African Journal of Biotechnology Vol. 10(19), pp. 3784-3789, 9 May, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1818
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Ultrastructural description of the corpora allata of *Pimpla turionellae L.* (Hymenoptera: Ichneumonidae)

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Accepted 9 March, 2011

The corpora allata, are endocrine glands which produced juvenile hormone. In this study, corpora allata of adult females of *Pimpla turionellae* were examined ultrastructurally by using the transmission electron microscopy. The gland is surrounded with thick fibrous capsule that penetrates into the gland as a stromatal ramification. The ultrastructure of gland cells had the same characteristic of protein-secreting cells. These cells had oval shaped nuclei, numerous granules, lysosomes, mitochondria and vacuoles. There are granules of electron-dense, electron-modarate and electron-lucent. These granules are actually membrane-limited secretory vesicles which are filled with the proteinaceous hormone produced by the gland cells. Microtubules and mitochondria were observed in the cytoplasm of axons of neurosecretory cells found among the gland cells.

Key words: Hymenoptera, *Pimpla turionellae*, corpora allata, ultrastructure, transmission electron microscopy.

INTRODUCTION

The cerebral neuroendocrine system of insects contains the brain and retrocerebral complex (Raina and Murph, 2008). The retrocerebral complex consists of a pair of each of corpora cardiaca and corpora allata (CA). The CA are known to be engaged in the release of hormonally active materials (Tombes and Smith, 2005). Juvenile hormone (JH), which is synthesized by the CA and secreted into the hemolymph, has an important role in the insect metamorphosis (Davey, 2000). Bonetti et al. (2006), pointed out that JH III plays an important role in the regulation of the metamorphosis, caste determination and age in bees. Bradley and Edwards (1979) reported that the ultrastructure of CA in the house cricket, *Acheta domesticus* is relatively uniform with one cell type predominating. Typical CA cells contain large nucleoli, golgi complexes, numerous mitochondria and occa-sionally microtubules.

In addition, the CA in insects are known to be involved in metabolic activities (Weaver and Edwards, 1990; King et al., 2005; Tombes and Smith, 2005). The neurosecre-tory material which is stainable with paraldehyde fuchsin positively was observed in the cytoplasm of CA cells of some insect species (Fletcher, 1969; Hoffmann, 1970; Awasthi, 1972; Singh and Narain, 1980; Khan et al., 1984). Although some authors demon-strated the presence of the neurosecretory material in these cells, only a few of them provided details about the functional significance of the neurosecretory material in the CA (Mordue, 1967; Karaçali and Geldiay, 1980; Sedlak et al., 1983; Deveci, 1998). Other studies showed that CA also has correlation with egg maturation (Toyoda et al., 1999; Huerta and Martinez, 2008).

As seen earlier, the relationship between the neuro-endocrine system of the insects belonging to different orders and the reproductive activities were studied and various results were obtained. The head endocrine system of *Pimpla turionellae* has been studied histo-logically and morphologically. The corpora cardiaca and the CA are present in the part where oeso-phagus enters the brain. The CA are paired, almost spherical bodies which lie on each side, ventro-posterior to the corpora cardiaca (Özlük, 1991). Also, the correlation between the brain neurosecretory system and the reproduction was investigated (Özlük, 1993). Although *P. turionellae* is one of the important parasitic hymenoptera species that are used extensively in biological control as agent for permanent suppression of pests, there is no study on the

