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Classification and mapping of neurosecretory cells in the optic, supraoesophageal and thoracic ganglia of the female spiny lobster *Panulirus homarus* (Linnaeus, 1758) and their secretory activity during vitellogenesis

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Abstract

The crustacean endocrine system consists of epithelial type of endocrine glands and endocrine structures of neural origin. The neurosecretory cells are of great significance in the crustacean endocrine system with respect to the number of neurohormones regulating reproduction. The study describes the general morphology of the central nervous system as well as the neurosecretory cells in the optic, supraoesophageal and thoracic ganglia of the spiny lobster, *Panulirus homarus*. The central nervous system of *P. homarus* follows the general arthropod pattern which consists of a ganglionated nerve cord extending from the cephalic region to the end of the abdomen. The neurosecretory cells are characterized by the presence of large nucleus, abundant cytoplasm, granules, vacuoles etc. in their perikarya showing differences in their size and shape. Based on these characteristics, the neurosecretory cells are classified into different cell types. The optic ganglia have six types of neurosecretory cells, whereas the supraoesophageal ganglion and the thoracic ganglia have eight neurosecretory cell types. Cyclic changes are observed in the perikarya of neurosecretory cells in relation to the synthesis of neurosecretory material. The secretory changes in the different neurosecretory cell types follow a basic pattern with four phases, *viz.* synthetic phase, vacuolar phase, secretory phase and quiescent phase. The secretory cycle of different neurosecretory cell types in the optic ganglia, supraoesophageal ganglia and thoracic ganglia were correlated with the ovarian developmental stages. The cyclic secretory activity of neurosecretory cells in the optic, supraoesophageal and thoracic ganglia points to secretion of certain hormones, which regulate the process of vitellogenesis.

Keywords: Lobster, ovarian development, vitellogenesis, neurosecretory cells, ganglia, secretory activities

Introduction

Knowledge of endocrine mechanisms controlling reproduction is useful in evolving techniques for stimulating reproductive process leading to increasing yield in aquaculture production systems. Crustaceans have a full range of neuronal structures modified for production and secretion of neurohormones and neuroregulators that control various physiological activities including reproduction. The neurohormones synthesised by the neurosecretory cells, which control female reproduction especially the process of vitellogenesis is mainly distributed in the optic ganglia, supraoesophageal ganglion and thoracic ganglia (Panouse, 1943; Adiyodi and Adiyodi, 1970). Panouse's discovery of a gonad inhibiting hormone was later demonstrated in many decapods (Deccaraman and Subramoniam, 1983; Radhakrishnan and Vijayakumaran, 1984). The presence of gonad stimulating hormone in brain and thoracic ganglia was reported in many decapods (Otsu, 1960; Gomez, 1965; Eastman-Recks and Fingerman, 1984). Quackenbush and Herrnkind (1981) partially characterized the eyestalk hormones controlling molt and gonadal development in the spiny lobster *Panulirus argus*.

For successful captive breeding of lobsters, precise knowledge of the source, nature and mode

of action of these neuroendocrine factors in relation to vitellogenesis is imperative. The spiny lobster, *P. homarus* was selected in this study for its commercial importance in India. Moreover the lobsters are indiscriminately exploited resulting in reduced catches. Keeping in mind the urgency of developing a viable hatchery technology, an investigation was carried out to study the morphology of the central nervous system; the structure, morphology, distribution and secretory activity of the neurosecretory cells in the optic ganglia, supraoesophageal ganglion and thoracic ganglia. The secretory cycle of the neurosecretory cells were correlated with the vitellogenic processes at the cellular and ultra-cellular level.

Material and Methods

Lobsters were collected from the southwest coast of India and transported alive to the laboratory. The optic, supraoesophageal and thoracic ganglia were excised freshly from the lobsters for histological studies. Histology was carried out as described by Nagarajalingam and Subramoniam (1982) with slight modifications. The neuroendocrine tissues were fixed in Bouine's fixative and washed in running tap water overnight for further processing as and when required. The tissues were serially dehydrated in alcohol, cleared in benzene and methyl benzoate and embedded in paraffin wax with a melting point of 58-60°C. Sections were cut at 6µ thickness using a rotary microtome. The neurosecretory cells were identified by staining the histological sections using Gomori's paraldehyde fuchsin method (Cameron and Steles, 1950) and Mallory's triple staining method (Mallory, 1944). The sections were photographed using a Nikon fdx 35 microphotography system. The transmission electron microscopy of neurosecretory tissues was carried out by the method suggested by Beams and Kessel (1963) and Spurr (1969). The ultra sections were cut in the LKB Ultratome NOVA and were stained in Uranyl acetate and Lead citrate, for double staining to enhance the contrast. The ultra thin sections were mounted and the images observed were photographed in Hitachi H 600 Transmission Electron Microscope.

The maturity stages of the female lobster were classified into five, namely; immature, primary vitellogenic, secondary vitellogenic, mature and spent, to study the secretory status of neurosecretory cell types in the optic ganglia, supraoesophageal ganglia and thoracic ganglia at different stages of ovarian development.

