RESEARCH ARTICLE

Effect of Induced Environmental Factors on the Neurosecretory Cells

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ABSTRACT

In the present study an experimental work was carried out to reveal the effect of induced environmental factors on the neurosecretory cells of *Spodoptera Litura* and the effect of temperature on neuroendocrine cells of *Spodoptera Litura*. Potassium chloride, sodium chloride and Distilled water were used as induced artificial factors. Male insects were used to find out the effects of Potassium chloride, sodium chloride and Distilled water on the neurosecretory cells of *Spodoptera Litura* while female insects were used to find out the effect on temperature on neuroendocrine complex. Four groups of insect were made for the experiments. 24 µl of 2 % potassium chloride solution was injected in first group. 25 µl of 2% sodium chloride solution was injected in second group.25 µl double distilled water was injected in third group. The fourth group served as control.

Effect of Potassium chloride: fair distribution of AF - positive secretory material was observed in the 'A' cells of the median neurosecretory cell group of pars intercerebralis of the brain. After six hours of injection, 'A' cells were deprived of the AF positive material.

Effect of sodium chloride: After six hours of injection the neurosecretory 'A' cells were flooded with AF positive material. After twenty four hours of injection The 'A' cells increased considerably. After forty eight hours of injection, 'A' cells showed only negligible amount of stainable granules. After seventy two hours stainable material was observed in the 'A' cells.

Effect of distilled water: After six hours of the injection the 'A' cells showed degranulation. After twenty four hours, decrease in the nuclear diameter was observed in 'A' cells. After forty eight hours of the injection the 'A' cells showed accumulation of AF positive material. After seventy two hours, diameter of the nuclei increased this differed than that of controls.

Effect of temperature: High temperature supports the production of high rate of neurosecretory material while low temperature decreases the production of neurosecretory material in the moth *Spodoptera litura*.

Keywords: *Spodoptera Litura*, Neurosecretory Cells, Environmental Factors, Potassium chloride, sodium chloride, Distilled water, Temperature.

INTRODUCTION

The control of osmosis by the neuroendocrine complex in insect was pointed out for the first time by pflugfelder (1937), in Dripus morossu. He had shown the accumulation of neurosecretory material in the brain when the insect was dissected. Nayar (1957) showed salt water as an induced factor while work in *Iphita limbata* bug and found stainable colloids in the neurosecretory cells. Highnam (1962; 1965) injected distilled water in *Schistocerca gregaria* and found rapid release of neurosecretory material from the neurosecretory cells of pars intercerebralis. Wall and Rulph (1962) in *Blaberus giganteus* when injected saline found clump of neurosecretory material in the neurosecretory cells of pars intercerebralis. Dogra and Ewen (1969) in *Melaolus sanguinipes* found identical results. The effect of temperature on the neurosecretory activities have been studied in detail. Highnam (1958) while working on Mimastiliae observed the effects of temperature on neurosecretory cells of the brain and his observations were supported by Awasthi (1969), in Nazara viridula. Clarke (1966) had reported the histological changes in detail after keeping the insect *Locusta migratoria* at different temperature. Raina (1974) reported the effects of temperature on the neurosecretory activity of Dysdercus similis. The effect of constant temperature on the survival rate, neurosecretion and endocrine cells and digestive enzymes in *Morimus funereus* larvae have been studied by Ivanoicvic *et al*. (1975). Bilapate and Thombre (1979) showed that the influence of temperature was much pronounced on larval and pupal development and concluded that 30^0C was the optimum temperature for development of *Spodoptera litura*. Bassal *et al*. (1980) had studied the water content and its distribution in the body of the *Spodoptera litura* during metamorphosis. In the present paper the study of induced artificial factors like potassium chloride, sodium chloride, distilled water on neurosecretory cells of *Spodoptera litura* in the brain and the effect of temperature on neuroendorcine cells have been described.

MATERIALS AND METHODS

The experiments were performed in the male insects as they do not show much fluctuation in their neurosecretory system during adult life. Females were used only in the experiments to observe the effect of temperature on neuroendocrine complex. The following substances were used to record, the effect of induced factors on neuroendocrine complex. Potassium chloride, sodium chloride and Distilled water. The insects were divided into four groups, age of insects were 5 days old age group, group one was injected with 24 µl of 2 % potassium chloride solution, group two of with 25 µl of 2% sodium chloride solution and the third group as control, double distilled water. The fourth group served as control. After the injection five insects from each group were dissected and then processed for drawing the conclusions.

