A preliminary study of the histochemical nature of neurosecretory material in different neurosecretory cell groups of the *Heterometrus fulvipes* (Koch) scorpion

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Abstract

Histochemical studies are essential for the understanding of biochemical reactions of any metabolic activity. It is therefore desirable to study the histochemical nature of the neurosecretory substance, which plays a pivotal role in the regulation of all metabolic activities. The neurosecretory substance produced by the neurosecretory cells of the cephalothoracic nerve mass viz., the brain and the sub-oesophageal ganglion, is tested for proteins (Mercuric Bromo Phenol Blue method), lipids (Sudan Black B), carbohydrates (Periodic Acid/Schiff), aminoacids tyrosine (Millon's reaction) and tryptophan (P-DMAB-No_2), protein bound $-NH_2$ groups (Ninhydrin/Schiff), –SH (Ferric Ferricyanide) and –SS groups (Performic acid/alcian blue) as well as Nucleic acids (Feulgen's reaction and Methyl green-Pyronin Y). The results indicated the presence of carbohydrates other than glycogen, lipids, basic proteins, protein bound amino groups, aminoacids tyrosine and tryptophan, –SS and –SH groups, RNA and trace amounts of DNA. Thus, it is apparent that the neurosecretory cells of *Heterometrus fulvipes* contain glycolipoproteinaceous secretory material and that the phloxinophilic globules/accumulations are 'Protein' in nature.

Keywords: histochemical nature, neurosecretory material, cephalothoracic nerve mass, glycolipoprotein, *Heterometrus fulvipes.*

1 Introduction

Studies on the histochemical nature of the neurosecretory substance among invertebrates have been mostly carried out on major groups like crustaceans, insects and mollusks (BARANYI, 1963; BONGA, 1970; COWDEN, 1972; ERRIBABU, SHYAMASUNDARI and RAO, 1979). Although there were many similar studies among arachnids, there is little information on the histochemical nature of the neurosecretory (NS) cells in the *Heterometrus swammerdami* scorpion (HABIBULLAH, 1971) and the *Argiope aurantia* spider (BABU, 1973). Therefore the study was undertaken in the *Heterometrus fulvipes* scorpion.

2 Material and methods

As a prerequisite for the histochemical study of NS cells, mapping of the distribution of NS cells in the cephalothoracic nerve mass (CTNM) viz., brain and sub-oesophageal ganglion, was carried out using the paraffin double embedding technique followed by serial sectioning and staining with Chrome-Haematoxylin-Phloxin (GOMORI, 1941b). Mapping the distribution of NS cells was based on the findings of earlier researchers (BABU, 1965; HABIBULLAH, 1970). The identification of cytomorphology of the NS cells was based on the work of Gabe (1955) and Babu (1973). Although two major types, viz., Type I and Type II, were identified on the basis of size, cytomorphology and nature of the neurosecretory material (BABU, 1973), we followed the same classification presented with regard to NS cell groups in *H. swammerdami* (Habibullah, 1970) for *H. fulvipes.* The histochemical nature of the neurosecretory substance of the cephalothoracic nerve mass in the *Heterometrus fulvipes* scorpion was studied by employing the following methods: mercury bromophenol blue for proteins (MAZIA, BREWER and ALFERT, 1953), Sudan Black B for lipids (McMANUS, 1946), standard Periodic acid/Schiff (PEARSE, 1968) for PAS positive substances, Millon's reaction (BAKER, 1956) for tyrosine, ninhydrin/Schiff reaction (YASUMA and ITCHIKAWA, 1953) for protein bound amino groups, Feulgen's reaction and Methyl green pyronin Y (KURNICK, 1952; PEARSE, 1968) for nucleic acids. The presence of –SS and –SH groups was identified by the ferric ferricyanide method (CHÈVREMONT and FREDERIC, 1943) and the Performic acid/alcian blue method (ADAMS and SLOPER, 1956). Suitable controls were employed for all the above-mentioned tests to identify the presence or absence of a specific chemical entity in the neurosecretory substance of the scorpion under study. Samples of the adult (both male and female) *H. fulvipes* scorpion, collected from the hilly areas of Tirupathi (13° 40' N and 79° 20' E) in South India, were brought in large well-aerated tin boxes to the laboratory, kept in suitable vivaria containing moist soil, and used for the study.

3 Results

Histological studies on the distribution of neurosecretory (NS) cells in the cephalothoracic nerve mass of the *Heterometrus fulvipes* scorpion revealed the occurrence of two groups of NS cells in the supra-oesophageal ganglion, designated group 1 and group 2, and metamerically arranged NS cells, designated group B, groups 3-9 and group C, in different neuromeres of the sub-oesophageal ganglion (SOBHA, 1994). The results presented here are applicable to the neurosecretory substance of NS cells belonging to both the brain and the sub-oesophageal ganglion. As there are no significant differences between different groups of cells with regard to sex and response to histochemical reactions, a general report is presented.

