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Consequences Due to Injection of Cerebral Ganglionic Extract on Neurosecretory Cells in Freshwater Bivalve: *Lamellidens Corrianus*

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Abstract:

Regarding the effect of neurosecretory cells as compare to marine pelecypodes less data is available on freshwater bivalves, considering this freshwater bivalve molluscs *Lamellidens corrianus* taken for the present study. This species abundantly distributed along the banks of Godavari river in Jayakwadi backwaters (Nathsagar) at Paithan, Maharashtra. These bivalves were collected, acclimatized and used for experiment. Sets were prepared, one control and another cerebral ganglionic extract injected group. Seasonal study of this revealed that cerebral ganglia play an important role, mostly inhibitory one in the regulation of gonad development and spawning. Neurosecretory cells were studied showed prominent type A and B cells.

Keywords: Cerebral ganglia, Freshwater bivalve, *Lamellidens corrianus*, Neurosecretory cells.

1. INTRODUCTION

Freshwater bivalve: *Lamellidens corrianus* has three different ganglia in pair, cerebral, viscera and pedal, these nerve centers play vital role in controlling and coordinating all body activity along with this it has neurosecretory cells (NSCs) which secrete neuro-secretion nothing but the hormones plays vital role in physiology of these molluscs. Evidences for the occurrence of a wide variety of neurotransmitters in different tissues of *Lamellibranchs* including the nerve ganglia have been discussed from the functional point of view (Leak and Walker, 1980). Under the light microscope, neurons are characterized by the presence of abundant secretary materials in their perikarya. This material is seen also in the axons which often end blindly adjacent to muscular spaces rather than innervating their target structures directly. These blindly ending terminals serve a storage release function and in the more advanced groups of animals such as crustaceans such compact structures are termed as neurohemal organ by Knowles and Carlisle (1956). The neurosecretory cells (NSCs) with their combination of neuronal and glandular capabilities are perfectly suited to translate a neuronal input into the hormonal output best suited to long-term process. In this capacity, the NSCs may produce hormones, which act directly upon the peripheral target or it may exert its effect indirectly by influencing the activity of other, non-neural, endocrine organs. In this later case, the NSCs organ may act via the production of blood-born hormone. Knowles and Bern (1966) stated that the significance of NSCs as connecting link between nervous and endocrine systems and neurosecretory neurons "participate either directly or indirectly in endocrine control and form all or part of endocrine organ". Hormones are consequently well suited to exert their effects over extended period of time, and the endocrine system control long-term process within the body such as the coordinated growth of organs or the maintains of appropriate metabolite concentration in the blood and tissues.

The significance of the differential staining affinities of neurons within the nervous system of both vertebrates and invertebrates was first appreciated by the Scharrers (review by Gabe, 1966). Their staining differences were used as the basics for first description of NSCs and to give original definition of neurosecretion (Scharrer 1977). The dangers of attributing neurosecretory function to a nervous on histological grounds have been pointed out on numerous occasions (Bern, 1966; Bern and Knowles,1966) and number of authors suggested vigorous criteria, including morphological and biochemical evidence, to be satisfied before ascribing neurosecretory function to a cell (Berlind, 1977). Histologically, neurosecretory cells were recognized by a number of specific histochemical techniques, including light microscopic stain such as chrome-haematoxylin-phloxene, paralhydide fuchsin and azan (review by Rowell, 1976). Light microscopic staining has been used extensively with paraldehyde fuchsin and chrome-haematoxylin-phloxene in molluscs (Simpson et. al., 1966; Gabe, 1966) and insects (Rowell, 1976 and Panov, 1980).

