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Endocrine regulation of ovarian maturation and cement glands activity in a stomatopod crustacean *Squilla holoschista*

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Abstract. In *S. holoschista*, the eyestalk is provided with four types of neurosecretory cells (NSC) while the thoracic ganglia and the brain each have three types. The activities of certain NSC types of the eyestalk (C and D), the thoracic ganglia (*B*) and the brain (*C*) show fluctuations in their secretory activity during the synchronous growth of the ovary and cement glands suggesting their involvement in the control of these organs. Eyestalk ablation experiments on the reproductively quiescent female revealed the inhibitory nature of an eyestalk hormone on the ovarian and cement glands growth. On the other hand, aqueous extract of thoracic ganglia from the mature reproductive females promoted ovarian and cement glands activities, when injected into the quiescent females. But brain extract injection failed to affect the activity of the ovary or cement glands.

Keywords. *Squilla holoschista*; reproductively quiescent and active stages; secretory granules; gonad inhibiting hormone and gonad stimulating hormone; ovary; cement glands.

1. Introduction

In decapod Crustacea, the reproductive hormones fall under two categories; an inhibiting principle originating from the eyestalk and a stimulating hormone emanating from the brain, or thoracic ganglia (Adiyodi and Adiyodi 1970). They have been shown to control the gametogenic activities in a variety of crustaceans. However, their activities, if any, in the functioning of accessory reproductive glands and the reproductive tract or in co-ordinating the post ovulatory processes, have not been understood properly. Several decapods incubate their eggs externally on the appendages. In a few cases, such as the crayfish, a distinct female accessory sex gland namely the cement glands are reported to release a mucoid substance that helps in the agglutination of the egg masses. Stephens (1952a) investigated the endocrine principle regulating the cement glands and ovarian activity in the crayfish, *Procambarus* and suggested a bihormonal mechanism to regulate both activities.

Different species of stomatopods inhabiting the tropical and temperate waters have been shown to possess cement glands whose activity is similar to that of crayfishes. In *Squilla mantis* inhabiting the Mediterranian waters, cement glands activity has been shown to be synchronous with the ovarian activity (Dochi 1975). In *S. holoschista* a similar cyclical activity of the cement glands correlated with the ovarian development has been reported recently. It was also shown that the cement glands secretion is highly mucoid sulphated acidmucopolysaccharide

substance, complexed with protein (Deecaraman 1980; Deecaraman and Subramoniam 1980). Here, we report the mechanism of neuroendocrine regulation of both cement glands and ovarian activity in a stomatopod crustacean *S. holoschista*.

2. Materials and methods

Mature females of *S. holoschista* (Woodmason), collected from the Madras coast were maintained in the laboratory in aquarium. The water was changed every day and sufficiently aerated. For eyestalk ablation, mature but reproductively quiescent females were selected from the daily collection. The quiescent females are identified by the absence of distinct patches of the cement glands on the sternal plates of the 6th, 7th and 8th thoracic segments.

Eyestalk ablation was performed by removing the stalks at the base with a fine sterilized scissors. This facilitates the removal of the sinus gland and the associated neurosecretory *X*-organ completely. This was followed immediately by cauterization with a hot needle to avoid bleeding. The destalked females were not fed during the course of this experiment. Similarly in the control, either the antennae or the antennules were removed. On the 15th day, the experimental and control females were sacrificed and weighed. Subsequent to dissection, the ovary and the cement glands were blotted dry and weighed in a monopan electric balance to the nearest milligram.

From the daily collection, both the mature females and reproductively quiescent females were isolated. The thoracic ganglia were removed from the two mature females and titrated with 1 ml of doubly-filtered seawater in an ice bath. This was later centrifuged at 3000 rpm for 15 min and 150 µl of the supernatant immediately injected into the quiescent females. The control groups received the same dosage of filtered seawater. On the 15th day, both the groups were sacrificed and the animal, ovary and cement glands were weighed.

The brain extract was similarly prepared and these animals were also treated like the previous one.

