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ACTIVITY OF VENTROMEDIAL NEUROSECRETORY NEURONS OF THE SUBESOPHAGEAL GANGLION (TYPE A) IN *MORIMUS FUNEREUS* MULS. DURING METAMORPHOSIS.

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During metamorphosis, holometabolous insects undergo dramatic alterations in external appearance and behavioral repertoire. This developmental process occurs by a combination of programmed cell death, cell proliferation and differentiation, and remodeling of old structures for new functions, along with major morphogenetic shifts (Riddiford, 1985). The given processes are under the control of neurohormones and hormones mainly belonging to the ecdysteroid class (Bollenbacher *et al.* 1981; Bollenbacher, 1988).

Increase in the haemolymph titer of juvenile hormone (JH) at the end of the last larval instar and during the prepupal stage stimulates the prothoracic gland to synthesize and release ecdysteroids (Cymborowski and Stolarz, 1982; Hiruma and Agui, 1982; Hiruma, 1986; Stanić *et al.* 1989). *Corpora allata* are not active during metamorphosis (Granger *et al.* 1985). However, JHs accumulated during the prepupal period are released successively during the pupal stage, enabling the differentiation of certain adult tissues to occur.

It is well known that, along with some biogenic amines, ecdysteroids stimulate diuresis in insects during the feeding period (Rafaeli and Mordue, 1982; Spring, 1990). Since the pupa is a non-feeding stage, the question arises as to the role of diuretic hormones during metamorphosis. The aim of the present work was to investigate the activity of a pair of ventromedial neurosecretory neurons (type A) during pupal development in *Morimus funereus*. It has been discovered that these cells synthesize a neuropeptide (arginine-vasopressin) that stimulates diuresis in the Malphigian tubules and/or retards reabsorption of water in the rectum (Maddrel and Nordmann, 1979; Remi and Girardie, 1980; Phillips, 1983; Spring, 1990; Gäde, 2004).

The pupae used in this experiment were obtained from larvae reared under constant laboratory conditions from hatching to pupation: temperature of 23°C, artificial diet for *Drosophila* (Roberts, 1989), relative humidity of 70%, and absence of light. Pupae were sacrificed on the 1st, 3rd, 7th, 13th and 15th day of pupal development, as well as on the 1st day of the adult stage. After decapitation, pupal heads were fixed in Bouin's solution. The chitinized surface and muscles were removed, and the neuroendocrine system (brain, subesophageal ganglion, *cardiacum-allatum* complex) was excised. Standard histological procedure was employed for embedding in paraffin (Merck, 57-59°C). Serial paraffin sections of 5 µm were stained using Alcian Blue Phloxine and Paraldehyde Thionine Phloxine (Panov, 1980). Five subesophageal ganglia were analyzed for each period of pupal development.

The activity of subesophageal neurosecretory cells was estimated using the following cytological parameters:

The size of neurosecretory neurons expressed as the mean of the products of the largest and smallest diameters of each neuron (a x b);

The size of the nucleus, expressed as the mean of the products of the largest and smallest diameters of each nucleus (a x b);

The amount of neurosecretory material (NSM), arbitrarily estimated as sparse, present, or abundant; and

The texture of NSM, described as powdery or fine-grained, medium-garined, or coarse-grained.

Changes in these parameters were used to indicate changes in the activity of neurosecretory neurons.

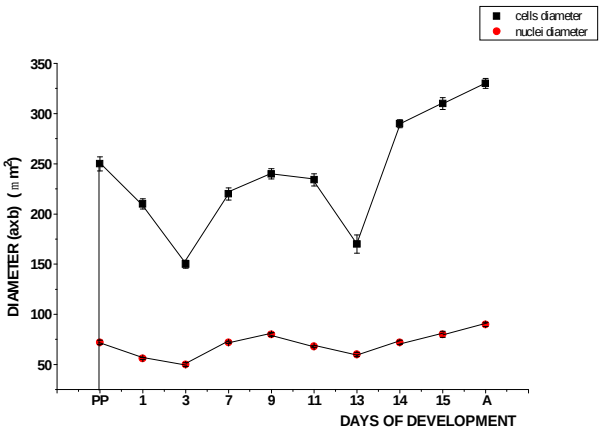

Fig. 1. Diameter of neurosecretory neurons and their nuclei during pupal development in *M. funereus.*

