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Precocene-induced Light and Electron Microscopic Changes in the Pars Intercerebralis Neurosecretory Cells of the Semilooper Caterpillar *Achoea janata* Linn.

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Light microscopic studies of the A-type pars intercerebralis neurosecretory cells of the brain (PINSC) in the 5th instar caterpillars of *Achoea janata* treated with Precocene-II show significant reduction in their activity as evidenced by a lower value of neurosecretory index, diameters of the cell and nucleus in comparison with acetone-treated controls. Ultra-structural observations such as a reduction in the number of mitochondria, elementary secretory granules, abundance of lysosomes and poor development of golgi complex which are suggestive of diminished activity of the secretory cells also confirm the light microscopic findings. It is suggested that Precocene-II inhibits the activity of A-type PINSC and also induces destruction and resorption of the secretory granules.

Key Words: Precocene, *Achoea janata*, **Neurosecretory cells, Ultrastructure**

Introduction

Recent reviews have dealt with the structural details of different types of neurosecretory cells in insects (Novak 1975, Rowell 1976, Raabe 1983). Electron-microscopic investigations have provided a new criterion for interpretation of data on neurosecretion. Raabe (1983) is of the opinion that multiplicity of neurosecretory cell types is not surprising in view of the number of neurohormones identified. She designates neurosecretory cells by their location and basic types as A, B, or Cr; additional categories within each basic type are designated by numbers (e.g. A1, A2, A3, B1, B2, Cr1, Cr2, etc.). Awasthi and Singh (1982) conducted a detailed light microscopic study of the neurosecretory cells in the brain of the larva, pupa and adult of the caster semilooper *Achoea janata*. They found A1, A2, A4 and B1 types of neurosecretory cells in the medical region of the brain.

Since the discovery of the biological activity of precocenes by Bowers (1976), effects of these chromene compounds on the corpora allata (CA) of insects have been studied (Pratt & Bowers 1977, Unnithan et al. 1977, Schooneveld 1979, Feyereisen et al. 1981). Unnithan et al. (1978) have suggested that the effect of precocene-II on CA might be mediated through A-type neurosecretory cells of the brain in *Oncopeltus fasciatus*. Soderlund et al. (1981) are of the opinion that CA of species which are not sensitive to precocene *in vitro* are intrinsically sensitive and they further stress the need for looking outside the CA for mechanisms of precocene sensitivity.

An attempt has been made here to study changes brought about by precocene-II on the A-type neurosecretory cells in the pars intercerebralis region of brain in the last instar larvae of *Achoea janata* as studied with light and electron microscope.

Materials and Methods

Caterpillars were selected from the stock colony maintained in laboratory on castor (*Ricinus communis*) leaves at 29±3°C, LD 12:12 and r.h. 90±3. Last instar (5th) larvae were separated on the day of emergence and maintained on fresh castor leaves. Healthy caterpillars of approximately uniform size were used for the experiments.

Precocene-II (6,7-dimethoxy 2,2-dimethyl Chromene); a gift from Prof. W S Bowers, Geneva, USA) was dissolved in acetone; the finalconcentration being 10 μg/μl. Effective dose in these caterpillars were determined to be 50 μg (Mohanakumar & Muraleedharan 1985). Five μl acetone containing 50 μg of precocene-II was topically applied on the dorsal side of mildly anaesthetised larva, using a calibrated microcapillary. Controls were treated similarly with acetone. After evaporation of acetone, caterpillars were allowed to feed on fresh castor leaves. As reported in our earlier publication (Mohanakumar & Muraleedharan 1985), maximum activity was noticed in the A-type PINSC from 4 p.m. to 6 p.m. and hence precocene was applied at 6 a.m. and the animals were sacrificed after 10 hr so that it coincided with the beginning of the period of maximum neurosecretory activity. Observations reported here are based on the results obtained from 10 experimental and 6 control insects for light microscopy and 5 experimental and 3 controls for electron microscopy.