For histological study, eyestalks, thoracic ganglia and brain of quiescent and reproductively active female were fixed in Bouin's or 10% neutral buffered formaldehyde. The eyestalks were also fixed in Perney's fluid (Mahoney 1966) and later neutralized in 5% sodium sulphate solution for 12 hr. Thin sections (6-8 µm thickness) were cut and stained in Mallory's and Masson's and haematoxylin. Sections were also treated with chrome-alum haematoxylin phloxine and paraldehyde fuchsin stains to study neurosecretory cell (Gomori 1941, 1950).

3. Results

3.1 *The X-organ*

The *X*-organ is located distal to the medulla terminalis is of a compact type having three lobes; proximal, middle and distal (figure 1). The *X*-organ appears with a characteristic shape of grape with different types of neurosecretory cells (NSC) in different lobes. These cells show variation in their staining affinity during the quiescent as well as the reproductively active periods. The *X*-organ is

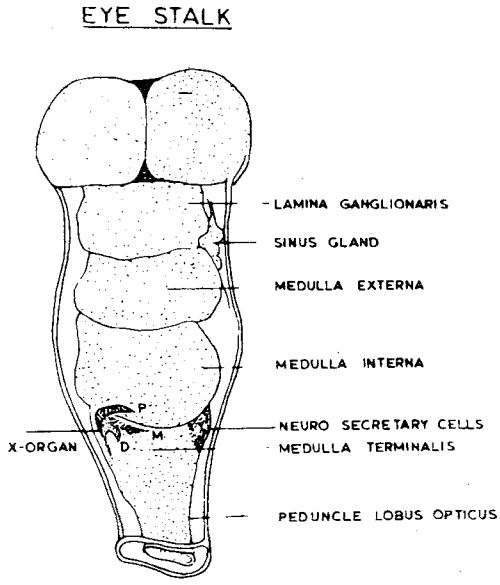

Figure 1. A composite diagram showing the *X*-organ/sinus gland complex of eyestalk with neurosecretory cells (not drawn to scale).

connected to the sinus gland by an axonal stalk called *X*-organ sinus gland tract. The *X*-organ found in the eyestalk has three types of NSC namely *A*, *B* and *D*. The *C* type of NSC are found only in the medulla terminalis. NSCs are identified and classified depending on the size and shape of the cell and nucleus, location, cyto-physiological and functional behaviour.

The *A* type of NSC are pear-shaped, few in number, unipolar and provided with distinct nucleolated nuclei (figure 2). These cells measure about 12 µm and are found mostly in the distal lobe of the *X*-organ. The cytoplasm is rich with dense secretory granules only at the reproductively active period. The *B* type of NSC are spherical in shape measuring about 8 µm and are moderate in number. They are unipolar and located in the peripheral region of the middle lobe of the *X*-organ. The nucleus is spherical and without a nucleolus (figure 2). The cytoplasm is free from dense granules during the quiescent state. The *C* type is located in the medulla terminalis and are moderate in number. They are oval to elliptical in shape, unipolar and measure about 5 to 6 µm in diameter. The cytoplasm is full of secretory granules with vacuoles during the quiescent period possibly indicating an active state of secretion. The *D* type of NSC are found in the proximal lobe of the *X*-organ. These cells are unipolar, measuring about 4 µm, moderate in number and the secretory granules are rich in the cytoplasm in the quiescent period compared to reproductively active period. The presence of secretory granules in the *C* and *D* cell types when cement glands and ovaries are quiescent suggests that they may be involved in the synthesis of a gonad inhibiting hormone (GIH).