Results

***Neuroendocrine system*:** The central nervous system of *P. homarus* follows the general arthropod pattern, which consists of a ganglionated nerve cord extending from the cephalic region to the end of the abdomen (Fig. 1). The thoracic region has a suboesophageal ganglion and five pairs of thoracic ganglia fused together and the abdominal region has six pairs of abdominal ganglia. The nerve cord remains ventral to the alimentary canal. In the cephalic region the two component cords of the chain separate from one another and after encircling the oesophagus (circumoesophageal connective)

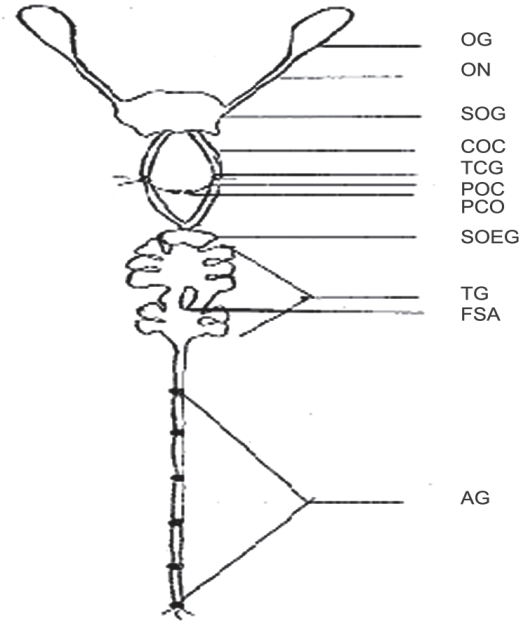

Fig. 1. The central nervous system of *P. homarus*; AG-Abdominal ganglia, COC-Circumoesophageal connective, FSA-Foramen for sternal artery, OG-Optic ganglia, ON-Optic nerve, PCO-Post commissural organ, POC-Postoesophageal commissure, SOEG-Suboesophageal ganglion, SOG-Supraoesophageal ganglion, TCG-Tritocerebral ganglion, TG-Thoracic ganglia

reaches the supraoesophageal ganglion (brain) dorsally. From the brain a pair of short nerves extends towards the eyestalk and forms the optic ganglia. In the circumoesophageal connective, a pair of tritocerebral ganglia is found. In the post oesophageal commissure a pair of post commissural organ is located. Scattered in the different ganglia there are neurosecretory cells (NSCs). These neurosecretory products stain purple with paraldehyde fuchsin stain and blue with Mallory's triple stain.

The NSCs are characterized by the presence of large nucleus, abundant cytoplasm, conspicuous granules, vacuoles and cell organelles like golgi complex, endoplasmic reticulum, ribosome, mitochondria etc. in their perikarya. The cytoplasm is filled with vesicles of varying size, shape and electron density. All NSCs are unipolar and nondendritic (Fig. 2a). Though the NSCs share the same basic characteristics they differ significantly in their morphological and cytological characteristics. Based on these, the neurosecretory cells are classified into different cell types. Separate classifications are given to NSCs in the optic, supraoesophageal and thoracic ganglia.

***Optic ganglia*:** The optic ganglia of *P. homarus* consist of four well defined ganglia *viz*. the medulla terminalis (MT), the medulla interna (MI) the medulla externa (ME) and the lamina ganglionaris (LG) (Fig. 2b). The ganglia are arranged in a consecutive manner behind the ommatidia. The medulla terminalis tapers to form the optic nerve. A neurohaemal organ known as the sinus gland (SG), which form the storage site of the neurosecretory products, is located on the dorsolateral side of the optic ganglia beneath the cuticle and between the MI and ME and at some points SG extends up to the MT. In the right eye stalk, the sinus gland is located on the right dorsolateral side and in the left eyestalk SG is located on the left dorsolateral side. The SG could be noticed with the naked eye as a very small bluish white opalescent body. The SG has an internal blood sinus and an external blood sinus with different axonic terminals containing the neurosecretory products.

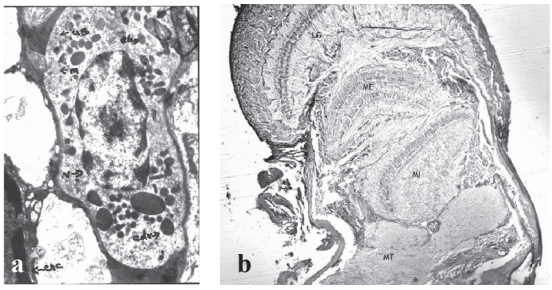

Fig.2a. Electron micrograph of a typical NSC in the supraoesophageal ganglion of *P. homarus*. elv: electron luscent vesicles, edv:electron dense vesicles,GB: Golgi body, M: Mitochondria, N: Nucleus & GC: Glial cell Magnification 8000X. b. Basic structure of the optic lobe *P.homarus*. MT: Medulla terminalis, MI: Medulla interna, ME: Medulla Externa & LG: Lamina ganglionaris. Magnification 50X

The neurosecretory cells were distributed only in MT, MI and ME and they were distributed three-dimensionally. Maximum numbers of neurosecretory cells were found in MT. The neurosecretory cells distributed in the MT was termed as MT X-organ while those in the MI and ME were termed as MI X-organ and ME X-organ respectively.

