Ultrastructural Aspects of Corpora Cardiaca in *Pimpla Turionellae* L. (Hymenoptera: Ichneumonidae)

Pimpla Turionellae L. Corpora Cardiaca'nın Ultrayapısal Yönleri (Hymenoptera: Ichneumonidae)

Research Article

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ABSTRACT

Pimpla turionellae has been known as an important parasitic hymenoptera species used in biological control for continuous suppression of pests. As an endocrine center, Corpora cardiaca are a pair of neuroglandular bodies that secrete neurohormones in endoparasitic *P. turionellae* were observed by transmission electron microscope. Brain samples were prepared and examined under transmission electron microscope. Cells of these glands were filled with generally round shaped numerous granules taht contain electron dense material, most probably proteinaceous hormone.

Key Words
Ultrastructure, transmission electron microscopy, *Pimpla turionellae*, corpora cardiaca

ÖZET

Pimpla turionellae haşerelerin kesintisiz baskılanmasında biyolojik kontrol amaçlı kullanılan önemli bir asalak hymenoptera türü olarak bilinmektedir. Endokrin merkezi olarak, Corpora cardiaca endoparazit *P. turionellae*'ya nörohormonlar salgılayan, transmisyon elektron mikroskobu ile gözlenebilen, bir çift neuroglandular organdır. Beyin örnekleri hazırlanmış ve transmisyon elektron mikroskobunda incelenmiştir. Bu bezlerin hücreleri muhtemelen protein hormon olan, elektronca yoğun materyal içeren genellikle yuvarlak şekilli sayısız granüller ile doludur.

Anahtar Kelimeler
Ultrayapı, Geçirimli elektron mikroskobu, *Pimpla turionellae*, Corpora cardiaca

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INTRODUCTION

While the ultrastructural features of corpora allata of *Pimpla turionellae* are fairly well known [1] those of corpora cardiaca (CC) have little investigated. A variety of histological techniques in different insects have been used to observe CC. Ontogenetically the corpora cardiaca must be considered as ganglia but their histology gives the idea that they act as secretory organs [2]. Tombes and Smith [3] pointed that CC are major neuroendocrine structures attached to the brain. CC directly or indirectly affects regulation of the endocrine-controlled functions such as moulting cycle, ovarian growth, ovoposition movements, heartbeat, the activity of regions of the central nervous system, colour change [4]. Researchers suggested that one of the effects of CC factors may be the suppression of carbohydrate utilisation by the flight muscle [5].

Corpora cardiaca of insects have known as neurohemal organ for the storage and release of neurosecretion material. Although a major portion of the glands are composed of axons whose cell bodies lie as nervi corporis cardiaci (NCC) in the brain, CC of several insect species also contain intrinsic neurosecretory cell bodies [4,6,7] which synthesize and secrete neurohormones. As an endocrine center, corpora cardiaca are a pair of neuroglandular bodies that are found behind the brain and on either sides of the aorta. They not only synthesize their own neurohormones but also store and release other neurohormones including prothoracicotropic hormone (PTTH), which stimula-tes the secretory activity of the prothoracic glands, playing an integral role in moulting [8]. For the regulation of diverse metabolic processes, the neurosecretory cells of CC release neurohormones [9-12]. Researchers have investigated the contents and quantities of these neurohormones. Iba et al. [13] reported that CC have a high content of octopamine in the cricket, *Gryllus bimaculatus*. Neuropeptide content of the CC was studied in *Locusta migratoria migratorioides* adults [14]. Another study revealed that there are quantitative differences between three neuropeptides (neuropasin, Lom-OMP, and APRP) in the locust phase polmorphism. El-Salhy et al. [15] also demonstrated that all the neuropeptide–immunoreactive cells emitted nerve fibers passing through the brain to the CC.

Pimpla are a worldwide genus of the parasitic wasp familiy Ichneumonidae. *Pimpla* species are idiobiont extoparasitoids of Holometabola, often the pupae of Lepidoptera. *Galleria mellonella* and *Pimpla* relationship is an example for this. As *Galleria* give damage to beehives, *Pimpla* gain economic value directing us to investigate this matter.

MATERIALS AND METHODS

Insects

Individuals of *P. turionellae* females was reared under laboratory conditions between years 2003-2005 at the Insect Culture Laboratuary in Department of Biology, Faculty of Science, Ankara University. Females were obtained from these stock culture. The continuity of the stock culture was supplied from greater wax moth, *Galleria mellonella*, reared in the semi-synthetic diet [16,17]. All *P. turionellae* females were kept at temperature of 25 ± 2°C with relative humidity of 75 ± 5% in a 12:12 (L:D) photoperiod. They were fed with cotton pieces absorbed 50% honey solution. Each insect was given a pupa of *G. mellonella* every two days in order to satisfy their host hemolymph needs.

