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Research Note

Studies on Chemotherapy of Parasitic Helminths (XXV) Neuropharmacological Action of Niclosamide on *Dipylidium caninum*

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Regarding the mechanism of anticestodal action of niclosamide, there have been a few studies which revealed inhibitory effects of the anthelmintic on energy metabolism such as glucose- and oxygen-uptake and oxidative phosphorylation (Strufe and Gönnert, 1960; 1967). Recently, in our study on the paralyzing action of hexylresorcinol, a phenolic anthelmintic like niclosamide, it was suggested that the drug inhibited the release of acetylcholine (ACh) from the cholinergic nerve endings of *Angiostrongylus cantonensis* (Terada *et al.*, 1985a). In previous studies, we have selected *Dipylidium caninum* as a model worm for detecting and determining anticestodal effects of drugs, and effects of various neuropharmacological agents on the motility of this worm were studied (Terada *et al.*, 1982). Thus, whether a neuropharmacological mechanism is involved in the anticestodal action of niclosamide or not was examined on this cestode in the present study.

Worms were obtained from dogs sacrificed at the Shizuoka Prefectural Dog Center. Using a mature or gravid proglottid as the experimental preparation, the isotonic transducer method was carried out as described previously (Terada *et al.*, 1982).

Niclosamide at the concentrations of 3×10^{-7} M and higher caused stimulatory effects on the motility of *D. caninum* (Figs. 1, 2). An increase of tone was remarkable at 6×10^{-7} M, and spastic paralysis was seen at 3×10^{-6} M. Though the stimulatory effects of the drug in lower concentrations were reversed by washing with Tyrode's solution, the contraction elicited by a 60 min-exposure to 3×10^{-6} M was not reversed and the tone remained increasing even at 12 hr after washing (Fig. 1A). Similar stimulatory and sustained mode of action of niclosamide (3×10^{-6} M) was seen in other cestodes as *Diplogonoporus grandis* (Terada *et al.*, 1985b).

From our results on the effects of various neuropharmacological agents on the motility of *D. caninum* (Terada *et al.*, 1982), inhibitory cholinergic and excitatory serotonergic mechanisms were suggested to be in this cestode which were similar to those reported in trematodes like *Schistosoma mansoni* (Bueding and Bennett, 1972) and *Fasciola hepatica* (Bueding and Bennett, 1972; Mansoure, 1984). That is, the motility of *D. caninum* was inhibited by various cholinergic agents including eserine (10^{-5} M, an inhibitor of acetylcholinesterase activity), N-methylcytisine (N-MC, 1.2×10^{-3} M, a stimulator of the release of ACh from the cholinergic nerve endings) and carbachol ($10^{-4}-3\times10^{-4}$ M, a stimulator of the nicotinic cholinergic receptors) (Terada *et al.*, 1982, 1985a). In the present study, however, eserine (10^{-5} M) didn't cause paralysis when this drug was

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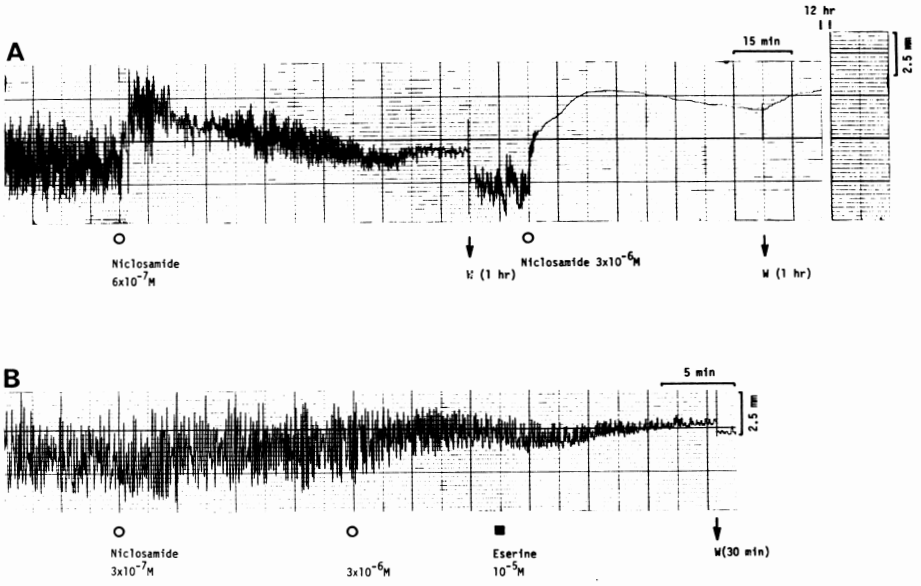

