CHAPTER - VI

NEUROSECRETORY CHANGES IN Polypheretima elongata
INTRODUCTION

The realization of the endocrine nature of nervous system is one of the outstanding developments in the biology. The endocrine portion of nervous system is composed of neurosecretory cells. The neurosecretory cells are the modified neurons which have developed secretory functions to such an extent that they become morphologically distinguishable from other neuronal cells (Jamieson 1981 a, b). Such glandular neurons synthesize substance that may act as neurohormone.

The neurohormones are retained in circulation for the longer time and evoke appropriate physiological responses at distant target organs (Robertson and Osborne, 1979). The concept of neurosecretion has undergone advancement with the development of histological techniques and electron microscopy.

The nervous system of earthworm is very simple and consists of the brain or cerebral ganglion, subpharyngeal ganglia, ventral nerve cord and peripheral nerve. The main trunk i.e. the ventral nerve cord runs below the gut in close contact with the coelom from the last segment of the body to the fourth segment from front. The ventral nerve cord is swollen into segmental ganglia in each segment. Anteriorly, the nerve cord passes into subpharyngeal ganglia and then bifurcates into circumpharyngeal connectives, which pass up encircling either side of pharynx and ultimately converge into a bilobed brain or cerebral ganglion at the dorsal surface of the pharynx in segment three (Patil, 2002) (Fig. 6.1 and 6.2)

Crawling locomotion in the earthworm may be controlled by a central pattern generator (CPG) (Delcomyn, 1980) located in the central nervous system (CNS). The CPG is a network of neurons capable of generating a rhythmic motor output in the absence of real movements provided by locomotors organ (Grillner, 1999), which is usually termed fictive locomotion (Buchanan, 1999). One way of inducing fictive locomotion is through the application of bioactive substances to the CPG. When an earthworm crawls over both rough and dry surfaces, locomotion is produced by waves of body wall contractions and elongations with
protraction and retraction of bristles (setae) on the lateral and ventral surfaces of the body (Friedländer, 1894; Gray and Lissmann, 1938). Peristaltic crawling in this animal was thought to be brought about by coordinated reciprocal contractions of longitudinal and circular muscle bands (Gardner, 1976), and the motor neuron regulation of muscle activity via the lateral nerves has previously been investigated (Knapp and Mill, 1968; Günther, 1970; Drewes and Pax, 1974). Assmé and Chang (1988) identified a rhythmically discharging neuron located in the VNC in the earthworm, whose frequency of discharge remained constant at about 100 Hz. The anatomy and function of the earthworm nervous system have been investigated in detail by Mill (1982).

As far as phylogeny of neurosecretion is concerned there exist two schools of opinion. According to the first school, neurosecretory cells represent specialized arm of nervous system elaborating neurohormone that are retained for longer time in circulation and act on distant target organs (Scharrer and Scharrer, 1954). Another school of investigators hold that neurosecretory acquired neuronal characteristics (Clark, 1956 a, b). From the physiological point of view, the neurosecretory cells are considered as the “connecting link” between the nervous system on one hand and the endocrine system on the other (Scharrer and Scharrer, 1973).