Effect of temperature on the NSC of *Spodoptera litura*:

The study of the effects of temperature on neurosecretory cells, moths of the same age group were kept at 10^0C and 30^0C for two hours, than tissue of brain fixed in Bouin's fluid dehydrated and stained with AF. The measurements of the neurosecretory cells nuclear cell diameter were calculated with the help of ocular micrometer. The averages of nuclear and total diameter of five neurosecretory cells from various sections were taken as standard measurements.

RESULTS

(a) Effect of potassium chloride (KCL) solution on the neurosecretory system:

In the initial control insects, moderate distribution of AF - positive secretory material (table 1) was observed in the 'A' cells of the median neurosecretory cell group of pars intercerebralis of the brain. The insects were injected with 25 µl of 2% potassium chloride solution and observed the changes on the nuclei of the median neurosecretory 'A' cells of the brain at different timings of adult *spodoptera litura*. In the control insects the brain were predominated by AF positive cells of pare - intercerebralis. After six hours of injection of 25 µl of 20% KCL solution a remarkable change was observed in the median 'A' cells of the brain of the moth. The 'A' cells were deprived of the AF positive material. After twenty four hours treatment there was a marked shrinkage in the nuclear wall. The nuclear diameter was decreased (diameter

6.20 ± 0.03 μ) and differs from the controls (diameter 6.60 ± 0.03 μ) and differs from the controls (diameter 6.60 ± 0.06 μ). The cells showed atrophic changes and had no stainable granules. Median neurosecretory products also naturally did not secret Stainable material. After forty eight hours treatment in the experimental insects the majority of the insects died. The survived insects showed the same condition of 'A' cells as seen in earlier stages. The nuclear diameter (6.18 ± 0.4 μ) did not differ much from the control (diameter 6.40 ± 0.09 μ) median neurosecrtory pathway also had no secretory material.In experimental insects which had survived, after seventy two hours treatment, showed marked granulation in the 'A' cells with nuclear diameter (6.60 ± 0.06 μ) while median neursoecretory pathway still had no secretory material.

(b) Effect of sodium chloride (NaC1) salt solution on neurosecretory system:

Insects were injected with 25 μl of 2% NaC1 solution and observed the change in the median neurosecretory 'A' cells of the brain of the adult moth *Spodoptera litura*.

1. After six hours injected with 25 μl 2% sodium chloride solution the neurosecretory 'A' cells were flooded with AF positive material whereas in initial control median N11 pathways (MNSP) showed higher degree of granulation. The nuclei in the experimental insects were seen almost of the same size (6.40 ± 0.10 μ) were bigger than those of the controls (6.55 ± 0.07 μ) median neurosecretory pathway showed dense of stainable secretory material.
2. After twenty four hours of treatment, with salt solution release of neurosecretory material was apparent. The 'A' cells increased significantly and their nuclei diameter (7.55 ± 0.10 μ) were bigger than those of the controls (6.55± 0.07 μ) median neurosecretory pathway showed dense of stainable secretory material.
3. After forty eight hours, treatment granulation reached its highest point in these insects, 'A' cells showed only negligible amount of stainable granules. Nuclei diameter (8.68 ± 0.15 μ) showed significant hypertrophy pathway did not take proper stain which indicate complete release of neurosecretory AF positive material.

After seventy two hours treatment insects a considerable predomination of stainable material was observed in the 'A' cells but their nuclei diameter (6.95 ± 0.07 μ) were still larger than that of controls diameter (6.57 ± 0.05 μ) in spite of this condition the median neurosecretory pathway showed a moderate amount of secretory material which did not differs with those of control insects.

C) Effect of distilled water on the neurosecretory system:

The moths injected 25 μl of double distilled water and changes were observed in the nuclei of the median neurosecretory 'A' cells of the brain of the pathway showed moderate in adult moth. The median `A' cells and median neurosecretory pathway showed moderate amount of AF positive material in the initial control insects.

