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Studies on Chemotherapy of Parasitic Helminths (XXI) Paralyzing Action of Hexylresorcinol on *Angiostrongylus cantonensis, Dirofilaria immitis* and *Ancylostoma caninum*

MAMORU TERADA, AKIRA I. ISHII, HIDETO KINO AND MOTOHITO SANO

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Introduction

Since Lamson *et al.* (1930) reported an anthelmintic effect of hexylresorcinol (HX) against hookworm disease, this drug has been used as a broad-spectrum anthelmintic against many parasitic worms including *Ascaris lumbricoides*, *Hymenolepis nana* and *Fasciolopsis buski* (Rollo, 1970, 1975). After the discovery of HX, many phenolic anthelmintics such as disophenol, niclosamide, dichlorophen and bithionol have been introduced as anthelmintics against nematodes, cestodes and trematodes (Cox, 1982).

Regarding mode of anthelmintic effects of phenolic drugs, it has been generally accepted that these drugs act as protoplasmic poisons (Krotov, 1970). Indeed, Lamson and Ward (1932) reported that HX acted primarily upon the nematode cuticle, since worms recovered from patients treated with the drug showed cuticular blistering. Del Castillo (1969) stated in his review on pharmacology of nematodes that HX had an ability to produce blisters and necrosis in the parasites because of its phenolic nature. However, Baldwin (1943) observed that the muscle strips of *Ascaris* were completely paralyzed long before any blistering could be detected. Then, he could not agree that HX owed its powerful nematocidal efficacy to its action upon the cuticle. Unfortunately, he did not study on the mechanism by which the paralysis was caused, probably because intact *Ascaris* or anterior part of it is influenced only slightly by the most of neuropharmacological agents (Baldwin, 1943; Del Castillo and Morales, 1969; Natoff, 1969). Thus, to define the mode and mechanism of action of HX, its effects on the motility of some parasitic nematodes including *Angiostrongylus cantonensis*, *Dirofilaria immitis* and *Ancylostoma caninum* were investigated by means of the isotonic transducer method (Sano *et al.*, 1981; Terada *et al.*, 1982a) in this study.

Department of Parasitology, Hamamatsu University School of Medicine, Hamamatsu 431-31, Japan.

Materials and Methods

Angiostongylus cantonensis was obtained from rats (Wistar strain) experimentally infected in our laboratory. *Dirofilaria immitis* and *Ancylostoma caninum* were obtained from dogs sacrificed at the Shizuoka Prefectural Dog Center. Whole worms of female *A. cantonensis* and *A. caninum* and anterior parts of female *D. immitis* (about 2.5 cm long) were used. The worm preparation was suspended in Tyrode's solution in a thermostatically controlled organ bath (glass, 7 ml in capacity) at 35°C and gassed slightly with air to diffuse the added drugs. Responses of the worms were 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 0.7 to 0.8 g. In all figures, a relaxation of worm

preparations is indicated by a downward deflection of the tracing recorder pen. Drugs in a single or cumulative dose were given successively at the points shown by the symbols in the figures; preparations were kept exposed to drugs until the end of experiments or until they were washed with Tyrode's solution for about 30 min at times shown by point W in the figures.

The following drugs were used: eserine salicylate, bicuculline (Sigma); 1, 1-dimethyl-4-phenylpiperazinium iodide (DMPP, Aldlich); guanidine hydrochloride, hexylresorcinol (HX, Wako); strychnine sulfate, dibenamine hydrochloride (Nakarai); picrotoxin, nicotine tartrate (Tokyokasei); phentolamine mesylate (Takeda); and pyrantel tartrate (Pfizer Taito). N-methylcytisine (N-MC), an alkaloid from *Sophora flavescens*, was kindly supplied from Dr. Tadataka Noro (Department of Pharmacognosy, Shizuoka College of Pharmacy). The bicuculline and N-MC