Apart from endogenous regulation in maturation and spawning in bivalve molluscs, as shown by the above workers, the data on such regulation in growth and metabolism, and several aspects of physiological are scanty. The work carried out by Lubet (1966) on *Mytilus edulis* and on *Perna perna* by Umiji (1969) stated that removal of cerebral ganglia has little or no effect on shell or body growth, or on glycogen metabolism and storage. However, ablation of cerebral ganglion does result in disorders of lipid metabolism, particularly a reduction in lipid accumulation (Lubet, 1965). Effect of injection of cerebral ganglionic extract to normal bivalve is still in shadow. Very little is known of the occurrence or functions of hormones in molluscs other than gastropods and cephalopods, considering the little work on NSCs present work deal with the study of NSCs and its effect on concern molluscs.

2. MATERIALS AND METHODS

Lamellidens corrianus (Lea) were collected from Jayakwadi backwaters (Nathsagar) at Paithan, 45 km. away from Aurangabad, Maharashtra. After brought to the laboratory, the shells of the bivalves were brushed and washed with fresh reservoir water so as to remove the fouling algal biomass and mud. The animals of 80-85 mm shell length were selected for experiment and they were acclimatized for 24 h. at laboratory condition in fresh aerated reservoir water (with renewal of water at the interval of 12-13 h.) and stocking capacity was given during this period and no food was given to the bivalves during laboratory acclimatization and subsequent experimentation.

After 24 h., reservoir water was once again renewed and aeration was given, after a lapse of 1h. Animals extended their organs (foot, mantle, siphons) to maximum and soon surgical operations were done. For injection of ganglionic extracts, cerebral ganglionic extract was prepared in ice cold distilled water, 10 ganglia in 1ml cold distilled water were centrifuged and the supernatant (0.2 ml/animal i.e. equivalent to 2 ganglia/animal) was injected into the foot (muscular region) of normal control bivalves. In sham operated control animals were injected by 0.2ml cold distilled water. The result for control and sham operated groups were similar and hence a comparison was made between extract injected to normal control group of animals only.

Animals were fixed in Bouins Hollande for histological study of ganglia. The animals were soaked carefully with the help of filter paper and flesh of the animals was fixed in Bouins Hollande fixative for 24 hrs. The fixative was renewed for next 24 hr. to facilitate better fixation of the tissues. During experimental period ganglia removed and processed for preparation of paraffin blocks. Dehydration of ganglia was done through serial grades of ethyl alcohol and tertiary butanol respectively while xylene was replaced by toluene during the process of dehydration. The tissues were embedded in paraffin wax at 58˚C-60˚C and the sections of ganglia were cut 5.0 – 6.0 µm thickness using Spence-rotary-microtome. The sections of cerebral ganglia were stained with Gomories Chrome alum haematoxylene–phloxine, Mallary triple stain and Thionine Paraldehyde after permagnate oxidation (as shown by Illanes and Lubet, 1980). All the sections were observed under the research binocular microscope and wherever necessary, measurements were made before microphotography.

3. RESULTS

Table 1: Changes in the neurosecretory cells of cerebral ganglia due to injection of cerebral ganglionic extracts in *Lamellidens corrianus* during summer season (All the values are µm)

Type of NSCs ↓	Seasons →	Summer		Monsoon		Winter	
	Experimental Set → / Cell Parameter ↓	Control	Injection of cerebral ganglionic extract	Control	Injection of cerebral ganglionic extract	Control	Injection of cerebral ganglionic extract
Type A Cell	Cell length	12.8571 ±0.8997	13.7142 ±0.4879	12.5714 ±0.5345	13.5714 ±0.5345	10.8571 ±0.6900	11.2857 ±0.7559
	Cell width	6.7142 ±0.4879	7.4285 ±0.5345	8.1428 ±0.6900	8.2857 ±0.7559	6.0000 ±0.5773	6.4285 ±0.5345
	Nucleus diameter	5.1428 ±0.3779	5.7142 ±0.4879	5.5714 ±0.5345	5.7142 ±4879	5.0000 ±0.5773	5.4285 ±0.5345
	Axon length	2.4285 ±0.5345	2.5714 ±0.5345	2.5714 ±0.5345	3.1428 ±0.6900	3.0000 ±0.8164	3.0000 ±0.8164
Type B Cell	Cell diameter	9.4285 ±0.5345	9.8571 ±0.6900	10.1428 ±0.6900	11.0000 ±0.5773	9.7142 ±0.7569	10.8571 ±0.6900
	Nucleus diameter	5.4285 ±0.5345	5.7142 ±0.4879	6.0000 ±0.5773	6.4285 ±0.5345	5.2857 ±0.4879	6.2857 ±0.4879