Table 1: effect of induced environmental factor on neurosecretory cells

Time in hous	Potassium Chloride	Sodium Chloride	Distilled Water
Initial control	Moderate distribution 0f secretory material AF positive	Cells showed higher Degree of granulation	Cells showed moderate Positive materials
After 6 hours	Cells deprive of AF Positive material	Cells flooded with AF positive material	Cells showed degranulation
After 24 hours	Marked shrinkage in Nuclear wall no Stainable granule	Neurosecretory material Apparent, cells increased significantly	Neurosecretory materials accumulation, decrase in nuclear diameter.
After 48 hours	No secretory material	Degranulation reached highet hypertrophy	Accumulation of atropy Of nuclear diameter
After 72d ed hours	Marked granulation	Predomination of Stainable material	Degranulation

1) After six hours treatment of distilled water the 'A' cells showed degranulation and so that median neurosecretory Pathway were seen with large stainable material. The nuclei showed significant hypertrophy in the experimental insects where they are larger in diameter (7.45 ± 0.10 µ) than the control (6.65 ± 0.04 µ).Similar results were noted even after twelve hours treatment with distilled water in insects. The nuclei (diameter 7.10 ± 0.10 µ) were also significantly larger than those of controls (diameter 6.65 ± 0.07 µ). The pre-sacrificed insects showed more AF-positive material than controls, median neurosecretory pathway showed comparatively less AF positive material. After twenty four hours, a decrease in the nuclear diameter (6.55 ± 0.13 µ) was noticed in 'A' cells after twenty four hours treatment in the experimental insects. The nuclear diameter did not differ in size markedly with that of controls insects (diameter 6.60 ± 0.13 µ). The AF positive choroid was seen to be accumulating in the cytoplasm of median neurosecretory pathway showed less secretory stainable material. After forty eight hours treatment of distilled water the 'A' cells showed accumulation of AF positive material. The nuclei showed much atrophy and indicated a stage of inactivation and their diameter (5.28 ± 0.07 µ) were smaller than that of controls (6.65 ± 0.06 µ).The cytoplasm was with AF positive colloids indicating inhibition in the release of stored material. The median neurosecretory pathway was filed with AF positive material; 'A' cells still showed sign of degranulation, after seventy two hours treatment with distilled water. The nuclei showed to increase in diameter (5.90 ± 0.07 µ) which differed than that of controls (6.65± 0.03 µ).

A) Effect of temperature on the neurosecretory system:

The insects of same age were selected to observe the effects of different temperatures on the neurosecretory system of *Spodoptera litura* adult moth. The insects were exposed to various experimental temperatures from 10 to 30^{0}C. The insects which were used as controls were kept at room temperature (26-27^{0}C) for two hours.

The insects at 10^{0}C for two hours become dull. The histological preparation showed no activity in their neurosecretory cells. The neurosecretory 'A' cells showed a release of stainable material after 2 hours exposure to 10^{0}C.Most of the cells having more granulated cytoplasm like those seen in the newly hatched adults. Only four to five cells are to be packed with stainable material. The remaining cells are seen with little amount of neurosecretory material with small nuclei. These cells were measured in nuclear diameter about 7.05 ± 0.13 µ while controls diameter was 6.60 ± 0.09 µ. The cells of the normal insets were however, bigger than these cells with large nuclei. The insects kept at 20^{0}C from the normal insect in their size and amount of neurosecretory material in the cell is concerned. The cell measured about in nuclear diameter (0.05± 0.05 µ) than the controls diameter (7.06± 0.09 µ).The neurosecretory 'A' cells of pars intercerebralis of the brain after 2 hours at 30^{0}C temperature showed an increase treatment in stainable material. The insects kept at 30^{0}C also showed marked difference in the cell size measuring (7.26 ± 0.11 µ). The secretory granules could be seen readily in neurosecretory 'A' cells. The nuclei show a significant rise differing from the controls temperature 6.68 ± 0.15 µ.The comparative amount of the temperature the cerebral neurosecretory cells of control and experimental insects is given in table. A conclusion can be drawn that high temperature favors the production of high rate of neurosecretory material while low temperature bring it to a minimum in the moth *Spodoptera litura*.

DISCUSSION

In *Dripus morossus* Pflugfelder (1937) for the first time pointed out that the osmotic process controlled by the neuroendocrine complex of the insect. He has drawn the attention to the accumulation of neurosecretory material in the neurosecretory cells of the brain when insect was dissected. On the contrary, a release of AF positive material is observed in *Spodoptera litura* after injecting the salt solution. The neurosecretory cells show degranulation of secretory material with increase in the nuclear diameter suggesting release of material during dehydration.

