

# Cholinergic and aminergic elements in the nervous system of *Pseudodactylogyrus bini* (Monogenea)

K. Buchmann<sup>1</sup>, P. Prentø<sup>2</sup>

<sup>1</sup>Department of Hygiene and Microbiology, Royal Veterinary and Agricultural University, Bülowsvej 13, DK-1870 Frederiksberg C., Denmark

<sup>2</sup>Institute of Cell Biology and Anatomy, University of Copenhagen, DK-2100 Copenhagen, Denmark

**ABSTRACT:** Presence and distribution of cholinesterases and catecholamines in the nervous system of the gill parasitic monogenean *Pseudodactylogyrus bini* from European eel *Anguilla anguilla* were detected by the acetylthiocholine iodide method and the glyoxylic acid method, respectively. Cerebral ganglia, opisthaptoral ganglia, longitudinal nerves and transverse commissures were positive for cholinesterase. The dorsal nerves – reacting only weakly for cholinesterase – some transverse nerves, cerebral and opisthaptoral ganglia and 2 anterior nerves were positive for catecholamines.

## INTRODUCTION

Cholinergic elements (AThChE, AchE, ChE) have been detected in the nervous system of monogeneans in several studies (Halton & Jennings 1964, Halton & Morris 1969, Lyons 1969, 1970, Tinsley & Wynne Owen 1975, Venkatanarsaiah 1981, Shaw 1982, Buchmann & Møllergaard 1988). A number of aminergic and peptidergic elements have been found in the nervous system of invertebrates (Van Noorden 1984, Falkmer et al. 1985, O'Shea & Schaffer 1985, Gustafsson et al. 1986) including some monogeneans (Reuter 1987, Maule et al. 1988). This paper reports the presence and distribution of acetylthiocholinesterase and catecholamines in the nervous system of the monogenean gill parasite *Pseudodactylogyrus bini* from the European eel *Anguilla anguilla*.

## MATERIALS AND METHODS

Specimens of *Pseudodactylogyrus bini* were collected from the gills of cultured European eels kept at 25 °C.

**Acetylthiocholinesterases (AThChE):** Parasites were fixed for 2 h in phosphate-buffered (0.1 M) 4% formaldehyde (3 °C), rinsed in 0.1 M phosphate buffer (2 h) and incubated at 20 °C for 4 h with the acetylthiocholin

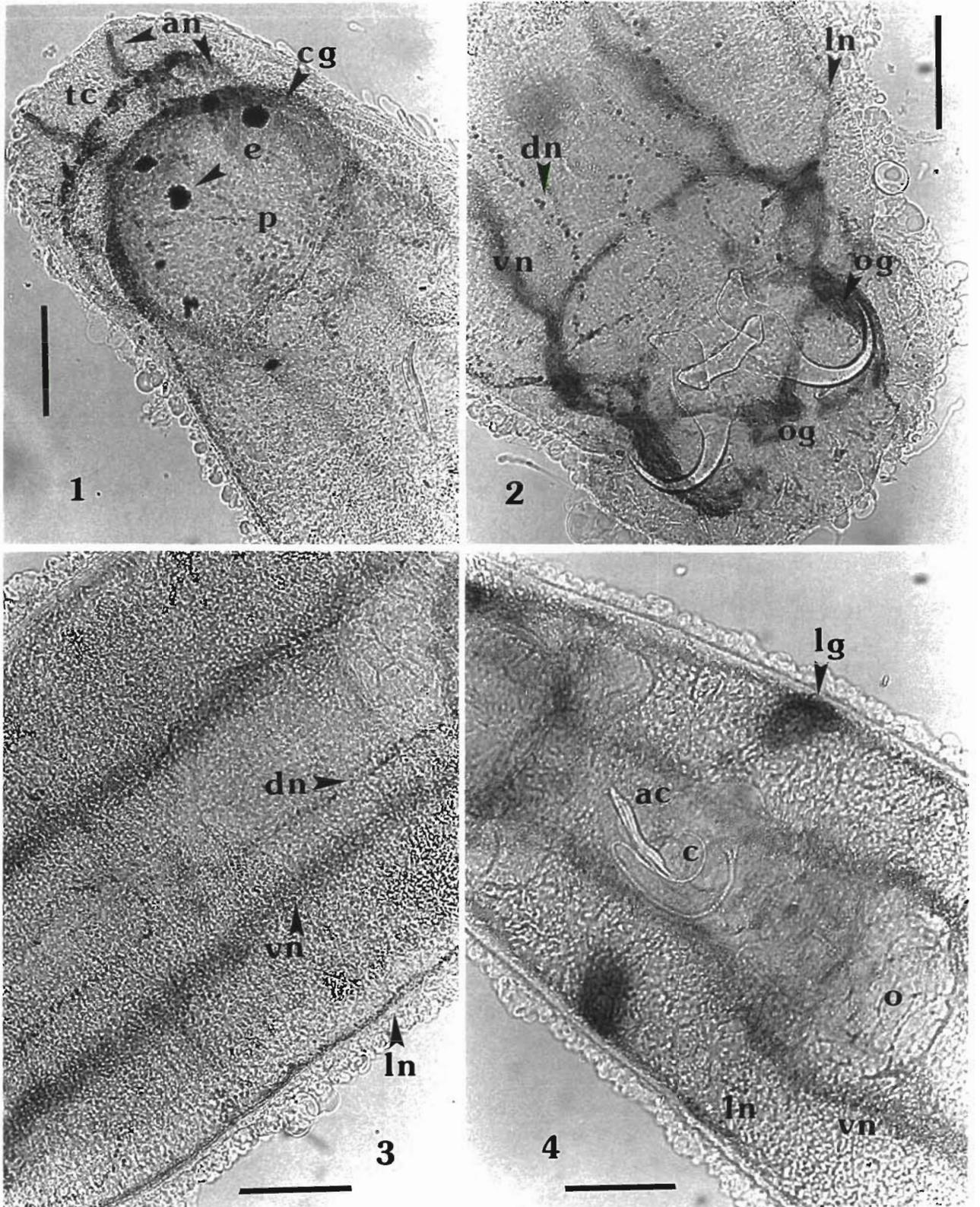
iodide method (Jennings & LeFlore 1972), and finally mounted in glycerine jelly. Incubation without substrate served as control for enzyme activity. Inhibitors for distinguishing between acetylcholinesterase and cholinesterase were not employed.