The NS cells and the neurosecretory substance (NSS) showed an intense positive reaction for basic proteins with the mercury bromophenol blue method (Figure 2, 3), a moderately positive reaction for tyrosine and tryptophan with Millon's reaction and P-DMAB-No_2 method, respectively. With the ninhydrin/Schiff reaction for protein-bound amino groups, the NS cells showed a faintly positive reaction, indicating the presence of few protein-bound amino groups.

The NS cells showed an intense positive reaction for lipids with the sudan black B method (Figure 4). With the performic acid/alcian blue technique, the NS cells stained moderately, demonstrating the presence of –SS groups, which are essential for the synthesis of the NS substance. The NS cells showed moderate to strong positivity to ferric ferricyanide reaction, indicating the presence of considerable amounts of –SH groups (Figure 5).

The NS cells showed a weak to moderately positive reaction for carbohydrates with the standard periodic acid/Schiff technique (Figure 6), and this reaction can be considered to be resistant to diastase digestion, although the intensity of staining is weaker, indicating the absence of glycogen. Negative result to PAS after acetylation and positive result after deacetylation suggest the presence of 1:2 glycol groups. A negative reaction to the treatment with chloroform/methanol mixture prior to staining with Schiff's reagent suggests the absence of glycolipids. There is no relation between the intensity of PAS reaction and the intensity of staining by methods specific for neurosecretory material.

With methyl green – pyronin Y, the NS cells showed moderate positive reaction for RNA, and with Feulgen's reaction, the NS cells showed faint response for DNA. Phloxinophilic globules transported from group 3 cells towards the dorsomedian region of the sub-oesophageal ganglion (Figure 3) and phloxinophilic accumulations (Figure 2) located near groups 5 and 6 (Figure 1) showed a strongly positive reaction for basic proteins with the mercury bromophenol blue method, and a moderately positive response to Millon's reaction and P-DMAB-No_2 method for tyrosine and tryptophan, respectively. They were negative to the sudan black B method for lipids and PAS for carbohydrates.

4 Conclusion

The results of the present study show that the neurosecretion in the *Heterometrus fulvipes* scorpion contains basic proteins, lipids, carbohydrates (other than glycogen), tyrosine, tryptophan, protein-bound amino groups, RNA, DNA, –SS and –SH groups.

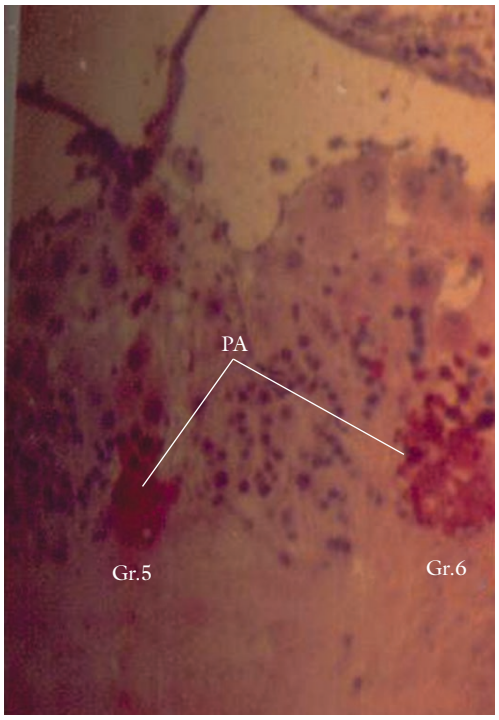

Figure 1. Localization of Groups 5 and 6 cells showing phloxinophilic accumulations of secretory product [Stain: CHP] X100; PA: Phloxinophilic accumulation.

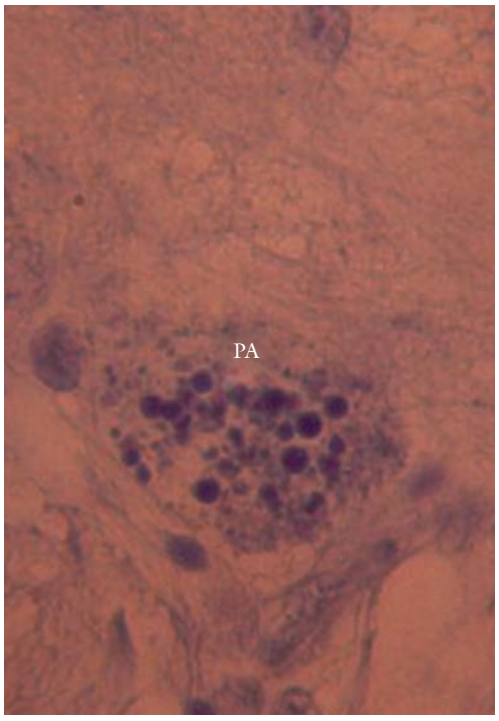

Figure 2. Phloxinophilic accumulation near Group 6 cells showing positive reaction to Mercury Bromophenol Blue (MBPB) X450. PA: Phloxinophilic accumulation.