Nayar (1957) working in *Iphita limbata*, injected salt water in the insect and found that neurosecretory cells were flooded with suitable

material and release of material was inhibited. Nayar (1960) again in the same insects i.e. *Iphita limbata* found characteristic lumped pattern of secretory material when the insects were fed with salt water. With this treatment the nucleus found to be highly distorted and shrunken, the same condition observed in *Spodoptera litura* when they are injected with salt water. The neurosecretory cells of *Spodoptera litura* were packed with decrease in the size of nuclei showing inhibition of releasing and synthesis phase. Highnam (1962) Highnam *et al*. (1965) observed again that when insects were injected with salt water, there was a rapid release of neurosecretory material from the cells. Wall and Ralhp (1962) in *Balberus giganteus* and Dogra and Ewen (1969) in *Melanoplus sanguinipes* have reported that the larger amount of secretory material was seen along the neurosecretory cells axons when injected the salt water in the insects. Stutinsky (1953) in Blabera fusca and Nayar (1962) in Periplaneta Americana on the contrary, found that dehydration was associated with lack of stainable material. In hydrated conditions the accumulation of secretory cells of the brain was observed by jarial and Scudder (1971) in Cenocorixa, whereas in dehydrated condition insect did not accumulate the neurosecretory material. When salt water injected in the moth *Spodoptera litura* it creates dehydrated conditions, because released of secretory products with increase the nuclear size of 'A' cells of the median pars intercerebralis. While distilled water causes hydration and show decrease in the nuclear size with accumulation of material in the neurosecretory 'A' cells of the brain. Delphin (1965) in *Schistoceraca gergaria* reported the view of antidicuresis staining at active release neurohormone occurs under certain conditions in which one would expect water conservation process to be active. Highnam *et al*. (1965) had observed that *Locusta migratoria* has developed very efficient water reaction reabsorption in rectum for continuous water conservation. Mordue (1971) stated that the amount of water lost in the feces is greater in insects with more active endocrine system. Walls and Ralph (1962) and Goldbard *et al*. (1970) assumed that the brain and the retro cerebral complex probably produce antidiuretic and diuretic factors which responsible for the regulation of in the haemolymph. A careful observation of the median neurosecretory cells, their paths after injecting of NaC1 and distilled water it is noted thoroughly and suggested that their neurosecretory material may be adaptive assistance to these effects.

The changes in the neurosecretory cells of *Spodoptera litura* provoked by different experimental modulated, are of great physiological significance at the ionic and water balance in *Spodoptera litura* are controlled by the neurosecretory cells the brain, which produce the antidiuretic mone, they release and retain in the neurosecretory 'A' cells during dehydration specitivley. Temperature also seems to have some effect the median neurosecretory cells. Highnam (1958) while studying the effect of temperature on neurosecretory cells reported in Mimas tilise that the activity of 'A' type of cells increased when they were subjected to high temperature. In Locusta migratioria (Clarke, 1966) in azara viridula Awasthi (1969) found less stainable material in corpora cardiac when kept at low temperature while stainable material in corpora cardiac, increase at high temperature. When kept the moth, *Spodoptera litura* at 10^0C the neurosecretory activity of the median cells diminishes, at 20^0C the activity in the cells is very low marked by decrease in the nuclear diameter where as at high temperature like 25^0C a very speedy synthesis and release of neurosecretion is observed. In contrast, Abraham (1966) in Dytiscus marginelis reported that there is no difference in the rate of synthesis of neurosecretory material by the medical 'A' cells at different temperature. Tewari and Awasthi (1968) has studied this factor in great details and showed that at low, temperature the synthesis of the neurosecretory Material in the medical 'A' cells in probably inhibited by the destruction of enzyme at low temperature. Banerjee (1969) in Acrida giganta had shown discharge of neurosecretory substance under pressure to high temperature and high amount of cytoplasmic arginine is produced, while less quantity is observed at low temperature. A conclusion can be drawn that high temperature is favorable production of high rate of neurosecretory, material while low temperature either op it completely or bring it to a minimum in this path.