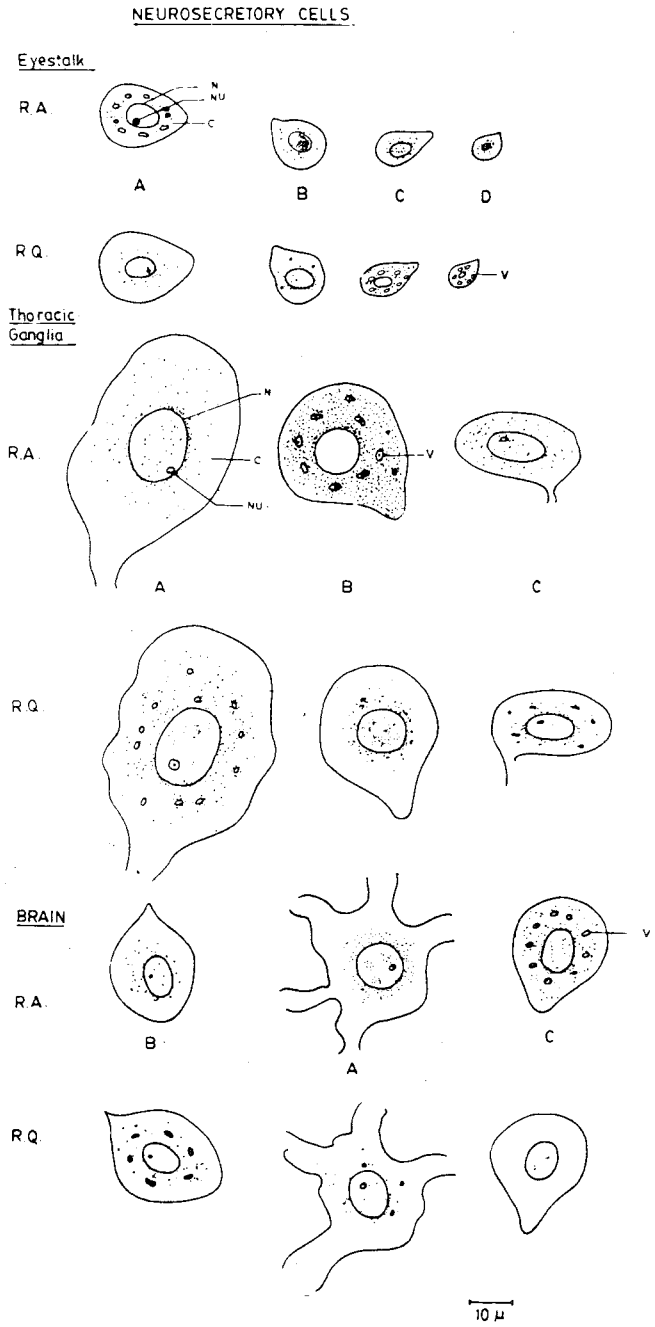

Figure 2. Camera lucida drawings showing the various types of neurosecretory cells in eyestalk, thoracic ganglia and brain at reproductively active and quiescent stages. (i) Eyestalk. Note secretory activity of *C* and *D* type of neurosecretory cells at reproductively quiescent stage. (ii) Thoracic ganglia. Note the active phase of *B* type of cell at reproductively active state. (iii)) Brain. Note the active phase of *C* type of cell at reproductively active state.

3.2 *Sinus gland*

The sinus gland is a neurohaemal organ storing neurosecretion from tne *X*-organ. It is located between the lamina ganglionaries and medulla externa (figure 1). The gland is somewhat triangular, having dorsal and ventral folds. Blood sinuses traverse these folds and surround the gland itself. The gland is filled with intensively staining granules during the reproductively quiescent state.

3.3 *Thoracic ganglia*

The thoracic ganglia are provided with three types of NSC namely *A*, *B* and *C*. The *A* type is large in size (32 µm in diameter) and hence called the "giant NSC" (figure 2). They are unipolar, few in number and located in the peripheral region of the thoracic ganglia. The nucleus is distinct with a nucleolus, which is provided with a central vacuole. The cytoplasm is filled with secretory granules only during the reproductively quiescent state. The *B* type is more or less spherical with a cone-like projection at one end and measures about 15 µm. The cells are unipolar and found towards the peripheral region of the thoracic ganglia. The cytoplasm shows more secretory granules during the reproductively active period than at quiescent state. The third type of NSC the *C* type, measures about 7 to 8 µm, and are unipolar having distinct nuclei. The cells are elliptical to oval, moderate in number and located laterally. The cytoplasm is rich with secretory granules, in the quiescent state than in the reproductively active state (figure 2). Among the three types of NSC in the thoracic ganglia only the *B* type shows more secretory activity during the reproductively active period suggesting involvement in the synthesis of gonad stimulating hormone (GSH).