A cytochemical study of the nature of neurosecretion in the *Portunus sanguino lentus* marine crab indicated that the NS material has a carbohydrate moiety and is rich in –SS groups, lipids, phospholipids and RNA, a small amount

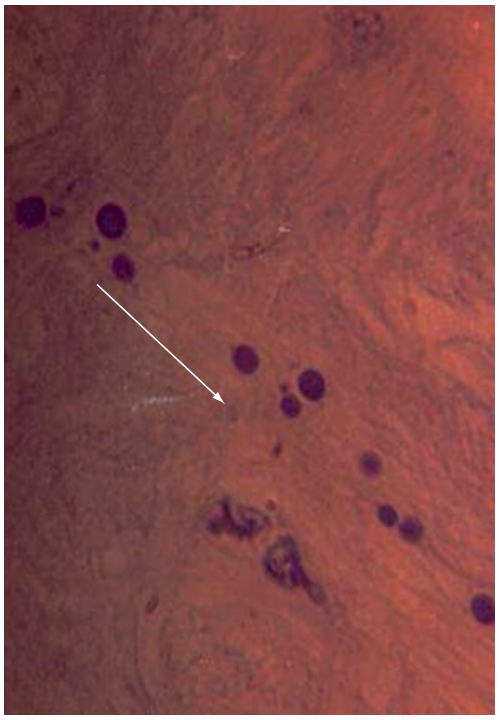

Figure 3. Phloxinophilic globules from Group 3 cells showing positive reaction to Mercury Bromophenol Blue (MBPB) X450; Arrow indicates the direction of transportation.

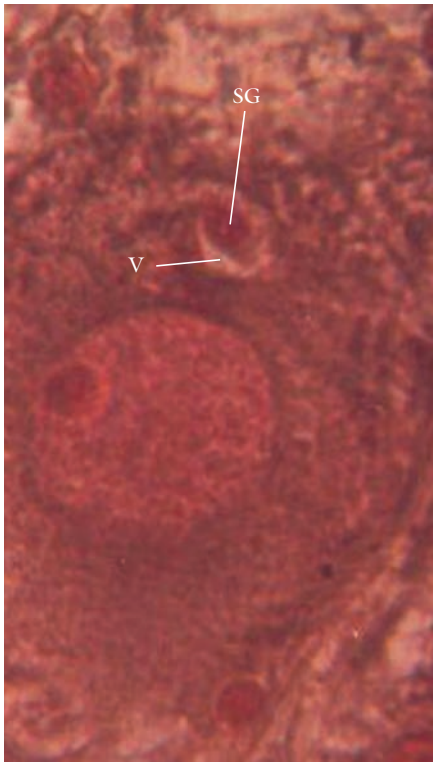

Figure 4. Intense staining of Neurosecretory cell with Sudan Black B X450. Note the negative reaction of secretory globule. V: Vacuole; SG: Secretory globule.

of –SH and protein-bound amino groups, but no tyrosine and tryptophan (TRINADHABABU, SHYAMASUNDARI and RAO, 1989). A cytochemical study of the NS cells in *Charybdis truncata* showed that they contained carbohydrates, 1:2 glycols, basic proteins, –SH and –SS groups, α-NH_2 groups, lipids, phospholipids, RNA and DNA (DEVI, SHYAMASUNDARI and RAO, 1987).

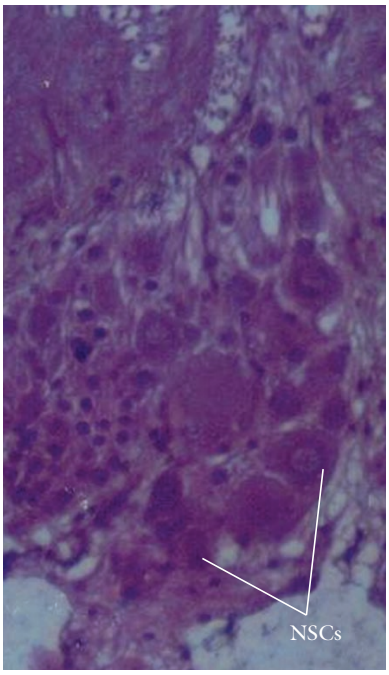

Figure 5. Neurosecretory cells (Group 3) showing positive reaction for –SH groups X450; NSCs: Neurosecretory cells.