The first credit of neuroendocrine investigation in oligochaete goes to Scharrer (1937) who demonstrated the presence of neurosecretory cells in the earthworm Lumbricus terrestris. Ultrastructural evidence has been presented substantiating the hypothesis that neurosecretory granules are packed within golgi complex in the earthworm Lumbricus terrestris (Tombes, 1970). The submicroscopic structure of the neurosecretory cells in the brain of Allolobophora calignosa was studied by Alonsa-Bedate and Sequeros (1983). They identified in Allolobophora caliginosa at least five to six types of neurons containing morphologically different neurosecretory vesicles. Aros and Vigh (1962) have demonstrated neurosecretory activity in the pharyngeal region of three Lumbricids, Lumbricus rubellus, Lumbricus hercules and Allolobophora foetida.
Drewes and Pax (1974) reported neuromuscular physiology of the longitudinal muscle of the earthworm, *Lumbricus terrestris*. Kulkarni (1989) studied the responses of neuroendocrine centers to some environmental factors like photoperiod, temperature, salinity, desiccation, starvation and to some pharmacological drugs. Csoknya *et. al*, (1996) have reported the occurrence of Octopamine in the central nervous system of Oligochaeta. Mizutani *et al*. (2000) noted the fictive locomotion induced by octopamine in the earthworm. Patil (2002) studied the responses of neuroendocrine centers to some agrochemicals like Ammonium sulphate, Urea, Suffala and Sampurna in the worm *Perionyx excavatus*. The available literature shows that not much is known about the effect of soil salination on the responses of neuroendocrine centers of *Polypheretima elongata* hence it is selected for this study.

**MATERIAL AND METHODS**

The earthworm *Polypheretima elongata* having approximately equal size and weight were collected from an upland non-irrigated field, Baramati area. The soil characteristics are the same as described in earlier chapter II. Earthworms were kept half immersed in glass petriplates containing 30ml of tap water at 25 ± 0.5°C for 24 hours to evacuate their guts as proposed by Dash and Patra (1977). The study was carried out in plastic culture pots under laboratory conditions.

The experiment was designed to study the histological effect of 24h sublethal dose of sodium chloride (1.05g/kg soil) on worms exposed for 5 days. The earthworms were dissected by taking an incision in the anterior six segments with the help of sharp scalpel. The brain was exposed and separated in two parts like supra and subpharyngeal ganglia by carefully severing circumpharyngeal connectives at lateral sides. Since the supra pharyngeal ganglion overlying the pharynx and connected to the ventral subpharyngeal ganglia the incision was extended up to the posterior end and ventral nerve cord was detached from the body wall and separated. All tissues were fixed in aqueous Bouins fluid for 24 h. After dehydration through different grades of alcohol and followed by cold and hot impregnations, the tissues were embedded in paraffin wax (M. P. 58-60°C)
and serial sections were cut at 5-7 µm in thickness mostly in transverse and a few in longitudinal planes. The ribbons containing sections were stretched on albuminized slides and dried.

For histomorphological studies Mallory’s triple strain (Mallory, 1944), chrome haematoxyline phloxin (CHP) (Gomori, 1941) and Paraaldehyde fuchsin (PAF; Meola, 1970) using counter staining with Halmi’s mixture, were used to stain the section. Of the three stains employed in the study Mallory’s triple strain yielded satisfactory results, so the same stain was used in future routine work. The histomorphological features and change in the neurosecretory cells of different ganglia were described after measuring the neurosecretory cell area (µm²) nuclear diameter (µm) and neurosecretory material staining intensity, using the methods described by Kulkarni and Fingerman (1985).

In brief: $D \times d \times \pi/4$ (where $D =$ longer diameter; $d =$ small diameter) for an oblong neurosecretory cells, and $4/3 \pi r^2$ for oval and round shaped cell. The neurosecretory material (NSM) staining intensity was judged visually by using the following index number:

0 - neurosecretory granules absent in the cell body
1 - very few neurosecretory granules
2 - Intermediate between 1 and 3
3 - a large number of granules present in the cell body

For every observation 100 randomly selected cells from different sections were examined under light microscope and results are averaged.

OBSERVATIONS AND RESULTS

A histological survey of serially sectioned supra and subpharngeal ganglia which were stained with Mallory’s triple stain showed the occurrence of some secretory cells with cytochemical profile distinct from normal neuronal cells. These secretory cells are bulkier and endowed with conspicuous nuclei and
copious amount of cytoplasm. The perikarya and axons are loaded with fine particles, brilliantly dyed by Mallory’s stain. These staining peculiarities are characteristics features of neurosecretory cells (Scharrer and Scharrer, 1945).