***NSCs in the optic ganglia*:** The different NSCs distributed in the optic ganglia are classified into six types; a', b', c', d', e' and f'. The distribution pattern of NSCs in the optic ganglia is shown in Fig. 3-5. The morphological and cytological characteristics of NSCs in the optic ganglia are given in Table 1. Type a', b' and c' NSCs are distributed mainly in the MT. Type d' cells are distributed in MT and ME. Type e' NSCs are found in abundance and they were distributed in all the three ganglia. Type f' NSC is also distributed in all the three ganglia. The percentage frequency distribution of NSCs in the optic ganglia is given in Fig. 6.

***Supraoesophageal ganglion*:** The supraoesophageal ganglion consists of three lobes, *viz*. protocerebrum, deuterocerebrum and tritocerebrum. The neurosecretory cells are distributed in these three lobes.

***NSC types in the supraoesophageal ganglion*:** The NSCs in the brain are classified into eight types - a'', b'', c'', d'', e'', f'', g'' and h'' NSCs

Table 1. Neurosecretory cell types in the optic ganglia of *Panulirus homarus*

Cell Type	Symbols used in the Fig. 3 and 4	Cell diameter (im±sd)	Nucleus diameter (im±sd)	Shape of the cell	Nucleus-cytoplasmic ratio
a'	❍	45.24±5.30	10.35±0.00	Oval	0.20-0.26
b'	●	30.48±2.43	10.80±1.28	Discoid	0.34-0.37
c'	❒	23.41±1.68	9.06±0.70	Round	2.60-0.36
d'	■	21.21±3.11	9.20±1.20	Pyriform	0.42-0.44
e'	∆	10.82±1.90	7.38±1.60	Round	0.65-0.70
f'	⬥	6.63±0.50	5.36±0.35	Elliptical	0.80-0.81

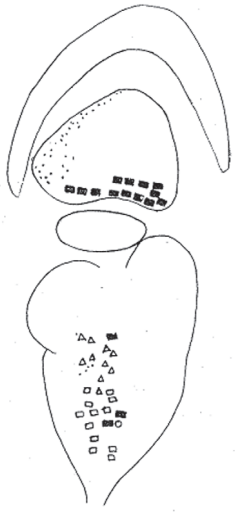

Fig. 3. Distribution of neurosecretory cells in the optic ganglia of *P. homarus* - dorsal view

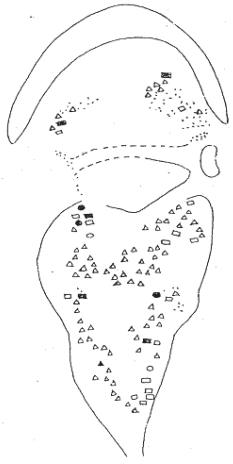

Fig. 4. Distribution of neurosecretory cells in the optic ganglia of *P.homarus* - median dorsal view

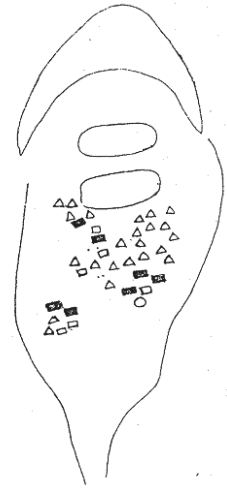

Fig. 5. Distribution of neurosecretory cells in the optic ganglia of *P. homarus* - ventral view

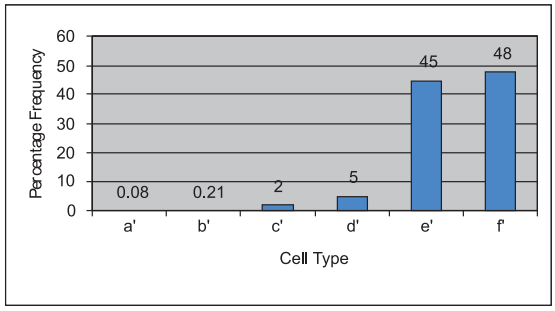

Fig. 6. Percentage frequency distribution of neurosecretory cells in the optic ganglia of *P. homarus*

(Table 2). Type e'' and h'' NSCs are usually found in clusters while the others are found scattered. The type g'' NSCs are concentrated more on the tritocerebrum while the others are seen throughout the ganglia (Figs. 7 and 8). Type a'' NSCs are not

found in the central part of the supraoesophageal ganglion (Fig. 9). Type b'' NSCs are found more on the protocerebrum and deuterocerebum. The distribution of c'' cells are limited at the protocerebrum and deuterocerebrum. The d'' NSCs are distributed more in the protocerebrum and deuterocerebrum. These cells are usually found in small groups. Type f'' NSCs are of limited occurrence. They are distributed among other cell types in the protocerebrum and deuterocerebrum. The distribution pattern of NSCs in the supraoesophageal ganglion is given in Figs. 7 and 8. The g'' cell type is the most abundant of all cell types. The percentage occurrence of different cell types is given in Fig. 9.

