Ultrastructural Study of Neurosecretory Cells in the Nervous System in the Cestode (Taenia hydatigena)

By
Bo LIU, Hidekazu WAKURI*, Ken-ichiro MUTOH* and Kazumi TANIGUCHI*

Department of Animal Anatomy, Changchun University of Agriculture and Animal Sciences, Xian Road 175, Changchun, Jilin 130062, P.R. China
* Department of Veterinary Anatomy, School of Veterinary Medicine and Animal Sciences, Kitasato University, Towada, Aomori 034, Japan

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Summary: The neurosecretory cells in nervous system of the Taenia hydatigena was studied light- and electron-microscopically. On the basis of the cytological structure they were divided into two types: type I neurosecretory cells, which containing large dense-cored vesicles, small elongated mitochondria and a large amount of free ribosomes, were located mainly in the central and peripheral nervous system; type II neurosecretory cells were characterized by their moderate amount of free ribosomes, endoplasmic reticulum which contacted with the membranes of perikarya, large ovoid mitochondria, large dense-cored vesicles and their localization in the musculature near nerve profiles. The synaptic and nonsynaptic contacts i.e. omega figure and exocytosis release sites were seen in the neuropile, musculature and excretory ducts. The present findings suggest that neurosecretory cells in nervous system may play an important integrative roles of both neuronal and endocrine in the flatworm.

In flatworms, which have not endocrine glands and a circulatory apparatus, the neurosecretory component of the nervous system is only system producing and transporting substance that can act in a hormone-like fashion, and so nervous system is more than just a nervous system, it may acts as an endocrine system via its neurosecretory component. Nerve cells with the characteristic of neurosecretory cells were first described by Turner (1946) in turbellarian Leptoplanida acticola, and their presence was documented later in Botheriocephalus scorpili, Hymenolepis nana, Diphyllobothrium dendriticum, Hymenolepis spp., Hymenolepis diminuta and Echinoecoccus granulosus (Davey & Breckenridge, 1967; Morseth, 1967; Featherston, 1972; Jones, 1975; Webb, 1977; Fairweather, 1979; Lumsden & Specian, 1980; Fairweather & Threadgold, 1981 & 1983; Gustafsson & Wikgren, 1981a; Wikgren, 1986 and Gustafsson, 1989). An distinct characteristic of neurosecretory cells is the presence of large dense-cored vesicles (>100 nm) which were called elementary granules of neurosecretion at ultrastructural level. Recent advances in immunocytochemistry have revealed the occurrence of a large variety of peptides in the nervous systems in tapeworms (Fairweather et al., 1988, 1990; Basch & Gupta, 1988; Maule et al., 1992; Gustafsson et al., 1995 and Liu et al., 1996). The peptidergic system is likely to form the predominant component of the nervous system (Reute & Gustafsson, 1989). These finding suggests that the secretory granules may particularly be peptidergic.

To our knowledge many researchers have accumulated attention to the morphological features of the electric dense vesicles. Litter have been known about morphological and functional feature of transport of messenger substances of neurosecretory cells to targets. In the present study, we focus on the fine structural observations which functionally characterize neurosecretory cells and the assumption of neurosecretory phenomena with transmission electron microscope and histochemical techniques.

Materials and Methods

Adult Taenia hydatigena which were obtained from small intestine of dog from China were used for this study. For light microscopy, the worms were fixed in 4% paraformaldehyde and embedded in histowax and sections of 2–7 μm thickness were cut from the scolex, neck region, immature proglottids, mature proglottids to gravid proglottid. The following staining methods were used: aldehyde-fuchsin, Gomori' aldehyde fuchsin and aldehyde-thionin.
For electron microscopical observation, specimens were fixed with 4% paraformaldehyde in 0.2 M PBS solution. After cutting into 1 mm cubes, they were fixed again in the 1.5% paraformaldehyde and 0.5% glutaraldehyde solution, and osmicated in a 1% OsO₄ solution for 2 hours, dehydrated in a gradual series of alcohol and embedded in Epok 812. Sections were cut on ultratome (Reichert-Nissei, Ultracut N, Tokyo), stained with uranyl acetate and lead citrate, and examined in a Hitachi 7000 electron microscope.