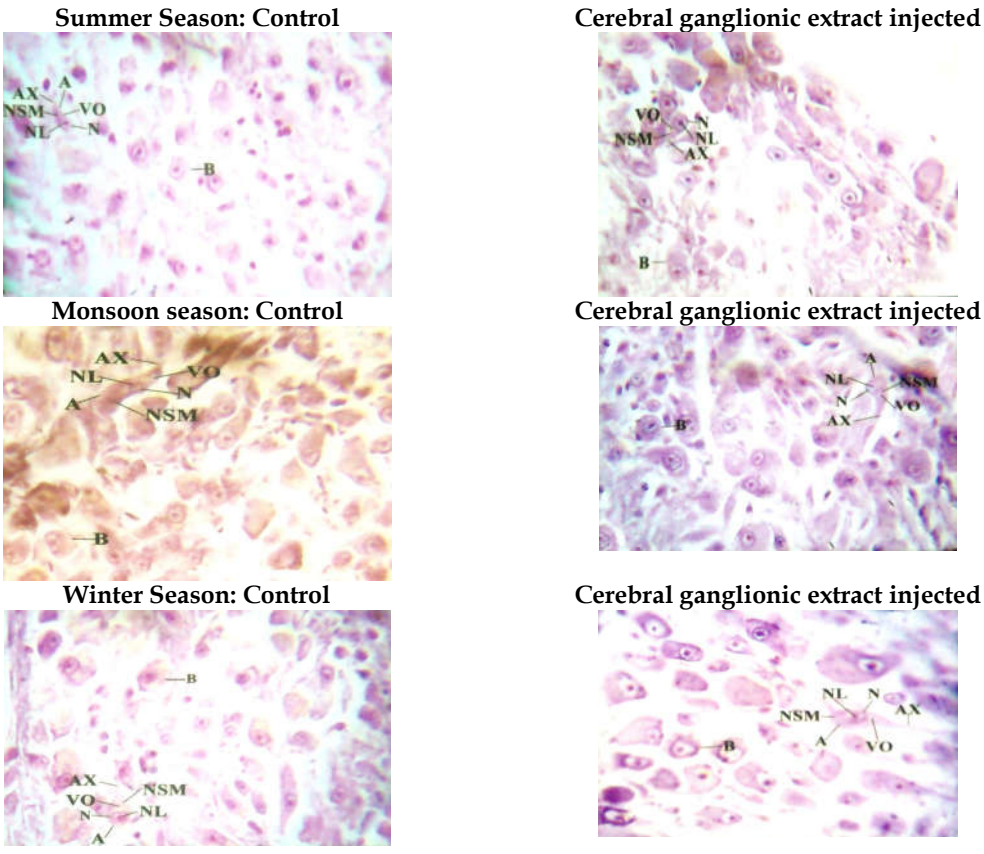

Figure 1: Histological changes in the neurosecretory cells of cerebral ganalia of *Lamellidens corrianus*. A = Type A cell, B = Type B cell, N = Nucleus, NL =Nucleolus, NSM=Neurosecretory material, AX =Axon, VO =Vacuole