***Thoracic ganglia*:** The thoracic ganglia of lobster are dorsoventrally flattened and consist of five pairs of ganglia fused into a large ganglionic mass. There is a perforation between the third and fourth pairs of thoracic ganglia for the passage of the sternal artery (Fig. 1).

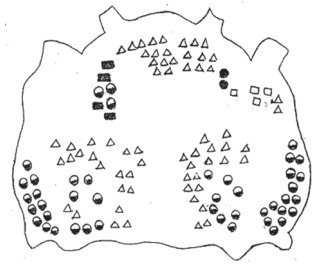

Fig. 7. Distribution of neurosecretory cells in the supraoesophageal ganglion of *P. homarus*-dorsal view

Fig. 8. Distribution of neurosecretory cells in the supraoesophageal ganglion of *P. homarus* - ventral view

Table 2. Neurosecretory cell types in the supraoesophageal ganglion of *Panulirus homarus*

Cell Type	Symbols used in used in the Fig. 7 and 8	Cell diameter (ìm±sd)	Nucleus diameter (ìm±sd)	Shape of the Cell	Nucleus-cytoplasmic ratio
a''	❍	102 ± 4.85	22.76±4.38	Oval	0.18 - 0.25
b''	●	53.00±6.18	14.98±2.75	Round	0.26 - 0.30
c''	❒	42.28±4.59	12.14±1.82	Oval	0.27 - 0.30
d''	■	23.48±4.59	11.33±1.44	Round	0.45 - 0.52
e''	∆	15.00±0.91	8.80±0.22	Round	0.53 - 0.64
f''	▲	13.37±1.30	7.74±1.15	Pyriform	0.54 - 0.60
g''	◒	10.09±.10	6.68±0.45	Round	0.61 - 0.70
h''	◓	8.60±.70	2.075±0.04	Hexagonal	0.22 - 0.25

***NSC types in the thoracic ganglia*:** There are eight neurosecretory cell types distributed in the thoracic ganglia; type a''', b''', c''', d''', e''', f''', g''' and h'''(Table 3). Compared to the optic ganglia of the eyestalk and supraoesophageal ganglion, the number of neurosecretory cells in the thoracic ganglia is very less. The size of NSCs in the thoracic ganglia is much greater than those in the other ganglia. Unlike in other ganglia the percentage contribution of larger NSCs is also higher in thoracic ganglia. Though type a''' cell is found in all the ganglia, this is mostly located in the fourth and fifth pairs of

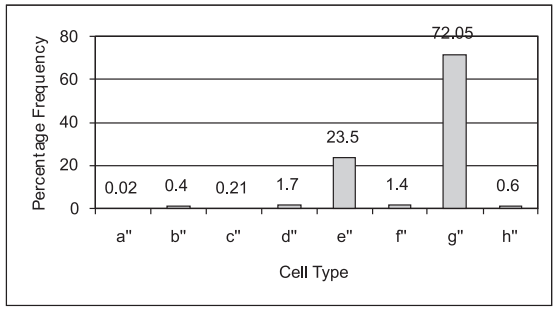

Fig. 9. Percentage frequency of neurosecretory cells in the supraoesophageal ganglion

ganglia. . Type b''', c''', d''', e''' , f''', g''' and h''' NSCs are seen in all the ganglia. The distribution pattern and percentage occurrence of different cell types are given in Figs. 10 and 11 respectively.

***Secretory cycle of NSCs*:** Cyclic changes are observed in the perikarya of neurosecretory cells in relation to the synthesis of neurosecretory material. The secretory changes in the different neurosecretory cell types follow a basic pattern which can be described in four phases based on the appearance of cytoplasmic cell organelles and inclusions like vesicles, granules and vacuoles. The different phases

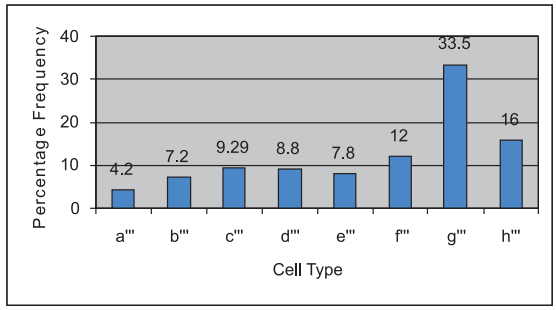

Fig. 11. Percentage frequency of neurosecretoy cells in the thoracic ganglia of *P. homarus*

Table 3. Neurosecretory cell types in the thoracic ganglia of *P. homarus*

Cell type	Symbols used in Fig. 10	Cell diameter (im±sd)	Nucleus diameter (im±sd)	Shape of the cell	Nucleus-cytoplasmic ratio
a'''	❍	133±11.57	6.39±0.54	Oval	0.05
b'''	●	102.84±3.28	23.33±2.47	Oval	0.20 - 0.24
c'''	❒	87.14±5.6	7.28±1.15	Oval	0.08 - 0.09
d'''	■	43.08±3.5	14.95±1.50	Round	0.34 - 0.35
e'''	∆	29.46±1.92	12.22±1.60	Oval	0.38 - 0.44
f'''	▲	18.6±1.6	9.06±1.50	Round	0.44 - 0.52
g'''	◒	14.64±2.10	8.05±1.00	Oval	0.54 - 0.56
h'''	◓	8.617±0.82	6.39±0.54	Hexagonal	0.73 - 0.75

