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Histology and Cytochemistry of the Neurosecretory Cells (NSC) of the Freshwater Snail *Lymnaea luteola* (Lamarck) Mollusca: Gastropoda

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ABSTRACT

Four types of neurosecretory (NS) cells are noticed in the cerebral, pleural, buccal and pedal ganglion of the freshwater snail *Lymnaea luteola*. According to the size and staining properties they are classified as A, B, C and D type cells. These are giant cells, medium cells, small cells and smallest cells. Type 'A' cells are largest of the all cells and measures about 0.0600-0.0800 mm in diameter. The B cells are smaller than 'A' cells and are ranges from 0.035-0.040 mm in diameter. The 'C' and 'D' cells are smaller than the 'A' and 'B' cells and more or less oval in shape. The histochemical observations reveal that the neurosecretory material is rich in carbohydrates, disulphides, sulphydryl group, protein bound amino groups, glycoprotein and lipids.

Key words: Cytochemistry, histology, *lymnaea luteola*, mollusca, neurosecretory cells

INTRODUCTION

The brain of a gastropod consists of three pairs of ganglia, all located close to the esophagus. In some primitive forms, these ganglia are relatively discrete, but in most species they have become so closely bound together as to effectively form separate lobes of a single structure (Barnes, 1982). The molluscan nervous system has been extensively studied by electro physiologists and more recently cytologists interested in the structure, formation and secretion of neurosecretory substances. In contrast to the other gastropods, relatively scanty attention has been focused on aspects of neurosecretion in prosobranchs. The presence of specialized types of cells of secretory nature in the central nervous system of gastropods and other molluscs was first noticed by Scharrer (1935) in opisthobranch snails. Scharrer (1935) observed secretory droplets in the neurosecretory cells of cerebral and visceral ganglia of *Aplysia limaciana*. Later on Scharrer and Scharrer (1954) have accumulated evidence for the existence of neurosecretory cells in the nervous system of vertebrates as well as invertebrates.

Gabe (1949, 1951, 1953a, b) reported the presence of neurosecretory cells in the prosobranch gastropods like *Dentalium entale* in the family pterotracheidae, monatocardae and in the opisthobranch gastropods. Neurosecretion has been reported in numerous molluscs primarily on the basis of morphological investigations involving the use of neurosecretory stains. The presence of prominent inclusions suggestive of secretory activity in the neurons of numerous gastropods has attracted the attention of number of investigators (Scharrer and Scharrer, 1954; Lemche, 1955; Chou, 1957; Ladislav, 1966).

Choquet and Lemaire (1969) have given cytological details of the nervous and ganglion complex in *Patella*. The presence of neurosecretory cells in the parietal ganglion of *Lymnaea stagnalis* was

reported by Yarmizina *et al.* (1968). Marchenko (1976) carried out morphological studies on the neurosecretory elements in molluscs. The anatomical and histological details of the buccal ganglia of *Helix pomatia* was furnished by Steffens (1980).

Histochemical observations on neurosecretory material of molluscs were made by some workers (Simpson *et al.*, 1966; Boer, 1965). Andrews (1968) has detailed the nervous system of *Bithynia tentaculata* with special reference to neurosecretion. Bonga (1970) furnished the details of the ultra structure of the neurohaemal areas in the pond snail *Lymnaea stagnalis*. Cowden (1972) made cytological and cytochemical examination of the central nervous system of pulmonate gastropods and some other molluscs. *Lymnaea luteola* is one of the freshwater gastropod pulmonates found abundantly in Visakhapatnam region and studies on the structural and functional aspects of neurosecretions are still wanting in this snail. Hence the present study was undertaken to study the histology and histochemistry of neurosecretory cells.

MATERIALS AND METHODS

Pulmonata to which the genus *Lymnaea* belongs to gastropods are widely distributed. For this study *Lymnaea luteola* has been selected. Live specimens were collected from Kondakarla Lake situated at a distance of 50 km from Visakhapatnam andhra Pradesh, East Coast of India. They were maintained in the laboratory in large aquarium tanks. Then the entire nerve complex was dissected out carefully with all the ganglia intact and was fixed immediately in Susa, alcoholic Bouin's, Carnoy, Zenker's and formal calcium (Bancroft and Stevens, 1990; Bancroft and Gamble, 2002). After usual procedure of dehydration in graded alcohols and clearing agent in Xylene, the material was embedded in paraffin wax and sections of 7-8 µm thick were cut and processed. For the detection of neurosecretory cells (Gomori, 1950), aldehyde fuchsin, chrome haematoxylin-phloxine, Mallory triple stain and Heidenhains Azan techniques were applied. The techniques employed for histochemical studies are presented in the Table 1.