Depending upon the morphological features (size, shape, vacuolization, stainability etc.) the neurosecretory cells are classified. The presence of 3 types of neurosecretory cells namely “A” cells and “B” cells in the brain of this earthworm *Polypheretima elongata* were observed. Hence in this study the response to various stimuli showed by larger cells A and B are enumerated. The morphological characters of these cells are- (Fig. 6.3 I & II).

**“A” Cells:**

These cells situated directly beneath the ganglion capsule. In the brain there appears to be a very heavy congregation of this type dorsally along the anterior-posterior axis. Inside the subpharyngeal ganglia A cells occupy chiefly dorsolateral particularly near the junction of circumpahryngeal connectives and subpharyngeal ganglion. Within segmental ganglia these cells are located along the ventral and lateral borders.

‘A’ cells are oval or rounded in shape with an average cell area oscillating between 15\(\mu\) x 10\(\mu\) to 30\(\mu\) x 10\(\mu\). They are in possession of a very prominent, centrally placed nucleus, which are rounded in shape. Nucleus is having only one conspicuous nucleolus, which has a strong affinity for acid fuchsin of Mallory’s stain. Inside the cytoplasm vacuoles are occasionally encountered.

Cytoplasmic granules of A cells take on deep red or violet coloration with Mallory’s triple stain. Secretory granules were found within the axonal tracts, which penetrated in to the neuropile. In transverse sections of the ganglia the axons are not usually visible. The axons of A cells are usually at right angles to the neuropile. It is interesting to record that at any one time while some of the cells are fully studied with neurosecretory material some may be absolutely empty and the remaining cells exhibit transitory stage between these two
extremes. This means that at any single time all the cells are not either active contributors or silent spectator (Fig. 6.3 I & II).

Cytoplasmic granules of “A” cells take on deep red or violet coloration with Mallory’s triple stain. Secretory granules were found within the axonal tracts which penetrated into the neuropile, hinting thereby the usages of axonal pathways for the transportation of neurosecretory material elaborated by these cells. In transverse sections of the ganglia the axons are not usually visible. The axons of “A” cells are usually at right angles to the neuropile (Fig. 6.4 A & B).

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“B” Cells:

“B” cells are predominant in the posterior part of the brain beneath “A” cells. In subpharyngeal ganglion they are distributed in all the groups. Similarly in the ventral nerve ganglia the B cells are dispersed in ventral and lateral cells groups (Fig. 6.5 I & II).

These cells are distinguished by an oblong or elongate or pear shaped pericarya. Nucleus is round and is usually near the axonhillock and sometime abaxonal. Nucleolus is single and prominent cytoplasm of B cells is heavily flooded with vacuoles of irregular shape and size. The secretory material is coloured pink with Mallory’s triple stain. The axons are distinctly longer than that of “A” cells and many a times neurosecretory material was located inside the axonal pathways. Their axons may be perpendicular to neuropile or may show a diagonal curvature before or after their entrance in to their terminus, the neuropile (Fig. 6. 4 A & B).
The number of “A” cells is more in brain, where as B cells are more in segmental ganglion. Neurosecretory material dispersed intercellularly and all along the circumpharyngeal connective tracts was frequently observed. There is a gradual decline in the number of neurosecretory cells from anterior to posterior segmental ganglion (Fig. 6.5 I & II). In *Polypheretima elongata* the axons do not form the axon bundle as in *Pheretima posthuma* (Dogra, 1968), insects and higher animals nor there were any axonal dilatations to form the axonal bulbs.

The results shown in table no. 6.1 and figure no. 6.10 & 6.11 indicate that after sublethal dose of sodium chloride (1.05g/kg soil) of *Polypheretima elongata* for 5 days exposure produced drastic changes in the neurosecretory material of both supra and subpharyngeal ganglion.