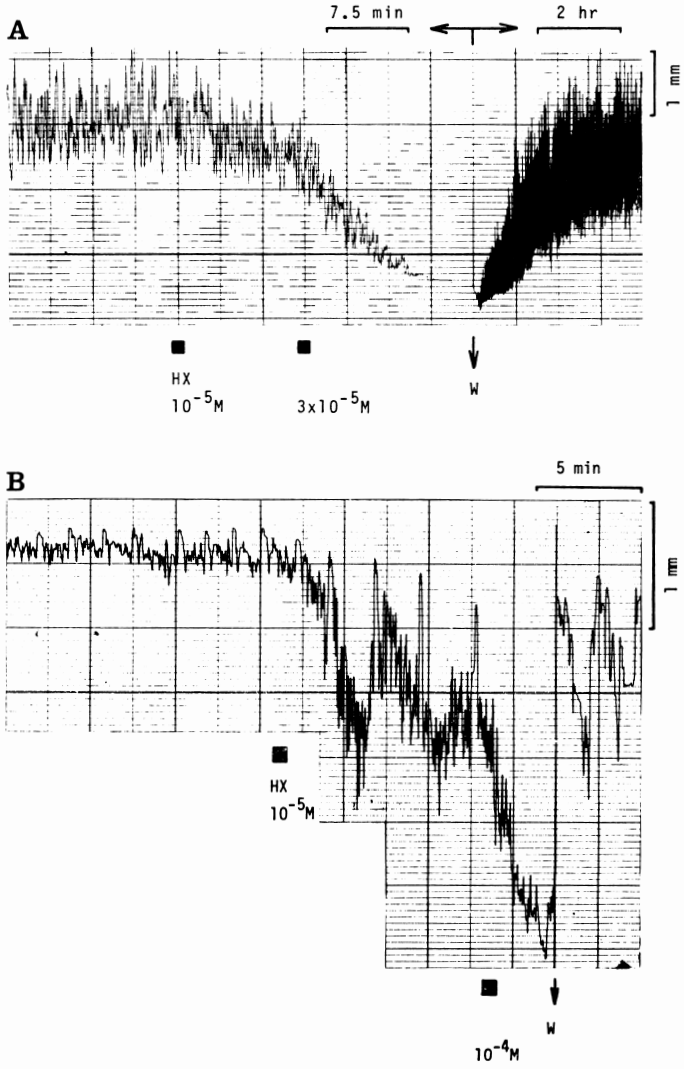

Fig. 1 Effects of hexylresorcinol (HX, ■) on the motility of *Dirofilaria immitis* (A) and *Ancylostoma caninum* (B).

