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EFFECT OF PAPER MILL EFFLUENT ON THE CEPHALIC NEUROSECRETORY AND MIDGUT PROTEASE ACTIVITIES IN THE LAST INSTAR LARVA OF *BRADINOPYGA GEMINATA* (RAMBUR) (ANISOPTERA: LIBELLULIDAE)

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The last instar larvae were treated with sub-lethal concentrations of paper mill effluent (PME) for 5 days. PME inhibits the synthesis of neurohormones in the A-type cells of the medial group of the brain and in the intrinsic neurosecretory cells of the corpora cardiaca. PME also causes histomorphological changes in the corpora allata and suppress protease activity of the midgut. These findings suggest that various metamorphic and intermediary metabolic alterations caused by the PME treatment is modulated by the changes in the synthesis and secretion of the neurohormones of the cephalic neurosecretory complex.

INTRODUCTION

Various industrial effluents contaminated with untreated waste are gradually proving to be a great hazard for terrestrial and aquatic ecosystem. The paper and pulp mill is a wet processing industry, discharging effluents in the surrounding water bodies. The paper mill effluent (PME) is rich in a variety of organic and inorganic material toxic to the environment (KSIBI et al., 2003). Release of PME has resulted in serious threats to various aquatic organisms, including insects (GHOSH & KONAR, 1980; CHANDRASEKARAN et al., 1989). In odonate larvae, the PME arrests moulting (SUBRAMANIAN & VARADARAJ, 1993), alters the biochemical components of the haemolymph (VARADARAJ et al., 1993), disintegrates the egg chorion and terminates embryogenesis (ANDREW et al., 2006). KHAN (1983) found that odonate larvae are characteristic of clean, non-polluted water. BUSS et al., (2002) classified them as a group which can tolerate moderate

level of pollution. Various experimental studies on these larvae have elucidated that the neurohormones of the cephalic neurosecretory complex not only control metamorphosis but most of the metabolic pathways mediate through these neurohormones (TEMBHARE & ANDREW, 1991a, 1991b, 1991c).

The present investigation has been undertaken to study the effect of PME on the secretory activity of various components of the cephalic neurosecretory complex viz. the neurosecretory cells of the brain, the corpora allata, the corpora cardiaca and the ventral (ecdysial) glands and also its effect on midgut protease activity.

MATERIAL AND METHODS

Penultimate instar larvae of *Bradinopyga geminata* (Rambur) were collected from the garden pond of SSESA's Science College, Nagpur, and acclimatized and reared in the laboratory at a photoperiod of 10 h light at 25±5°C. They were fed ad libitum on mosquito larvae. After moulting, the last instar larvae were separated and reared individually in small plastic containers. The 96 h LC 50 value for PME was found to be 40%. Two sub-lethal concentrations of 20% (group-1) and 30% (group-2) of PME were prepared as per the method of SUBRAMANIAN & VARADARAJ (1993).

5-day old last instar larvae were used as control and experiment of each group. Six larvae were sacrified on the second, third and fifth day of treatment. The cephalic neurosecretory complex was dissected, fixed and processed for wax-block preparations. The sections of 6 µm thick were cut and stained with Aldehyde Fuschsin (AF) and Chromalum Haematoxylin Phloxin (CAHP) (PANOV, 1980). While the midgut protease activity was determined by the method of ISHAAYA et al., (1971).

RESULTS

Paper mill effluent has a profound effect on most of the components of the cephalic neurosecretory complex (Tab. I).

Table I

The effect of PME on the synthesis of neurosecretory material of the cephalic neurosecretory complex and midgut protease activity in the larva of *Bradinopyga geminata*

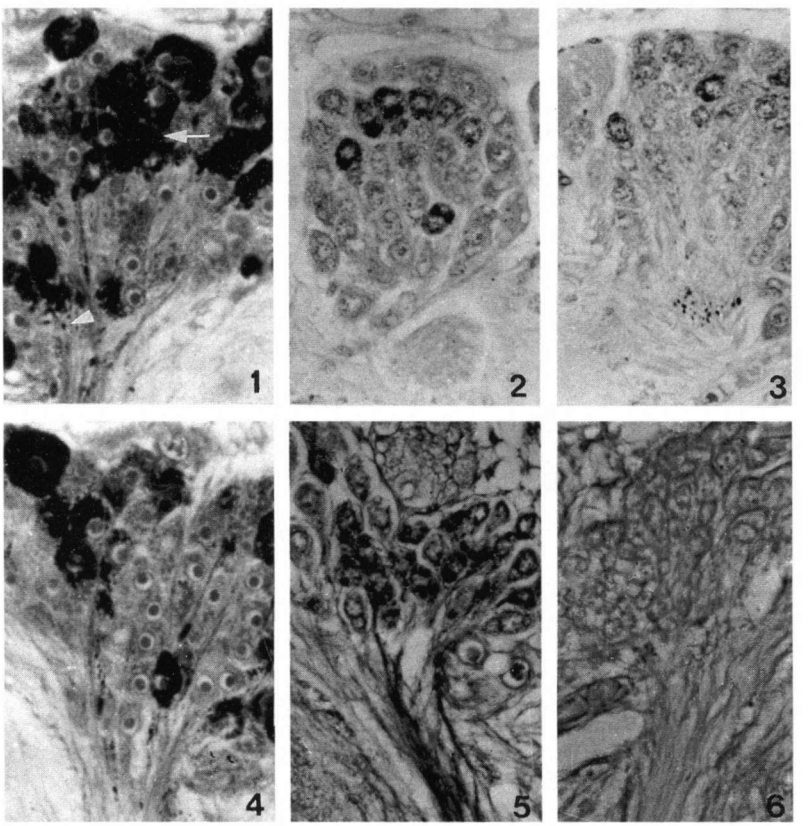
% PEM	days	A-cell		CC		INC		Protease activity– (OD)	
		C	E	C	E	C	E	C	E
Group-1	2	+++	+++	+++	+++	+++	++	0.85±0.02	0.42±0.02
(20%)	3	++	++	+++	++	++	+	0.78±0.06	0.32±0.04
	5	+++	-	++	+	+++	-	0.80±0.05	0.25±0.03
Group-2	2	+++	+	+++	+++	+++	++	0.84±0.02	0.43±0.06
(30%)	3	++	-	+++	+	++	-	0.91±0.05	0.28±0.02
	5	+++	-	++	-	+++	-	0.85±0.06	0.18±0.08