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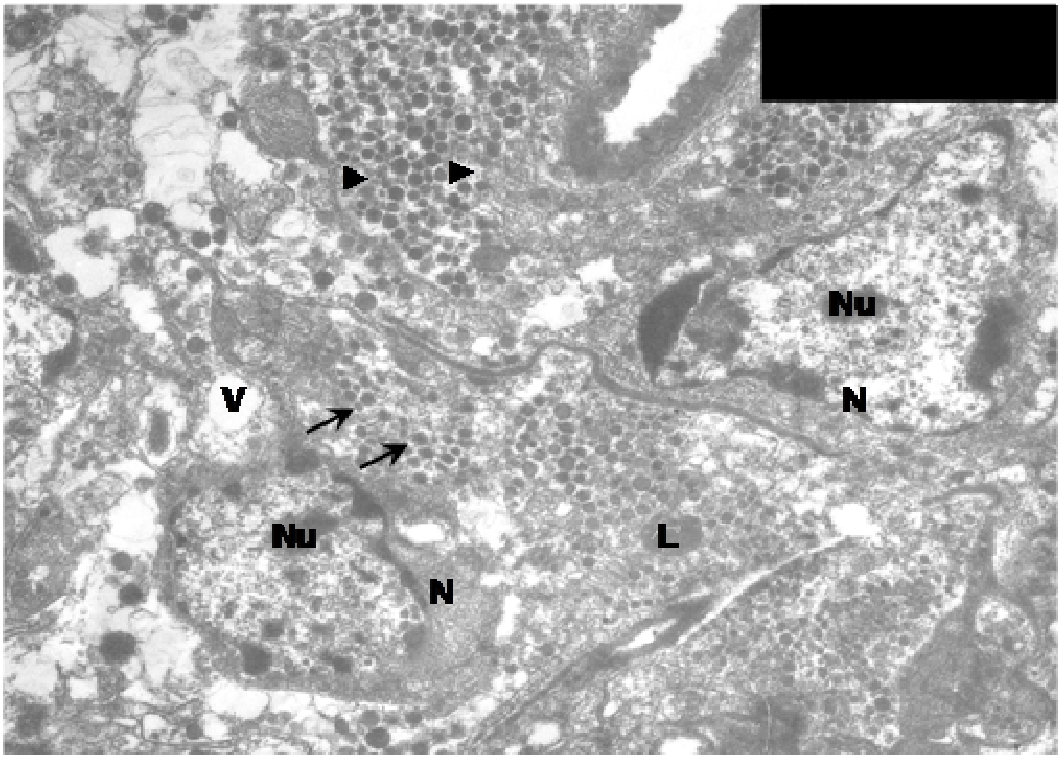

Figure 1. Cells of CA in *P. turionellae.* nucleus (N), nucleolus (Nu), electron dense granules (►), electron moderate granules (→), lysosome (L), vacuole (V); 15.000x.

ultrastructural features of CA. Thus, the present study aimed to investigate the ultrastructural properties of CA cells of this species.

MATERIALS AND METHODS

Experimental ınsect

Adult female individuals of *P. turionellae* (n = 10) were reared under laboratory conditions between years 2003 and 2005 at the Insect Culture Laboratoary in Department of Biology, Faculty of Science, Ankara University, Turkey. The raising of the stock culture was needed to be provided with the greater wax moth, *Galleria mellonella*, reared in the semi-synthetic diet (Bronskill, 1961). The culture of the present insect *P. turionellae* was kept at temperature of 25 ± 2°C and relative humidity of 75 ± 5% under 12:12 (L:D) photoperiod. The insects were fed on cotton pieces soaked in 50% honey solution. Each insect individual was given a pupa of *G. mellonella* every two days in order to satisfy their host hemolymph needs.

Transmission electron microscopy

Insects were anaesthetised in CO_2 then killed and head capsules were immersed in 2.5% glutaraldehyde buffered to pH 7.4 with 0.1 M sodium phosphate, and kept cold for 2 h. After rinsing several times in cold sodium phosphate buffer, the tissues were postfixed in 1% osmium tetroxide solution for 2 h. Fixed tissues were dehydrated in a series of graded ethanol, placed into propylene oxide and embedded in araldite (Hayat, 1981; Mrdakoviæ et al., 2005). Ultrathin sections (60 µm thick) were taken by Ultramicrotom (REICHERT 700141). Five sections from each brain samples were held on grids, and then stained with uranyl acetate and lead. The sections were examined by transmission electron microscopy (TEM) (JEOL 100CX II) at 80 kV.

RESULTS

In the present study, the CA cells of *P. turionellae* had typical secretion cell-like organelles and cytoplasm (Figure 1). Nuclei of cells had generally oval shape and homogeneous or peripherally located chro-matin material. Nucleoli were seen as a distinctive mark in the karyoplasms. As seen in Figure 1, the cells of CA had nuclei and the cytoplasm was filled with numerous granules of different sizes. The granules of these endocrine cells of CA had electron dense or electron moderate spheres bound by a unit membrane. Also, lysosomes and vacuoles were observed in the cytoplasm.

Other sections of CA showed that cytoplasm of cells had numerous electron dense endocrine granules (Figure 2). As shown in the electromicrograph, these granules were homogenously placed in the cytoplasm. These endocrine cells are located either in the middle of the gland or near the capsule. Some of the endocrine cells had oval shaped nuclei. Also, stromal ramifications were seen in the gland area (Figure 2).