RESULTS

Histology of NS cells: The central nervous system of *Lymnaea luteola* includes the following ganglia: Buccal ganglion, Cerebral ganglion, Pleural ganglion, Pedal ganglion, Parietal ganglion and Visceral ganglion. Each ganglion is clearly distinguishable and well separated spatially from the other. Generally all ganglions having the neurosecretory cells. There are four types of neurosecretory (NS) cells present and are distinguished into NS cells type 'A' 'B' 'C' and 'D'. These

Table 1: Histochemical reactions carried out in neurosecretory cells in *Lymnaea luteola*

Histochemical test applied	Reference	Results			
		'A' cell	'B' cell	'C' cell	'D' cell
Aldehyde-fuchsin	Gomori (1950)	+++	+++	+++	+++
Periodic acid/ Schiff (PAS)	McManus (1946)	+++	+++	+++	+++
Alcian Blue (AB) 1.0 pH	Mowry (1956)	-	-	-	-
Alcian Blue (AB) 2.5 pH	Mowry (1956)	-	-	-	-
Bromophenol blue	Mazia <i>et al.</i> (1953)	++	++	++	++
Millons' reaction	Baker (1956)	-	-	-	-
Ninhydrin/ Schiff	Chevremont and Frederic (1943)	+++	+++	+++	+++
Ferric ferricyanide	Yasuma and Itchikawa (1953)	-	-	-	-
Sudan black B	Chiffelle and Puft (1951)	++	++	++	++
Copper phthalocyanine	Kluver and Barrera (1953)	+++	+++	++	++
Gallocyanine technique	De Boer and Sarnakar (1965)	++	++	++	++

+++: Strongly positive, ++: Moderately positive, +: Faintly positive, -: Negative

are giant cells, medium cells, small cells and the smallest cells. Generally, NS cells type 'B', 'C' and 'D' are found in all ganglia where as NS Type-A cells are present in the cerebral and pleurals. Type-A cells also known as giant cells.

The NS Type 'A' cells are bigger in size and are found in brain. They are mostly round to oval in shape. The size of the giant cells is ranging from 0.600-0.800 mm. It has big nucleus and the size ranging from 0.056×0.080 mm in diameter. Giant cells in all the ganglia are generally situated towards the periphery. Nevertheless, these neurosecretory cells are found to be of the giant category with respect to the size of the other neurosecretory cells. Giant cells contain stainable material in the cytoplasm as well as in the nucleus by chrome haematoxylin phloxine.

The NS Type-B cells are medium sized neurosecretory cells. The cells also big in size and are oval in shape. The cytoplasm is granular in nature. Conspicuous nucleoli are seen inside the nucleus. The size of the cells ranges from 0.035-0.040 mm in diameter. Most of the medium sized cells are towards the neuropil. The NS Type 'C' cells are small in size when compared to the 'A' and 'B' cells. These are round bodied with round to oval shaped nucleus. These cells are also known as globuli cells. It ranges about 0.016-0.017 mm in diameter. The cytoplasm is granular. These cells are present in all the ganglia. Nucleus is fairly big and occupies 1/3-2/3rds of the cells. Finally, the fourth type of NS Type 'D' cells is smallest of all neurosecretory cells. They are circular in shape. The nucleus is big when compared to the size of the cells. Cytoplasm is granular and is present as a thin layer around the nucleus. The diameter of the cell varies from 0.088-0.11 mm and the size of nucleus varies from 0.005-0.008 mm. single conspicuous nucleolus and several nucleolar spherules are seen inside the nucleus. In the cerebral ganglion, all the four types of cells are seen and 'A' and 'B' types of cells are more in number than the other ganglia.

Histochemistry of NS cells: The histochemical localization of various organic substances in the neurosecretory material reveals the presence of glycogen in all the four cell types. Neurosecretory cells take a characteristic purple color with Gomori (1950) paraldehyde fuchsin violet chromohematoxylin phloxine and red with Heidenhain's azan stains. With PAS (Fig. 1, 2) all types



Fig. 1: Sagittal section of *Lymnaea luteola* through cerebral ganglia (control) showing neurosecretory cells (Periodic Acid Schiff's) PAS



Fig. 2: Sagittal section showing enlarged view of neurosecretory cells (Azan)

of neurosecretory cells showed an intense positivity indicating the presence of carbohydrate containing groups and a reaction which is resistant to diastase digestion indicating the absence of glycogen. The PAS reaction is abolished after acetylation and restored after deacetylation suggesting the presence of 1:2 glycol groups. The non secretory neurons in the ganglion also showed positivity to this reaction but it is not so marked as in secretory cells. The neurosecretory cells exhibited moderate PAS reactivity, which could partly be abolished by prior diastase digestion. With alcian blue (1.0 and 2.5 pH) the neurosecretory cells and non-secretory neurons showed a negative response suggesting the absence of acid mucopolysaccharides. Neurosecretory cells are negative to toluidine blue.