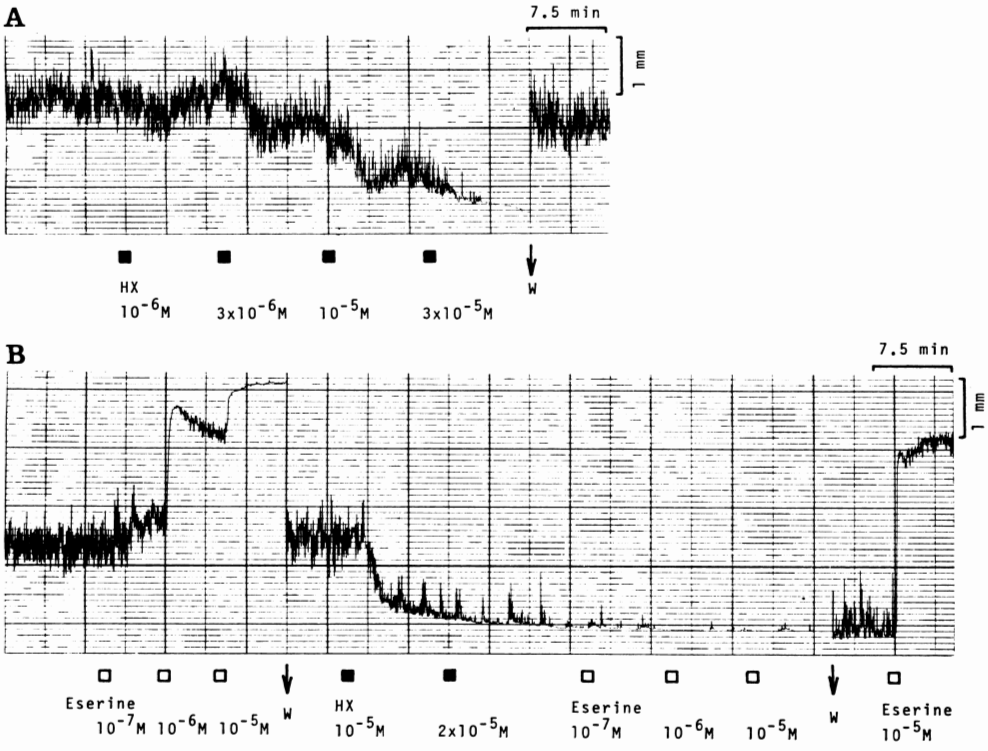

Fig. 2 Effects of hexylresorcinol (HX, ■) on the motility (A) and on the contraction elicited by eserine (□, B) in *Angiostrongylus cantonensis*.
Eserine was used as an inhibitor of acetylcholinesterase.

were dissolved in dimethyl sulfoxide and ethanol, respectively, and added to the organ bath. Final concentrations of the solvents were 0.5 % or less, which had little effect on the motility of the worm preparation. Other drugs were dissolved in a 0.9 % NaCl solution and the concentrations refer to the weight of the salts.

Results and Discussion

Similarly to the paralyzing action on the muscle strips of *A. suum* at the concentrations of 10^{-4}–2×10^{-4} g/ml (5.2×10^{-4}–10^{-3} M) (Baldwin, 1943), HX (10^{-5}–10^{-4} M) caused a paralyzing effect on the anterior preparation of *D. immitis* and on the whole worm preparation of *A. caninum* (Fig. 1). On the whole worm preparation of *A. cantonensis*, the paralyzing effect was seen at concentrations of 10^{-6} M and higher, and a complete paralysis was caused at 2×10^{-5}–3×10^{-5} M (Figs. 2–5). A decrease of tone was commonly caused by HX in these preparations, and a decrease of both amplitude and frequency was also seen in the paralyzed preparations of *D. immitis* and *A. cantonensis* (Figs. 1 and 2). In all preparations, the effect was reversed by washing with Tyrode's solution (Figs. 1–5). Thus, paralysis is thought to be a common mode of action of this anthelmintic on nematode worms.

The mechanism of the paralyzing action of HX was then studied using the whole worm preparation of *A. cantonensis*, which has been selected by present authors instead of *Ascaris* as a suitable parasitic nematode for detecting and determining antinematodal effects of drugs (Sano *et al.*, 1981; Terada *et al.*, 1982a). As described in previous papers (Terada *et al.*, 1982b, c, 1983, 1984), paralysis in *A. cantonensis*