**Catecholamines:** About 15 lining parasites were immersed for 5 min in the glyoxylic acid solution (20 °C) (de la Torre & Surgeon 1976), dried in cool air on microscopic slides for 1 h, reacted in an oven at 80 °C for 5 min, mounted in paraffin oil, coverslipped and placed at a hot plate (80 °C) for 90 s. Specimens were studied with an incident fluorescence microscope (Nikon-Microphot-FX 360 to 460 nm: violet-blue light).

## RESULTS

### Acetylthiocholinesterases (AThChE)

Activity of these enzymes was distributed in cerebral ganglia, anterior longitudinal nerves and an anterior terminal commissure from which 2 anterior nerve processes originated. Two ventral and 2 lateral longitudinal nerve cords extended from the cerebral ganglia to the opisthaptor. Transverse commissures including a posterior terminal commissure occurred between the pharynx and the opisthaptor. At the level of the cirrus a ganglion was situated at the lateral cord in both sides.



Figs. 1 to 4 *Pseudodactylogyryrus bini* Wholemounts, acetylthiocholinesterase, scale bars = 60  $\mu$ m. **Fig. 1.** Anterior part showing cerebral ganglia (cg), anterior nerves (an), terminal anterior commissure (tc), e: eye spots; p: pharynx. **Fig. 2.** Posterior part including the opisthaptor (ln), lateral nerve (vn), ventral nerve; dn: dorsal lightly stained longitudinal nerves; og: opisthaptoral ganglia. **Fig. 3.** Median part showing lightly stained dorsal nerves (dn), ventral nerves (vn) and lateral nerves (ln). **Fig. 4.** *P. bini* at the level of the cirrus (c), accessory cirrus (ac), ovary (o), showing lateral ganglia (lg), ventral nerves (vn) and lateral nerves (ln)

Large ganglia extending from the junction between the longitudinal posterior ventral and lateral cords were seen in the opisthaptor. From the cerebral ganglia 2 dorsal pointed patterns of esterase activity went posteriorly to the haptor (Figs. 1 to 4).