The nuclei of the cells are enormous with chromatin

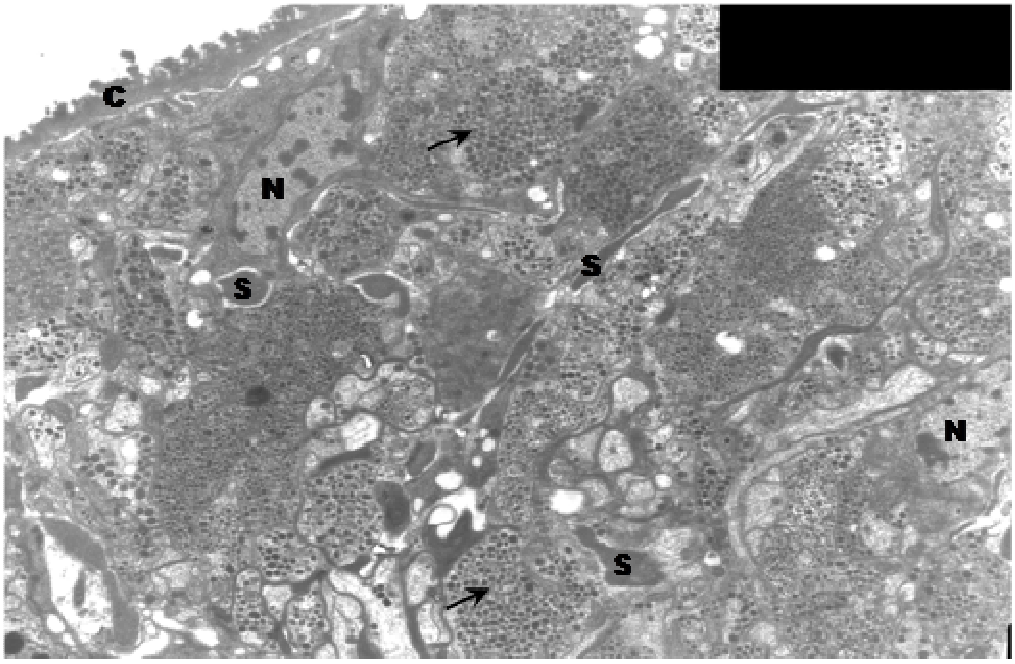

Figure 2. The cells full of granules. Nucleus (N), electron dense granules (→), stromal ramification (S), capsule (C); 10.440x.

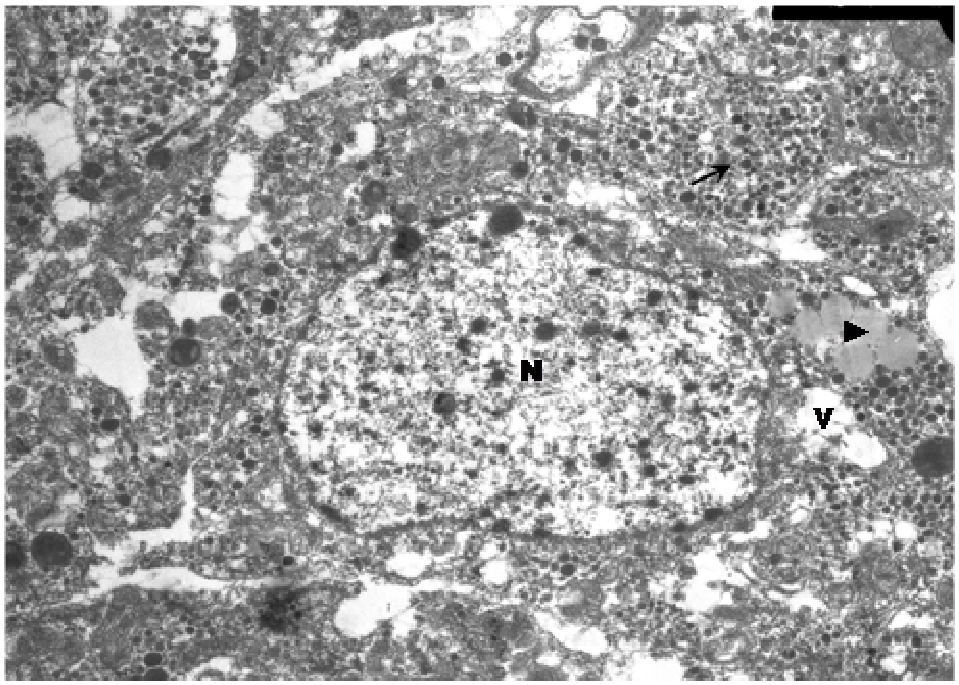

Figure 3. Large nucleus of neurosecretory cell in CA. Nucleus (N), electron lucent granules (►), electron dense granules (→), vacuole (V); 12.000x.