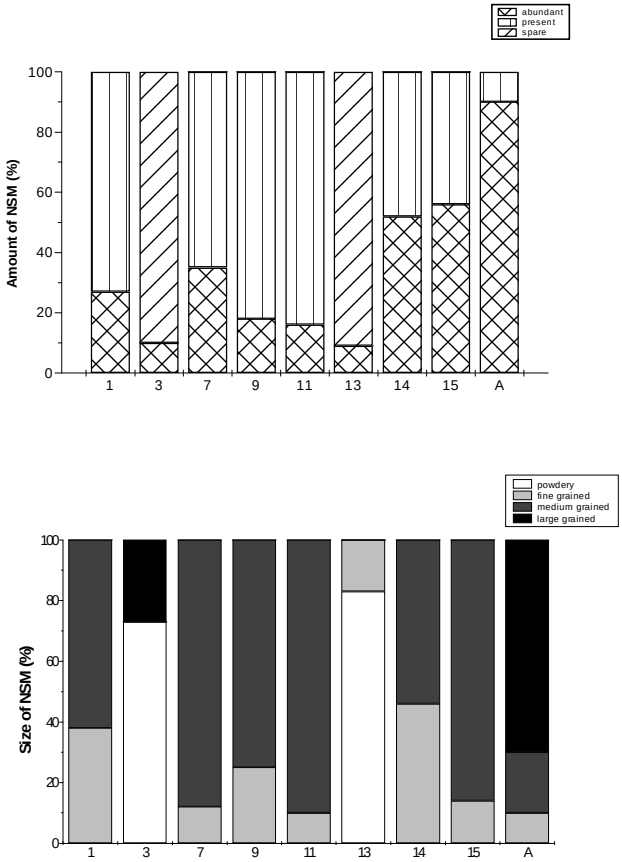

Fig. 2. Size and amount of neurosecretory material in neurosecretory neurons.

The activity of subesophageal ventromedial neurons is high during the last larval instar. However, it decreases significantly in the pupal stage. Low activity in 3-day-old pupae was indicated by reduced diameter of the nucleus and perikaryon, the presence of one small nucleolus, and sparse coarse-grained NSM in the perikaryon (Figs. 1 and 2).

Pupal development lasts 15 days on average. Increased activity of subesophageal ventromedial neurons was noticed in the middle of the pupal stage, i.e., between the 7^{th} and 11^{th} day. The decrease in activity on the 13^{th} day was followed by an increase in activity until the end of pupal development (Fig. 1). The presence of two large nucleoli in the nucleus indicated increased synthetic activity. However, the release of NSM stopped, which was revealed by a high quantity of NSM in the perikaryon (Fig. 2). At the beginning of the adult stage, the size of nuclei and perikarya increased and the presence of a large quantity of coarse-grained NSM was noticed (Figs. 1 and 2).

It has been shown that the activity of ventromedial cells during metamorphosis of ***M. funereus*** is correlated with the activity of A_1 medial protocerebral neurosecretory neurons (Nenadović, 1992).

An ecdysteroid maximum in the middle of the pupal stage is characteristic of ***M. funereus*** and other holometabolous insects (Cymborowski and Stolarz, 1982; Hiruma and Agui, 1982; Hiruma, 1986; Stanić ***et al.*** 1989). The hemolymph titer of ecdysteroids is highest during the intensive cytodifferentiation of adult tissues in ***M. funereus*** (Stanić ***et al.*** 1989; Nenadović, 1992).

Increased activity of ventromedial neurosecretory neurons has be found to be correlated with the ecdysteroid maximum in ***M. funereus***. Accordingly, it could be supposed that, along with ecdysteroids, neurohormones synthesized in ventromedial cells also take part in cytodifferentiation of adult Malphigian tubules and hindgut (Maddrel and Normann, 1979).

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