The histological details of neurosecretory cells in cerebral ganglias of *Lamellidens corrianus* during different seasons were given in table (1) and figure (1). In cerebral ganglia Type A cell showed cell length (12.8571 ± 0.8997 µ) in control, (13.7142 ± 0.4879 µ) in injection of cerebral ganglionic extract on 12th day during summer season. The cell width of Type A cell was (6.7142 ± 0.4879 µ) in control, (7.4285 ± 0.5345 µ) in extract injected group. The nuclear diameter in control was (5.1428 ± 0.3779 µ); in extract injection was (5.7142 ± 0.4879 µ). Axon length in Type A cell in control was (2.4285 ± 0.5345 µ), in ganglionic extract injected was (2.5714 ± 0.5345 µ). Type B cell showed the diameter (9.4285 ± 0.5345 µ) in control, (9.8571 ± 0.6900 µ) in injection of cerebral ganglionic extracts. Nuclear diameter in Type B cell was (5.4285 ± 0.5345 µ) in control, (5.7142 ± 0.4879 µ) in extract injected group.

During monsoon season on 12th day in cerebral ganglia Type A cell showed cell length (12.5714 ± 0.5345 µ) in control, (13.5714 ± 0.5345 µ) and in injection of cerebral ganglionic extracts. Cell width in Type A cell was (8.1428 ± 0.6900 µ) in control, (8.2857 ± 0.7559 µ) in injection of cerebral ganglionic extracts. Nuclear diameter in control was (5.5714 ± 0.5345 µ), in injection of cerebral ganglionic extracts was (5.7142 ± 0.4879 µ). Axon length of Type A cell in control was (2.5714 ± 0.5345 µ), injection of cerebral ganglionic extracts was (3.1428 ± 0.6900 µ). The cell diameter of Type B cell was (10.1428 ± 0.6900 µ) in control and in injection of cerebral ganglionic extracts was (11.0000 ± 0.5773 µ). Nuclear diameter in control was (6.000 ± 0.5773 µ), in ganglionic extract injected was (6.4285 ± 0.5345 µ).

During winter season on 12th day in cerebral ganglia, Type A cell showed cell length (10.8571 ± 0.6900 µ) in control, (11.2857 ± 0.7559 µ) in extract injection. The cell width of A Type cell was (6.0000± 0.5773 µ) in control, (6.4285 ± 0.5345 µ) in cerebral ganglionic extract injected group. The nuclear diameter in control was (5.0000 ± 0.5773 µ) and in ganglionic extract injected was (5.4285 ± 0.5345 µ). Axon length was (3.0000 ± 0.8164 µ) in control and ((3.0000 ± 0.8164 µ) in extract injected group. Type B cell showed the cell diameter (9.7142 ± 0.7559 µ) in control and (10.8571 ± 0.6900 µ) in extract injected group. In B Type cell nuclear diameter in control was (5.2857 ± 0.4879 µ) and in extract injected was (6.2857 ± 0.4879 µ).

4. DISCUSSION

Presence of NSCs (Neurosecretory cells) has been demonstrated by classic histological studies on a number of species. Their number and location vary among species. NSCs are located in the central ganglia. In higher lamellibranchs, NSCs are less numerous and more localized. These cells are generally found in the dorsal caps of cerebral ganglia and dorsal cell layer of visceral ganglia (Lubet, 1955a, 1959; Nagabhushanam, 1963, 1969; Nagabhushanam and Mane, 1973; Mane, 1986). The NSCs have been reported to occur in all the ganglia of freshwater mussels, *Unio tumidus* (Fahrmann, 1961), *Dreissina polyrnoroha (*Antheunisse, 1963), *Lamellidens marginalis* and *Lamellidens corrianus* (Muley, 1985). In *Lamellidens corrianus* the NSCs in the ganglia are located on dorsal and lateral periphery in cerebral ganglia.

