International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 8 | Issue 2 | APR-JUN | 2018 | 762-768
Research Article | Biological Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

TEMPERATURE INDUCED BEHAVIORAL CHANGES IN THE CEREBRAL NEUROSECRETORY ELEMENTS IN *LYMNAEA (RADIX) LUTEOLA*

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ABSTRACT
The physiological basis of environmental regulation on reproduction is readily identifiable at cellular level in the nervous system of molluscs. In the present investigation temperature induced behavioral nature of the neurosecretory elements in Lymnaea (Radix) luteola has been identified. This paper relates to certain changes in dorsal body cells with respect to their secretory dynamics and the neuro endocrine cells controlling reproduction. Ultra-structurally the cyto anatomical profile portray quanta of secretory granules, ribosomal content and their criteria, extent of axoplasamic flow, availability of the electron dense granules, abundance of heterochromatin bodies that relate to secretory dynamics of the neurosecretory cells .In the species under observation the lateral lobes of the brain produce a factor that stimulates in males spermatogenesis and are in the sharp contrast with the female where the hormone of the Dorsal Body is instrumental in the production of ripe oocytes and vitellogenesis and this can be a reference that dorsal body cells produce hormones which provide ground for cellular differentiation.

KEY WORDS
Molluscs, neuroendocrine regulation, dorsal body cells, neurosecretor[NS] neurosecretory material, seasonal influence, Nucleoplasmic indices.

1. INTRODUCTION
Changes of environmental temperature depict unique characteristics of ectothermic organisms especially amongst molluscs where the capacity of thermoregulation is far less than in many other invertebrates. Temperature along with other hydrological factors is proved to act on the biochemical machinery of several organs including neurosecretory system. The sensitivity of the neuroendocrine centers to thermal stress is really very striking. The changes in the kinetic energy of the environment are characteristically (rapidly and quantitatively) transmitted to the cellular chemistry of the organism and provoke detectable micro anatomical alterations. This is imperative in case of pulmonate molluscs like *Lymnaea (Radix) luteola* that inhabits in freshwater (pond water).

The principal ganglionic whorl of Central Nervous System of *Lymnaea (Radix) luteola* is comprised of peri-oesophagial ring with three pairs of ganglia (cerebral, pedal and pleural ganglia) and a pair of buccal ganglion and a visceral loop composed of a pair of parietal and visceral ganglion and connections intercommunicating the pleural ganglion. Two conspicuous paired symmetrical bodies (dorsal bodies) just over cerebral ganglion as well as in confluence with the cerebral commissure are present. The involvement of dorsal body cells (DBCs) is considered as small groups of endocrine cells in the dorsal part of connective sheath surrounding the cerebral ganglion. In fresh water snail, DBCs form well defined structures and undergo seasonal changes in size being largest in the reproductive season. These cells are believed to undergo secretory cycles and packaging. The cellular architecture of the neurosecretory cells basically follow three steps-synthesis, axonal transport and release. Temperature augments this cellular behaviour and the same reflection can be seen in the different temperature regimes electron microscopically.

The objective of this proposed investigation is to spell out in details the functional status of dorsal body cells (DBCs) that remain in close proximity with one of the components (cerebral) of the ganglionic whorl in aquatic *Lymnaea (Radix) luteola.* More emphasis is stressed upon the causo -mechanism of the effects of temperature regimes ranging above, below and near ambient temperatures on vital processes and the cellular structures in the biological system. This is designed in view of seasonal changes in terms of hydro ecology indeed.

2. MATERIALS AND METHODS

Materials to be used in the proposed investigation are full-grown *Lymnaea (Radix) luteole* size ranging from 1.2cm to 1.5cm (**Fig.1**). They are collected from the adjoining pond within Vivekananda College Campus, Thakurpukur, Kolkata-700063 during summer (April-June), monsoon (July-Sept) and winter (Oct-Dec).

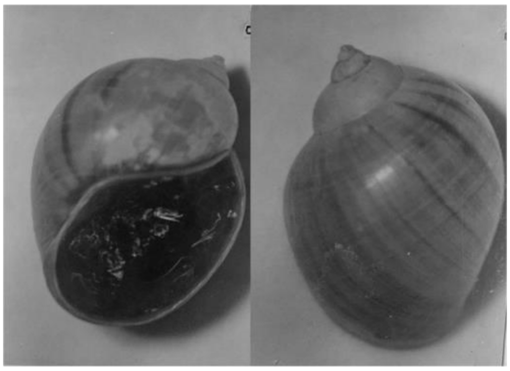


Figure 1. Ventral and Dorsal view of *Lymnaea (Radix) luteola.*

Exposures to seasonally acclimatized individuals are to be selected as summer (April, May and June), monsoon (July, August and September) and winter (October, November and December) groups. Ten individuals for each seasonal regime are kept in the BOD incubator for 24 hours at hypothermal (10 ºC), near ambient (25ºC) and hyper thermal (35ºC) condition to probe the activities of DBCs and relevant neuroendocrine cells.