Transmission electron microscopy

Brain tissues of *P. turionellae* adult females were prepared for Transmission Electron Microscope (TEM) as follows. The dissected brain tissues 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 at +4°C. Fixed tissues were dehydrated in a series of graded ethanol, placed into propylene oxide and embedded in araldite [18]. Ultrathin sections were stained with uranyl acetate and lead citrate and examined by TEM (JEOL 100CX II) at 80 kV.

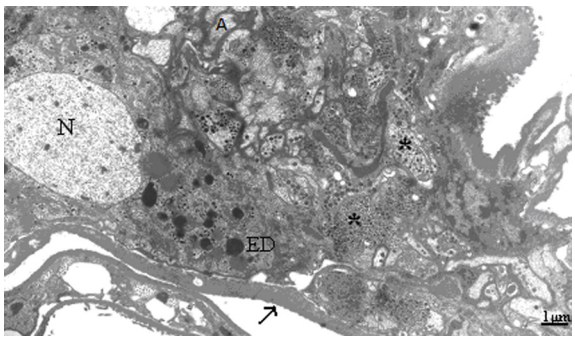

Figure 1. Micrograph representing cells and axons (A) of corpora cardiaca in *Pimpla turionellae*. The cell was full of small granules (asterisk) along electron dense granules (ED). Cell with a clear nucleus (N) was enclosed with capsule (arrow).

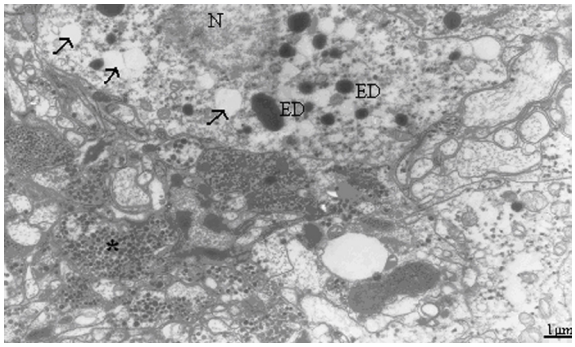

Figure 2. Secretion cells of corpora cardiaca showing nucleus (N), granules with electron dense (ED) and electron lucent material (arrow). Numerous small granules (asterisk) were also evident.

RESULTS

Observations depicted that cells of corpora cardiaca in *Pimpla turionellae* had organelles and cytoplasm typically like secretion cells. Cells of CC had large nuclei full of euchromatin and separately placed chromosentrum bodies. These cells were seen to have numerous small and large granules. Large granules were full of electron dense material and on the contrary small granules were full of moderate electron dense material. Axons of NCC were observed to contain microtubules. These glands were covered with thick capsule consisting of connective tissue (Figure 1).

Observations revealed that, intrinsic neurosecretory cell was very large in size and the cytoplasm of this cell was full of granules in various sizes. Some of these granules were seen to contain electron dense material and the others were discharged of its material (Figure 2).

Electron lucent granules were located near the plasma membrane. A great deal of CC cells were observed to contain numerous small sized granules. These electron dense granules had a unique shape (Figure 3).

Another section of CC in *P. turionellae* adult females showed that intrinsic neurosecretory cell had enlarged nucleus with chromosome and small-large mixed vacuoles in the cytoplasm. Around this cell, there were a great number of neurosecretory endings and their cytoplasms were filled with electron dense material (Figure 4).

These granule shapes were oval and round in cytoplasm of neurosecretory endings of the cell. Intrinsic neurosecretory cells and axons of NCC were observed in CC. The cytoplasms of these cells were filled with electron dense material considered as neurosecretory granules. The axons of NCC were placed among the neurosecretory cells. Cytoplasm of axons had

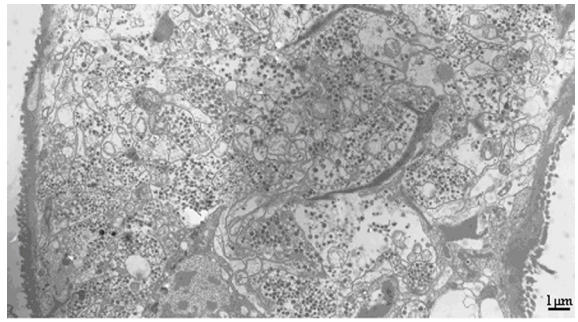

Figure 3. Small granules with electron dense material in the cytoplasm of neurosecretory ending cells were demonstrated in CC.