Results

A number of neurosecretory cell bodies were demonstrated in the cerebral ganglia and main nerve cords, arranged peripherally around a neuropile. It was possible to identify nerve cells and neurosecretory cells on the basis of the cytological structure, especially according to vesicles in the perikarya and processes. Nerve cell bodies contained numerous clear vesicles (30–50 nm) and occasional dense-cored vesicles (50–100 nm). The mitochondria were ovoid or elongated. The basic components of neurosecretory cells were almost similar to those of nerve cells. There were, however, some differences, the most apparent of which was distinct existence large dense-cored vesicles. Two types of neurosecretory cells can be distinguished according to their structure and localization: type I neurosecretory cells were found that their perikarya commonly associated with the neuropiles of the cerebral ganglia and main nerve cords (Fig. 1), occasionally among the muscles. Their fibres extended along the main nerve cords and terminated the neuropiles or targets. The peripheral cytoplasm contained active Golgi complexes with clear vesicles (50–100 nm in diameter) and large dense-cored vesicles (100–250 nm in diameter), and a few electron dense-cored located eccentrically within the vesicles (70–100 nm). In addition, they contained large amount of free ribosomes and ovoid or elongated mitochondria. The edge of the cell bodies were not smooth, cytoplasm extending many processes which containing electron dense granules. Type II neurosecretory cells were mainly distributed amongst the muscles in vicinity of the main nerve cords. They were multipolar cells filled with a small amount large dense-cored vesicles (100–220 nm in diameter) in association with the Golgi complexes. Prominent ovoid nucleus was surrounded by cytoplasm containing mitochondria, ribosomes and peripherically located cisternae of granular endoplasmic reticulum (Fig. 2). Mitochondria were conspicuous large, particularly ovoid in form. Some granular endoplasmic reticulum contacted with the membranes of perikarya. Free ribosomes were moderate amount.

In the main nerve cords and cerebral ganglia, neurosecretory processes were packed with large dense vesicles. They occurred frequently in the neuropile in which was made up of unmyelinated axons, dendrites and synapses and also constitute a considerable part of the peripheral nervous system innervating the musculature and the subtegumental region. The vesicles in processes of neurosecretory cells were distinguished into three types: type A contained round large dense-cored in 100–250 nm in diameter (Fig. 3); type B contained round small dense-cored vesicles in 70–100 nm in diameter (Fig. 4); and type C contained a heterogeneous population of granules ranging from 100 to 300 nm in length and 70 to 160 nm in width (Fig. 5). The processes of myocyton were scattered among the axons and dendrites in the main nerve cords (Fig. 6). A few empty omegashaped figures were found (Fig. 13 A & B). A few processes contained electron dense granules which sparsed in the glycogen.

Variety of contacts were observed in the main nerve cords and the cerebral ganglia which included synaptic and nonsynaptic contacts. Nonsynaptic contacts were by far the commonest pattern. The large dense-cored vesicles touch the membrane of terminals (Fig. 7). The omega figures were seen on their membranes (Fig. 5). A few synapse were observed in the neuropile. They were characterised by the presence of prominent coating of dense material on postsynaptic cytoplasmic face and a relatively less dense presynaptic membranes. The process terminals commonly contained a small clear round vesicles and large dense-cored vesicles (Fig. 8). Occasionally, nonsynaptic adherens were seen on the membranes of neuropiles filled with large dense-cored vesicles (Fig. 9).