Amount of neurosecretory material: +++ completely filled; – ++ moderately filled; – + poorly filled; – - absent; – CC: corpora cardiaca; – INC: intrinsic neurosecretory cells; – C: control; – E: experimental; – (OD) optical density

NEUROSECRETORY CELLS OF THE BRAIN

There are three types of neurosecretory cells in the brain depending upon their staining affinity and cytomorphological characteristics. The A-cell stain darkly and is pyriform in shape; the B-cell stain with the counter stain and are spherical whereas the C-cells are amphibious in staining affinity and elliptical in shape. Of these, PME predominantly interfered with the secretory activity of the A-cells of the medial group (Figs 1-6).

In control of both the groups, the perikarya of the A-cell is filled with neurosecretory material which is also evident along the axons of the cells indicating



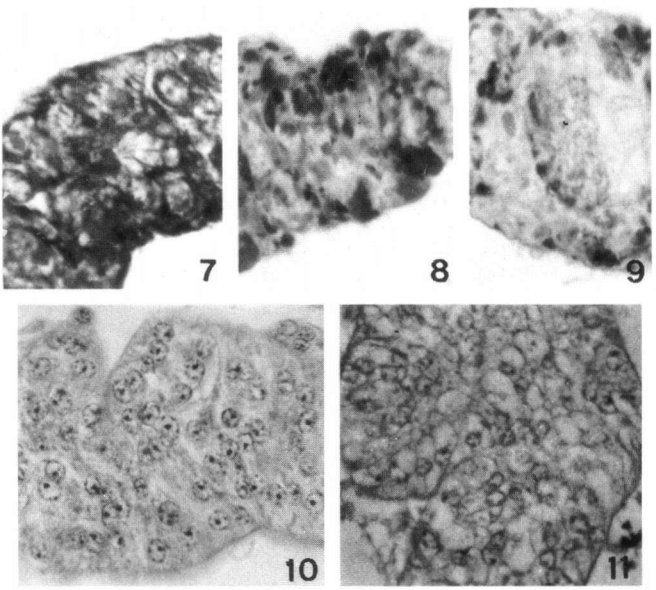
Figs 1-6. Section of brain through the medial group of neurosecretory cells (AF, ×1000): (1) control on first day (5-days old): note the abundance of neurosecretory material (NSM) in the A-cells (arrow); – (2) 20% PME treated larva after 2 days. Note reduction in the synthesis of NSM; – (3) 20% PME treated larva after 5 days exhibiting negligible amount of NSM; – (4) control on third day (8-days old larva) showing synthesis of NSM; – (5) 30% PME treated larva after 2 days showing active release of the axons containing NSM in the axons and pathway; – (6) 30% PME treated larva after 5 days: note empty A-cells without NSM in the perikarya and axons.

active synthesis and release of neurosecretory material (Figs 1, 4). But within two days of PME treatment in group-1 there is a marked decline in the synthesis of neurosecretory material by these cells (Fig. 2), whereas in group-2, there is a sudden release of neurosecretory material and the axons are filled with the neurosecretory material (Fig. 5).

With increase in the concentration of PME and period of exposure, there is a corresponding decrease in the synthesis of neurosecretory material (Figs 2, 5). By the fifth day, the A-cells and their axons are completely devoid of neurosecretory material in both groups of PME treated larvae indicating blockage of its synthesis (Figs 3, 6) (Tab. I).