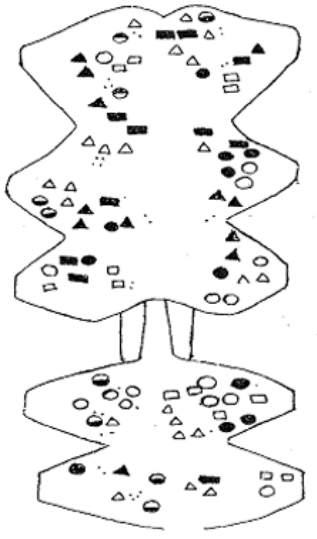

Fig. 10. Distribution of neurosecretory cells in the thoracic ganglia of *P. homarus*

are the synthetic phase, vacuolar phase, secretory phase and the quiescent phase.

***Synthetic phase*:** Synthetic phase of the neurosecretory cells are characterized by the stained granular heterogeneous cytoplasm (Fig. 12a, b). Nuclear membrane shows perforations through which the nuclear material oozes out. Membrane bound vesicles are seen originating from the endoplasmic reticulum. They move to the Golgi complex which encircles and fabricates them into membrane limiting dense units. Such packed neurosecretory products are seen pinching off from the end of the Golgi complex. Other cell organelles like ribosome, mitochondria etc. are also present in the cytoplasm.

***Vacuolar phase*:** This phase is characterized by numerous vacuoles and vesicles of varying size and shape. The cytoplasm has taken the stain and it is heterogeneous. Individual vacuoles that tend to

merge to form larger vacuoles around the nucleus are also observed in the cytoplasm. The vesicles, which are produced by the Golgi complex clump

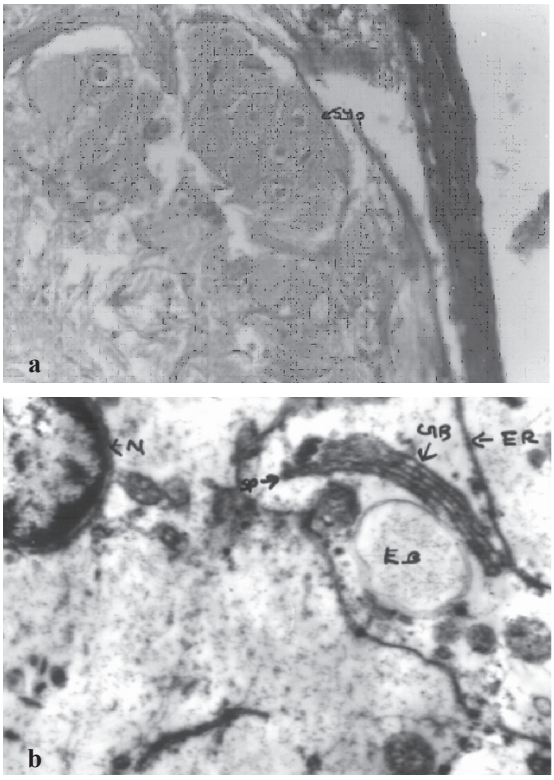

Fig. 12. a. Synthetic Phase of a NSC in the optic gangla of *P. homarus*. Syp: Synthetic Phase. Magnification 100X. b. Electron micrograph of a NSC in the supraoesophageal ganglion *P. homarus* during the Synthetic Phase. ER: Endoplasmic reticulum, EB: ER bound vesicles, GB: Golgi body & N: Nucleus. Magnification 10000X

together and start filling the vacuoles. During this time the membranes of different vesicles unbind and join together to form a larger one (Fig. 13a, b). In the cytoplasm, Golgi complex is seen in abundance while other cell organelles are very few.

***Secretory phase*:** The NSCs are intensely stained at this phase.

Numerous vesicles filled with the neurosecretory material are found abundantly in the cytoplasm. Neurosecretory material is seen flowing through the axon. At the time of axonal transport changes occur