The musculature of typeworm consists of two major components: myofibril and myocyton which they are frequently some distance away from each other, connected thereto by tendrilled cytoplasmic processes of myocyton. The neurosecretory terminals which were filled with crowds of dense-cored vesicles were found widely in association with the musculature of strobila and scolex. The dense-cored granules in the terminal were commonly at 100–250 nm in diameter. The structure of motor end-plate of the nerve terminal in vertebrates was not observed. However, three types of the neuromuscular connections were consistently identified: Type 1 connection showed very close contact between the membranes of terminals and myocytons or myofibrils (Fig. 10). Type 2 showed that the peripheral cytoplasm of myofibril containing mitochondria extending the processes toward the nerve ending filled the dense-cored vesicles (Fig. 11). Type 3 showed that the terminal surrounding by the connective tissue, not
contact with the myocytons and myofibrils, however, within the endings the dense-cored vesicles touching the membranes of the terminals in which they became thicken and densities, and the dense-cored vesicles released the materials toward the connective tissues arround them (Fig. 12).

In addition, the neurosecretory terminals containing the dense-cored vesicle connected with the duct of excretory were seen in the parenchyma. Their membranes were seen to connect with the membranes of syncytial cytoplasm of collecting ducts in the excretory system, and each membrane was thicken (Fig. 12).

With aldehyde-fuchsin, Gomori' aldehyde fuchsin and aldehyde-thionin staining, a amount of positive granules were filled in perikarya, processes and the peripheral net from the head to the tail of worm. Cells were defined at 3–7 μm in shortest diameter, with short or long processes, mainly bipolar or multipolar in form.

A regular distribution of positive cells and fibres was demonstrated in the worm. In the scolexes numerous cell bodies were observed in the cerebral ganglia (Fig. 15) and the ganglionated transverse commissures. Accumulation of the varicose fibres were scattered in the musculature of suckers. Positive fibres also occurred in the main nerve cords along the length of the cords (Fig. 16), and in the transverse commissures where they extending laterally to the surface of the tegument, giving a distinctive laddered appearance. Largest amount of the cells with varicose processes and fibres were found in the regions between the longitudinal and transverse muscle layers, forming a distinct nerve plexes (Fig. 17).

Discussion

The present study gives evidence for the existence of a large number of neurosecretory cells in the nervous system and the musculature of its vicinity in Taenia hydatigena. At the electron microscope level, the presence of dense-cored vesicles which have been termed elementary neurosecretory granules, both within perikarya and processes, can be interpreted to indicate neurosecretory functions which have been detected to be present in Hymenolepis diminuta, Hymenolepis microstoma and Hymenolepis nana (Webb, 1976; Lusden & Specian, 1980; Basch, 1986). The presumption of existence of neurosecretion in Taenia hydatigena is supported by the positive staining with paraldehyde-fuchsin and aldehyde-thionin which demonstrate perikarya as well as processes in the nervous system in this study. Both stains were supposed to reveal the presence of peptidergic neurosecretory cells in Diphyllobothrium dendriticum (Gustafsson & Wikgren, 1981b). The present study, however, has offered some additional data to contribute to the detailed distribution, besides nervous system, accumulation of positive perikarya and processes was observed between the longitudinal and transverse muscle layers, forming intermuscular nerve plexes and extending their processes to the musculature organs. It is interesting that a number of neurosecretory elements in the scolex were stained with aldehyde-fuchsin, which was obtained also in PRL immunocytochemical staining in some area in our previous study (Liu et al., 1996). Moreover, they showed almost similar shape, size and localization which the cells located individually or in group.