Treated caterpillars were slightly ether-anaesthetised and the brains were dissected out in insect Ringer (Ephrussi & Beadle 1936). Some of the whole brains were stained with Paraldehyde fuchsin (Cameron & Steele 1959) or Performic acid-Victoria blue (Dogra & Tandon 1964). Either paraffin sections (6 μ) or Spurr low viscocity resin-embedded thin sections (1μ) were stained with Chrome-haematoxylin-Ploxine (Gomori 1941). Neurosecretory index was calculated according to Jalaja and Prabhu (1977). All measurements were made with a calibrated ocular micrometer and the significance of the data was calculated using Student's "t" test.

For electron microscopic investigations, brains from experimental and control insects were fixed in *situ* with a few drops of phosphate buffered saline containing 2.5% gluteraldehyde and subsequently fixed in fresh amounts of the same fixative for 3 hr at 4°C, as described by Unnithan et al. (1971) and Tembhare (1980) with minor modifications. After repeated buffer wash (*p*H 7.2), tissues were post-fixed in 1% buffered O_sO_4 for 1 hr at 4°C, then subjected to rapid dehydration in acetone series and embedded in Spurr resin (EMscope, England). Semi-thin sections (1μ) were stained with Azure-II-methylene blue (Pearse 1964) and used for orientation purpose. Ultra-thin sections were cut on LKB-Nova microtome with glass knives. Sections were stained with uranyl acetate and lead citrate and examined with a Carl Zeiss 109S electron microscope at an accelerating voltage of 60 Kv.

Results

Light Microscopy

Neurosecretory index as well as the secretory content in the A-cells of pars intercerebralis of precocene-treated larvae is much lower than that noticed in the acetone-treated control as is evident from table 1 and figures 1a to 1d. Statistical analysis of the data showed that the difference was significant ($P< 0.05$). Differences between the cell and nuclear diameters in A-cells in the treated individuals and those of the control are also statistically significant ($P<0.05$).

Electron Microscopy

Electron micrographs of the A-cells in the control insects reveal the presence of elementary neurosecretory granules in the axonal region (figure 2a) suggesting rapid release of neurosecretion from these cells while this is not to be seen in the precocene-treated individuals. Abundance of mitochondria with well-defined cristae noticeable in the A-cells of control insects (figure 2a) are lacking in the precocene-treated animals (figure 2c). However, chromatin material in the A-cells of both the treated and control insects did not show any significant difference (figures 2a & 2c). The number of neurosecretory granules noticed in the A-cells of treated individuals are significantly lower (figure 3d) than that observed in the control animals (figures 3a & 3b) even though the granules of treated animals were more electron-dense

Table *Effect of Precocene-II on the A-type pars intercerebralis neurosecretory cells in the 5th instar larvae of* A. janata

Time after Precocene treatment	Neurosecretory index	Cell diameter (in μ)	Nuclear diameter (in μ)
10 hr-EXP (50 μg)	16.00±2.4	23.63±0.7	9.3±0.5
10 hr-CON	22.75±1.3	25.28±0.9	10.1±0.3

Mean values of experimental and control of each column are significantly different ($P>0.05$)

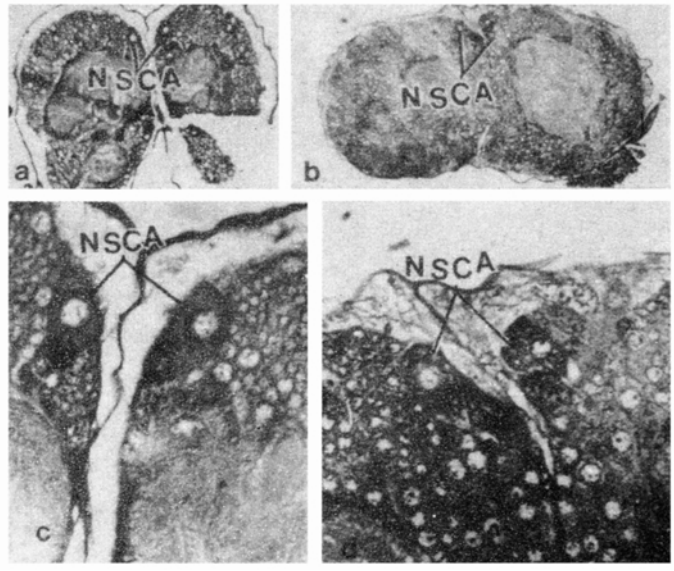

Figure 1 a-d Photomicrographs of the brain showing A-type PINSC of the brain in the 5th instar larvae of *A. janata*. **a,** A-cells of the acetone-treated control (PAF) (× 67); **b,** A-cells of the precocene-treated (PAF) (× 67); **c,** A-cells of the acetone-treated control (CHP) (× 267); **d,** A-cells of the precocene-treated (CHP) (× 267)