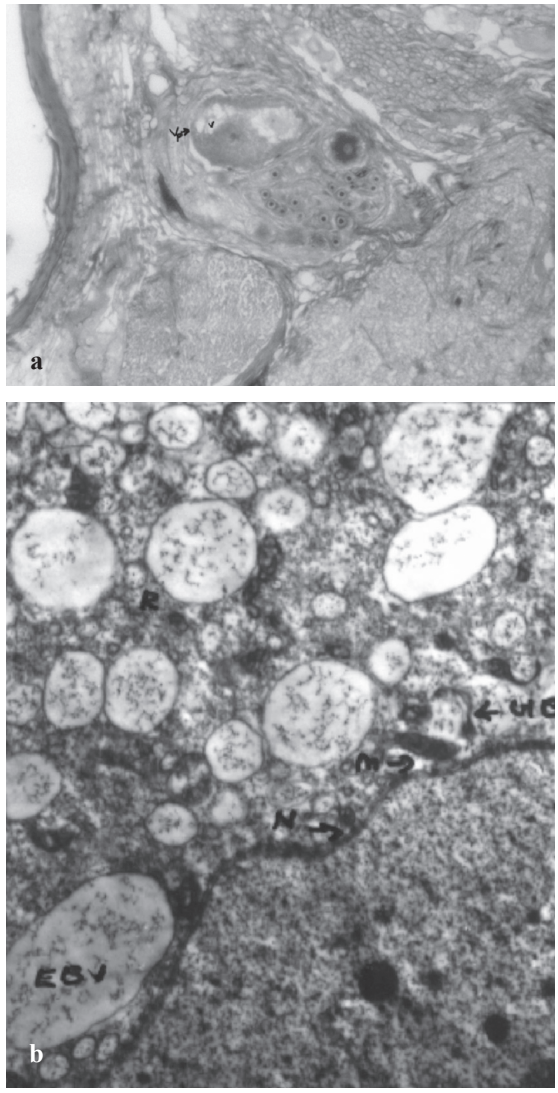

Fig.13. a. Vacuolar phase of a NSC in the optic ganglia of *P. homarus* V: Vacuoles. Magnification 50X. b. Electron micrograph of a NSC in the thoracic ganglia of *P. homarus* during the vacuolar phase. EBV: ER boundvesicles, GB: Golgi body,M: Mitochondria, N: Nucleus & R: Ribosomes. Magnification 10000X

in the size and electron density of the vesicles. The products are released through the axonal endings (Fig. 14 a).The diffusion of the neurosecretory products are also noticed through the cell body. At this stage cell organelles are very few in number or almost absent.

***Quiescent phase*:** This is the inactive or resting phase of the NSCs when synthesis of neurosecretory material is not visible. The cytoplasm is very lightly stained and homogeneous (Fig: 14b). The cytoplasm is seen shrunken at this stage. The cell organelles and other cytoplasmic inclusion are very scanty.

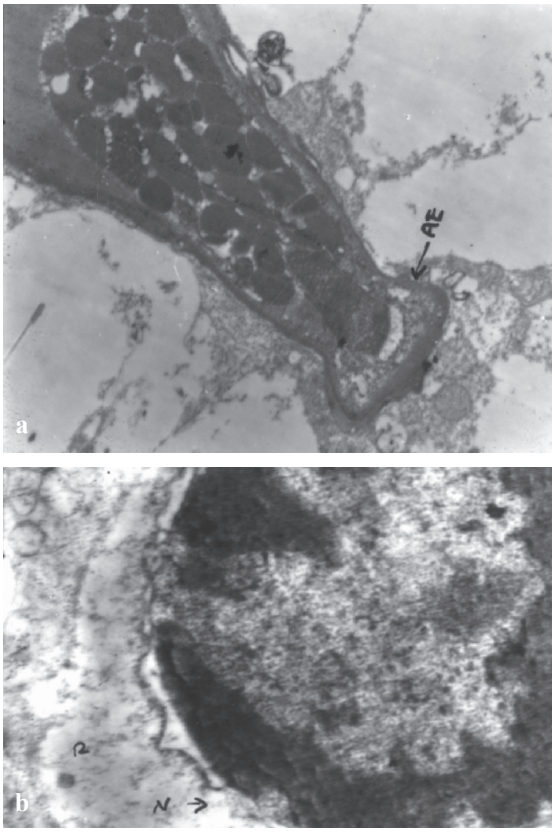

Fig. 14. a. Electron micrograph of the axonal ending of a NSC in the optic ganglia of *P. homarus* during secretory phase. AE: Axonal ending. Magnification 8000X. b. Electron micrograph of a NSC in the supraoesophageal ganglion of *P. homarus* during Q - phase. R: Ribosomes & N: Nucleus. Magnification 25000X

***Secretory status of NSCs at various developmental stages of ovary*:** The secretory status of various neurosecretory cell types in the optic ganglia, supraoesophageal ganglia and thoracic ganglia at different stages of ovarian development are described below:

***Optic ganglia*:** At immature stage the NSC type b' and d' cells were in the synthetic phase and c' and e' cells were in the quiescent phase. The a' and f' NSC cells were in the secretary phase and synthetic phase, respectively. At the primary vitellogenic stage, except a', all other cell types were in the secretory phase. Type a' cells were in vacuolar phase. At secondary vitellogenic stage, when active vitellogenesis takes place, type a', c', and d' cells were in secretory phase and the rest were in quiescent phase. In the mature stage, a' and b' cell types were in secretory phase, whereas type c', d' and e' were in the synthetic phase. In the spent condition, all the cells were in secretory phase.

***Supraoesophageal ganglion*:** In the immature stage, four types of NSCs, a'', c'', e'' and g'', were in the secretory phase. The NSCs in the vacuolar phase included only type b'' while type f'' and g'' were in the synthetic and quiescent phase respectively. In the primary vitellogenic stage, three cell types e'', g'' and h'' were in the secretory phase; type b'', d'' and f'' were in the vacuolar phase and type a'' and c'' were in the quiescent phase. At the secondary vitellogenic stage, all the cell types, except a'' and c'', were in secretory phase. Type a'' was in synthetic phase while type c'' was in quiescent phase. At mature stage b'', c'', f'', g'' and h'' were in the secretory phase whereas the other three cell types (a'', d'' and e'') were in quiescent phase. In the spent condition, most of the cell types were in quiescent phase. This included type a'', d'', e'', f'' and h''. Type b'' and c'' were in secretory phase while g'' was in synthetic phase.

