

Putative neurosecretory cells in the monozoic cestode, *Lytocestus indicus* (Caryophyllidea)

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Abstract. In *Lytocestus indicus* putative neurosecretory cells (pNSC) are recognized on the basis of phloxinophilic and fuchsinophilic nature of their cytoplasm; pNSC in *L. indicus* are dimensionally small. Morphologically, there are four types of pNSC: a-, uni-, bi-, and multipolar cells.

Key words: *Lytocestus indicus* (Cestoda), neurosecretory cells

Introduction

In the absence of a coelom and a circulatory system in platyhelminth group of worms, the neurons and endocrine cells form a coincident and continuous spectrum of cells and constitute the neuroendocrine system (Hakanson and Sundler 1983) in which the neurosecretory cells (NSC) emerge as an important intermediate type (Fugita et al. 1982). The neurosecretory neuron identified as a nerve cell that is involved in secretion to an extent which greatly exceeds that of the conventional neurons (Scharer and Weitzman 1970) and secretes hormones' that are active at a remote site' (Gustafsson 1985). Hence, the nervous system in flatworms would take over an endocrine function by producing neurosecretory (peptidergic) molecules and releasing these signal substances into the inter-cellular spaces adjacent to or far from the target organ/cell (Gustafsson and Reuter 1992).

With the aid of classical methods of staining for neurosecretion, the peptidergic nervous system of turbellaria, trematodes and cestodes has been investigated and attempts to classify the NSC on morphological basis have been made (Vendrick 1963, Lender 1964, Morita and Best 1965, Morseth 1967, Harris and Cheng 1972, Webb 1977, Specian et al. 1979, Gustafsson and Wikgren 1981, Mehrotra and Bhutia 1981). Based on the immunocyto-

They are found both in the cortical and medullary parenchyma of the neck and strobilar regions; in the latter the pNSC occur in close vicinity with the longitudinal muscles and components of the reproductive system.

chemical activity against the antisera of many vertebrate and invertebrate peptides, the presence of many peptidergic nervous elements has been reinforcingly demonstrated in several members of the platyhelminth group (Wikgren 1986, Gustafsson et al. 1986, Reuter and Gustafsson 1989, Smyth and McManus 1989, Fairweather and Halton 1991, Gustafsson 1992, Halton et al. 1992). Recently the physiological significance of NSC in flatworms has been reviewed (Fairweather and Skuce 1995). Regarding cestodes the available information mainly pertains to the pseudophyllidean and cyclophyllidean parasites and that relating to the monozoic cestodes is rather scanty. The occurrence of NSC has been reported in *Archigetes* and *Glaridacris* spp. (Clegg and Smyth 1968, Hayunga and Mackiewicz 1988). The nervous organization of the caryophyllids, *Lytocestus indicus* and *Djombangia penetrans*, common parasites of the siluroid catfish, *Clarias batrachus*, has been described in detail (Lyngdoh and Tandon 1992, 1994). The present study aims to demonstrate the presence of NSC in *L. indicus*.

Materials and methods

For the demonstration of the NSC the adult specimens of *Lytocestus indicus* (Moghe, 1925) Woodland, 1926 were recovered in 0.7% saline from the intestine of freshly