(figures 2b & 3c). The golgi complex in the A-cells of the control were well-equipped with stacks of smooth membranes and vesicles (figure 3a) indicating their involvement in the formation of the neurosecretory granules while these features were less evident in the cells of the precocene-treated individuals. An abundance of lysosome and the ill-defined nature of granular endoplasmic reticulum (figure 3d) suggested the rapid degradation and lower rate of synthesis of neurosecretory material in the treated larvae.

Discussion

Raabe (1982) opines that it is difficult to interpret the quantitative variations in the neurosecretory products stored in the somata—a heavily loaded cell can be in a period of intense secretory activity accompanied by hormone release, but it may also be in a storage phase. Simultaneous study of variations in nuclear size and neurosecretory material or the product density of the perikarya gives evidence to support this view. Present investigations show that compared to control, topical application of Precocene-II brings down the activity of A-type PINSC significantly as evidenced by the reduction of the neurosecretory index and the diameter of the cell and nucleus. Unnithan et al. (1978) have also demonstrated that in *O. fasciatus* precocene treatment results in a significant reduction in the amount of stainable material in the A-cells of pars intercerebralis and within 24 hr of JH treatment of precocene-treated individuals, a significant increase in the PF stainable material was again noticed, concluding therefrom that the reduction is caused by an inhibition in the synthetic activity of the cells. Martinez Carrau et al. (1981) observed an accumulation of more secretory material in the precocene-treated *O. fasciatus* and *Lygaeus militaris* and suggest that to check whether this is due to a high rate of synthesis of the secretory material, electron-microscopic investigations should be performed.

Present studies establish that precocene-treatment brings down the activity of A-cells of *A. janata* to a significant level. The presence of elementary neurosecretory granules at the axonal region of the control animals and their absence in the precocene-treated individuals indicate that synthesis of neurosecretory material is inhibited by precocene treatment. Further reduction in the number of well-defined mitochondria in the treated insects suggests the comparatively inactive nature of the cells of the treated individuals. The smaller number of the secretory granules and their dense nature in the A-cells of the experimental individuals indicate a reduced rate of synthesis and release of the secretory material. The nature of ER and golgi complex in the A-cells of the treated individuals also indicates a reduced activity of A-cells in the experimental insects. Schooneveld (1974) believes that the difference in the density of the elementary neurosecretory granules in the protocerebral neurosecretory cells of the Colorado potato beetle