SUMMARY AND CONCLUSIONS

The present study of, *Spodoptera litura* (Fabricius) (Noctuidae: Lepidoptera) commonly known as tobacco caterpillar is a serious polyphagus insect species attacking on a wide variety of food plants, covers the morphology, histology and histochemistry of retrocerebral endocrine complex and histomorphology of ventral nervous system. It deals with the histomorphology and histochemistry of female reproductive system of moth. Moreover the effects of intrinsic and extrinsic factors on ovarian development and effects of induced environmental factors are also studied. The cephalic endocrine system in this moth includes neurosecretory cells in the brain, a pair of corpora cardiac, a pair of corpora allata and a neurohaemeal organ aorta. The brain of this insect is transversely elongated with well developed three lobes, protocerebrum, deutocerebrum and tritocerebrum. The optic lobes are well developed and narrowed at the bases. The suboesophageal ganglion is also present in head and marked the beginning of ventral nerve cord which postersiorly with two thoracic and four abdominal ganglia ventral nervous system. In the caterpillar the general nervous system includes a bed brain, a suboesophageal ganglion in the head capsule three thoracic and eight abdominal ganglia in the ventral house system. There are generally four types of neurosecretory cells A, B, C, and D, recognized in the basis of their staining behavior and cell measurements. 'A' type of neurosecretory cells is restricted only in medial group in the brain of the animal which is purplish AF staining technique. 'B' type of neurosecretory cells are smaller in size, sent in all groups of neurosecretory cells in brain as well in ventral ganglion of the neuroendocrine system. These cells take the deep purple with AF staining technique. 'C' type of cells neurosecretory cells is larger in size, present in tritocerbrum. These cells take the deep red stain as well as the granular inclusions are also stained by AF techniques. 'D' type of neurosecretory cells is present only in cerebral, region and stained dull purple by AF technique. All these types of neurosecretory cells are functionally act types and not the different stages in the secretory. The size and number of neurosecretory cells increase with growth of the larval instar, but 'A' type of secretory cells maintain their number constant throughout development of the insect. The brain increases in size due to multiplication of cells. The brain of caterpillar in earlier instar shows only group of cells whereas lateral groups of neurosecretory develops from IIIrd instar onwards. The increase in the size of the brain, the larval instar may be attributed to the plait of neurosecretory cells, to adjust with the logical changes in the moth. The neurosecretory material is transported to the neurohaemal organs. These axons come out of the as NCC I and NCC II to reach the storage and release to organs. The corpora cardiac and corpora allata are in close tact with the aorta. Carpora allata are larger than corpora cardiac and oval in shape, lie posterior and very close to corpora cardiac. The corpora cardiac contain two types of cells viz. mophobic cells and chromophilic cells. The histochemical methods have shown the presence of proteins and carbohydrates in the neurosecretory cells. The suboesophageal ganglion lies in head capsule and has 'B' and 'C' types of neurosecretory cells, they are elliptical spherical in shape and reacted with AF, bluish green and purple, 'A' and 'D' type of cells are absent. Prothoracic ganglia and meso-metathoracic ganglion as 'B' and 'C' type of neurosecretory cells. They are elliptical and spherical in shape, getting bluish green and purple by AF stain. The abdominal ganglia have 'B' and 'C' type of neurosecretory cells. They are also elliptical and spherical in shape and reacted bluish and purple with AF stain. The paired polytrophic ovaries are present in abdominal cavity; each ovary consists of four polytrophic ovarioles, which open in to the oviduct, to form the common oviduct of ovarioles. Each ovariole is divided into three recognizable basal pedicel, the middle egg tube, which stores eggs and terminal filaments. The egg tube consists of terminal filaments, the vitellarium, where ooctye and trophocytes alternately, the germarium where oogonia are formed germ cells. Spermatheca consists of terminal capsule or storage of sperms after copulation connects with the oviduct. A pair of accessory glands is present with association of female genital organs. Each egg follicle of an ovariole consists of a seven cytes cells and an oocyte which is surrounded by nucleate follicular epithelium in different stages of congenesis. The neurosecretory cells and corpora allata play the role in the egg maturation, A cells of pars cerebralis show cyclical activity with ovarian competent. The effect of temperature on ovarian,

development shows activity of 'A' cells of low temperature the low rate of maturity of eggs in ovarioles ads compare to high temperature the maturity of eggs at higher rate is noticed. 72 hours, injection of potassium chloride (KCL) solution showed 'A' cells marked granulation 'A' cells to conserve when moths are induced by injecting 25 µl of sodium (NaC1) solution showed predomination of stainable against to that when the insects are injected with distilled water the 'A' cells retain degranulation. The neurosecretory cells activity diminishes at very low nature whereas at 10^0C marks the accumulation of material cells. But at 30^0C rapid synthesis and release of secretory material is observed. This is noted by the use in the number of diameter and in the cell size.

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