Requisite number of animals is deshelled and their dorsal bodies with ganglions are removed [1]. Dissections under stereoscopic biunocular are made to locate the dorsal bodies and the cerebral ganglion using suitable molluscan ringer solution. Relevant tissues (Cerebral ganglion) are dissected out in suitable ringer solution and fixed in a mixture of 3 percent gluteraldehyde, 2 percent formaldehyde, in 0.1 M cacodylate buffer at 4^0 c. The samples are washed in 0.1 M cacodylate buffer 3 times at ten minutes interval. The tissues collected are then post fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer at 4^0 c for one and half hours. The tissues are then dehydrated in the graded alcohols for scheduled time slots, cleared by graded propylene oxide and are embedded in pure plastic blocks, polymerized at 60^0 c in an incubator overnight. The blocks are carefully trimmed initially at 1 micrometer thin and cut in LKB ultratome 8800 using glass knives and stained in 1% toludine blue for the selection of the desired area. Ultra-thin sections mounted on the grids are stained for 30 minutes with 1% uranyl acetate and for 5-10 minutes with lead citrate in CO_2 free atmosphere. The samples are examined under Philips TEM 416 LS at an accelerating voltage of 80KV.

3. RESULTS AND DISCUSSIONS

3.1. Effect of near ambient temperature (25^0C)

The DB cell bodies are 12- 17 µm in diameter and their processes remain slender and may branch and terminate after short distance near to the connective tissue of the perinurium or close to the cerebelar neurosecretory cell complements (**Fig.2a**). The components of the cell are demonstrable with variable quanta of lipid droplets, semispherical mitochondria, glycogen and circular stacks of rough endoplasmic reticulum (RER). On some occasions, stacks of RER may undergo fragmentation. Golgi structures are moderate in numbers and some electro lucent vesicles may be noted amongst these complexes. Sometimes prominence of smooth endoplasmic reticulum could be visualized in active cells (**Fig.2b**). Sporadic distribution of electron dense granules in the cell body is not ruled out apart from having RER. Sometimes the mitochondria may also contain electron dense granules. Distribution of periodic nucleopore all around the nuclear membrane is striking and seemed to be the common feature. Moreover, the conformity of the Neuro Secretory Material (NSM) is of normal appearance on the basis of their size and electron density. Indications are there to identify the presence of pores all around the nuclear membrane with periodic interruptions as well as evenness of hetero chromatin areas underneath it.

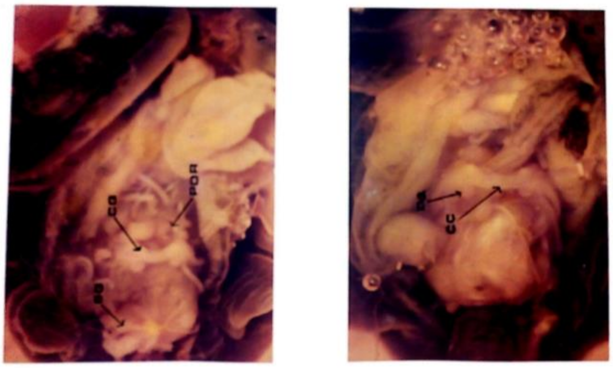


Figure 2: *In-situ* projection of the central nervous system of *Lymnaea (Radix)luteola* [CG- Cerebral ganglion CC- Cerebral Commissure BG-Buccal Ganglion]

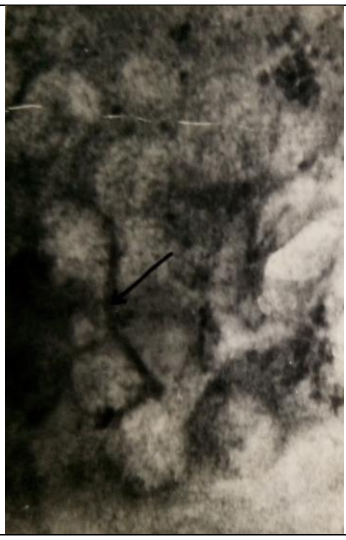


Figure 2a: Electron micrograph exposing some of the dorsal body cells (x2000).	**Figure 2b: Electron micrograph of NS cells showing the smooth endoplasmic reticulum (x20000).**