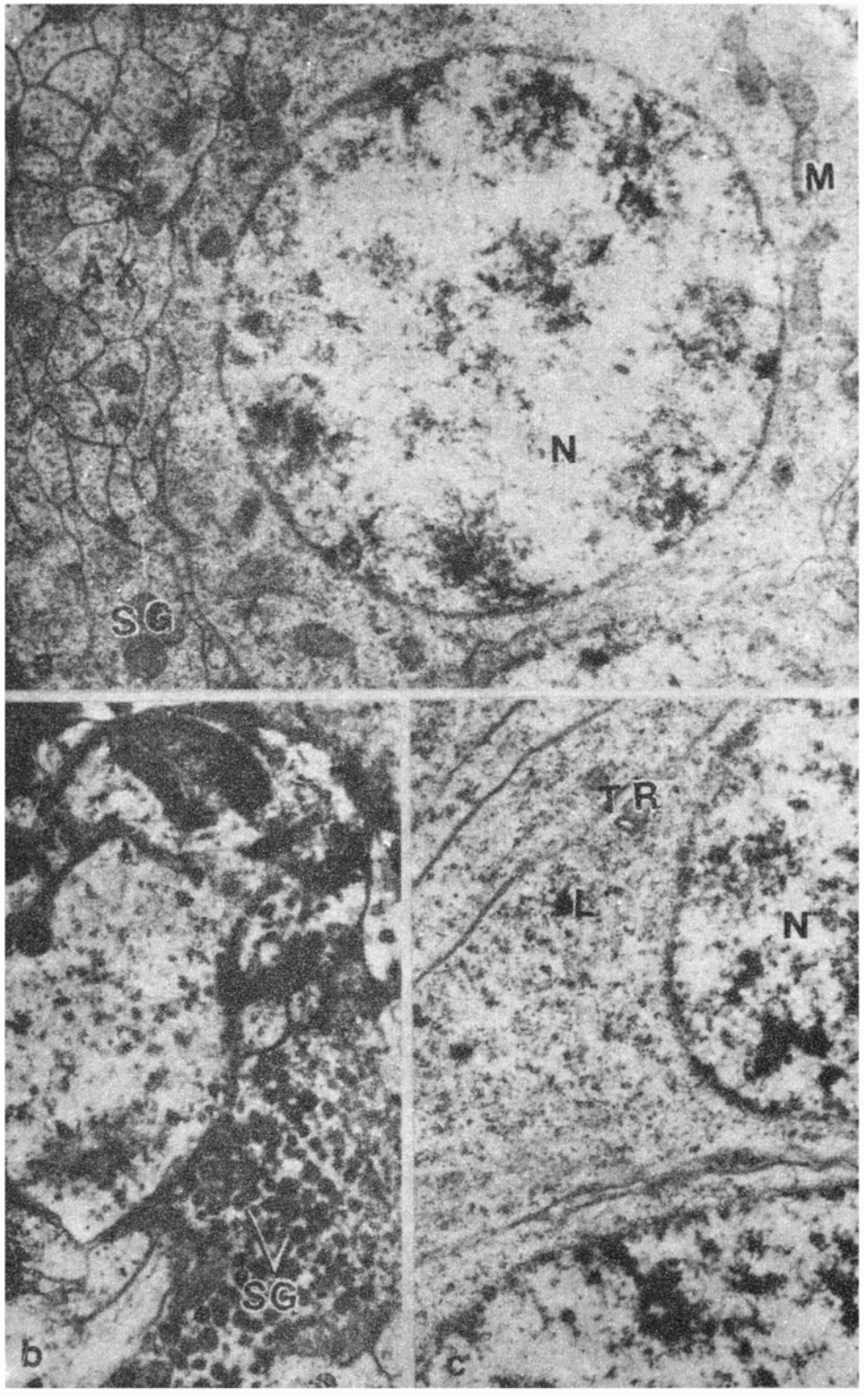

Figure 2 a–c. Electron micrographs showing different portions of A-type PINSC of the 5th instar larva of *A. janata*. **a,** Nucleus and axonal region of the A-type cell of acetone-treated control (×13000); **b,** Portion of A-type cell of the Precocene-treated larva showing electron-dense secretory granules (×20000); **c,** Nuclei of A-type cell in the precocene-treated larva (×15000)

