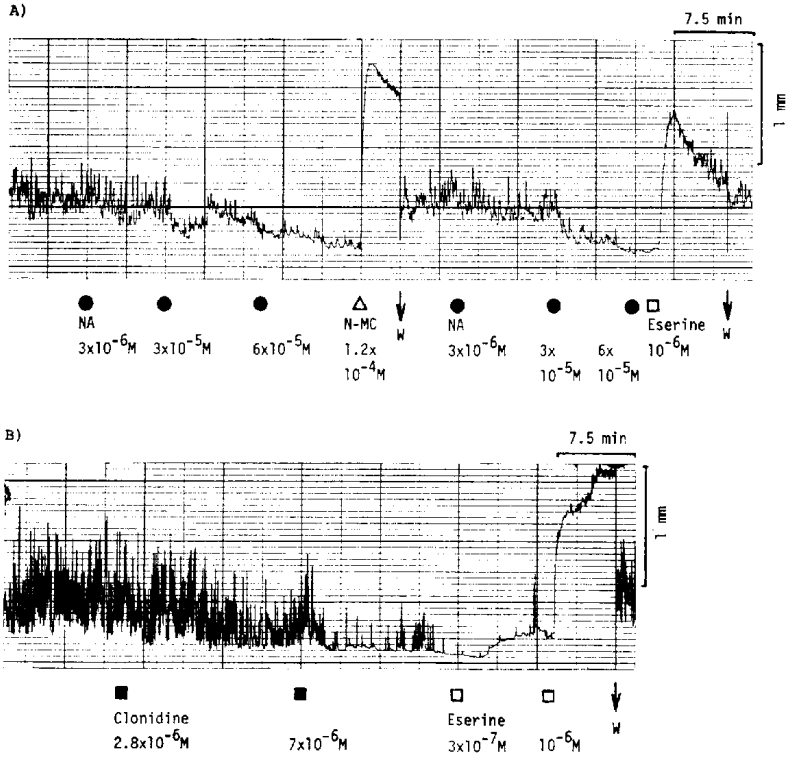

Fig. 7. Effects of N-methylcytisine (N-MC) and eserine on the preparation paralyzed with noradrenaline (NA) or clonidine in *A. cantonensis*. Some drugs were given cumulatively. A): N-MC (1.2×10^{-4} M) or eserine (10^{-6} M) was given after the treatment with NA (3×10^{-6}–6×10^{-5} M). B): Eserine (3×10^{-7}–10^{-6} M) was given after the treatment with clonidine (2.8×10^{-6}–7×10^{-6} M). At the point W, preparations were washed by Tyrode's solution.

of mammals and stay there to develop to young adult worms for about 20 days in their life cycle (16), the above-mentioned suggestion is rather likely. In the present study, however, other tentative neurotransmitters in the central nervous system such as glycine, glutamate, aspartic acid, taurine, and substance P showed little effect on the motility of *A. cantonensis*.

Thus, from these findings on the neuropharmacological properties of *A. cantonensis*, it is suggested that this worm is useful as an excellent model of nematodes for the investigation of anthelminthics. It seems also to be worthwhile studying the effects of various drugs on this worm from other viewpoints such as comparative pharmacology (8, 14). Comparison to neuromuscular junctions both in vertebrates including mammals and in invertebrates is probably interesting. In addition, this worm may also have use as one of the screening models for drugs affecting the central nervous system in mammals. Though we reported that Av-Bia paralyzed the motility of *A. cantonensis* through activating the GABA mechanism (17), it is also reported that this drug affects the GABA mechanism in the brain of mammals (18, 19) and influences the actions of drugs such as diazepam (20, 21). α-Adrenergic agonists such as clonidine and methoxamine paralyzed the motility of *A. cantonensis*, while there is interest in these drugs as cardiovascular depressants which are as-