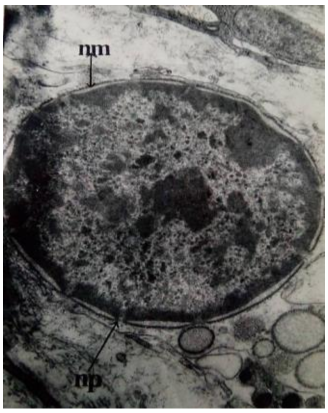


Figure 2c: Electron micrograph of NS cells showing nuclear periphery with pores and sporadic distribution of electron dense particles close to nucleus (x20000). [nm = nuclear membrane, np = nuclear pore]

3.2. Effect of low temperature (10^{0}C)

Impact of low temperature displays accumulation of cytoplasmic vesicles containing fluctuating electron dense secretory inclusions (**Fig. 3a**). Cell processes are not well demonstrable despite the existence of electron dense granules in the cytoplasmic mass. The nucleus also shows the possession of less heterochromatin substances when comparison is made with the individuals exposed at 25^{0}C. The nucleoli however do not always show such distinction. Some non-neurosecretory elements may be visible and in that case their nuclei are in possession of heterochromatin substances. Ribosomal materials are available in abundance when cytoplasmic portion of the cell is being examined (**Fig. 3b**).

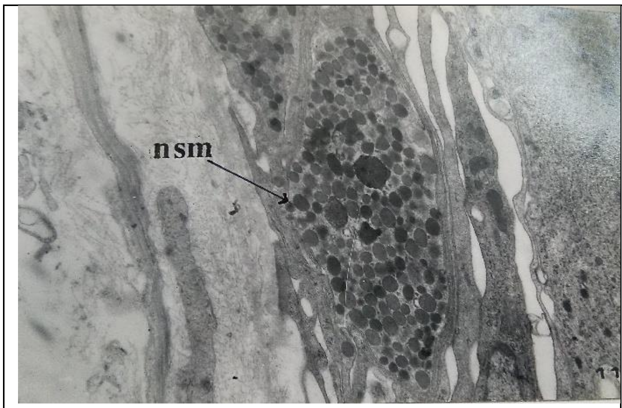


Figure 3a: Electron micrograph of NS cells showing dense NS material accumulation at axon endings (x20000).[nsm = neurosecretory material]

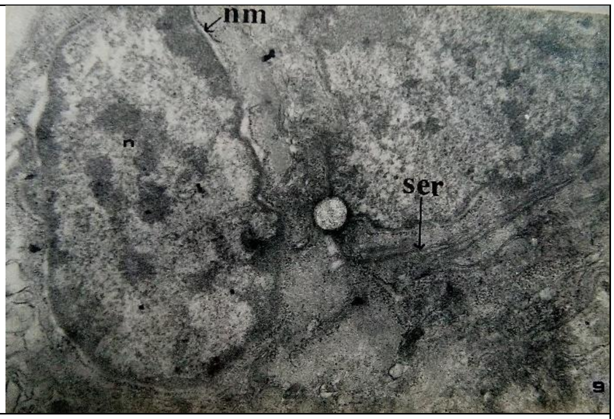


Figure 3b: Electron micrograph of NS cells showing smooth endoplasmic reticulum and less abundance of electron dense granules & ribosomal inclusion (x15000). [ser = smooth endoplasmic reticulum]

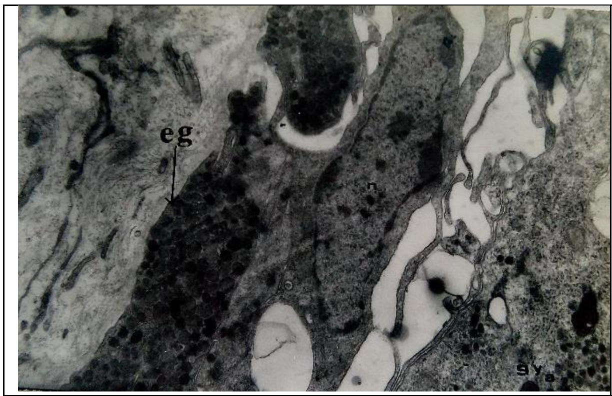


Figure 4a: Electron micrograph of NS cells showing amassing of electron dense granules & ribosomal inclusion. Note: Nucleus with less chromatin material (x20000). [eg = electron dense granule]