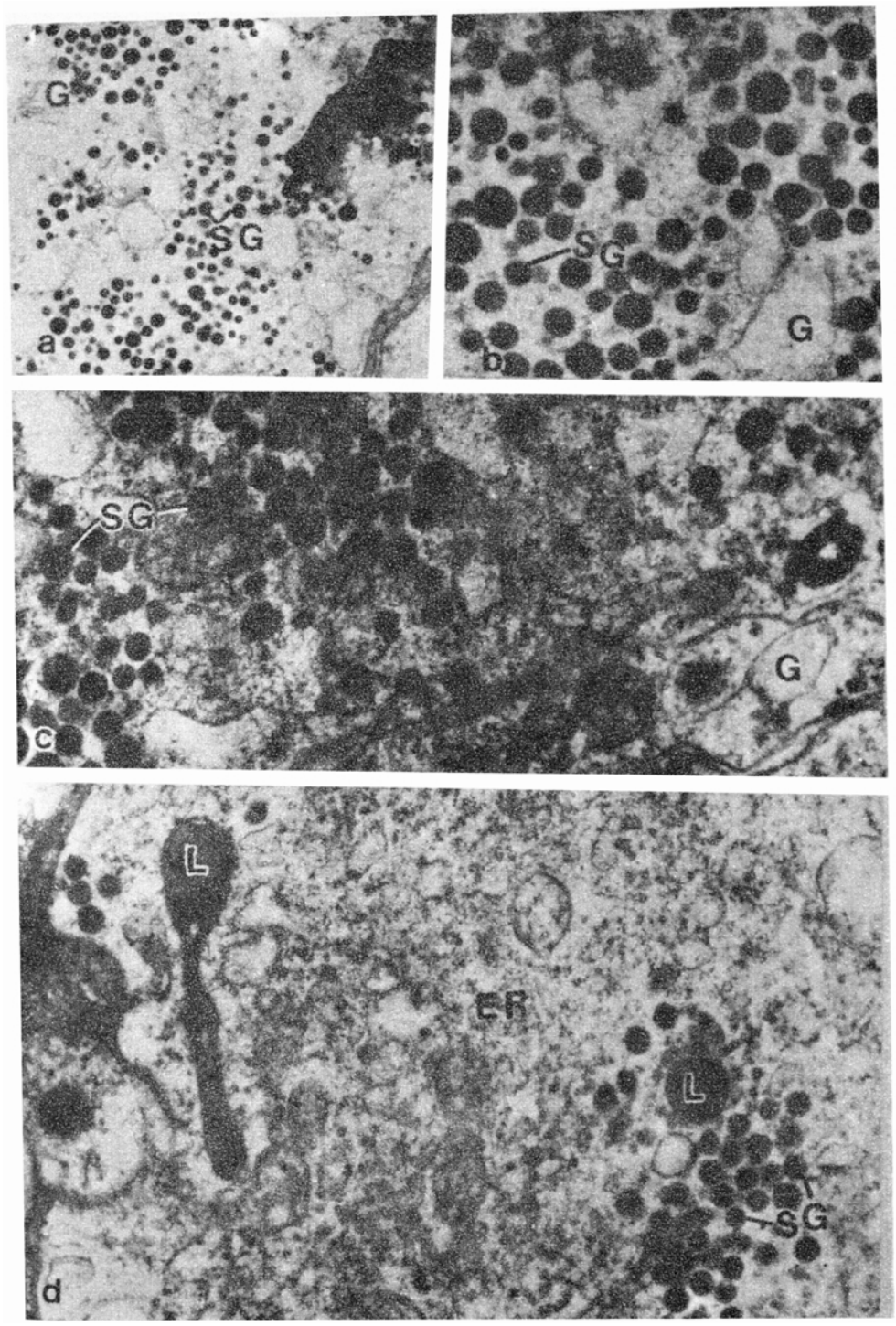

Figure 3 a–d. Electron micrographs showing different parts of A-type PINSC of instar larva of *A janata*. **a,** Cytoplasmic region showing secretory granules in acetone-treated control (×5318); **b,** Magnified portion of the Cytoplasmic region showing abundance of secretory granules in acetone-treated control (×21272); **c,** Cytoplasmic region of the A-cell showing secretory granules in precocene-treated larva (×21272); **d,** Cytoplasmic portion of A-type cell showing abundance of lysosome and the nature of endoplasmic reticulum in precocene-treated larva (×24820)

NSCA-A type Neurosecretory cell; N-Nucleus; M-Mitochondrion; SG-Elementary secretory granules; TR-Trachea; L-Lysosome; G-Golgi complex; ER-Endoplasmic reticulum

Leptinotarsa decemlineata indicates different phases of the synthetic cycle. In the light of available literature Raabe (1983) explains the mechanism of secretion as initiated in the rough ER and continued in the golgi apparatus. Subsequently, this material is gradually condensed so that electron dense and membrane-bound granules are budded off from the extremities of golgi saccules. She is also of the opinion that the lysosomes or multivesicular bodies are involved in the destruction of secretory granules. So the ill-defined nature of ER and abundance of lysosomes and multivesicular bodies as evident in figure 3 in the present studies indicate that the A-cells of precocene-treated insects are less active and destroy the secretory granules to a large extent.

It may be suggested that in the 5th instar caterpillars of *A. janata,* Precocene-II brings down the neurosecretory activity in A-type PINSC to a significant level. Further, treatment of this anti-JH induces destruction or resorption of the secretory material.

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