Fig. 1 Effects of nicolsamide on the motility of *Dipylidium caninum*. Proglottid preparations of *D. caninum* were used with a tension of 0.40 to 0.45 g. In B), the effect of eserine on the stimulatory action of niclosamide was also examined.

given after the treatment with niclosamide ($3\times10^{-7}-3\times10^{-6}$ M) (Fig. 1B). Whereas niclosamide ($3\times10^{-7}-3\times10^{-6}$ M) showed only a slight effect when it was added to the preparation pretreated with carbachol (10^{-4} M) (Fig. 2A) and the paralyzing action of N-MC was seen when the drug (1.2×10^{-3} M) was given to the preparation contracted by niclosamide (3×10^{-6} M) (Fig. 2B). These results may be more reasonably understood when we assume that niclosamide inhibits the release of ACh from the cholinergic nerve endings of this worm. In other words, under conditions in which the release of ACh was blocked by niclosamide, eserine couldn't cause paralysis by accumulating endogenous ACh, but carbachol could paralyze the worm by simulating directly the cholinergic receptors and N-MC also could antagonize the action of niclosamide through stimulation of the release of ACh. In relation to this assumption, there were some reports describing the effects of phenolic agents including phenol, *p*-nitrophenol, picric acid and hexylresorcinol on the release of ACh from the cholinergic nerve terminals of various animals (Otsuka and Nonomura, 1963; Takagi and Takayanagi, 1965; Terada *et al.*, 1985a).

As described above, if the cholinergic mechanism in *D. caninum* is inhibited by niclosamide, contraction should be caused by a resultant stimulation of the serotonergic mechanism. As to this mechanism, it was reported that the stimulatory action of 5-hydroxytryptamine (5-HT, serotonin, 10^{-4} M) was antagonized by tryptophol (10^{-4} M, a tryptophan metabolite of African trypanosomes, Stibbs and Seed, 1973) (Terada *et al.*, 1982). Tryptophol (10^{-4} M) also paralyzed the preparation contracted by niclosamide (3×10^{-6} M) (Fig. 2C). Thus, this strong paralysis is probably elicited through inhibition of both cholinergic and serotonergic mechanisms with these two agents.

Conclusively, it is suggested from these results that niclosamide has a neuropharmacological action besides one on energy metabolism and that the anthelmintic may contract *D. caninum* by inhibiting the cholinergic mechanism, though a possibility of a

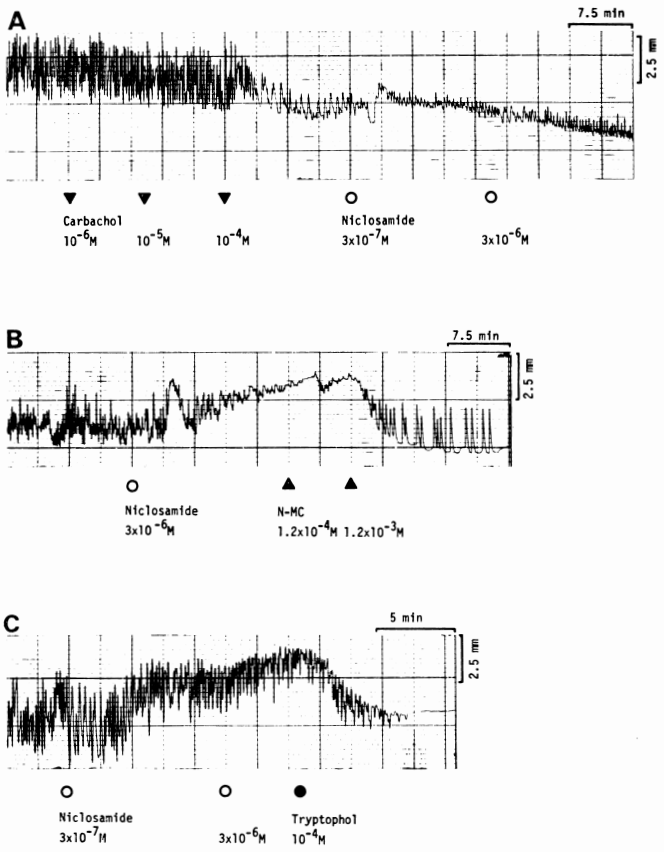

Fig. 2 Effects of carbachol (A), N-methylcytisine (N-MC, B) and tryptophol (C) on the stimulatory action of niclosamide in *D. caninum*.

direct stimulation on the serotonergic mechanism by this drug can't be excluded.