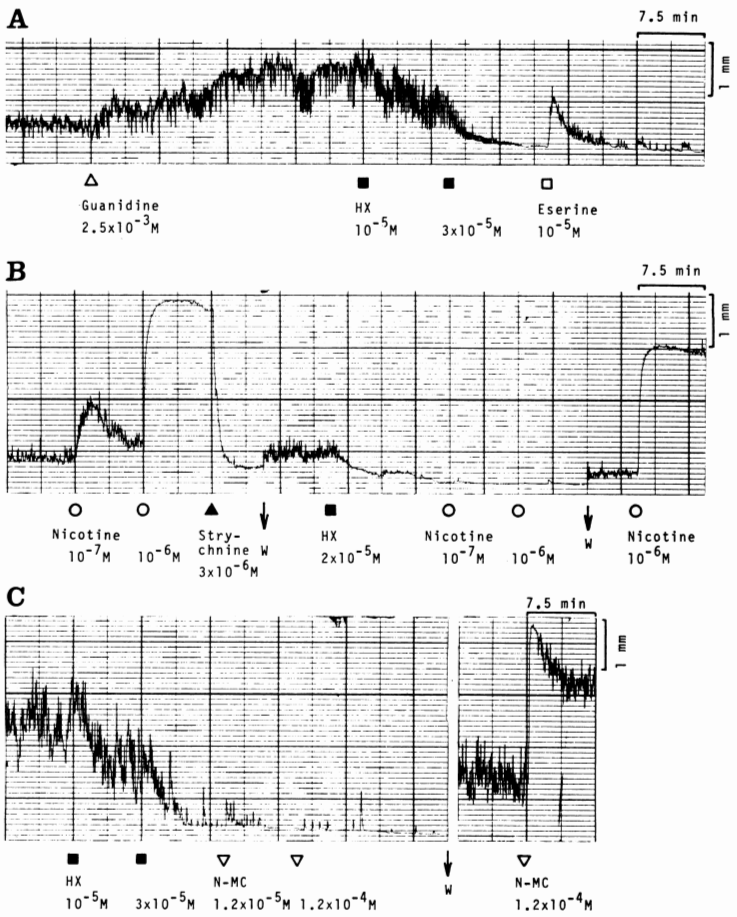

Fig. 3 Effects of hexylresorcinol (HX, ■) on the contraction elicited by guanidine (△, A), nicotine (○, B) or N-methylcitisine (N-MC) (▽, C) in *Angiostrongylus cantonensis*. Drugs were used to stimulate the release of acetylcholine (ACh) by the worm (guanidine, nicotine and N-MC), as an inhibitor of the release of ACh by the worm (strychnine, ▲), and as an inhibitor of acetylcholinesterase (eserine, □).

could be caused by either stimulation of the gabergic and/or α-adrenergic mechanisms, or by inhibition of the cholinergic mechanism in the worm's nervous system.

It is well known in various animals that eserine inhibits the acetylcholinesterase activity and accordingly acetylcholine (ACh) endogenously released from the cholinergic nerve endings is accumulated. When eserine (10^{-7}–10^{-5} M) was given to the whole worm preparation of *A. cantonensis*, a remarkable contraction was caused probably through the accumulation of ACh. But eserine (10^{-7}–10^{-5} M) did not cause contration in the paralyzed preparation by HX (10^{-5}–3×10^{-5} M) (Fig. 2B).

In the worm preparation, some agents such as guanidine, nicotine and N-methylcitisine (N-MC) are said to stimulate the release of ACh from the cholinergic nerve terminals (Terada *et al.*, 1982a, b). It is also reported that the contraction elicited by these agents is antagonized by strychnine, an inhibitor of the release of ACh in the worm (Terada *et*

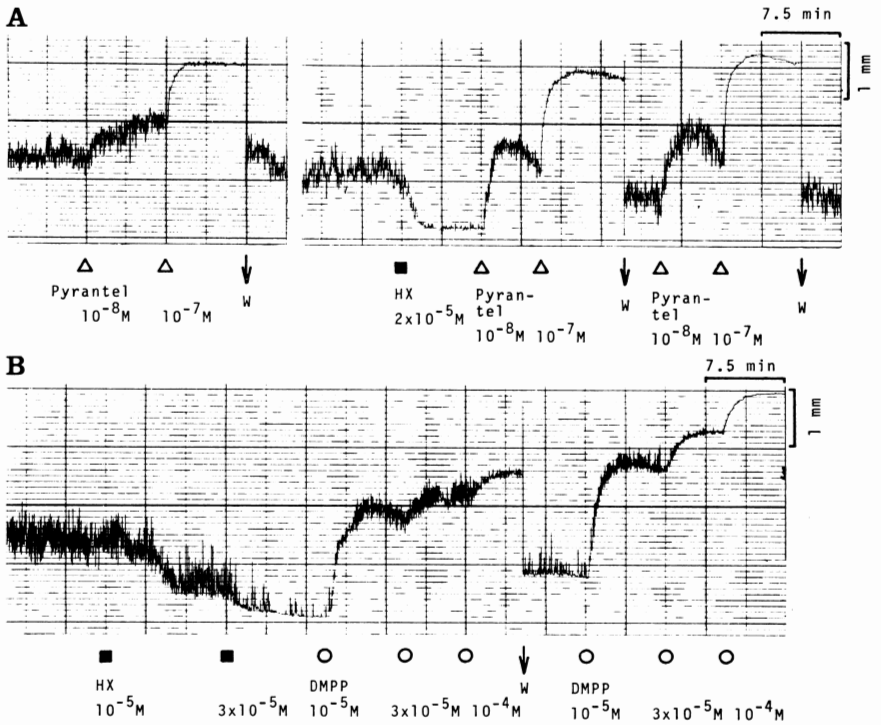

Fig. 4 Effects of hexylresorcinol (HX, ■) on the contraction elicited by pyrantel (△, A) and 1, 1-dimethyl-4-phenylpiperazinium (DMPP) (○, B) in *Angiostrongylus cantonensis*. Pyrantel and DMPP were used as a nicotinic cholinergic agonist.