material homogenously dispersed (Figure 3). The cytoplasm contained limited numbers of organelles but numerous secretion granules. Some of the granules of the cells had electron dense materials and the others which have electron-lucent granules were discharged of their materials. Due to secretion, CA cells of *P. turionella* had moderate and electron dense secretory granules in cytoplasm of endocrine cells. The axonal cytoplasms of

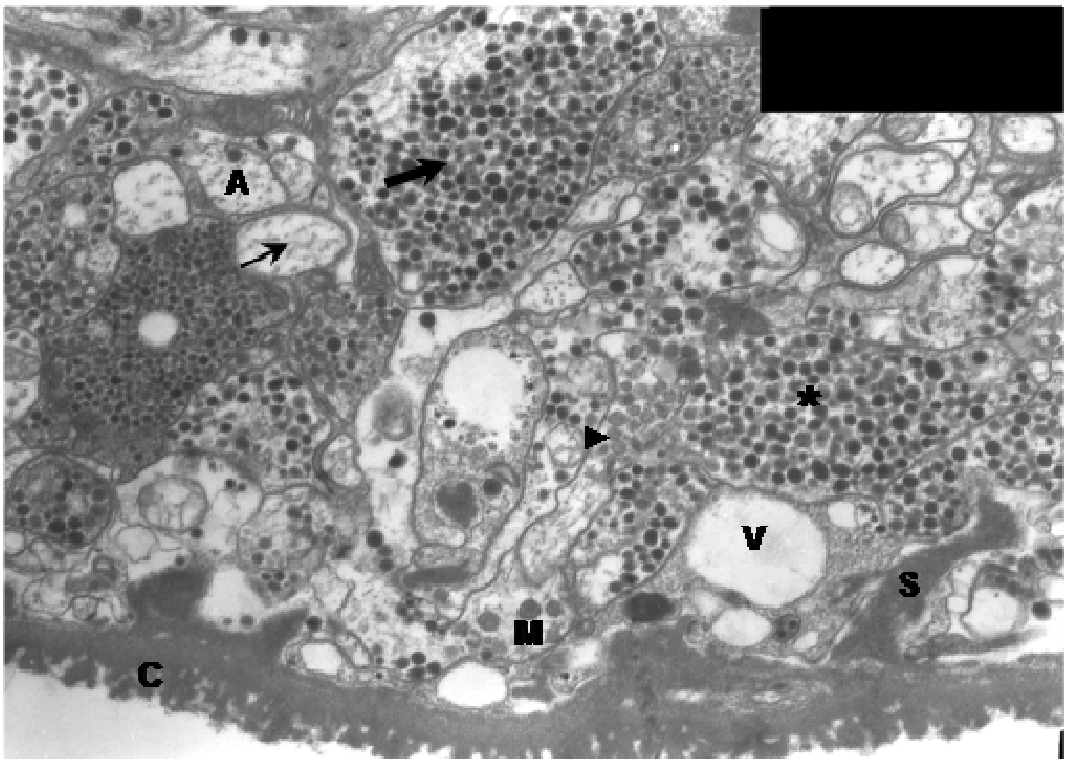

Figure 4. Secretion cells and axons of CA. Mitochondria (M), axon (A), microtubules (→), electron dense granules ( ), moderate electron granules (►), capsule (C), stromal ramification (S), granules (*); 15.000x.

CA had several mitochondria and microtubules (Figure 4). Electromicrograph showed that CA cells contained numerous granules (Figure 5). Mitochondria were observed in the endocrine cells. The gland was surrounded by a capsule of dense connective tissue which appeared as thick and contained fibrous material. This capsule was dispersed as a stromal ramification into the gland.