Recferences

1) Bueding, E. and Bennett, J. (1972): Neurotransmitters in trematodes. Comparative Biochemistry of Parasites, Academic Press, New York and London, 95-99.
2) Mansour, T. E. (1984): Serotonin receptors in parasitic worms. Ad. Parasitol., 23, 1-36.
3) Otsuka, M. and Nonomura, Y. (1963): The action of phenolic substances on motor nerve endings. J. Pharmacol. Exp. Ther., 140, 41-45.
4) Stibbs, H. H. and Seed, J. R. (1973): Chromatographic evidence for the synthesis of possible sleep-mediators in *Trypanosoma brucei gambiense*. Experientia, 29, 1563-1565.
5) Strufe, R. and Gönnert, R. (1960): Experimentelle Untersuchungen mit N-(2'-Chlor-4'-nitrophenyl)-5-chlor-salicylamide, einem neuen Bandwurmmittel. Arzneimittel-Forsch., 10, 886-890.
6) Strufe, R. and Gönnert, R. (1967): Über die Beeinflussung des Bandwurmstoffwechsels druch Arzneimittel. Z. Tropenmed. Parasitol., 18, 193-202.
7) Takagi, K. and Takayanagi, I. (1965): Effects of aromatic nitrocompounds and phenol derivatives on the cholinergic nerve ending of smooth muscle and skeletal muscle. Arch. Int. Pharmacodyn., 155, 373-380.
8) Terada, M., Ishii, A. I., Kino, H. and Sano, M. (1982): Studies on chemotherapy of parasitic helminths (VI). Effects of various neuropharmacological agents on the motility of *Dipylidium caninum*. Jpn. J. Pharmacol., 32, 479-488.

9) Terada, M., Ishii, A. I., Kino, H. and Sano, M. (1985a): Studies on chemotherapy of parasitic helminths (XXI). Paralyzing action of hexylresorcinol on *Angiostrongylus cantonensis*, *Dirofilaria immitis* and *Ancylostoma caninum*. Jpn. J. Parasitol., 34, 79-86.

10) Terada, M., Fujiu, Y. and Sano, M. (1985b): Effects of some neuropharmacological agents and anthelmintics on the motility of mature proglottids of *Diplogonoporus grandis*. Jpn. J. Parasitol., 34, 517-519.

短　報

寄生蠕虫症の化学療法に関する研究（XXV）；瓜実条虫に対する niclosamide の神経薬理学的作用について

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Niclosamide の抗条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，瓜実条虫の自動運動に対する niclosamide の作用を検討した．(1) 瓜実条虫の自動運動に対し，niclosamide は 3×10^{-7} M 以上の濃度で興奮作用を示し，3×10^{-6} M では収縮性麻痺を生じた．3×10^{-6} M を 1 hr 作用させた場合の収縮作用は，タイロード液による洗浄によっても回復しなかった．(2) Niclosamide による収縮はアセチルコリン（ACh）の遊離促進を介して虫体を麻痺させる薬物（N-MC）とかアセチルコリン受容体に直接作用して虫体を麻痺させる薬物（carbachol）により拮抗されたが，アセチルコリンエステラーゼ活性の阻害により内因性 ACh の蓄積を介して虫体を麻痺させる薬物（eserine）によっては拮抗されなかった．一方，本駆虫薬による収縮は，瓜実条虫においてセロトニンと拮抗作用を示す薬物（tryptophol）によっても拮抗された．これらの結果から，niclosamide は，瓜実条虫のコリン作動機構の抑制（恐らく，ACh の遊離の阻害）の結果生ずるセロトニン作動機構の優位状態を介して虫体を収縮させることが示唆された．