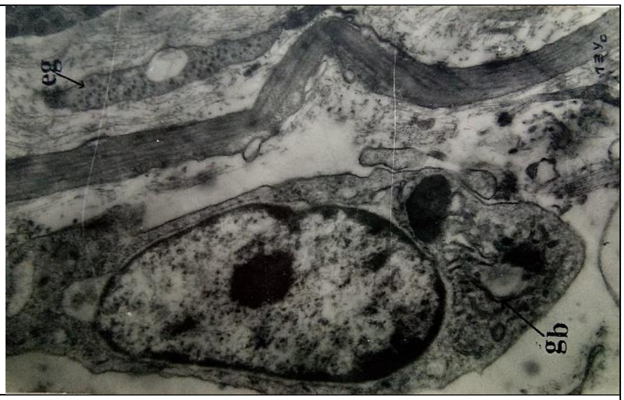


Figure 4b: Electron micrograph of NS cells showing electron dense granules to one of the branches at axon terminal end; golgi comples seen at the apex of nucleus in non-neurosecretory neuron (x15000). [eg = electron dense granule, gb = golgi body]

3.3. Effect of high temperature (35^{0}C)

This exposure reveals poor distribution of cytoplasmic inclusions which are mostly electron dense. The cell processes too, particularly the microtubules, are found to contain vesicles that have variable electron density (**Fig.4a**). Some fibrillar structures are also demonstrable within the cytoplasm which may be due to intra cytoplasmic changes following hyperthemic stress. Existence of large dense bodies of variable dimensions in the vicinity of the nucleus may be encountered. And their relation with reference to the secretory nature of the cell concerned is rather obscured. Sometimes electron-dense-granule accumulation may be observed in some of the branches of axon- terminals. Indications for the deployment of these granules from cytoplasmic pool are obvious. The nuclei of non-neurosecretoy elements too bear rich heterochromatin substances although nucleoli remain most conspicuous (**Fig.4b**).

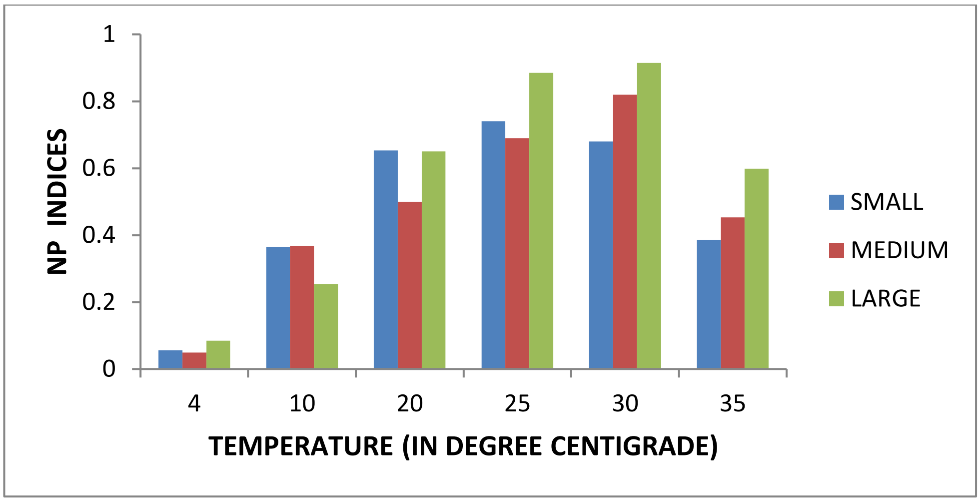
Environmental temperature changes depict unique characteristics for molluscs where the capacity of thermoregulation is far less than many other invertebrates. Indeed, the changes in the kinetic energy of the environment are characteristically (rapidly and quatitatively) transmitted to the cellular chemistry of the organism. In the present study, the near ambient temperature demonstrates near normal features as and when extent of accumulation of the electron dense granules at the axon terminals, their conformity and relative abundance of heterochromatin substances are referred to. Distribution of periodic nucleopore all around the nuclear membrane is likely to indicate a compromise in the functional aberrations of the nurosecretory elements with respect to physiology of secretion. DB cells contain cell cluster that produce DB hormones which are very likely necessary for controlling growth and reproduction in *Lymnaea (Radix) luteola*. The presence of the lipid droplets in the cells and production of DB granules coincide to establish the evidence of the steroid secretion [2]. Similar attribute has been given by Miksys and Saleuddin (1988) [3] and their observations are based on the intracellular smooth endoplasmic reticulum. Electron micrographically, the DB granules display membrane bound and electron dense properties to consider them as being peptides. Indirect evidence proves that they are steroids. Keeping these two views in consideration it is likely to concede that DB granules may contain a binding protein for steroids [4]