DISCUSSION

Bonetti et al. (2006) reported that the CA cells of queens and workers of *Mellipona quadrifasciata* had large nuclei with dispersed chromatin. Queen, but not workers', CA cells contained electron dense secretory bodies that were absent in worker cells. Similar characters such as large nuclei and dispersed chromatin, like in queen's electron dense granules were observed in CA cells of the present experimental insect, *P. turionellae* in transmission electron microscopy. The CA of *P. turionellae* were covered with thick connective tissue having fibrous materials. The same properties of CA were seen in house cricket of *Acheta domesticus* (Bradley and Edwards, 1979) and *Carausius morosus* (Smith and Smith, 1966). Cytoplasm of CA cells of *P. turionellae* was filled by round-shaped secretion granules. Similar result was obtained by Rankin et al. (1998) because the CA cells of earwig (*Euborellia annulipes)* are full of unique shape secretion granules. The secretion granules of CA were electron dense in both insects. In the present study, the CA cells of *P. turionellae* had poor organelles such as mitochondria, Golgi complex, rough endoplasmic reticulum, whereas the other insects had more organelles types such as Golgi complex, smooth endoplasmic reticulum and mito-chondria. Neurosecretion cells of *P. turionellae* had large and oval nuclei. This characteristic feature was similar to those reported in the literature for other insects (Smith and Smith, 1966; Bradley and Edwards 1979; Yin and Chippendale, 1979). The axons of endocrine cells in the gland were not covered with myelin but they included microtubules and mitochondria. Similar characteristic properties of axons were seen in other insects such as stable fly (*Stomoxys calcitrans*) and tsetse fly (*Glossina morsitans*) (Meola et al., 1999). Kou et al. (2005) observed very large numbers of mitochondria, abundant whorled smooth endoplasmic reticulum, irregularly shaped nuclei, Golgi bodies and free ribosomes in the CA cells of adult females of *Leucania ioreyi*; whereas, the CA cells of the present insect *P. turionellae* had a few mitochondria, Golgi bodies, rough endoplasmic reticulum and oval shaped nuclei.

In conclusion, the present study extends the knowledge of the cell morphology of the corpora allata in *P.*

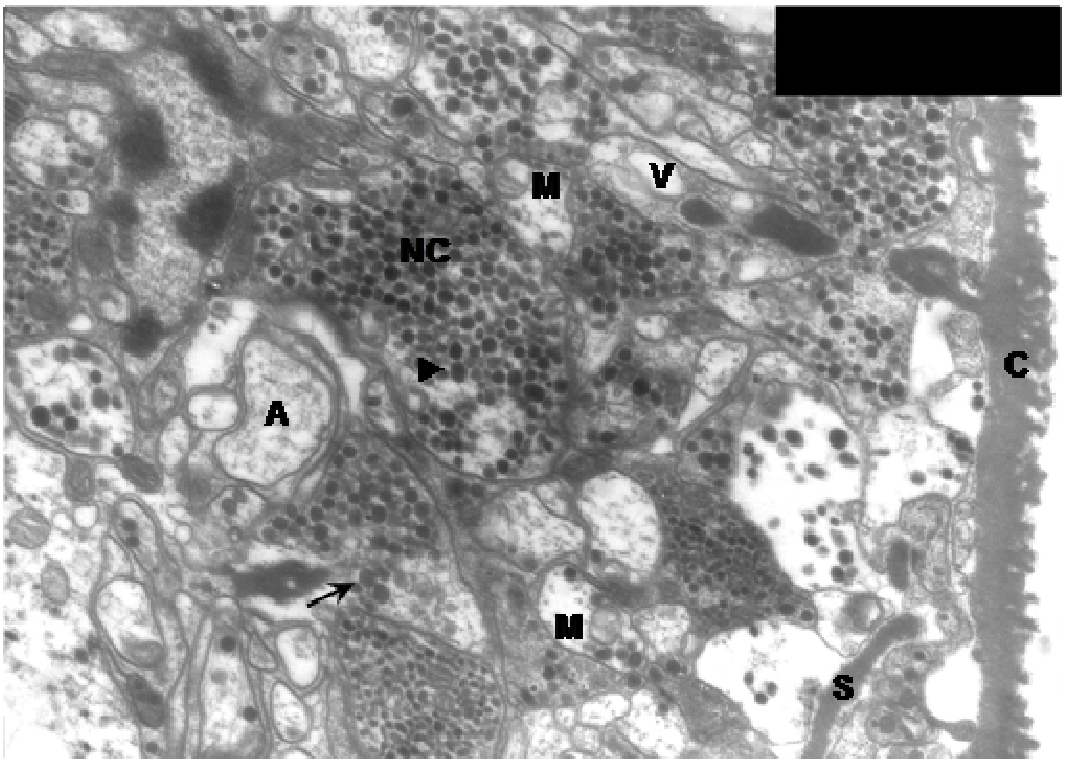

Figure 5. Secretion cells of CA in *P. turionellae*. Vacuole (V), axon (A), mitochondria (M), electron moderate granules (→), capsule (C), stromal ramification (S), electron dense granules (►), nuerosecretion cells (NC); 18.000x.

turionellae to the ultrastructural level. The CA cells had oval shape nuclei, numerous neurosecretory granules but rarely had lysosome, mitochondria and vacuoles. We recommended that ultrastructure of CA in *P. turionellae* should be studied in different physiologic conditions.

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