Gregor Mendel Foundation Proceedings 2007: 36-38.

Histological changes in the cerebral neurosecretory cells during larval-pupal transformation of *Spodoptera mauritia Boisd.* (Lepidoptera: Noctuidae).

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Abstract: *The structure of neuroendocrine organs of sixth instar larvae of Spodoptera mauritia Boisd. (Lepidoptera:Noctuidae) consists of the neurosecretory cells of the brain as well as the retrocerebral organs like the corpora cardiaca and corpora allata. Neurosecretory cells are intact cells in the brain. Neurosecretory cells have been identified by their characteristic staining properties with histochemical stain (Paraldehyde-Fuschin). Three types of neuroscretory cells are present in Spodoptera mauritia which includes medial, lateral and posterior group. Different sub types of medial and lateral neurosecretory cells are also present in Spodoptera mauritia . These different sub types of cells show difference in cell volume in different days of sixth instar larva. The present study shows that in Spodoptera mauritia the median neuroscretory cells of the brain show an increase in volume and cellular contents on day two and day four of sixth instar larval stadium. The increase in the synthetic activity of neurosecretory cells on day two and day four of last larval stadium of Spodoptera mauritia possibly represent the biphasic release of neurohormones needed for larval –pupal metamorphosis.*

Key words: larval-pupal transformation, *Spodoptera mauritia*

INTRODUCTION

Endocrine system plays an influential role in the sequential events taking place during insect metamorphosis. The major hormones controlling insect development and metamorphosis are the pro thoracicotropic hormone secreted by the neurosecretory cells of the brain, ,juvenile hormone secreted by the corpora allata and ecdysone secreted by prothoracic glands. The neurosecretory cells of the brain in Lepidoptera have been described by several authors (Mc Leod and Beck, 1963; Singh and Arif, 1978). The main objective of the present investigation was to study the structure of neuroendocrine organs of sixth instar larvae of *Spodoptera mauritia* and the changes undergone by the neurosecretory cells in different days of development of the sixth instar larvae.

MATERIALS AND METHOD

The studies were conducted on rice army worm *Spodoptera mauritia* which is a widely distributed sporadic pest of paddy in South India. The adult moths were collected at night using fluorescent lamps and kept in glass chimneys, closed at both ends by muslin cloth. The first instar larvae hatched from the eggs after three days. Larvae were fed daily with fresh tender leaves of *Ishaemum aristatum* which was collected from paddy fields. The first instar larvae molted into sixth instar larvae within 2-3 weeks. The sixth instar larvae transformed into a wandering stage during which they did not feed. These wandering larvae became prepupae on the fifth day and pupated after 24 hrs.

The sixth instar larvae of *Spodoptera mauritia* were chosen for the present study. The larvae used for the experiments were taken from the laboratory stock culture. The age of the larvae was designated as day n where day 0 indicates the day of ecdysis to this stage. Newly ecdysed larvae were considered as day 0, the larvae 24hrs old as day 1 and so on.

Larvae were anaesthetized in specimen tubes containing diethyl ether and they were dissected in cold insect saline using sterilized instruments. The brain was separated together with the suboesophageal and prothoracic ganglia under a stereozoom dissection microscope. The dissected out tissue was fixed in Bouin's fluid for 24-48 hrs. The fixative was washed out in running water and brought to 70%ETOH.Then the tissue was hydrated and oxidized for thirty minutes by using $KMnO_4H_20$.

The oxidant was removed with 4% aqueus sodium bisulphate. Then the tissue was brought to 70% ETOH and stained with Paraldehyde-Fuschin for 24 hrs. The stained tissue was differentiated in tap water, dehydrated through different grades of ETOH, passed through 100% ETOH-Acetone mixture, Acetone cleared in Methyl benzoate-acetone mixture and Methyl benzoate for 20 minutes and the mounted in DPX.

RESULTS AND DISCUSSION

In sixth instar larvae of *Spodoptera mauritia*, the major neuroendocrine organs consist of the neurosecretory cells of the brain as well as the retrocerebral neuroendocrine organs like the corpora cardiaca and corpora allata. The corpora cardiaca of the larvae are paired, slender fusiform and oblong organs lying on each side ventral to the brain and lateral to the oesophagus. The corpora allata are discrete spherical bodies lying ventrolateral to the oesophagus.

Neurosecretory cells can often be seen as intact cells in brain. Neurosecretory cells have been identified by their characteristic staining properties with specialized histochemical stain *ie*.,Paraldehyde Fuschin. This dye stains a different group of neurosecretory cells. In the present study it is found that the neurosecretory cells are located in three groups *ie*., medial group in the pars intercerebralis, lateral group in the pars lateralis and the posterior group in the posterior region of the brain. In the stained whole mount preparation of the brain, all the three groups of neurosecretory cells could be classified into subtypes like A (medial) cells L(lateral) cells and P (posterior) cells.

The neurosecretory cells could be further classified based on the topographical features, volume of cytoplasm and nucleus as well as on the amount and stainability of the neurosecretory material. In the larvae each hemisphere of the brain contained ten stainable materials in the medial group. Of these two were distinguished as A1 cells, two as A2 cells, two as A3 cells and four as A4 cells. In the newly molted sixth instar larvae, A1 cells were darkly stained neurons and were the largest volume of 1.322*10-6 mm^3. A2 cells were small irregularly shaped and moderately stained with a volume of 0.6721*10-6 mm^3.A3 cells were smaller and darkly stained neurons with a volume of 0.2938*10-6 mm^3. A4 cells showed least sensitivity and had a volume of 0.918*10-6 mm^3.

The lateral group contained five cells of L1 type which were smaller in size and nuclear volume. The other five cells were of L2 type which were larger in size and cell volume. There were 4 posterior groups of P cells with two on each side of the medial tissue and they are moderately stained.

In the present study, medial, lateral and posterior groups of neurosecretory cells differed in cell and nuclear volumes as well as in the amount of stainable material in the perikaryon. In the day 0 larvae A1, A2, A3 and A4 cells were found to have smallest nuclear and cell volumes and also the amount of stainable material.

The amount of neurosecretory material in the perikaryon was sparse in day 0 larvae as compared to the other days of the instar. Day 1 and day 2 larvae showed moderate amount of neurosecretory material. A considerable increase of cell volume and nuclear volume were observed in day 3 larvae. The maximum accumulation of neurosecretory material was observed in day 4 larvae. A decrease in cell and nuclear volume was observed in day 5 (prepupa). The neurosecretory cells of day 2 larvae showed an initial increase in volume and an exponential increase in the volume of neurosecretory cells was observed in the brain of day 4 larvae.

During larval-pupal development of all the Lepidoptera examined, ecdysteroid release occurs twice. The first small peak of ecdysteroids is involved in the change of developmental commitment of epidermal cells and in the induction of gut purge and wandering behaviour. A second major peak of ecdysteroids preceeds pupal development (Bollenbacher *et al*.,1975) since PTTH released by the neurosecretory cells of the brain stimulate the release of ecdysteroids, a biphasic secretion of PTTH has also been reported during larval pupal development. Earlier studies have demonstrated that increase in cell volume and stainability of neurosecretory material in the cytoplasm represent higher synthetic activity of neurosecretory cells (Granger and Bollenbacher, 1981; Santha and Nair, 1991).The increase in the synthetic activity of neuroseccretory cells on day 2 and day 4 of last larval stadium of *Spodoptera mauritia* possibly represent the biphasic release of neurohormones needed for larval –pupal metamorphosis.

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