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tomorphic studies on the cerebral ganglion of a diplopod, Trigoniaulus lumbricinus (Gerst) with particular reference to neurosecretion

by

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(Plate 38)

Abstract

Cytomorphic studies on the cerebral ganglion of adult Trigoniaulus lumbricinus reveal the existence of CAHP- and AF-positive neurosecretory neurones. They are classified into large, medium and small types of cells. Large and small types of cells are principally located in the dorsal and ventral regions of the brain, respectively. The medium types of cells, however, are found in both the regions. Furthermore, both these planes could morpho-anatomically be demarcated due to the presence of a median delicate canal passing through the antero-posterior axis of the brain. Evidence for axoplasmic flow and various secretory phases amongst the neurosecretory cell types are conspicuous. Neuropilar accumulation of NSM transported axopically from antero-dorsal and dorso-lateral neurosecretory cell groups substantiates the occurrence of intracerebral-neurohaemal organ, hitherto undescribed, in this species.

Introduction

Existence of neurosecretory cells in the protocerebral lobes of diplopod brain and transport of neurosecretion through intracerebral pathways in order to be stored retrocerebrally located cerebral glands had been determined (Gabe 1954, Sahli 1958, 1961, Prabhu 1962, Shukla and Tripathi 1976). Additional sites for tracerebral storage of secretion have also been well documented (Prabhu 1959,
1962, Juberthie-Jupeau, cited by Sehnal 1971). Resemblance of such stoni-
sites in *Gonoplectus malayus* with the sinus glands of crustacean has recently be-
suggested by Shukla and Tripathi (1976). Neurosecretory pathways in of-
areas like the periesophageal ring of strongylomodid, *Orthomorpha gracilis* h-
also been traced out by Nair (1973) and Sahi and Petit (1975). The present
vestigation on the neurosecretory system of spiroboloid millipede *Trigoni-
lumbreinus* has revealed the structure, nature and especially the distribution
neurosecretory cells in the cerebral ganglia. Attempts have also been made to tr-
out additional neurosecretory storage sites within the brain apart from the \nknown extra-cerebrally located bodies like cerebral glands, connective bodies
hypocerebral organs.

**Material and methods**

Local diplopods *Trigonulina lumbreinus* (Gerst) (Order *Spirobolida*, Fam-
*Trigoniulidae*) ranging from 43 mm to 50 mm in length were collected dur-
monsoon of 1975 and 1976 from the neighbourhood of Calcutta. Animals w-
kept in the laboratory at room temperature (29°C to 31°C) and housed in plai-
containers with soil from the site of collection. Water was sprinkled to the s-
periodically as a source of moisture. Food consisting of dry hay and cowdung et-
was made continuously available. Brains were fixed in aqueous Bouin's fixati-
dehydrated, cleared and embedded in paraffin (56°—58°C). Serial frontal and ce-
sections of 7 micra thick were stained with both Gomori's chrome alum haema-
xylene phloxine as modified by Bargmann (cited by Pearse 1960) and simplif-
aldehyde fuchsin (Cameron and Steele 1959) stain techniques. Material stain-
blue black by the former and purple red by the latter will be referred to as CAI
positive and AF — positive respectively.

**Results**

**Morphology**

Brains of the millipedes under study exposed typical bilobed appearance a-
each lobe is triangular in shape with its apex facing posteriorly. The perieso-
communicating nerve finds its origin from the apex of each triangle. Distal extrem-
dorsolateral extension of the protocerebrum, resembling the optic peduncles
hexapods, are beset with ample sessile pigmented blotches. The axis of the br-
however, has been found to be traversed by a median delicate intracerebral ca-
that runs anteroposteriorly. This is well conceived when the posterior lobes of t-
brain are deflected more dorsally at the time of dissection under a stereosco-
binocular.
Cytomorphology

Cytomorphic studies reveal characteristic distribution of distinct types of neurosecretory cells in the brain of this millipede. These secretory neurons are mainly characterised by their morphological peculiarities, tinctorial responses and patterns of distribution.

There are three types of cells which could be designated as large, medium and small types.

Large cells are unipolar and pear-shaped measuring an average of 12.6 μm in length. Nuclei are, in general, centrally placed and contain sparsely distributed chromatin granules. Nucleoli are prominent. The secretory products are stainable with both CAHP- and AF-methods (Figs. 1a and 1b). But the former does not pose a good picture of either the internal architecture of these cells or their products when being compared with other arthropods, especially in hexapods.