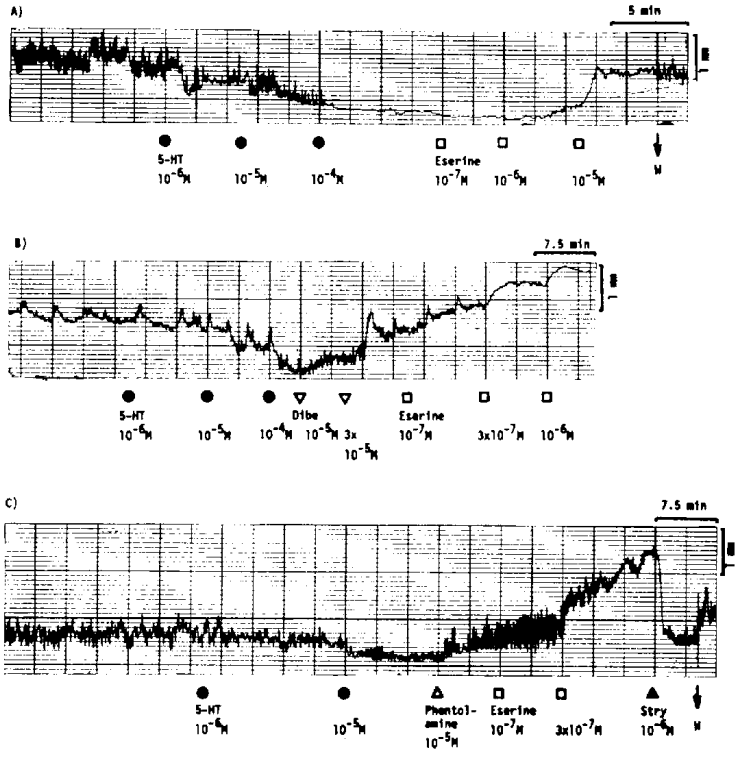

Fig. 8. Effects of 5-hydroxytryptamine (5-HT) and other agents on the motility of *A. cantonensis*. Drugs were cumulatively given. A): Eserine (10^{-7}–10^{-5} M) was given to the preparation paralyzed by 5-HT (10^{-6}–10^{-4} M). B) and C): Eserine (10^{-7}–10^{-6} or 3×10^{-7} M) was given to the preparations paralyzed by 5-HT (10^{-6}–10^{-4} M) after the treatment with dibenamine (Dibe, 10^{-5}–3×10^{-5} M) or phentolamine (10^{-5} M). At the point W, preparations were washed by Tyrode's solution.

sociated with central α-adrenoceptors and presynaptic α-adrenoceptors (22).

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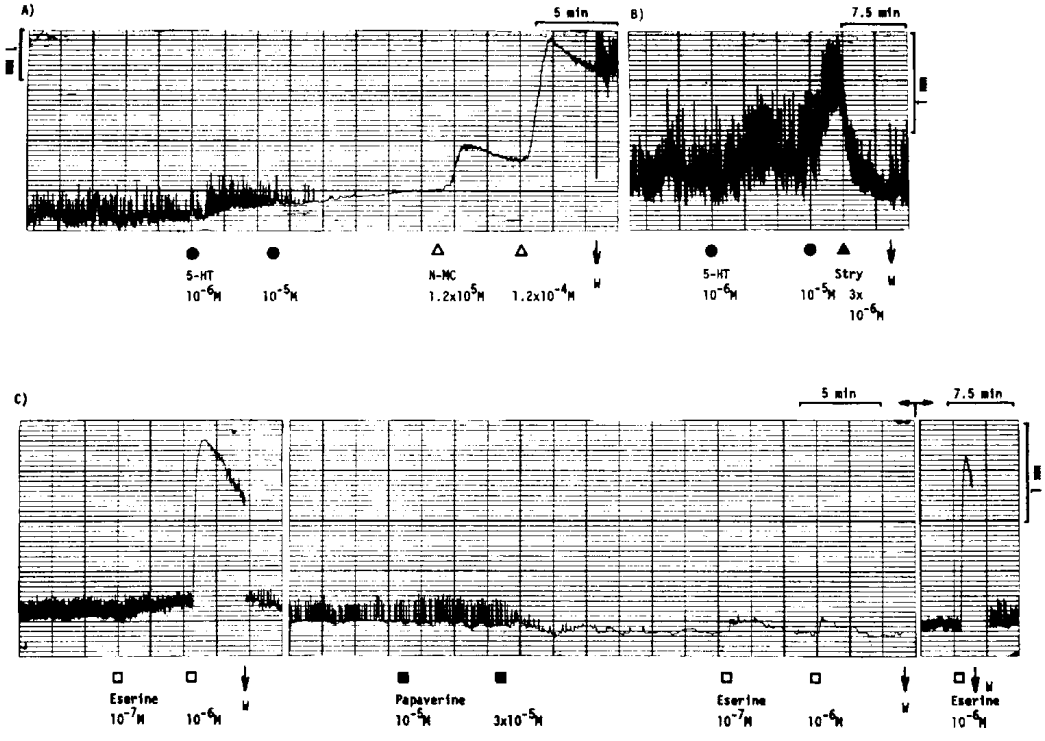

Fig. 9. Effects of 5-hydroxytryptamine (5-HT), papaverine, and other agents on the motility of *A. cantonensis*. Drugs were cumulatively given. A) and B): N-methylcytisine (N-MC, 1.2×10^{-5}–1.2×10^{-4} M) or strychnine (3×10^{-6} M) was given to the contracted preparation by 5-HT (10^{-6}–10^{-5} M). C): Eserine (10^{-7}–10^{-6} M) was given with or without the treatment with papaverine (10^{-5}–3×10^{-5} M). At the point W, preparations were washed by Tyrode's solution.

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