The suprapharyngeal ganglia of A cell neurosecretory material (NSM) intensity was decreased by 66.66%, cell area and nuclear diameter increased by 8.6 % and 29.16 %, whereas B cell neurosecretory material (NSM) intensity was increased by 66.66%, cell area and nuclear diameter increased by 20 % and 36.84 %. In the T. S. of suprapharyngeal ganglia of control worm stained with Mallory’s Triple stain showed two distinct lobes with healthy appearing neurosecretory cells A and B (Fig. 6.6), whereas in experimental worms exposed to 24 h sublethal dose of sodium chloride for 5 days showed damaged structure with distorted neurosecretory cells (Fig. 6.7).

The subpharyngeal ganglia of A cell neurosecretory material (NSM) intensity was decreased by 66.66%, cell area and nuclear diameter increased by 20.14 % and 42 %, whereas B cell neurosecretory material (NSM) intensity was increased by 66.66%, cell area and nuclear diameter increased by 22.36 % and 69 %. In the T. S. of subpharyngeal ganglia of control worm stained with Mallory’s Triple stain showed two distinct lobes with healthy appearing neurosecretory cells A and B (Fig. 6.8), whereas in experimental worms exposed to 24 h sublethal dose of sodium chloride for 5 days showed damaged structure with distorted neurosecretory cells (Fig. 6.9).
DISCUSSION

The present investigation covering the study of histomorphological characteristics of the neurosecretory cells of the earthworm *Polypheretima elongata* proves beyond doubt the neuroendocrine status of type A and B cells from various ganglia as per the criteria of Bern and Hagadorn (1965).

The salt and water balance in various invertebrate groups appears to be under the regulation of neurosecretory factors. In the earthworm, a nervous control of permeability to water has been suggested by Maluf (1939). The possible role of neurosecretory system in regulation of salt and water has been studied in the worm *Lumbricus terrestris* by Kamemoto et al., (1966) and reported a brain factor influences the salt and osmotic dose of the blood and coelomic fluid. Takeuchi in a series of papers (1965, 1967, 1968a, b.) described the structural relation between the neurosecretory granules and intracellular membranous cell organelles as well as histochemistry of the neurosecretory cells of the Japanese earthworms Viz. *Pheretima communissima*, *Pheretima hilgendorfi* and *Pheretima vittata*. In spite of these investigations, very little is known about the influence of salinity stress on the neurosecretory cells of the earthworm.

The influence of salinity stress on the neurosecretory cells of supra and subpharyngeal ganglia the earthworm *Polypheretima elongata* after exposure to sodium chloride produced a considerable reduction in the quantity of NSM. Depletion of NSM suggests that probably NSM plays a significant role in maintaining the hydrostatic and salt balance in this earthworm.

The present study on earthworm *Polypheretima elongata* revealed that there was an increase in cell area of A and B neurosecretory cells of supra and subpharyngeal ganglia. The nuclear diameter of both the cells was increased. It may be because of sodium chloride stress and toxic effect on cellular mechanics of neurosecretory cells. From the data it emerges that the sodium chloride is probably decreasing the rate of synthesis of NSM intensity of A and B cells as
diminished nuclear sizes. It may be possible that rate of transport and release of neurosecretory material in “A” and “B” cells was increased cell area and nuclear diameter.

The functional status of neurosecretory element is linked with changes in the size of nucleus may be considered as the index of cell activity (Ghosh et. al., 1981). The changes observed in neurosecretory cells of earthworm *Polypheretima elongata* suggests that perhaps the cells themselves may be acting as osmoreceptors and chemically linked with osmoreceptor cells. Sodium chloride, therefore, appear to stimulate the neurosecretory cell to release NSM through the axons, and this accounts for a drastic decrease of NSM intensity in A and B cells. Hence it is quite possible that the increased release of neurosecretory material in A and B cells cause cell osmosis. It is quite possible that the increased release of NSM from brain (Supra and Subpharyngeal ganglia) of earthworm *Polypheretima elongata* is a physiological protest to stress of toxic action. These observations are supported by Hanumante (1975), Bharathi *et.al.*, (1985) and Kulkarni (1989) who observed the effect of salinity, desiccation and osmotic stress on the neurosecretory activities of earthworm *Perionyx excavatus* and *Lampito mauritii*. These reports are also well in accordance with the reports made by Orlovsky *et al.*, (1999) in the worm *Drawida wellisi*. Chaung *et. al.*, (2006) reported that after exposure of earthworm *Lampito mauritii* to different abiotic factors produced a destruction of intranuclear structure and vacuolization in cytoplasm of “A” and “B” cells in the brain.