Cells of medium size are more or less unipolar, elliptical or oval measuring an average of 7.9 μm in length. Nuclei may be centric or eccentric. Chromatin granules are few and not so discrete. Secretory inclusions of the cytoplasm may be feebly positive with CAHP-method which shows acidophilic nature of cytoplasm (Fig. 2a).

Small cells are more or less oval and rounded. They reach a maximum size of 6 μm long. Nuclei are centrally placed with ill defined chromatin granules. They are variably stained blue black with Giemsa CAHP-method (Fig. 4).

In so far the distribution of various types of cells in the brain of this diplopod concerned, it is found that the dorsal most part bears large to medium types of cells having variable secretory contents and less defined axonal processes. An accumulation of neurosecretory material (NSM) (both AF- and CAHP-positive) in the form of a streak or band has been found to extend transversely in the mid-dorsal region of the protocerebrum (Fig. 3). Instances for direct deposition of NSM to this region by some of the adjacent cell groups (dorsal and dorso-lateral) have also been detected during the course of this investigation. Such localised concentration of secretory product is always found in the vicinity of the globuli cells. Besides these, regular transport of secretory material elaborated both from dorso-lateral and lateral neurosecretory perikarya through axonal pathways is prevalent as observed by other workers (Sahli 1958, Prabhu 1962). Discharge of the neurosecretory material into the blood is not thoroughly understood but seems to occur by "gradual diffusion" as found in other invertebrates (Prabhu 1961). Drop in the number of neurosecretory neurones as well as complete disappearance of intracerebral accumulation of colloids are the usual features observed in the late dorsal part of the brain.

Ventral profile of the brain could be ascertained with the appearance of a median intracerebral canal. Two lobes of the brain, in consequence, remain segregated temporarily which however, become reunited more ventrally; coupled with these, distinct groupings of smaller to medium types of cells (Fig. 2b), variable stainability of cytoplasmic inclusions and their explicit intracerebral transportation (Fig. 4) as possible extracerebral storage are some of the salient characteristics visualised in this plane.
Discussion

Occurrence of neurosecretory cells in the cerebral ganglia of diplopods has been established by several investigators (Gabe 1954, Sahli 1958, 1961, Prabhu 1962). In their studies, categorisation of neurosecretory cells after employing various staining techniques yielded conflicting results when staining responses are taken into account. In fact, all the observations were mainly based upon the shape, size, and the tinctorial affinity of the cell concerned and accordingly they have been designated as A—B- and C-types and their respective subtypes (Prabhu 1962). The neurosecretory cells with their secretory contents of the millipede under study have shown general staining response to both CAHP- and AF-methods as observed by Gabe (1954). In fact, variable staining reactions of the secretory inclusions expose „differential shades of colours” are due to acidophilic nature of the product indicative of the secretory state of the neurosecretory perikarya (Gabe 1966). Indeed, several subtypes in Jonespellis (Prabhu 1962) may have some relevance with the secretory phases of single type of cells in the event of their activation.

Facts like exaggeration of particular types of cells in morphologically distinct areas of the brain, localisation of intracerebral sites for NSM accumulation, hitherto underscribed, intensification of ventral groupings of neurosecretory cells and presence of median intracerebral canal demarcating the dorsal half of the brain from the ventral, are some of the interesting features observed in this species as opposed to the findings made in other diplopod like Polydesmus (Gabe 1954), Jonespellis (Prabhu 1962), Schizophillum (Sahli 1961). Such variations are obvious as profiles of neurosecretory system may undergo modifications in different species. In so far, the intracerebral axonal pathways are concerned, there is not much difference from that of either Jonespellis (Prabhu 1962) or Iulus (Gabe 1954, Sahli 1961) although traceable axons are well demonstrated in mid-dorsal and ventral parts of the brain especially when CAHP-method has been employed.

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References

Neurosecretion in the cerebral ganglion of a diplopod


Explanation of Plate 38

1. Section showing a large type of neurosecretory cell at the dorsolateral region of the brain. Note evenly distributed secretory material in the cytoplasm and transport of NSM along the axonal processes.

2. Section showing medium types of neurosecretory cells. Note the pattern of distribution of neurosecretory cells in the dorsal (a) and ventral (b) parts of the brain. a) CAHP-reaction, b) AF-reaction.

3. Section showing the intracerebral accumulation of CAH-positive secretory material in the vicinity of the globuli cells. Note the transverse extension of the intracerebral accumulation in the form of a streak.

4. Section showing medium and small types of CAH-positive ventral cells with their discrete intracellular architecture and axonal processes. Note secretory cycle amongst the cell types and overall deep inviability in the small cells.