CORRELATION BETWEEN NEUROSECRETION AND SOME PHYSIOLOGICAL FUNCTIONS OF THE SCORPION *HETEROMETRUS SWAMMERDAMI*

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Abstract

The diverse types of neurosecretory cells located in the brain and suboesophageal ganglion of the scorpion *Heterometrus* have shown morphological evidence of activity in relation to diverse physiological functions. Some of the functions investigated were the process of delivery of the young, apolysis, ecdysis, growth, maturation, water relations, diurnal rhythms of locomotor activity and temperature adaptation. Continuous light and continuous darkness reflected change in the activity of specific groups of neurosecretory cells. In this Arachnid also, neurosecretion seems to play a central role in the regulation of many physiological activities as in insects and other organisms.

With the discovery of neuroendocrines in invertebrates, many physiological activities were correlated with neurosecretion, particularly, in insects (Gabe, 1966). Although the group Arachnida has received lesser attention, a few reports on neurosecretion are available (Gabe, 1954, 1955; Junqua, 1963; Legendre, 1958; Habibulla, 1961, 1962, 1970, 1971 *a*). In spite of this, functional relations of the neurosecretory system in Arachnida are hardly known. An attempt is made here to see if neuroendocrine control operates in some of the diverse physiological activities of the less studied archaic Arachnid *Heterometrus swammerdami*. Since the mapping of the diverse neurosecretory cell types in the brain and suboesophageal ganglion has recently been reported (Habibulla, 1970), involvement of neurosecretion in some of the physiological activities of this scorpion will be reported here.

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Material and Methods

Involvement of neurosecretion was evaluated before, during and after a specific physiological function, by fixing and staining selectively for the neurosecretory product, and examining the diverse neurosecretory cell types of the brain and suboesophageal ganglion. The methods of selective staining for neurosecretion, rearing the scorpions, and the classification of the neurosecretory cells are all same as reported earlier (Habibulla, 1970). Mapping of the cells is shown in Table I, to facilitate easy reference.

Process of delivery.—These archaic scorpions are viviparous. The fully formed young are born alive, often as many as 48. Since the breeding season of *Heterometrus swammerdami* is from late June to early October, and the delivery season from about May to July (Habibulla, 1962), it was not difficult to fix the cephalothoracic nerve mass (brain + suboesophageal ganglion) according to the stage of 'pregnancy'. The females were cut open and examined for the size and stage of embryos before fixing the cephalothoracic nerve mass. The nerve mass of gravid females of different stages until up to 12 days after delivery were examined.

Apolysis, ecdysis and growth.—In scorpion apolysis and ecdysis are interrelated with growth because of the inelastic nature of the cuticle. Apolysis and ecdysis of different stages up to the adult stage of both the sexes were studied for the neurosecretion.

Water relations.—Since the relationship between water content and neurosecretion has been shown in insects (Nayar, 1960), an attempt was made to see if similar relationship exists in the scorpion. Three sets of male scorpions of nearly the same size and weight were subjected to (1) desiccation, (2) injection of sodium chloride and (3) injection of distilled water, separately. Each set consisted of at least one dozen scorpions.

Scorpions were withdrawn from desiccation at an interval of 6, 8 and 12 hours and thereafter every 24 hours till 7 days, and examined for neurosecretion after fixing and staining the cephalothoracic nerve mass. From the second set into which up to 0·5 ml of 0·5 % Sodium chloride was injected (into each scorpion) examination for neurosecretion was made for every 15 minutes until 4 hours. From the third set of scorpions into each of which up to 0·75 ml of distilled water was injected, examination was made initially at the interval of every 15 minutes and thereafter at an interval of 30 minutes until 4 hours.