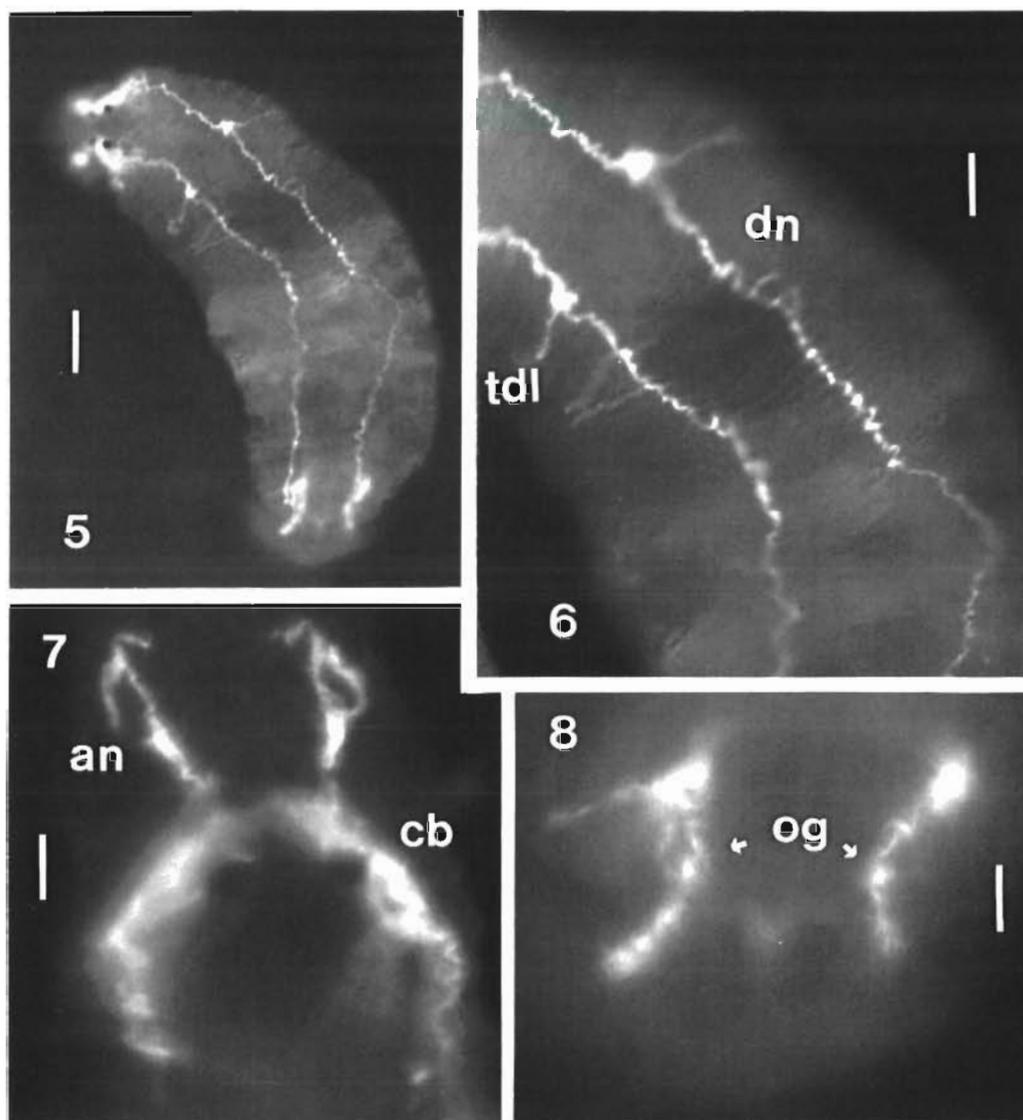
### Catecholamines

Extensive activity of catecholamines was seen in the cerebral ganglia from which 2 anterior longitudinal nerves extended and in the opisthaptor. The 2 dorsal nerves were the only longitudinal posterior fibres showing activity (Figs. 5 to 8). A swelling – probably a

cell body – from which transverse dorso-lateral fibres descended were found in both sides at the level of the cirrus. Weaker reactions were seen in other branchings from the dorsal cords. The green fluorescence indicated that the activity was due to dopamine or norepinephrine. No yellow fluorescence indicating the presence of serotonin was found.

### DISCUSSION

The distribution of acetylthiocholinesterase in *Pseudodactylogyrus bini* corresponds to that in *P. anguillae* (Buchmann & Møllergaard 1988) except that



Figs. 5 to 8. *Pseudodactylogyrus bini*. Wholemouts, glyoxylic acid. Fig. 5. Total helminth showing the dorsal aminergic nerves; scale bar = 120  $\mu$ m. Fig. 6. Median part of *P. bini* showing dorsal nerves (dn), transverse dorsolateral nerves positive for catecholamines; scale bar = 60  $\mu$ m. Fig. 7. Anterior part with anterior nerves (an) and cerebral ganglia (cb); scale bar = 30  $\mu$ m. Fig. 8. Opisthaptor showing opisthaptoral ganglia (og) positive for catecholamines; scale bar = 30  $\mu$ m

the lateral ganglia at the level of the cirrus in *P. bini* are strongly developed, while they were not detected in *P. anguillae*. The main structure of the nervous system comprising cerebral ganglia, posterior and anterior nerve trunks and connecting commisures is analogous with its composition in other monogeneans (e.g. Halton & Morris 1969, Venkatanarsaiah 1981, Reuter 1987). However, it is possible that the acetylthiocholinesterase activity only reveals a part of the total nervous system as a number of other neurotransmitters occur in invertebrates (Van Noorden 1984, Falkmer et al. 1985, O'Shea & Schaffer 1985, Gustafsson et al. 1986). It is worth noting that the dorsal longitudinal nerves in *P. bini* – with only a weak acetylthiocholinesterase activity – exhibited a strong catecholamine fluorescence. Possibly other neurotransmitters may be present; this is for future research to clarify. Thus Reuter (1987) demonstrated immunoreactivity for FMRF-amide, enkephalin, urotensin GRF and serotonin in the monogenean *Gyrodactylus salaris* and Maule et al. (1988) found a number of regulatory peptides in *Diclidophora merlangi*, a monogenean fish parasite. The anterior aminergic nerves in *P. bini* end at the level of the cephalic gland cells, which may indicate an aminergic regulation of the cephalic glands. The presence of catecholamines in the nervous system of *P. bini* suggests that anti-adrenergic drugs should be investigated for possible effects on this helminth.

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