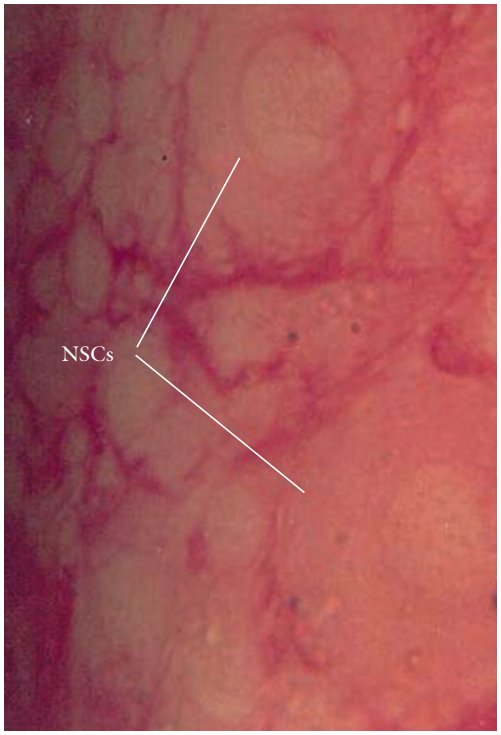

Figure 6. Faint staining of Neurosecretory cells with Periodic Acid Schiff (PAS) X450; NSCs: Neurosecretory cells.

Histochemistry of the NS system in the *Metapenaeus affinis* penaeid prawn revealed that the NS material is of proteinaceous nature. The positive reaction of NS material to PF and alcian blue indicates the presence of cystine and cysteine rich material (RAO, SAROJINI, JAYALAKSHMI et al., 1988).

Histochemical studies on the NS cells of the *Dysdercus* insect demonstrated the presence of lipids, proteins and carbohydrates. RNA was also apparently present in a very low quantity. The NS cells appeared to have mainly lipids, although they have a mixture of proteins (GUPTA, 1971). Histochemical analysis of the 'A' cell NS material in the *Oncopeltus fasciatus* Dallas milk weed bug showed neither carbohydrates nor lipids. The results suggested that the neurosecretory material is a protein rich in cystine and cysteine (SCHREINER, 1966).

In the *Argiope aurantia* spider, it is reported that the secretory product of type I cells present in the protocerebral and all sub-oesophageal ganglia and type II cells confined to cheliceral ganglia is proteinaceous, while that of type III cells present in cheliceral and sub-oesophageal ganglia is a polysaccharide, in particular glycogen (BABU, 1973).

As is evident from the results, the neurosecretory material in the *Heterometrus fulvipes* scorpion is a glycol-lipoprotein complex. Habibullah (1971) reported that in the *Heterometrus swammerdami* scorpion, the carbohydrate component of the neurosecretory material is glycogen. But in the present study, the carbohydrate component is apparently something other than glycogen. This is similar to the situation in insects where it is reported that the sugar component is either absent (WIGGLESWORTH, 1956) or, if present, it is other than glycogen (GABE, 1955). As noticed in *H. fulvipes*, after treatment with diastase, the weaker staining with PAS was reported in *Helix aspera* (CHOU, 1957). Habibullah (1971) reported the occurrence of large amounts of –SHs, characteristic of vertebrate NS cells, in the neurosecretory material of the *H. swammerdami* scorpion. Also in *H. fulvipes,* fairly large amounts of –SHs were noticed. The neurosecretion in *H. fulvipes* contained considerable amounts of –SS groups, which are essential for the synthesis of NS material.

The NS substance in *H. fulvipes* was apparently rich in basic proteins just as in many invertebrates and vertebrates. Active portion of the NS substances in vertebrates was presumed to be the glycolipid component of the glyco-lipoprotein complex, and that the protein component has no hormonal activity (ORTMAN, 1958). Although the protein component of the neurosecretion has no hormonal activity by itself, association of the glycolipid component of the complex with the protein is evidently essential for activity as in the case of insect ecdysone.

Interestingly, in the *H. fulvipes* scorpion, the phloxinophilic secretory globules that are transported from group 3 cells towards the dorso-median region of sub-oesophageal ganglion and the phloxinophilic accumulations near groups 5 and 6, which are presumed to play a significant role in the reproductive cycle, showed strong positive reaction for basic proteins and a moderate positive reaction for tyrosine and tryptophan. They showed a negative reaction to lipids and sugars. Therefore, it may be said that the "Hormone" or the "Carrier-coated hormone" released in the form of globules from group 3 cells and accumulations near groups 5 and 6 is 'Protein' in nature. On the contrary, Habibullah (1971) reported the sudanophilic, in addition to the proteinaceous, nature of the phloxinophilic accumulations in the *H. swammerdami* scorpion. In short, the NS cells of *H. fulvipes* contain glycolipoproteinaceous secretory material, and the phloxinophilic globules from group 3 cells and accumulations near groups 5 and 6 are 'Protein' in nature.

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