TABLE I

Types of neurosecretory cells found in the brain and suboesophageal ganglion of the scorpion Heterometrus swammerdami

Classification of cell type	Size of cell	Staining reactions	Distribution
Group I Set 1 ..	38–48 μ	Deep blue with chromic haematoxylin and purple with paraldehyde fuchsin	In brain. Behind the globuli in the neighbourhood of caryochrome cells of central body.
Set II ..	33–38 μ	do.	In brain. Ventro-lateral to Set I
Set III ..	30 μ	do.	In brain below and behind Set II
Group A..	30–35 μ	Cytoplasm feebly stained. Secretory product stained with orange-G. Secretory globule size (upto 12 μ)	In brain, anterior and dorsal to the globuli cells by the stalks of the lateral eyes
Group 2 ..	45–50 μ	Bluish with paraldehyde fuchsin. Reddish to deep blue stained with Heidenhain's haematoxylin	Near the complex of stomatogastric ganglion.
Group B	80 μ	Stainable with chromic haematoxylin. Secretory product phloxinophilic, and also stainable with Heidenhain's haematoxylin and occurs inside the vacuoles.	Near the origin of the pedipalpal nerves, from the suboesophageal ganglion.
Groups 3–9	40 μ	Stainable with chromic haematoxylin and paraldehyde fuchsin	Metametrically arranged with concentration at the posterior region of suboesophageal ganglion
Group C..	40–75 μ	Chromic haematoxylin and fuchsinophilic and phloxinophilic	Hind region of suboesophageal ganglion

TABLE II

Table showing relationship between the neurosecretion and physiological function

Function	Neurosecretory cell groups involved														
	Group 1 Set I	Group 1 Set II	Group 1 Set III	Group 2	Group 3 Lat.	Group 3 Med.	Group 4 Lat.	Group 4 Med.	Group 5 Lat.	Group 5 Med.	Group 6 Lat.	Group 6 Med.	Groups 7–9 Lat.	Groups 7–9 Med.	Neuro-secretory storage center	ABC
1. Process of delivery		+	+	+	+	+	+		+	+	+	+	+	+	+	
2. Growth ..	+	+	+			+		+		+		+		+	+	
3. Apolysis ..	+	+	+													
4. Ecdysis ..	+	+	+													
5. Maturation ..			+			+		+		+		+		+	+	
6. Water relations:																
(*a*) Desiccated								+		+		+		+	+	+
(*b*) NaCl injected								+		+		+		+	+	+
(*c*) Distilled water injected								+		+		+		+	+	
7. Continuous light					+		+		+							
8. Continuous darkness		+			+		+		+							
9. Diurnal rythms of locomotor activity					+		+		+							
10. Temperature adaptation						+		+		+		+		+	+	

+ = indicated activity; Lat. = Lateral cells; Med. = Median cells.

Effect of photoperiodism.—Since in nature these scorpions are found easily from dusk onwards and seldom seen during the day, it was suspected that the neurosecretery phenomenon might be involved in its locomotor activity. Such rhythms in locomotor activity in insects are well known. Two types of experiments were performed for observing the neurosecretory change. One set of scorpions were sacrificed at an interval of every 4 hours for at least 3 days, to see if there is a natural neurosecretory rhythm. The other set was subdivided into two groups. One group was subjected to continuous light, and the other to continuous darkness up to 15 days, the neurosecretion being examined at an interval of every 4 hours. Only adult males of about the same weight were utilized.

Effect of temperature.—Since it is known that any considerable variation in temperature of the environment is perceived by the animal and an effort is made to compensate metabolically, it was expected that neurosecretion might be involved in such a control. Adult male scorpions of the same weight range were separately subjected to $20 \pm 0{\cdot}5°$ C (cold), and $35 \pm 0{\cdot}5°$ C (warm) for a period of up to 8 days (the normal temperature of the terraria was $25 \pm 0{\cdot}5°$ C). The scorpions were sacrificed at 24-hour intervals and the neurosecretion examined.

RESULTS AND DISCUSSION

The classification, mapping and staining characteristics of the neurosecretory cells of the cephalothoracic nerve mass are summarized in Table I. Details of these cells and photographs have been published earlier (Habibulla, 1970). The responses of specific neurosecretory cells to specific functions are provided in Table II.

Group 2 cells appear to be concerned exclusively with the delivery process, since in no other case examined, a change in their secretory activity was apparent. Except the set I of group I, and A, B and C groups, all the other neurosecretory cells show an increase in secretory activity. This is not specific because such changes are seen during the performance of other functions also. The neurosecretory storage centers in the suboesophageal ganglion, which are not usually found empty, are almost completely devoid of secretory product just preceding and immediately after delivery. This may suggest the involvement of this neurosecretory product in the complex phenomenon of viviparity.