The secretory nature of neurosecretory cells becomes evident from the fact that the dense-cored vesicles occurred abundantly both in the perikarya and processes. In a previous report by Webb and Davey (1976), dense-cored vesicles (55–90 nm in diameter) were observed in putative neurosecretory neurons in Hymenolepis microstoma metacestodes. While Gustafsson and Wikgren (1981b) described that the peptidergic neurons were filled with large dense vesicles (77–322 nm) in Diphyllobothrium dendriticum. In this study, large dense-cored vesicles 100–250 nm and heterogeneous dense vesicles (usually spherical in shape) 100–300 nm in diameter were observed in neurosecretory perikarya and processes in Taenia hydatigena. The observations in various cestodes varied from different researchers. However, it is generally accepted that the small clear synaptic vesicles about 50 nm in diameter contain cholinergic while small dense-cored vesicles in synapses were regarded as aminergic (Gustafsson, 1984). Accordingly, large dense-cored vesicles were regarded as peptidergic. The typical peptidergic type granules consist of relatively electron-dense and homogeneous-looking contents bound by a smooth membrane, usually of spherical shape, ranging in diameter from 80–300 nm. It is interesting to note that the neurosecretory terminals with large dense-cored granules in neuropil also contained small clear vesicles in Taenia hydatigena. These vesicles have diameters of about 30–50 nm and resemble those in synaptic ending. Their effect in this situation is unknown, but they may be either residual vesicles left after discharge of the granular contents or the coexistence of neurosecretory granules and aminergic or cholinergic in neurosecretory cells. The finding in Taenia hydatigena of neuronal synaptic contact with neurosecretory processes suggests the possibility that in cestodes neurosecretion may be regulated by integration through nervous system.

The neurosecretory cells, with processes associating with the muscles where lack the sarcoplasmic contact zone have been extensively observed in
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Explanation of Figures

Plate I

Fig. 1. Neurosecretory cell close to the neuropile of the cerebral ganglia. Note the large dense-cored vesicles (L), a large number of free ribosomes (R), mitochondria (M) and nucleus (N).

Fig. 2. Neurosecretory cell amongst muscles (Mu) in vicinity of the main nerve cord. Note moderate amount free ribosome (R), large mitochondria (M) and large dense-cored vesicles (L).

Fig. 3. Processes of neurosecretory cell containing large dense-cored vesicles (L) scattered amongst the nerve fibres (Nf) in the main nerve cord.

Fig. 4. Process of neurosecretory cell containing small dense-cored vesicles (S) located amongst the nerve fibres (Nf) in the main nerve cord. Note mitochondria (M) in the process.

Fig. 5. Heterogeneous vesicles ranging from 100 to 300 nm in length and 70 to 160 nm in width in process of the neurosecretory cell. Note a omega figure (arrow) on the membrane and a vesicle contacting the membrane of omega figure.

Fig. 6. Many processes of myocyton (Mp) were scattered among the axons, dendrites and processes of neurosecretory cell in the main nerve cords.
Plate II

Fig. 7. Large dense-cored touching the membrane of the process (arrow head).

Fig. 8. Synapse between both endings containing large dense-cored vesicle (L) and small clear vesicles (s) in the main nerve cord.

Fig. 9. Nonsynaptic adherens (arrows head) of process with the dendrite.

Fig. 10. Terminal of neurosecretory cell locating amongst myocytom (M) and processes of myocytom (Mp). Showing very close contact (arrow head).

Fig. 11. Myofibrils sending their processes (Mp) containing mitochondria (M) to the neurosecretory ending.

Fig. 12. Large dense-cored vesicles touching the membrane and releasing the materials toward the connective tissue (arrow).

Fig. 13 A. & B. Showing omega-shaped figures (arrow head) on the membrane of process containing heterogeneous vesicles.

Fig. 14. Neurosecretory terminal connecting to syncytial cytoplasm of collecting duct in excretory system (E). Note membrane becoming thickened (arrow head).

Fig. 15. In the scolexes numerous aldehyde-fuchsin stained cell bodies (arrow head) in the cerebral ganglia. e: excretory duct. Aldehyde-fuchsin stain.

Fig. 16. Varicose fibres occurred in the main nerve cord (MC) along the length of the cords (arrow head). Aldehyde-fuchsin stain.

Fig. 17. Aldehyde-fuchsin positive cells (large arrow head) with varicose processes (small arrow head) in a nerve plexes between the longitudinal and transverse muscular layers. Aldehyde-fuchsin stain.