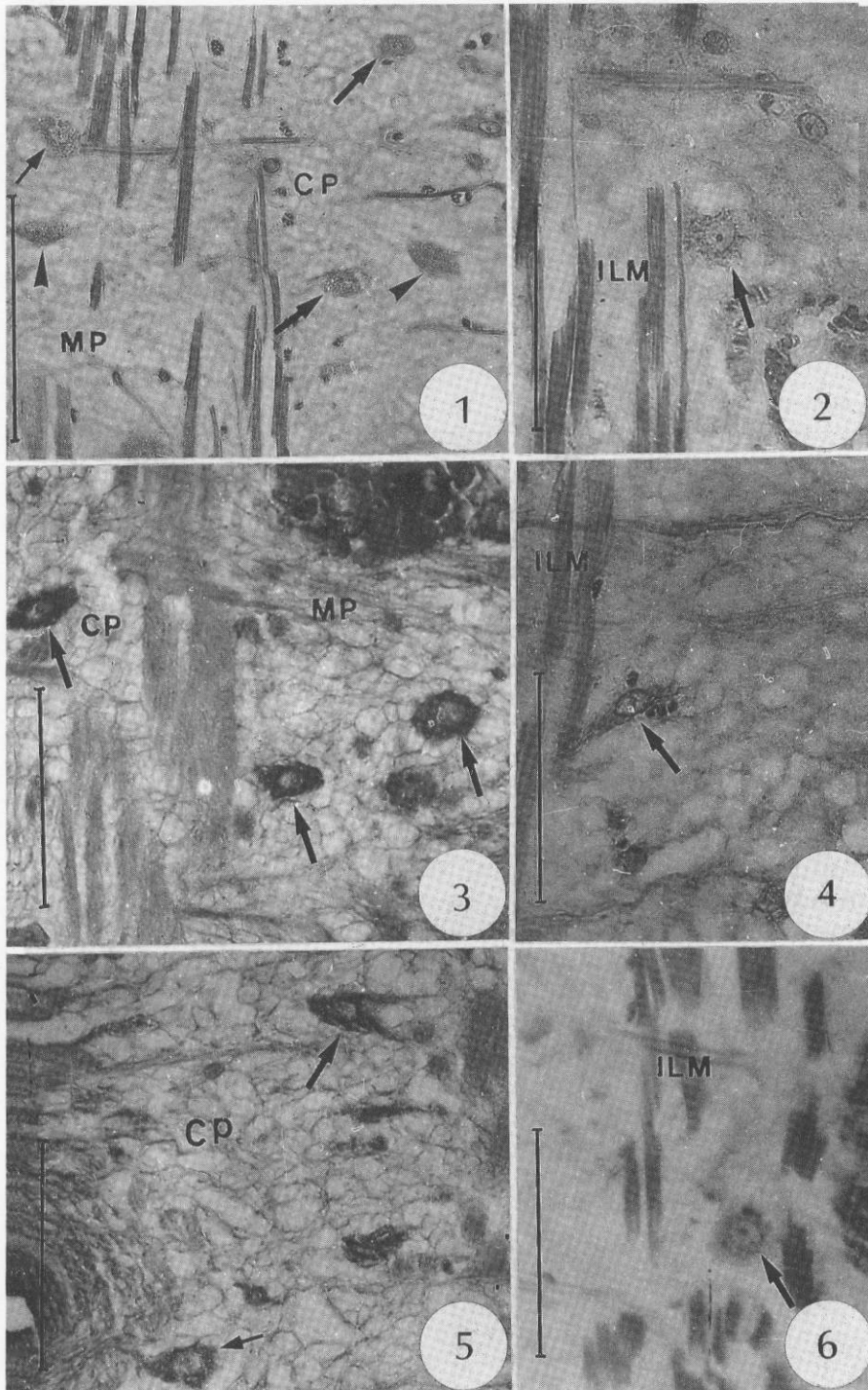
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killed fish, *Clarias batradus* (L.). The worms were fixed in Bouin's or Helly's fluid and were processed for paraffin sectioning. Serial sections were cut at a thickness of 6–8 μ m. For the localization of NSC the sections were stained according to Panov (1980) with Bargmann's chrome haematoxylin phloxin (CHPh), Gabe's paraldehyde fuchsin (PAF), Herlant's alcian blue phloxin (AbPh) and Raabe's modified Heidenhain's Azan methods. Visual criteria of

specific histochemical staining were adopted for determining the neurosecretory nature of the cells.

Results

On the basis of phloxinophilic and fuchsinophilic nature of their cytoplasm, cells could be recognized as neurosecretory cells (pNSC).



Figs. 1–6. Neurosecretory cells in *Lytocestus indicus* (photomicrographs): **1** – an apolar (small arrow), unipolar (large arrows) and bipolar (arrowheads) cells in the medullary (MP) and cortical (CP) parenchyma of the neck region, AbPh (scale bar = 0.1 mm); **2** – a multipolar cell (arrow) in the cortex of the main body close to inner longitudinal muscles (ILM), CHPh (scale bar = 0.05 mm); **3** – bipolar cells (arrows) in the medullary and cortical parenchyma of the mid-body region, also in association with longitudinal muscles, PAF (scale bar = 0.05 mm); **4** – a bipolar cell (arrow) approximating the inner longitudinal muscles in the mid-body region, AbPh (scale bar = 0.05 mm); **5** – unipolar (small arrow) and multipolar (large arrow) cells in the cortical parenchyma of the mid-body region, PAF (scale bar = 0.05 mm); **6** – an apolar cell (arrow) between the inner longitudinal muscle strands in the main body region, AbPh (scale bar = 0.05 mm)

All the four tests employed, viz. CHPh, PAF, AbPh and Heidenhain's Azan could stain the pNSC.

The pNSC in *L. indicus* are small in dimension, their size is $15 \times 7 \mu\text{m}$ and that of the nucleus $6 \times 5 \mu\text{m}$.

