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Histochemical Demonstration of the Inhibitory Effect of Nuvan® and Neguvon® on Cholinesterase Activity in Pseudodactylogyrus anguillae (Monogenea)

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Buchmann, K. and S. Mellergaard: Histochemical demonstration of the inhibitory effect of Nuvan and Neguvon on cholinesterase activity in Pseudodactylogyrus anguillae (Monogenea). Acta vet. scand. 1988, 29, 51-55. – The nervous system of Pseudodactylogyrus anguillae, a gill parasite from European eel (Anguilla anguilla) is demonstrated histochemically using the acetylthiocholine iodide method. It was shown that the staining of the nervous system was reduced or eliminated due to the inhibitory effect of Nuvan® and Neguvon®.

European eel; Anguilla anguilla; acetylthiocholine iodide method.

Introduction

Infections with the monogenean gill parasites *Pseudodactylogyrus* spp., have been a problem in commercial eel farming (*Ogawa & Egusa* 1976, *Egusa* 1979, *Chan & Wu* 1984, *Buchmann* et al. 1987). Different chemicals have been recommended for the treatment of pseudodactylogyrosis. Among these are Metrifonate (trichlorfon) (*Imada & Muroga* 1979, *Chan & Wu* 1984). However this drug is reported to be ineffective under Danish eel farming conditions (*Buchmann* et al. 1987).

The nervous system of a number of monogeneans has been demonstrated due to its content of cholinesterases by histochemical methods such as the indoxyl acetate, acetylthiocholine iodide or butyryl thiocholine iodide methods (see *Halton & Jennings* 1964, 1965, *Halton & Morris* 1969, *Lyons* 1969, *Tinsley & Wynne Owen* 1975, *Venkatanarsaiah* 1981, *Shaw* 1982).

The inhibitory effect of the cholinesterases by organophosphorous compounds such as Nuvan® (dichlorvos) and Neguvon® (trichlorfon) should result in an inhibition of the staining of the nervous system by the above mentioned methods.

This paper gives a description of the nervous system of P. anguillae and demonstrates that the inhibitory effect of these drugs on the parasites can be shown histochemically.

Materials and methods

Specimens of P. anguillae were removed from cultured European eels (Anguilla anguilla) and exposed in vitro to Nuvan® (0.0-dimethyl - 0 - (2.2 - dichlorovinyl -)phosphonate or Neguvon® (0.0-dimethyl -(2.2.2 - trichloro-hydroxyethyl -)phosphonate for 1 h at 20°C. The number of parasites and the drug concentrations used are shown in Tables 1 and 2. After exposure the monoge-

Table 1. Reduction of staining of the nervous system of *P. anguillae* and motility loss of parasites at various concentrations of Neguvon® (Acetylthiocholine iodide staining).

Neguvon conc. (ppm)	0 (control)	1	5	10	50	100
No. of parasites	10	9	11	10	12	10
No. of parasites immobilized within 1 h	0	0	0	0	0	0
Staining of cerebral ganglia	+++	++	++/+	++/+	+	-
Staining of longitunidal fibres	+++	++	+	+	-	-

+++ : strong reaction
++ : moderate reaction
+ : weak reaction
- : no reaction

Table 2. Reduction of staining of the nervous system of *P. anguillae* and motility loss of parasites at various concentrations of Nuvan® (Acetylthiocholine iodide staining).

Nuvan conc. (ppm)	0	0.5	1.0	1.5	2.0	5.0	50
No. of parasites	10	12	9	10	9	7	10
No. of parasites immobilized within 1 h	0	0	0	3	4	5	10
Staining of cerebral ganglia	+++	++	++/+	++/+	++/+	++/+	-
Staining of longitunidal fibres	+++	+/-	+/-	-	-	-	-

+++ : strong reaction
++ : moderate reaction
+ : weak reaction
- : no reaction

neans were fixed in 4% phosphate buffered formaldehyde (pH 7.0) at 3°C for 2-3 h.
After rinsing in distilled water the monogeneans were incubated for 18 h at 20°C in the acetylthiocholine iodide medium for detection of acetylcholinesterase as described by *Jennings & LeFlore* (1972). Controls were kept in tap water at 20°C for 1 h. Parasites exposed to 1.0% periodic acid for 10 min to eliminate enzyme activity served as negative controls.
After staining parasites were rinsed in distilled water and mounted in glycerine jelly.

Results

Staining of P. anguillae with the acetylthiocholine iodide method demonstrates the nervous system of the parasite. As shown in Fig.

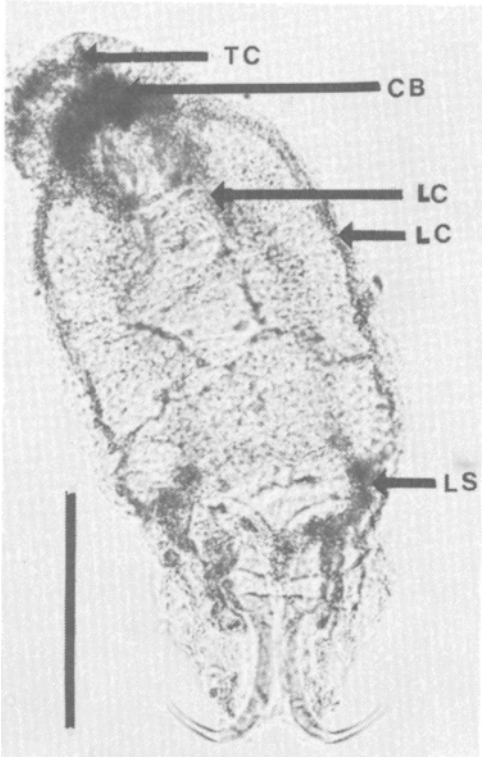

Figure 1. Whole mount of P. anguillae showing the location of the cerebral ganglia (CB), the terminal commisure (TC), the posterior longitudinal nerve cords (LC), the posterior lateral nerve swellings (LS). Untreated specimen. Scale bar = 100 µm.