3.4 *Brain*

The brain of *S. holoschista* is provided with three types of NSCs namely *A*, *B* and *C* (figure 2). These cells also show variation in their cyclical activity with regard to reproductively active and quiescent states. The *A* type of NSC are multipolar, 15 µm in size, few in number and found at the anterior end of the brain. The cytoplasm is rich in granules only at the quiescent state. The *B* type is oval, unipolar and located at the lateral sides of the brain. These cells measure about 10 µm, and are moderate in number. The cytoplasm shows less secretory activity during the reproductively active period than at quiescent state. The *C* type of NSC are 7 to 8 µm, unipolar and are more numerous than the other types. They are mainly distributed in the distal portion of the brain. The staining activity of cytoplasmic granules is greater during the reproductively active period than at the quiescent state.

3.5 *Eyestalk ablation*

The destalked females after 15 days showed a remarkable increase in the weight of the ovary and cement glands. The cement glands were in the form of lobate clusters, charged heavily with dense mucoid secretion and the ovary was yellow with mature oocytes. The mean weight and SD values of the ovary (0.2600 gm, 0.084) and cement glands (0.0375 g, 0.012) as related to the total body weight

showed a great difference to that of the control group (ovary 0.025 g, 0.012; cement glands 0.0006 g, 0.011). The statistical analysis revealed a significant increase in the proportion of (a) weight of the animal to the weight of the ovary, (b) weight of the animal to the weight of cement glands and (c) weight of the ovary to the weight of the cement glands between the control and the experimental groups (tables 1, 2A,B,C).

Table 1. Mean and standard deviation.

	Source	Weight of the ovary	Weight of the cement glands
Eyestalk ablation	Experimental	0.26 ± 0.084	0.037 ± 0.012
	Control	0.025 ± 0.01	0.0006 ± 0.011
Thoracic ganglia extract injection	Experimental	0.323 ± 0.191	0.032 ± 0.021
	Control	0.33 ± 0.020	0.0006 ± 0.0016
Brain extract injection	Experimental	0.035 ± 0.015	0.0044 ± 0.0013
	Control	0.023 ± 0.021	0.0012 ± 0.0024

(50 animals are used for each experimental and control group).

Table 2A. Summary of analysis of variance.

	Source	Observed value	Expected value at 5%
A. Eyestalk ablation	animal/ovary	15.24	
	animal/cement glands	32.29	Significant
	ovary/cement glands	81.09	
B. Thoracic ganglia extract injection	animal/ovary	26.43	
	animal/cement glands	80.60	3.96
	ovary/cement glands	52.79	
			Non-significant
C. Brain extract	animal/ovary	0.01	
	animal/cement glands	1.80	
	ovary/cement glands	2.76	

Note: (50 animals for experimental and control groups).

Table 2B. Test of significance (only for the experimental groups).

Source	*t* observed ovary	*t* observed cement glands
Eyestalk ablation × Thoracic ganglia extract injection	2.46	0.034
Eyestalk ablation × Brain extract injection	14.13	15.60
Thoracic ganglia extract injection × Brain extract injection	11.66	3.42

T expected at 5% in all cases is 1.96.

Table 2C. Correlation (r) only for the experimental groups.

Source	r	df	p
Eyestalk ablation experiment	0.91	48	0.05*
Thoracic ganglia extract injection	0.86	48	0.05*
Brain extract injection	0.052	48	0.05 (NS)

* — Significant; NS — Non-significant.

