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Research Note

Gabergic Mechanism in *Angiostrongylus costaricensis* in Connection with Sensitivity to Gabergic Drugs*

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In our studies using *Angiostrongylus cantonensis* and *A. costaricensis* as model worms for studying chemotherapy of parasitic diseases caused by blood nematodes, it has become apparent that abrupt killing of worms often leads adverse systemic reactions in the host, elicited by allergens released from killed worms and distributed through the blood system. For example, when adult worms of *A. costaricensis* were killed with mebendazole, an inhibitor of glucose uptake (Vanden Bossche, 1972), more host mice died more rapidly than in non-treated group (Terada *et al.*, 1988). Thus, it seems reasonable to kill worms as gradually as possible by indirect means such as by a paralyzing action of neuropharmacological anthelmintics. Inhibition in a stimulatory cholinergic mechanism and/or stimulation in an inhibitory gabergic mechanism in the worm will make nematodes paralyzed neuropharmacologically (Del Castillo, 1969; Terada *et al.*, 1984). In the case of *A. cantonensis,* such a gradual lethal effect was attained by successive daily administration of milbemycin D, a gabergic stimulant (Terada *et al.*, 1987). We have reported in the previous paper that *A. costaricensis* was, however, little sensitive to gabergic drugs among various anthelmintics examined (Terada *et al.*, 1986). The difference in the sensitivity may be attributable to the age of worms, because we used 3 to 6 month-old worms of *A. cantonensis* and 30 to 40 day-old worms of *A. costaricensis* in the experiment. Then we studied the gabergic mechanism comparatively in both species of *Angiostrongylus* at various ages for the sensitivity to gabergic drugs and the present note deals with these results.

Adult worms of *A. costaricensis* (Costa Rica strain) at 10 and 15 week- and 7 month-old, and *A. cantonensis* (Hawaiian strain) at 5, 7 and 10 week-old were obtained from experimentally infected ddY mice and Wistar rats, respectively. The isotonic transducer method previously described (Terada *et al.*, 1984) was used for monitoring the motility of the worms.

As compared with insensitivity in younger worms of *A. costaricensis* of 30 to 40 day-old in the previous study (Terada *et al.*, 1986), all worms of *A. cantonensis* of 5, 7 and 10 week-old responded well to GABA (3×10^{-5} to 3×10^{-4} M), and paralysis was caused, though the response of 5 week-old worms was slightly less (Fig. 1). On the other hand, when *A. costaricensis* at older ages was treated with GABA (10^{-4} to 6×10^{-4} M or 10^{-3} M), all worms of 10 week- to 7 month-old responded only slightly. That is, the motility of 10 week-old worms was slightly stimulated, 15 week-old worms were *little* influenced, and 7 month-old worms were slightly inhibited. Ivermectin (2.5×10^{-8} g/ml) paralyzed *A. cantonensis* completely within 7 min in 70, 90

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and 93% of 5, 7 and 10 week-old worms (n = 10 to 17), respectively. Remaining worms of every age showed marked inhibition in the motility within 15 min by the treatment. When *A. costaricensis* was treated with ivermectin (2.5×10^{-7} g/ml), however, the motility was stimulated at 22, 33 and 50% in 10 and 15 week- and 7 month-old worms (n = 8 or 9), respectively. In remaining worms of all ages, complicated reactions of a slight inhibition and/or stimulation in tone, rate and/or amplitude were seen at various probabilities. From these results, we may conclude that regardless of their age, *A. costaricensis* has extremely less sensitivity to gabergic agonists than *A. cantonensis* does.

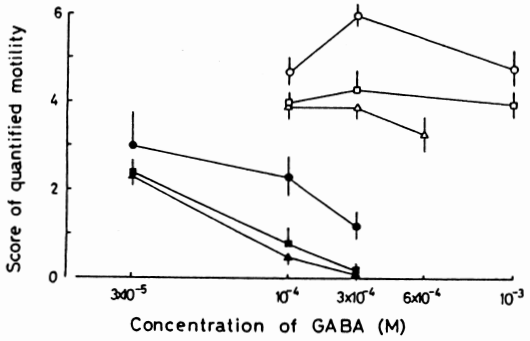

Fig. 1. Effects of GABA on the motility of *A. costaricensis* and *A. cantonensis*. Quantification of the motility was made by estimating the motility as follows; 0, complete paralysis; 2, moderately inhibited in tone, rate and/or amplitude; 4, little influenced; 6, moderately stimulated in tone, rate and/or amplitude and 8, complete spastic paralysis. Intermediate score of 1, 3, 5, 7 was given to intermediary changes between them, respectively. Each point represents mean ± SE.
A. costaricensis (n = 8 or 9): ○, 10 week-old; □, 15 week-old; △, 7 month-old, *A. cantonensis* (n = 10 to 17): ●, 5 week-old; ■, 7 week-old; ▲, 10 week-old.