1 an intense staining of the cerebral ganglia, on which the eye spots are located, and from where anterior nerves pass to the anterior terminal commisure are clearly demonstrated. A weakly stained ventral commisure connects the cerebral ganglia with the subpharyngeal ganglia. Two broad ventral nerve cords pass from the brain posteriorly. Two posterior lateral nerve trunks arise from the cerebral ganglia and pass to the junction between the body proper and the opisthaptor. Here they end into two lateral intensely stained ganglia, which are interconnected by a transverse posterior terminal commisure. One pair of hardly discernible posterior nerves pass from the nerve ring and unites with two lightly stained transverse nerve fibres.

From the posterior lateral nerve swellings, two nerves pass posteriorly to the level of the connecting bar on the opisthaptor. From the same swellings nerves were seen to arise and pass to the marginal hooklets. It is shown (Tables 1 and 2) that both Nuvan® and Neguvon® totally inhibit the cholinesterase activity of the nervous system in P. anguillae at concentrations of 50 and 100 ppm respectively.

The staining of the cerebral ganglia generally persists stronger drug concentrations than the longitudinal fibres (Fig. 2). It is notable that no parasites lost motility totally (Table 1) during the exposure to Neguvon® although the acetylcholinesterase activity was reduced or eliminated.

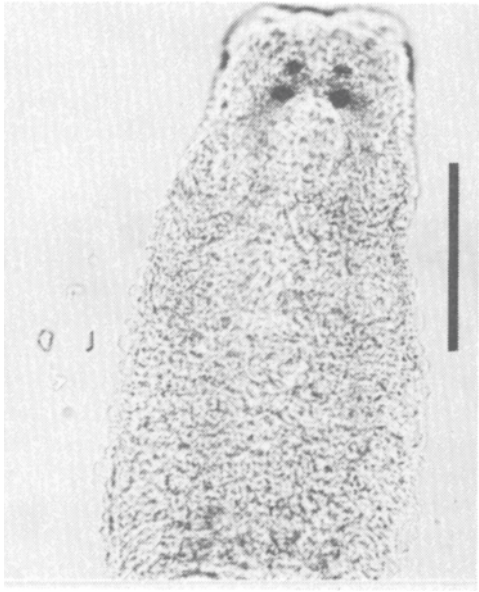

Figure 2. Whole mount illustrating the staining of the nervous system in a specimen of P. anguillae exposed to 1.0 ppm Nuvan® for 1 h. Scale bar = 100 µm.

However, the exposure to Nuvan® in concentrations of 1.5 ppm or stronger were found to reduce motility in the parasites (Table 2).

Discussion

Description of the nervous system of P. anguillae or of the genus Pseudodactylogyrus has not previously been given. In the description of P.bini Kikuchi (1929) only mentioned that the nervous system of this parasite presented »nothing peculiar«. The location of the brain was indicated as done by *Gussev* (1965) in his redescription. In the present work it was shown that the nervous system of P. anguillae could be demonstrated histochemically and inhibition of its staining after in vitro exposure of Neguvon® and Nuvan® was clearly illustrated. *Goven* et al. (1980) suggested that unsuccessful trichlorfon treatment of monogenean infestation in cultured goldfish might be due to drug resistance.

The in vitro exposure of monogeneans to different concentrations of the drug followed by a histochemical detection of cholinesterase activity as described here would demonstrate if the drug actually penetrated the helminth and inhibited its cholinesterases.

Ramisz & Szankowska (1970) suggested that histochemical methods could be used to determine satisfactory doses of drugs. However, as it was demonstrated in the present work, the drugs could inhibit the staining of the nervous system without eliminating motility of the parasites. Therefore these methods alone should not be considered adequate for determination of therapeutic concentrations.

This histochemical method seems to be suitable to trace activity mechanisms of drugs. Such use of histochemical methods has been seen previously. Thus *Ramisz* et al. (1967) tried to detect the effect of Tiguvon (0.0-dimethyl - 0-(4-methyl-mercapto-3-methylphenyl) thionophosphate) administered in vivo on *Trichinella spiralis* infected mice by using histochemical methods for esterases, and *Bueding* et al. (1967) elucidated the effect of Tris (p-alminophenyl) carbonium salts (TAC) administered in vivo on *Schistosoma mansoni* infected mice by using similar methods.

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Sammendrag

Histokemisk påvisning af den hæmmende effekt af Nuvan® og Neguvon® på cholinesterase aktivitet i Pseudodactylogyrus anguillae (Monogenea).

Nervesystemet i den monogene gælleparasit *P. anguillae* fra *Anguilla anguilla* er påvist ved hjælp af den histokemiske metode acetylthiocholin-iodid-metoden. Det blev demonstreret, at in vitro behandling af *P. anguillae* med Neguvon og Nuvan hæmmede cholinesterase i parasitterne, så farvningen af nervesystemet forhindredes.

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