al., 1982b, 1983, 1984). Contraction elicited by guanidine (2.5×10^{-3} M), nicotine (10^{-7}–10^{-6} M) or N-MC (1.2×10^{-4} M) was antagonized by the treatment with HX (10^{-5}–3×10^{-5} M). (Fig. 3A, B and C).

Pyrantel and 1, 1-dimethyl-4-phenylpiperazinium (DMPP) are reported to stimulate directly the nicotinic cholinergic receptors in nematode and mammlian preparations (Aubry *et al.*, 1970; Chen *et al.*, 1951; Terada *et al.*, 1982a, 1983). These agents did contract the paralyzed preparation by HX (2×10^{-5}–3×10^{-5} M) (Figs. 4A, B and 5A), and the amplitude of contraction elicited by pyrantel (10^{-8}–10^{-7} M) or DMPP (10^{-5}–10^{-4} M) was influenced slightly by HX (Fig. 4A and B). Though the contraction elicited by drugs which are related to the accumulation of the endogenous ACh was blocked by HX (Figs. 2B and 3), this anthelmintic did not block the contraction induced by drugs directly acting on the nicotinic cholinergic receptors (Fig. 4). These results suggest that HX probably paralyzes the worm through inhibiting the release of ACh from the cholinergic nerve terminals but not through blocking the nicotinic cholinergic receptors on the muscle of *A. cantonensis*.

It was reported that the paralyzing action of α-adrenergic agonists such as noradrenaline and phenylephrine or gabergic agonists such as γ-aminobutyric acid (GABA) and ivermectin were antagonized by their specific antagonists, i.e., α-adrenergic antagonists such as dibenamine and phentolamine and gabergic antagonists such as picrotoxin and bicuculline (Terada *et al.*, 1982c, 1984). The paralyzed preparation by HX (3×10^{-5} M) was not reversed by bicuculline (3×10^{-5} M) and phentolamine (8.1×10^{-6} M) in the presence of eserine (10^{-5} M) (Fig. 5A), and also by dibenamine (10^{-4} M) and picrotoxin (10^{-4} M)