A histological survey of serially sectioned cerebral, sub pharyngeal and segmental ganglia, which were stained with Mallory’s triple stain showed the occurrence of some secretary cells with cytochemical profile distinct from normal neuronal cells. These Secretory cells are builkier and endowed with conspicuous nuclei and copious amount of cytoplasm. The pericarya and axons are loaded with line particles, brilliantly dyed by Mallory’s stain. These staining peculiarities are characteristic features of neurosecretory cells (Scharrer and Scharrer, 1945).
Depending upon their morphological features the neurosecretory cells can be classified. The presence of three types of neurosecretory cells namely A cell: B cells and C cells in the brain of the earthworm has been reported by Hanumante (1975). Out of these three cells C cell are very small and appear to be non-functional. Hence in this study the response to various stimuli showed by large cells A and B are innumerate.

The number of A cell is more in brain where ‘B’ cell are more in segmental ganglion. Neurosecretory material dispersed intercellular and all along the circumpharyngeal connectives tracts was frequently observed. There is a gradual decline in the number of neurosecretory cells from anterior to posterior segmental ganglion.

Occasionally, it was observed that while the pericarya are filled with neurosecretory material, the axons are totally devoid of it and vice versa. Very rarely was it found that the entire neurosecretory cell is packed to capacity with secretory material where as the neuropile region is denudes of the secretory granules and vice versa.

In this earthworm *Polypheretima elongata* it is established that neurohormones synchronize a few physiological activities reproduction, excretion etc. Thus if the neurosecretory cells are disturbed then the other physiological activities which are controlled by neurohormones are also disturbed. In the present study the effect of sodium chloride induced vacuolization in the neurosecretory cell pericarya of supra and subpharyngeal ganglia of earthworm *Polypheretima elongata*. The sodium chloride produced a destruction of intracellular structure, decrease in neurosecretory material and damage to cell wall of A and B cells.
Table No. 6.1: Changes in the activity of neurosecretory cell types ‘A’ and ‘B’ of suprpharyngeal (cerebral) and subpharyngeal ganglia of *Polypheretima elongata* after exposure to 24 h sublethal dose of sodium chloride for 5 days.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Suprpharyngeal ganglia</th>
<th>Subpharyngeal ganglia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A cell</td>
<td>B cell</td>
</tr>
<tr>
<td></td>
<td>NSM Intensity</td>
<td>Cell area (µm²)</td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>20.7</td>
</tr>
<tr>
<td>Experimental (Sodium chloride 1.05g/kg soil)</td>
<td>1.002* (-66.66%)</td>
<td>18.92* (+8.6%)</td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>19.56</td>
</tr>
<tr>
<td>Experimental (Sodium chloride 1.05g/kg soil)</td>
<td>1.002* (-66.66%)</td>
<td>15.62* (+20.14%)</td>
</tr>
</tbody>
</table>

* - Original values  
NSM – Neurosecretary material  
+ - % increase  
- - % decrease
**Figure 6.10** Changes in the activity of neurosecretory cell types ‘A’ and ‘B’ of suprapharyngeal (cerebral) ganglia of *Polypheretima elongata* after exposure to 24 h sublethal dose of sodium chloride for 5 days.

**Figure 6.11** Changes in the activity of neurosecretory cell types ‘A’ and ‘B’ of subpharyngeal ganglia of *Polypheretima elongata* after exposure to 24 h sublethal dose of sodium chloride for 5 days.
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