In most species NSCs are small or medium-sized; with an approximate diameter of 18µ. Neurosecretory perikarya are ovoid or pyriform. Different categories of NSCs have been distinguished based on their size and morphology. In *Mytilus edulis* and *Chlamys varia* some NScs are pear-shaped, unipolar, and upto 25µ, while others are small and multipolar (Lubet, 1959). Pear-shaped (Type I) and oval-shaped (Type II) NSCs were distinguished in *Crassostrea virginica* and *Meretrix casta* (Nagabhushanam, 1963, 1969) and *Katelysia opima* (Nagabhushanam and Mane, 1973). Different categories of NSCs have also been reported in the freshwater mussel, *Unio tumidus* (Fahrmann, 1961). In the present study, ganglia of *Lamellidens corrianus* showed two types of NSCs which are in accordance with those observed in *Teredo* (Gabe and Rancurel, 1958), in *Mytilus* and *Chlamys* (Lubet, 1955b), in *Crassostrea virginica* (Nagabhushanam, 1963) and in *Meretrix casta* (Nagabhushanam, 1969) for Type A cells and in *Crassostrea virginica* (Nagabhushanam, 1963) and in *Katelysia opima* (Nagabhushanam and Mane, 1973) for Type B cells.

The appearance and position of neurosecretory products within the perikarya vary with the stage of the secretion (Lubet, 1955a, b; 1959; Gabe, 1966; Gabe and Rancurel, 1958; Blake, 1972; Nagabhushanam *et al*., 1972). In some cells, neurosecretory granules are few, while in others they are abundant and remain discrete. Endocrine glands or neurohemal organs have not been identified in bivalve molluscs. Umiji (1969) has reported that a neurohemal area exists on the cerebral commissure of *Perna perna.* In the present study on *Lamellidens corrianus* axonal transport of the neurosecretory product is distinct in Type A cells in winter. In Type B cells probably the transport is likely to be by diffusion. Vacuoles in both the type of cells are also distinct when the neurosecretory products disappear. The transport of neurosecretory substances by axons, intermediate cells, and possibly glial cells has been suggested by Lubet (1955b) and Umiji (1969). Several authors have suggested that glial cells play a role in storage and transport and that glial cells and epineurons can function as neurohemal organs (Fahrmann, 1961; Antheunisse, 1963). However, the chemical nature, transport, and fate of neurosecretory products is not clearly established in bivalves.

Investigation by Lubet (1959) demonstrated distinct annual neurosecretory cycle in the pear-shaped NSCs of the cerebral ganglia in temperate species, *Mytilus edulis* and *Mytilus galloprovincialis*. The annual neurosecretory cycle and gametogenic cycle in these mussels appear to be closely correlated. Secretary material is accumulated in the cerebral ganglia during gametogenesis, and evacuated from the cells when the gametes become fully mature. The small multipolar neurons and the NSCs of the visceral ganglion in these muscles showed continuous activity throughout the year. These observations were confirmed in oyster *Crassostrea virginica* by Nagabhushanam (1963, 1964). In freshwater mussel, *Dreissina polymorpha*, the neurosecretory material begins to accumulate in the cerebral ganglia in autumn; maximum activity takes place during winter. A period of inactivity occurs in summer. Neurosecretory product begins to accumulate in the Type I cells with the initiation of gametogenesis and reaches a maximum when the animals are mature (Nagabhushanam and Mane, 1973). Secretary granules in the NSCs decrease with spawning and are not seen in resting animals.

In the present study, histological sections of cerebral ganglia in different seasons revealed accumulation of neurosecretory material in Type A cells in summer, which increased in monsoon, but in winter the material was comparatively very less. In summer and winter vacuoles were conspicuous. The nuclei and nucleoli were more prominent in summer and monsoon. Type B cells revealed accumulation of neurosecretory material in monsoon. Experiments carried out on *Lemellidens corrianus* by giving cerebral ganglionic extracts, revealed pronounced changes in both the type of NSCs from the existing ganglia. In extract injected group, in cerebral ganglion cell length of Type A cells increased in monsoon but in summer and winter there was no change. The cell width and nuclear diameter did not change in any season. The axonal length significantly increased in monsoon. In Type B cells, the diameter decreased in monsoon and winter but in summer, there was no change. The nuclear diameter in summer and monsoon did not change but in winter, it decreased. The neurosecretory material was comparatively less in all the seasons, particularly in winter and summer.

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