When tested for basic proteins, the cells are moderately positive to BPB and this reaction is abolished completely by deamination with Van Slyke's reagent. Protein tests like p-dimethyl aminobenzaldehyde nitrite and Millon's yielded negative results. The NS cells showed a positive response to ferric ferricyanide, ninhydrin/ Schiff and KMnO₄/AB technique. A strong response to Congo red was obtained. With pyronin Y for RNA, the neurosecretory material showed a faint positivity. With Feulgen reaction for DNA, the nuclei stained in dark purple color indicating the presence of DNA in the nucleus. When stained with Sudan black B for lipids and copper phthalocyanine for phospholipids these neurosecretory cells in the ganglion reacted very strongly indicating the presence of both lipids and phospholipids.

From aforementioned histochemical reactions, it may be concluded that the neurosecretory material has high quantities of lipid, phospholipids, di-sulphide groups, sulfhydryl groups and carbohydrates like 1:2 glycol groups, protein bound amino groups and amino acid like tyrosine.

DISCUSSION

Evolutionary rank may be assigned on the basis of dominance that the central nervous system exerts over peripheral events, the mollusca certainly head the list of all invertebrates. Most of the neurons in the central ganglia show signs of some form of glandular nature of the substance. These cells with glandular substance are known as secretory cells. In *Lymnaea luteola* four types of cells are distinguishable according to their size, number of nucleoli and the amount of neurosecretory

material present in the cell. They are designed as 'A', 'B', 'C' and 'D' cells or giant cells, medium size cells, small and smallest cells. The 'B', 'C' and 'D' types of cells are found in all five ganglia. 'A' cells or the giant cells are present only in the cerebral, parietal, pedal and visceral ganglia but are absent in pleural ganglia. In *Lymnaea* (or) *Aplysia* giant neurosecretory cells are present (where they can be 800 μm). They are much greater than the remaining pulmonates. In this species, they measure about 0.030×0.025 mm in diameter. Price (1977) has not found any giant cells in the pleural ganglia of *Melampus bidentatus*. Both parietals and viscerals however have a number of large cells. The 'B' cells are analogous to the conventional neurons of Cowden (1972).

She observed cells filled with secretory droplets. Lever (1957) distinguished five types of NS cells in cerebral ganglion of *Ferrisia* species. Van Mol (1960) reported the presence of large NS cells forming two dorsal caps in the mid brain of *Arion rufus*. Krause (1960) observed two types of NS cells in the pharyngeal nerve ring of *Helix pomatia*. Observations of Lever *et al.* (1961) revealed that in *Lymnaea stagnalis* all ganglia possess NS cells but pedal ganglia possess the smallest number of cells. Seven types of NS cells have been reported in *Lymnaea stagnalis* by Bonga (1970). Swindale and Benjamin (1976) distinguished three neurosecretory cell types in the central nervous system of the gastropod *Lymnaea stagnalis* by using AB and AY techniques. The present result also indicate that the secretions of all the cells ('A', 'B', 'C' and 'D') do not differ in their chemical properties. Only variations in the quantity of NS products were observed. Type 'A' cells showed the highest quantity of secretory material than other NS cells.

There have been several reports on the presence of PAS positive material in NS cells. Chou (1957) reported the presence of PAS positive neurosecretory substance in *Helix aspersa*. After digestion with saliva the reaction is weaker (though it still exists). This suggests the presence of carbohydrates in it. Boer (1965) in *Lymnaea* and Cook (1966) in *Succinea* suggested that the NS material in these pulmonates is lipoprotein whereas, Andrews (1968) reported it as glycoprotein in *Bithynia*. In *Lymnaea luteola*, in addition to carbohydrate moiety and SS and SH groups it is thought to be a glycoprotein and lipid. Phospholipids are also present. Lipid globules do, indeed occur in the neurons of mammals and these contain phospholipids (Casselman and Baker, 1955). Boer (1965) observed the Sudan Black B positive material in the NS cells of Gomori positive and Gomori negative cells in Molluscs. SS and SH groups are present in NS material of all the cells.

CONCLUSION

It is generally accepted that the nervous system of higher gastropods is simple an anatomical constitution. The central nervous system of *L. luteola* is composed of paired ganglia, these adjoining very closely to one another. Four types of neurosecretory cells (A, B, C and D) have been encountered in these ganglia. These are found to be cytologically different and much larger than the neurons. The categorization is based on the size, number of nucleoli and distribution. They stain characteristically purple with Paraldehyde-fuchsin and red with Heidenhain's Azan stain. The histochemical studies revealed that the NS material in *L. luteola* rich in s-s bonds of cystine, phospholipids and glycoprotein. The NS material is positive to PAS technique which is resistant to pretreatment with diastase. The glycogen is absent in the NS material and 1: 2 glycol groups predominant. The NS material also contains free aldehyde some S-H groups sufficient quantities of protein bound amino groups and glycoproteins.

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