The importance of neurosecretion in apolysis and ecdysis is well known in insects as a result of knowledge acquired during the last 20 years. In

the scorpion, group I neurosecretory cells are clearly concerned with apolysis in later stages of postembryonic development. First morphological signs of secretion in these cells are evident only from stage 4 onwards (*i.e.*, not earlier than 4 months after birth) although the protocerebral neurosecretory cells are already differentiated. However lack of visible cytological signs of secretion need not mean that they are physiologically inactive. For example, in the case of chick embryo, it was possible to demonstrate the presence of posterior lobe hormone in hypothalamic region on 9th or 10th day of incubation, although the neurosecretory substance was visible only on 13th or 14th day (Wingstrand, 1954). Although group I neurosecretory cells appear to be involved with ecdysis also, distinct correlation as in the case of apolysis was not possible.

Attainment of secondary sexual characters and maturation in both the sexes is preceded by the activity of most of the neurosecretory cells (Table II) including the ones from suboesophageal ganglion. Such distinct relationship between the state of neurosecretory cells and maturation of gonocytes in Araneida is already known (Legendre, 1959; Kuhne, 1959). The involvement of neurosecretion in oocyte maturation in insects is well known, although relationship with testicular functions have apparently been completely overlooked.

Both in the desiccated and the sodium chloride injected scorpions, immediate change in the neurosecretory storage center is seen, which is succeeded by the group B-cells. The situation seems to be different in scorpion when compared to insects like the *Iphita limbata*. Under conditions of dehydration more of neurosecretory colloid was seen in protocerebral neurosecretory cells in *Iphita limbata* and when the insect was loaded with water the protocerebral neurosecretory cells were devoid of secretory colloid (Nayar, 1960). In scorpion, neurosecretory cells from the suboesophageal ganglion, predominantly group B, and to a lesser extent median cells of group 4 through 9 appear to be involved in water regulation. However it is morphologically evident that neurosecretion is involved in water regulation of the scorpion also.

Diurnal rhythm of neurosecretory activity in lateral cells of groups 3, 4 and 5 could be correlated with the circadian type of locomotor activity (active phase from about 6 in the evening to about midnight) of this scorpion. It was also possible to induce locomotion in the scorpion at mid-day (when the neurosecretory cells are inactive) by injecting an extract of these specific cell groups isolated during their peak of activity at 6 in the evening even

after storing the extract in the refrigerator for 18 hours. Further details about the Kymographic recording technique for the locomotor activity (Naylor, 1958), extraction and injection of neurosecretory regions would be reported separately. A method for isolating living neurosecretory cells has been reported earlier (Habibulla, 1970). Both continuous light, and continuous darkness have shown changes in the secretory activity of these particular neurosecretory cell groups. However Set I of group I cells of protocerebral neurosecretory cells showed an increase in activity when exposed to darkness continuously. This set is also concerned with apolysis and ecdysis in scorpion. Whether manipulation of the photoperiodism has any interference with the interval of ecdysis between two stages was not investigated.

Only the median cells of groups 3 to 9 showed neurosecretory changes during temperature adaptation. When the scorpions are subjected to low temperature continuously, the secretory product of these cells is changed from pink to blue with chrome alum haematoxylin-phloxin staining method. Presumably the blue staining material represents a chemically different product. Reversal of this blue staining product to pink could be brought about by exposing the scorpions to a continuous warm temperature. However, there appears to be a critical period at about the 4th day before this change could occur in either direction. Whether this neurosecretory change is responsible for the increase in proteins and free amino acids of the cephalothoracic region on warm adaptation (Habibulla, 1971 *b*) although indicative, is not known with certainty.

Although the neurosecretory involvement in the control of diverse physiological functions in the scorpion is indicative, it is at present not known how many of these could be direct without involving intermediate mechanisms. Three types of correlations could be formulated between neurosecretion and physiological function. (1) Single groups of neurosecretory cells being involved in the control mechanism of a single function, (2) more than one group of neurosecretory cells being involved in a single function, and (3) single group being involved in control mechanism of more than one function.

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