***Thoracic ganglia*:** At the immature stage, the NSC types are found in all the phases of secretory cycle except the quiescent phase. Five cell types, a''', b''', d''', f''' and h''', were found in the vacuolar phase. Type e''' was in quiescent phase while c''' and g''' were in secretory phase and synthetic phase respectively. At the primary vitellogenic stage, b''', f''' and g''' NSCs were in the vacuolar phase, a''' and d''' in the synthetic phase and the rest (c''', e''' and h''') in the secretory phase. At the secondary vitellogenic stage all NSCs except d''' and h''' were in the synthetic phase. Type a''', b''' and f''' NSCs were in quiescent phase in the mature stage. But c''', d''' and e''' were in secretory phase while the rest (g''' and h''') were in vacuolar phase. Spent stage was represented by

all the four stages. Only type a''' was in the secretory phase. Type b''', e''' and f''' were in quiescent phase. Two of the NSC types (c''' and f''') were in the vacuolar phase while d''' and h''' were in synthetic phase.

Discussion

***Morphology and cytology of the neuroendocrine system*:** The morphology of the central nervous system of *P. homarus* has close similarity with that of other spiny lobsters, *Panulirus polyphagus* (George *et al*., 1955) and Jasus lalandii (Paterson, 1968) and also with that of the clawed lobster, *Homarus americanus* (Bullock and Horridge, 1965). In *P. homarus* the NSCs in each ganglion are significantly different and therefore the NSCs of the optic ganglia, supraoesophageal ganglia and thoracic ganglia were classified separately. Description of NSCs in various ganglia of the shrimps, *Penaeus indicus* (Mohamed, 1989) and *Penaeus monodon* (Joseph, 1996) were based on a common pattern. Similarly, a common classification was followed for describing NSC types in five species of crabs except for one cell type in the optic ganglia (Matsumoto, 1958). However, the NSC types described in these crustaceans differ from the cell types found in the central nervous system of *P. homarus* as it has a large number of NSC types. Furthermore, most of the NSC types are comparatively larger in *P. homarus*. The microanatomy of the optic ganglia of *P. homarus* is identical to the optic ganglia of other crustaceans such as the crabs, *Eriocheir japonicus, Chionectes opilio, Potaman dehanni, Neptunus trituberculatus, Sesarma intermedia* (Matsumoto, 1958); cray fish, *Orconectes nais* (Shivers, 1967); the shrimps, *P. indicus* (Mohamed, 1993), *P. monodon* (Joseph, 1996) and the lobster, *Nephrops norvegicus* (Giulianini *et al*., 1998). Micro anatomical studies conducted by Rotllant *et al*. (1995) shows that embryonic eyestalk of *Homarus gammarus* also consists of four ganglia viz. MT, MI, ME and LG along with the neurohaemal organ the sinus gland. The sinus gland of *N. norvegicus* is not as well defined as in *P. homarus*.

In *P. homarus*, different groups of NSC types in the medulla terminalis - X organs are identified. They are fused together to form a continuous structure and hence these are collectively called as MT - X organ. This is different from the MT - X organ 1 and MT - X organ 2 of *P. indicus* and *P. monodon*. In the crabs, *E. japonicus, C. opilio, P.dehanni, N .trituberculatus, S. intermedia* (Matsumoto, 1958) (Matsumoto, 1958) different groups of NSCs are fused together as observed in *P. homarus*.

The observations in the present study are contrary to the observation in the shrimp, *P. indicus* (Mohamed *et al*., 1993) in which NSCs in the thoracic ganglia showed a different distribution pattern. It had the same pattern of arrangement of NSCs in all five pairs of thoracic ganglia. But in *P. homarus*, NSC types showed a different distribution pattern in all the five pairs of ganglia. The results agree with the studies conducted on five species of crabs (Matsumoto, 1958).

***Secretory activity*:** Durand (1956) and Matsumoto (1962) classified secretory activity of the neurosecretory cells into two phases whereas Mohamed *et al*. (1993) and Joseph (1996) reported that the secretory cycle had three phases *viz*., vacuolar phase, secretory phase and quiescent phase. In the present study, a fourth phase, a synthetic phase, is also included in *P. homarus*. Most of the studies considered the presence of peripheral vacuoles as the initial phase. In *P. homarus* synthetic phase is considered as the first phase followed by the vacuolar phase during which the vacuoles are filled up by the secretory material.