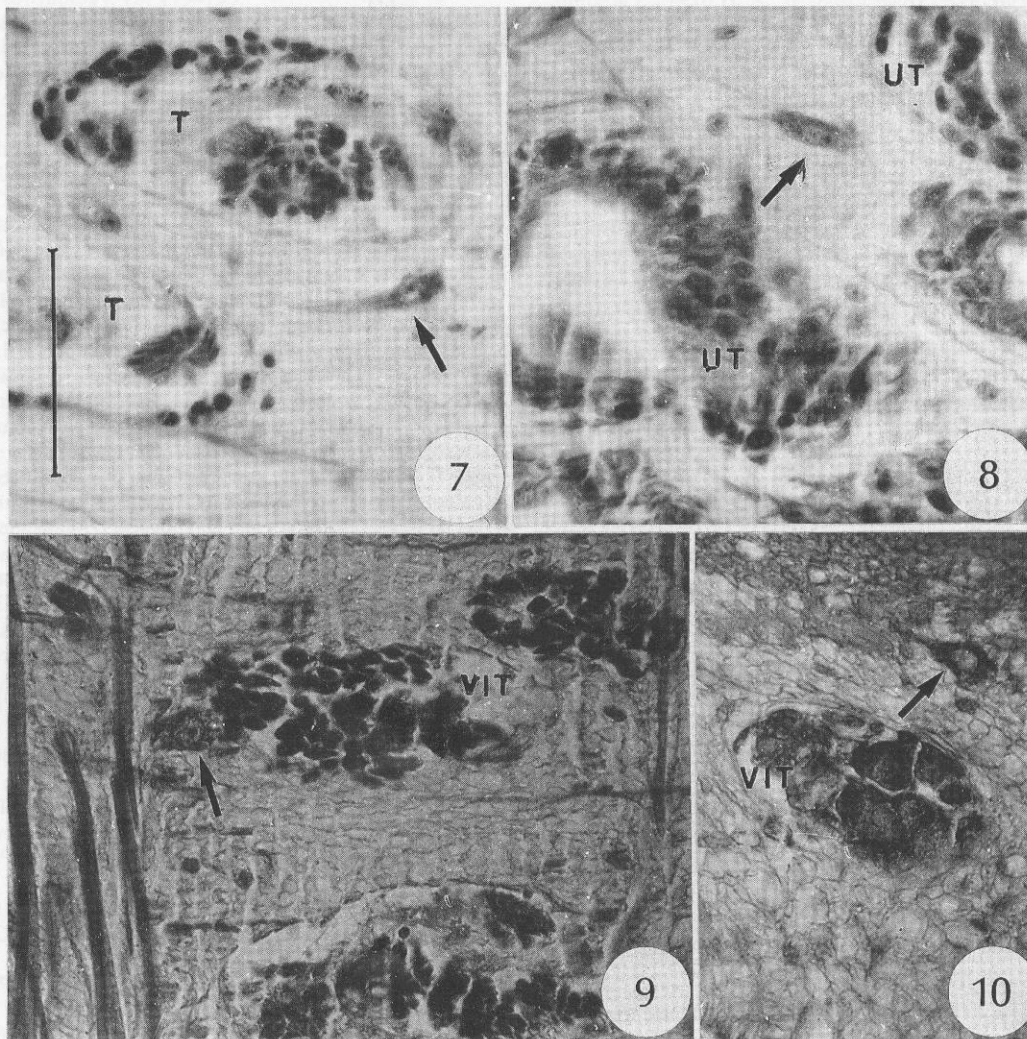
On the basis of the morphological appearance, mainly of the peripheral processes, the pNSC appear to be mainly of four types. They are a-, uni-, bi- and multipolar cells. The aporal pNSC are spherical or pyriform without any axonal or dendritic extension (Figs. 1 and 6), the unipolar cells are mostly oval or elongated with an axonal extension (Figs. 1, 5, 7, 8 and 10), the bipolar cells are oval or elongated with one axonal and dendritic extension each (Figs. 1, 3 and 4) and the multipolar cells are spherical or polygonal with an axonal and more than one dendritic extensions (Figs. 2, 5 and 9).

All the four types of pNSC are with granular cytoplasm, showing a high intensity of stain. A-, uni- and bipo-

lar cells are abundantly found in the cortical and medullary regions of the neck as well as in the neighbourhood (Fig. 1). All the four types of pNSC are detectable in the main body region both in the cortical as well as medullary parenchyma. While a-, bi- and multipolar cells in the main body are found in association with the longitudinal muscles (Figs. 2-6), the unipolar cells are detectable in close vicinity with the testes, uterus and vitellaria (Figs. 7, 8 and 10). The vitellaria are also consorted by multipolar cells (Fig. 9).