During hypothermic exposure, ultra-microscopical revelation of the fluctuating contents of electron dense granules, abundance of ribosomal materials, appearance of smooth endoplasmic reticulum and possession of less heterochromatin material has been amenable to lesser neurosecretion in context with reduced reproductive activity.

However, during hyperthermic regimes, possession of sparse electron dense granules, availability of several vesicles and rich distribution of nuclear heterochromatin substances within neurosecretory perikarya might due to impaired secretory cycles of the neurosecretory cells [5, 6]. Over and above existence of demonstrable fibrillar structures within cytoplasm may be the consequence of hyperthermic stress.

The relationship between DB and cerebral ganglion proves to be intimate and likely to have ontogenic bearing as and when cephalisation of the CNS is called for. Anterodorsal cell mass at each hemisphere of the cerebral ganglion demonstrates a parallelism with hexapod brain corpora cardiaca allata system. It is nonnervous and mesodermal in origin and has been attributed as the endocrine organ. The cytomorphology and organization of the endocrine dorsal body cells as well as cerebral ganglionic NSC_S are being elicited to highlight their physiological interdependence. Wendelar Bonga (1972) [7] in his studies on *Lymnaea stagnalis* has also emphasized the particularization of the specific neurosecretory cells with respect to water influx and salt loss. Such criteria are tenable in the species where environmental change in respect to temperature fluctuation seems not to be due to intervening neuronal message [8]. Importance of cerebral ganglion pertaining to control of growth and carbohydrate metabolism was established [9] in *L. stagnalis*

Infliction of different temperature regimes elicit more or less similar trend in ascribing the fluctuating pattern of the nucleoplasmic indices amongst three categories of cells in the brain of the species under study. Indeed, the most spectacular the both Large and Medium types when subjection to variable temperature regimes is specifically up to 30^0C. Such situation is not demonstrable in case of small types of cells, exhibit low peak at 30^0C when being compared to preceding one at 25^0C. However, they portray identical trend at hypothermic temperature regimes that is at 35^0C. Moreover, fluctuations in the nucleoplasmic indices depends upon the susceptibility of the cells.



CONCLUSION

A variety of environmental factors affect the neurosecretory rhythms in *Lymnaea sp.* and amongst them temperature is one of the most important factor. The present investigation on the effects of temperature gradients over the neurosecretory elements of the cerebral gangliona in *Lymnea* (Radix) *luteola* provides evidence for the involvement of direct environmental parameter i.e. temperature in the neurosecretory system. Temperature stress along with other hydrological factors is proved to act on the biochemical machinery of several organs including the neurosecretory system. Present study further demonstrates the existence of reactive responses amongst the neurosecretory elements under inhospitable situation and in that event certain biochemical components like free aminoacids, proteins and nucleic acids may play an important role in this.

Tendency for the accumulation of the neurosecretory materials at the axon hillock regions amonst some of the members of perikarya is considered to be due to stress and is consistent with ultramicroscopic observations [10].

Influence of near ambient temperature renders moderate cytoanatomocal alterations amonst cerebral neurosecretory components of *Lymnaea(Radix)luteola* with reference to their staining affinities , morphological contours of the secretory contents, axoplasamic flow, nucleolar sizes etc. Besides images of the secretory cycles in the neurosecretory components especially amongst the large types have become obvious. Electron microscopic profile also demonstrate near normal features as and when the extent of accumulation of the electron dense granules at the axon terminals, their conformity and relative abundance of the heterochromatin substances are considered. Distribution of the periodic nucleopores all around the nuclear membrane is striking and seemed to be a common feature.

Coupled with this nucleoplasmic ratios amongst all the types of neurosecretory neurons undergo moderate elevations and seemed to be at per with the rise of temperature. Such features likely to indicate a compromise with the functional aberrations of the neurosecretory elements with respect to the physiology of the neurosecretion. Indeed, the clarity of the morphological enlargement of both nuclei and nucleoli substantiate the observations of Andersen [11].

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