THE CORPORA CARDIACA

The corpora cardiaca is a neuroendocrine as well as a neurohaemal organ since it contains intrinsic neurosecretory cells, and also stores the neurosecretory material produced by the neurosecretory cells of the brain. The corpora cardiaca of the control is filled with a large amount of neurosecretory material and the perikarya of the intrinsic neurosecretory cell is also loaded with stainable neu-



Figs 7-9. Sections of corpora cardiaca (AF, ×460): (7) control showing abundance of NSM; – (8) 20% PME treated (group-1) larva after 3 days showing decreased amount of NSM; – (9) 30% PME treated (group-2) larva after 5 days showing negligible amount of NSM.
Figs 10-11. Sections of corpora allata (CAHP, ×460): (10) control exhibiting large nuclei and the syncytial condition of the gland; – (11) 30% PME treated larva after 5 days showing cellular condition of the gland with small nucleus and scanty cytoplasm.

rosecretory material indicating active synthesis of neurohormones (Fig. 7). But in PME treated larvae of both groups there is a gradual decrease in the amount of neurosecretory material in the body of corpora cardiaca and intrinsic neurosecretory cells (Fig. 8). By the fifth day, the corpora cardiaca and intrinsic cells contain negligible amount of neurosecretory material (Fig. 9).

THE CORPORA ALLATA

The corpora allata is composed of a large number of small, spherical epithelial cells with a prominent, centrally placed nucleus (4.7±0.4 μm diameter). In the control larva, the corpora allata exhibits syncytial condition since the cell walls are indistinct (Fig. 10). In larvae treated with PME, there is a marked increase in the cell wall formation along with reduction in the size of the nucleus (3.85±0.34 μm diameter) and by the fifth day in both the groups of treatment, the histomorphology of corpora allata exhibits a complete cellular structure (Fig. 11).

THE VENTRAL GLANDS

The ventral glands are composed of polygonal epithelial cells with a large nucleus and nongranular cytoplasm. Histomorphological changes are not observed in the glands of control and treated larvae of both the groups.

MIDGUT PROTEASE ACTIVITY

The midgut protease activity in control larvae measures 0.85±0.2 OD at 280 nm. By the second day the PME causes a significant decline in the midgut protease activity and by the fifth day of treatment, it drops down to 0.25± 0.09 and 0.18±0.08 OD at 280 nm in the first and second groups, respectively (Tab. I).

DISCUSSION

In Odonata, the neurosecretory cells of the brain exhibit a cyclical secretory activity during the development of the last instar larva and the neurohormones secreted by the medial neurosecretory cells are predominantly involved in the metamorphosis of the larva into an adult dragonfly (SCHALLER, 1959; SCHALLER & CHARLET, 1970; TEMBHARE & ANDREW, 1994). In larva of the dragonfly *Tramea virginia*, the A-cells secrete altatotrophic neurohormone to stimulate the corpora allata and prothoracicotropic neurohormone to stimulate the ventral glands (TEMBHARE & ANDREW, 1994). In *B. geminata,* A-cells exhibit active synthesis and secretion of neurohormones but in PME treated larvae, the cells gradually stop their secretory activity. In odonates, the active corpora allata exhibit syncytial condition, whereas inactive corpora allata are cellular with dis-

tinct cell walls (SCHALLER & CHARLET, 1970; TEMBHARE & ANDREW, 1994). After PME treatment, the corpora allata become inactive and change from syncytial to cellular. The present observations on the A-cells and corpora allata indicate that PME probably causes inhibition of synthesis and secretion of allatotrophic neurohormone by the A-cells, which in turn results in the regression of the corpora allata.

The corpora cardiaca is devoid of stainable neurosecretory material in PME treated larva because the corpora cardiaca acts as a neurohaemal organ and stores the neurosecretory material produced by the neurosecretory cells of the brain (TEMBHARE & THAKARE, 1976; ANDREW, 1989; TEMBHARE & ANDREW, 1994). The cessation of secretory activity by the A-cells of the brain's neurosecretory cells results in the depletion of the stored neurosecretory material in the corpora cardiaca. The PME also causes depletion in the secretary activity of the intrinsic neurosecretory cells of the corpora cardiaca. VARADARAJ et al., (1993) reported that PME causes a significant decline in the haemolymph sugar level. This is probably due to the non-production of neurohormones by the intrinsic cells, which control haemolymph sugar level in the dragonfly larva (TEMBHARE & ANDREW, 1991c).

The ventral glands are active at the beginning and end of the inter-moult period in the last instar larva of Odonata (SCHALLER & HOFFMANN, 1973; TEMBHARE & ANDREW, 1994). The present study did not observe any histological changes in the control and experimental larvae probably because these experiments were conducted in the mid-intermoult period (5-10 days) when the ventral gland is in an inactive state.

The protease activity of the midgut is also affected by the PME treatment since protease activity is controlled by the secretion of the A-cells of the medial neurosecretory cells of the brain (TEMBHARE & ANDREW, 1991a) and PME has a direct inhibitory effect on these cells as observed in the present study.

In larvae, PME treatment causes major changes in the metamorphic (SUBRAMANIAN & VARADARAJ, 1993) and intermediary metabolic processes (VARADARAJ et al., 1993). The present report indicates that these changes are manifested due to the profound negative effect of the PME on the secretory activity of the cephalic neurosecretory complex.

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