The paralytic response in *A. cantonensis* to GABA and ivermectin was blocked well with gabergic antagonists such as picrotoxin and bicuculline as previously reported (Terada *et al.*, 1984). On the other hand, in the preparation of *A. costaricensis* against which GABA (6×10^{-4} M) had little influence, paralysis was caused by addition of strychnine (10^{-5} M), an inhibitor of the release of acetylcholine in the worms (Terada *et al.*, 1984). The paralysis was partially recovered by further addition of picrotoxin (5×10^{-5} M) (Fig. 2A). A stimulated motility was caused when

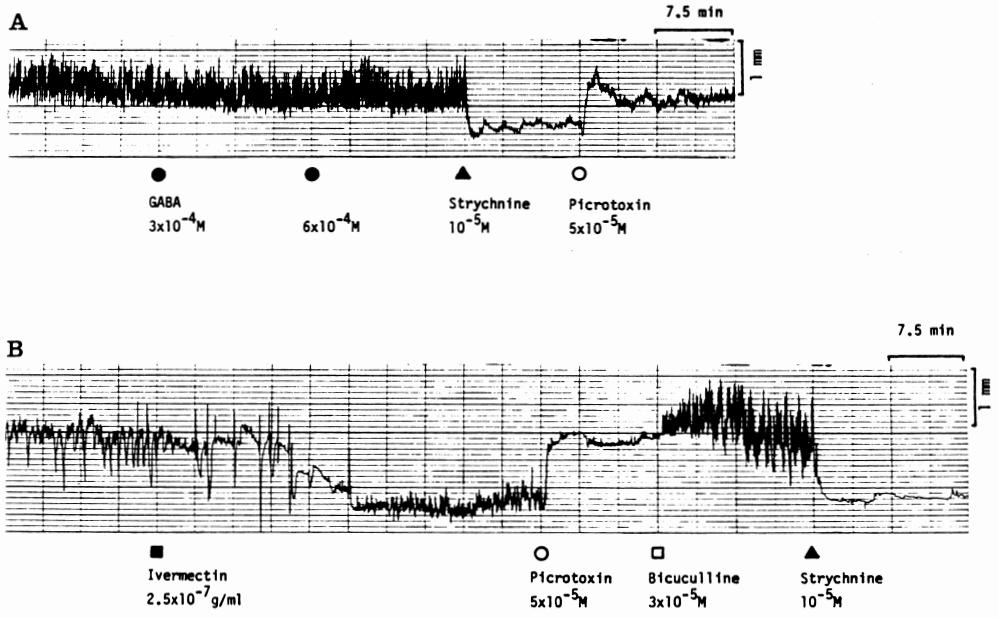

Fig. 2. Effects of some gabergic and cholinergic drugs on the motility of *A. costaricensis* of 7 month-old.

picrotoxin (5×10^{-5} M) and bicuculline (3×10^{-5} M) were given to the slightly paralyzed preparation with ivermectin (2.5×10^{-7} g/ml). The motility was then paralyzed by further treatment with strychnine (10^{-5} M) (Fig. 2B). These results suggest that *A. costaricensis* has an inhibitory gabergic mechanism in itself, but it seems less significant in maintaining the motor activity probably because a stimulatory cholinergic mechanism is extremely predominant in the worm. This imbalance of two mechanisms in *A. costaricensis* may explain in part why this species is less sensitive to gabergic drugs and also why the worm shows extremely active motility *in vitro* (Terada *et al.*, 1986).

It is well known that gabergic anthelmintics such as piperazine, ivermectin and milbemycin D are markedly effective against parasitic nematodes including *A. cantonensis* (Vanden Vosshe, 1985; Rew & Fetterer, 1986; Terada *et al.*, 1984). More effective and useful anthelmintics could be found through studies aimed at the gabergic mechanism in nematodes as a target. In the case of *A. costaricensis*, however, the results in the present study suggest little possibility that promising anthelmintics along this mechanism will be found. There remains a possibility to look for cholinergic inhibitors like tuberostemonine, a plant principle, which paralyzes *A. cantonensis* similarly to strychnine (Terada *et al.*, 1982).

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