3.6 *Thoracic ganglia extracts injection*

The quiescent females receiving the aqueous extract of thoracic ganglia showed an enhancement in the activities of ovary and cement glands, while the control animals did not show any such increase. A comparison of the mean weight and SD values of the ovary (0.3230 g, 0.191) and cement glands (0.0326 g, 0.0215) of the experimental group to that of the control (ovary 0.0334 g, 0.0206; cement glands 0.0334 g, 0.026) reveals the differences between these groups. This is further substantiated by the statistical analyses (tables 1, 2A,B,C).

3.7 *Brain extract injection*

The reproductively quiescent females that received the brain extract failed to show any significant development of the cement glands and ovary. The mean weight and SD values of ovary (0.0350 g, 0.01566) and the cement glands (0.0044 g, 0.0013) of the experimental group, when compared to the control values (ovary 0.0238 g, 0.0218; cement glands 0.0012 g, 0.0024) fails to show much difference. These differences were also statistically insignificant (tables 1,2A,B,C).

4. Discussion

Many of the malacostracan crustaceans brood their eggs in their appendages. Oviducal secretions are the secretions from the tegumental glands, which help in the agglutination of the eggs as well as in the attachment of the eggs to the pleopods. In stomatopods such secretions are produced by the distinct cement glands, the chemical composition of which has been shown to be an acid-mucopolysaccharide-protein complex and is protective in function (Deecaraman and Subramoniam 1980).

As against the information available on the hormonal control of ovarian function in Crustacea, very little is known about the accessory reproductive glands (Subramoniam 1981). Available information, however, indicates that the activities of these glands are controlled by the same reproductive hormone that influences the gonadal activity. For example, Stephens (1952a) showed in the crayfish, by the classical eyestalk ablation methods and the implantation of brain, that the cement gland activity is not only parallel to ovarian development but also controlled by the same hormones regulating the ovarian development. The results now reported corroborate the view of Stephens (1952a). Of the NSCs present in the eyestalk of

S. holoschista only types *C* and *D* exhibit cyclical fluctuations in their secretory activity correlated to cement gland and ovarian development suggesting that they may be directly involved in the inhibition of the activity of these organs. These NSC cells may be homologous to the *B* type of cells described by Matsumoto (1958) and *E* type of cells by Adiyodi (1967) involved in the production of GIH in brachyurans. Eyestalk ablation studies in *S. holoschista* now support this hypothesis and suggest the presence of inhibitory hormones in the eyestalk for gonad as well as cement glands activity. Similar results have also been reported in the cement glands activity of crayfish (Stephens 1952a).

A survey of NSC types that are distributed in the thoracic ganglia and brain has also indicated that certain cells types show cyclical variation in *S. holoschista*. The *B* and *C* types, respectively of the thoracic ganglia and the brain show cyclical variations in their secretory activity suggesting that they may be involved in the synthesis of gonad as well as cement glands stimulating hormones. The *B* and *C* types of NSC of the thoracic ganglia and the brain of *S. holoschista* may be homologous to the *A* and *A'* cells of thoracic ganglia and *A* and *E* cells of brain of the brachyuran crab, which have been implicated with the release of GSH (Matsumoto 1958, 1962). However, injection of aqueous extracts of the thoracic ganglia and brain indicates that only those animals which received thoracic ganglia extract showed acceleration in the growth rates of the ovary and cement glands. In this connection it is of interest to compare the data of Stephens (1952b) who found that the brain along with the circumoesophageal connectives induce ovary and cement glands in the crayfish. That the thoracic ganglia contains the NSC responsible for the secretions of GSH in a number of crustacean species has been indicated in the work of Otsu (1963) and Matsumoto (1958, 1962). Generic differences may thus exist in the distribution of NSC in the central nervous system for controlling the activities of both ovary and cement glands in Crustacea (Subramoniam 1981). The present data on the neuroendocrine coordination of the ovarian as well as cement glands function further high-lights the importance of the synchronous activity of these two organs. Furthermore, the present observation confirms the earlier reports on the regulation of accessory gland by the gonadotrophic hormone.

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