Cytological changes were observed in the NSCs at various secretory phases in *P. homarus*. During the synthetic phase, the endoplasmic reticulum, along with the ribosomes, is engaged in the synthesis of the raw material for the neurosecretory products which in turn is processed by the Golgi complex. In the vacuolar phase, individual vacuoles tending to merge to form larger vacuoles were observed. The membranes of different vesicles unbind and join together to form a larger one. These membranes may have a role in the modification of the vesicle content after it has passed from the Golgi complex. Neurosecretory products are processed even at the time of its axonal transport i.e., at the

secretory phase by adjustment in the quality and quantity of material within the vesicle and this is detected by the changes that occur in size and electron density of the vesicles. The changes observed in the synthetic, vacuolar and secretory phases in *P. homarus* are in conformity with the observations of Bern (1963) and Bern and Hagadorn (1965) regarding the synthesis and transportation of neurosecretary materials in invertebrates.

The optic ganglia were considered as the site of the synthesis of gonad inhibiting hormone (GIH) in crustaceans and its presence was first demonstrated by Panouse (1944) and later confirmed by many authors (Adiyodi and Adiyodi, 1970; Herp and Payan, 1991; Subramoniam, 1999; Huberman, 2000). The GIH has also been named as Vitellogenin Inhibiting Hormone (VIH) as the hormone is inhibitory to vitellogenesis in females. The presence of this hormone has also been confirmed by the isolation, purification and characterization of VIH from the eyestalk of the lobster *H. americanus* (Meusy *et al*., 1987; Meusy and Soyez, 1991; Soyez *et al.*, 1991).

Since VIH has an inhibitory action on the ovarian development, it is reasonable to assume that the NSCs involved in the VIH secretion will be in the S-phase in primary vitellogenic and spent stages and in the Q-phase in secondary vitellogenic phase when active vitellogenesis takes place. During this period, VIH in the haemolymph is at the lowest (Adiyodi and Adiyodi, 1970). In *P. homarus*, three neurosecretory cell types in the optic ganglia were observed to follow such a pattern. They are b', e' and f' NSC types (Table 4). In *P. indicus* (Mohamed, 1993) and in *P. monodon* (Joseph, 1996) A & B cells exhibited heightened activity in the immature stage whereas C cells were in the Q-phase. But in the fully mature stage, reduced secretory activity was observed in the GN, B & C cells and invariably most of the A cells were in the Q-phase.

The Gonad Stimulating Hormone (GSH) is presumed to be present in the supraoesophageal ganglion and thoracic ganglia of crustaceans, but till now it has not been characterized (Otsu, 1960; Eastman - Reks and Fingerman, 1984; Takayangi *et al.,* 1986; Yano *et al.*, 1988; Kulkarni *et al*., 1991; Joseph, 1996; Zacharia, 2001). Supraoesophagel ganglion and thoracic ganglia are considered as the sites of synthesis of GSH while the optic ganglia are designated as the site for the synthesis of the Gonad Inhibitory Hormone (GIH). In the immature and primary vitellogenic stages of *P. homarus*, the NSCs involved in the production of GSH were in the synthetic or vacuolar phase and in the secondary vitellogenic stage they were in the S-phase and in the Q-phase. In the spent stage, the NSCs were in the Q or S-phases or even V-phase. In the supraoesophageal ganglion only d'' cell types follow such a pattern and in the thoracic ganglia, b''' and f''' cell types follow this pattern (Table 4). These NSC types could be the site of synthesis of GSH in the supraoesophageal and thoracic ganglia. In studies conducted in *P. monodon*, GN or B cell types are presumed to be involved in the production of GSH. In the crabs *E. japonicus, P. dehaani, S. intermedia, N. trituberculatus* and *C. opilio*; the A, B' and E cells appear to be active when the ovary is generally in its early stage of maturation

Table 4. Secretory status of NSCs involved in the synthesis of VIH in the optic ganglia and GSH in the supraesophageal and thoracic ganglia, Synthetic phase - Sy, Vacuolar phase - V, Secretory phase - S and the Quiscent phase - Q

Ganglia	Cell type	Immature stage	Primary vitellogenic stage	Secondary vitellogenic stage	Mature stage	Spent
Optic	b'	Sy	S	Q	S	S
Optic	e'	Q	S	Q	Sy	S
Optic	f'	S	S	Q	S	S
Supraesophageal	d''	V	V	S	Q	Q
Thoracic	b'''	V	V	S	Q	Q
Thoracic	f'''	V	S	Sy	V	Sy

(Matsumoto, 1958). Experimental studies of the neurosecretory activities of the thoracic ganglion of crab *Hemigraspus* sp. show that B'- cells are in the active state at the time of ovarian development (Matsumoto, 1962). In *P. homarus*, it could be assumed that, three cells types; b', e' and f' NSCs in the optic ganglia may have a prominent role in the production of VIH. Similarly d'' cells in the supraoesophageal ganglion and b''' and f''' cells in the thoracic ganglia are likely to be the probable site of synthesis of GSH.

The cyclic secretory activity of neurosecretory cells in the supraoesophageal and thoracic ganglia points to secretion of certain hormones, which accelerates vitellogenesis. Whether the vitellogenesis stimulating hormones from brain and thoracic ganglia are of the same chemical nature needs clarification. These clarifications can pave the way for a major breakthrough in the captive breeding of lobsters and crustaceans by the isolation, purification and production of analogues of neurohormones, which can stimulate the reproductive processes as in finfishes.

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