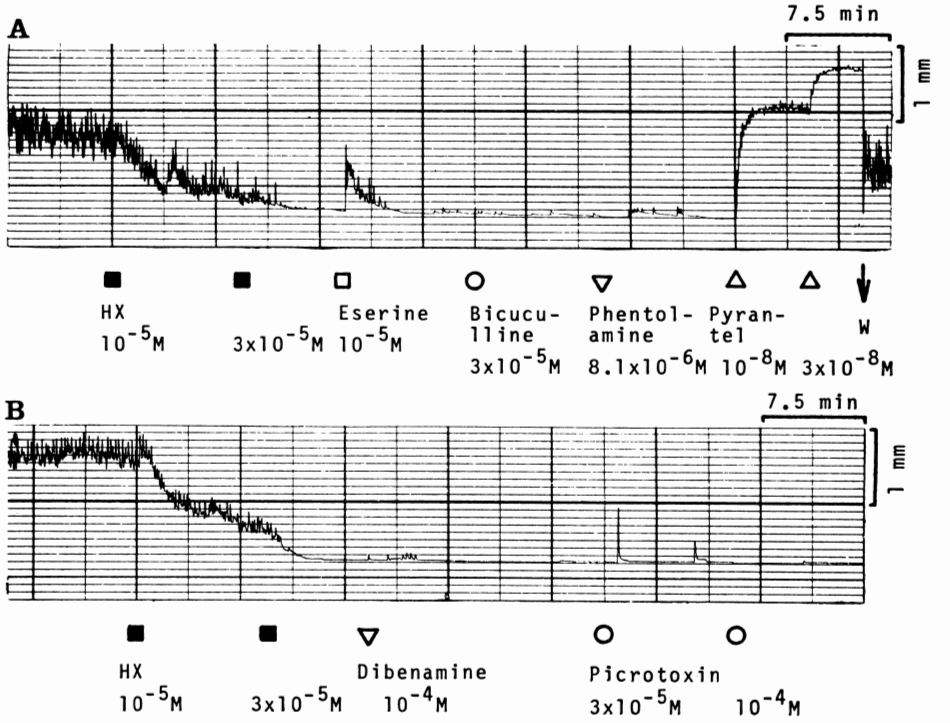

Fig. 5 Effects of gabergic and α-adrenergic antagonists and cholinergic agonists on the paralyzed preparations by hexylresorcinol (HX, ■) in *Angiostrongylus cantonensis*. Drugs were used as an inhibitor of acetylcholinesterase (eserine, □), as gabergic antagonists (picrotoxin and bicuculline, ○), as α-adrenergic antagonists (dibenamine and phentolamine, ▽) and as a nicotinic cholinergic agonist (pyrantel, △).

(Fig. 5B). From these results, it is likely that the paralysis due to HX is caused through inhibiting the cholinergic mechanism above mentioned, but not caused through stimulating α-adrenergic and/or gabergic mechanisms.

These results strongly support the opinion of Baldwin (1943) that a powerful nematocidal efficacy of HX is due to its paralyzing action. There have been some excellent antinematode anthelminthics which paralyze worms by stimulating the gabergic mechanism of nematodes, such as piperazine and ivermectin (Del Castillo 1969; Del Casillo and Morales, 1969; Terada *et al.*, 1984). Therefore, drugs such as HX which inhibit the cholinergic mechanism possibly act synergistically with the gabergic anthelmintics, and cause stronger paralysis in nematode worms.

Summary

Effects of hexylresorcinol (HX) on the motility of some nematode preparations were examined using the isotonic transducer method. HX paralyzed *Angiostrongylus cantonensis*, *Dirofilaria immitis*, and *Ancylostoma caninum*, and the effect was reversed by washing with Tyrode's solution. The mechanism of the paralyzing action of HX on *A. cantonensis* was examined in detail. The contraction elicited by drugs such as eserine, guanidine, nicotine and N-MC was blocked by the treatment with HX, but this anthelmintic did not block the contraction induced by pyrantel and DMPP. The paralyzed preparations by HX were not reversed by gabergic antagonists such as picrotoxin and bicuculline, or α-adrenergic antagonists such as dibenamine and phentolamine. It is con-

cluded that HX paralyzes *A. cantonensis* by inhibiting the cholinergic mechanism (probably inhibiting the release of acetylcholine from the cholinergic nerve terminals), but not by stimulating the gabergic and/or α-adrenergic mechanisms.

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寄生蠕虫症の化学療法に関する研究（XXI） 広東住血線虫，犬糸状虫および犬鉤虫に対する hexylresorcinol の麻痺作用について

寺田 護 石井 明 記野秀人 佐野基人
（浜松医科大学寄生虫学教室）

Hexylresorcinol（HX）の抗線虫作用に，神経薬理学的機序が関与するか否かを追究するため，アイソトニック・トランスデューサー法を用いて，3種線虫標本に対する HX の作用を検討した．(1) HX は広東住血線虫，犬糸状虫および犬鉤虫のいずれに対しても，麻痺的に作用し，その作用は可逆的であった．(2) 広東住血線虫では，eserine，guanidine，nicotine および N-MC（内因性 ACh の蓄積と関連して収縮をひき起こす薬物）による収縮は HX で阻害されたが，pyrantel および DMPP（直接，ACh 受容体を刺激して収縮をひき起こす薬物）の作用は HX でほとんど影響されなかった．HXで麻痺した標本は，GABA 拮抗薬（picrotoxin, bicuculline）ないし α-アドレナリン拮抗薬（dibenamine, phentolamine）で回復しなかった．これらの結果から，HX は線虫類に対し麻痺的に作用し，その麻痺は，GABA 機構とか α-アドレナリン作働機構の促進よりも，むしろ，コリン作働機構の抑制（恐らく，ACh の遊離の阻害）によることが示唆された．