Discussion

A nerve cell is considered as neurosecretory when only the following criteria are agreed upon: (1) the presence of a morphologically demonstrable secretory product, typically stainable by CHPh or PAF or by visualization of electron-



Figs. 7-10. Neurosecretory cells in *Lytocestus indicus* (photomicrographs - scale bar 0.05 mm for all the figures): **7** - unipolar cell (arrow) in association with the testes (T), CHPh; **8** - unipolar cell (arrow) between the uterine coils (UT), CHPh; **9** - multipolar cell (arrow) in association with the vitellaria (VIT), CHPh; **10** - unipolar cell (arrow) in the region near the vitellaria, PAF

dense granules, called elementary secretory granules (Bern 1962); (2) the presence of α -glycogen in the neurites (Webb 1977); (3) morphological changes in the neurosecretory cells correlating with the physiological changes in the organism; and (4) an identifiable physiological hormonal effect of the product of the NSC (Simpson et al. 1966). However, in the present study, only the first criterion, i.e. that of stainability, is satisfiable. Hence, the cells herein are designated as putative.

The pNSC in *L. indicus* revealed a phloxinophilic and fuchsinophilic nature of their cytoplasm, showing an affinity for CHPh, PAF, AbPh and Heidenhain's methods. Positive PAF staining, however, is not considered as unequivocal indication for cells under question being NSC. In *Hymenolepis diminuta*, the rostellar glands are PAF positive but are still considered non-neurosecretory (Specian and Lumsden 1980); these cells were earlier thought as neurosecretory (Davey and Breckenridge 1967). Furthermore, the PAF technique is known to reveal substances other than neurosecretory material too (Pearse 1968).

The size of the cell body and nucleus in *L. indicus* is small; pNSC in cestodes generally appear to have small dimensions. In the pseudophyllid, *Diphyllobothrium dendriticum*, the pNSC body measures $5 \times 8 \mu\text{m}$ with a nucleus of the size $3 \times 5.5 \mu\text{m}$ (Gustafsson and Wikgren 1981).

In *L. indicus* four types of pNSC, are recognizable. In *D. dendriticum* bi- and multipolar cells with long varicose processes were observed (Gustafsson 1990); the multipolar cells in the cortical parenchyma send processes towards the basement membrane of the tegument and the release of neurosecretory material may take place in direct apposition to the acellular stroma between the cells (Gustafsson and Wikgren 1981). Thus, a vascular system does not play an obligatory part of pNSC to reach their target organs. However, PAF-positive rostellar gland cells in *Hymenolepis* species were considered as non-neurosecretory since they were always located far away from the main nervous system (Specian et al. 1979). In trematodes, these long processes were regarded as dendritic (Bhatnagar et al. 1980). Extensive branching of the cells providing an increase in the surface area for the exchange of material is also regarded yet another characteristic of NSC (Berlind 1977).

The cell cytoplasm is observed in the pNSC to the full of granular material in *L. indicus*; in addition, these cells are found distributed both in the cortical and medullary parenchyma. In *D. dendriticum*, the presence of granular material was observed in the perinuclear cytoplasm as well as in the processes; the multipolar pNSC in the cortical parenchyma send processes towards the tegument (Gustafsson and Wikgren 1981).

The different types of pNSC in *L. indicus* are localized in close association with the muscle layers. This observation agrees with that of Davey and Breckenridge (1967) in the cyclophyllidean cestode, *H. diminuta* and of Gustafsson (loc. cit.) in the pseudophyllidean, *D. dendriticum*.

Direct apposition of the neurosecretory terminals to the muscle fibres has been reported by Scharrer (1976).

In *L. indicus* pNSC were also observed in association with the reproductive structures. The involvement of NSC through neurosecretory material directly or indirectly in the maturation of gonads and growth has been reported by several workers in trematodes (Clegg and Smyth 1968, Grasso and Quaglia 1972, Harris and Cheng 1972, Mehrotra and Bhutia 1981) and planarians (Webb 1988). In nereid annelids, the maturation of the gonads is controlled by an inhibitory action of the neurosecretory cells which only continued their activity when the secretion decreased (Durchon 1960).

The presence of secretory neurons as a part of nervous system in caryophyllids is indicative of their ancient position at the base of the phylogenetic sequence in the evolution of Bilateria as proposed by Barnes et al. (1988).

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