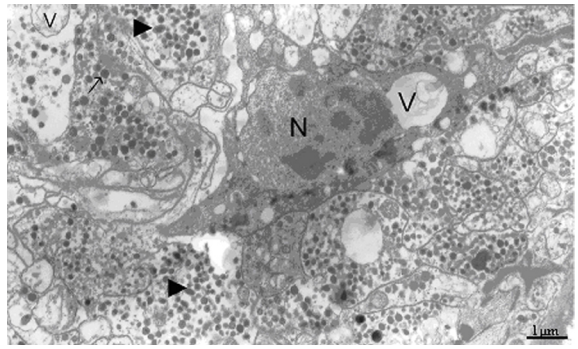

Figure 4. Vacuole (V), oval shaped (arrowhead) and round shaped (arrow) granules with large nucleus (N) of intrinsic neurosecretory cell were prominent features in this micrograph.

several mitochondria and microtubules. Stromal ramifications of capsule were seen in the area of CC (Figure 5).

Axons of NCC in CC were seen to contain numerous mitochondria and microtubules in their cytoplasms (Figure 6).

The cytoplasm of axons (axoplasm) of NCC in CC had numerous peripheral located mitochondria and homogenically dispersed microtubules. The neurosecretion material of NCC has been released drop by drop out of the cell as covered with the plasma membrane of the neurosecretory cells (Figure 7).

DISCUSSION

In this paper ultrastructural features of a neurosecretory center, corpora cardiaca were investigated in a hymenopter, *Pimpla turionellae* with transmission electron microscope. Sections of cerebral neuroendocrine system glands of *P. turionellae* were studied earlier [1]. In addition to previous studies, corpus cardiaca glands were observed by transmission electron microscopy in current study. Observations revealed that glands had capsule, neurosecretory cells, neurosecretory endings and axons of NCC. The neurosecretory endings of the neurons *P. turionellae* adult females were filled with the secretory granules like Rankin et al. [19] reported in cockroach. These authors pointed out that the nuclei of neurons were enormous. We observed that nuclei of neurons of CC in *P. turionellae* had the same dimensions in cockroach. Bradley and Edwards [6] reported that the axons of CC in *Acheta domesticus* had electron lucent vesicles in cytoplasm however, parallel findings were not seen in our study. Unlike other authors we did not observe the similar structures in the organelles such as Golgi complexes, rough endoplasmic reticulum etc. We think that these findings may be caused by thick sectioning. The secretion granules of CC in tsetse fly, *Glossina morsitans* were observed in varied shapes such as spheres, angular, spindle either square or rectangular by the researchers [20]. We observed that the granules of CC of *P. turionellae* had oval and spheres in shape.

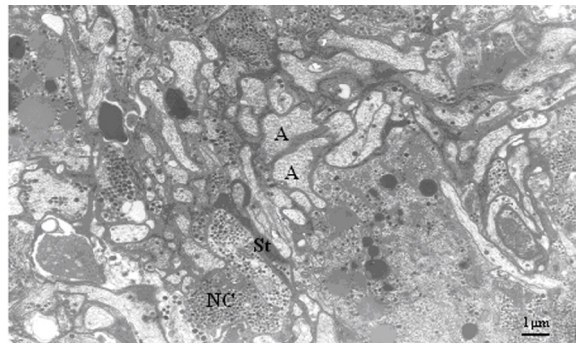

Figure 5. The ultrastructure of CC showing stromal ramification (St), axon (A) and a neurosecretory cell (NC) full of granules.

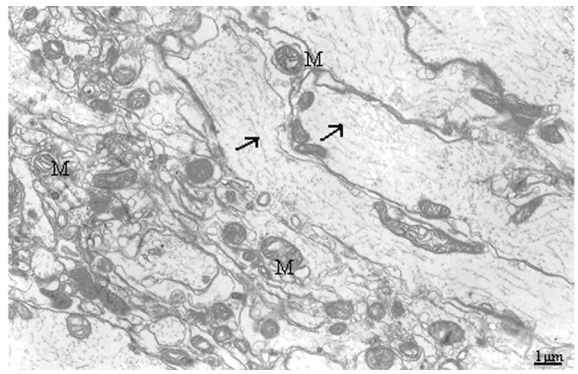

Figure 6. Transverse section demonstrating axons of neurosecretory cell. Microtubules labelled with arrow and a great number of mitochondria (M) were depicted.

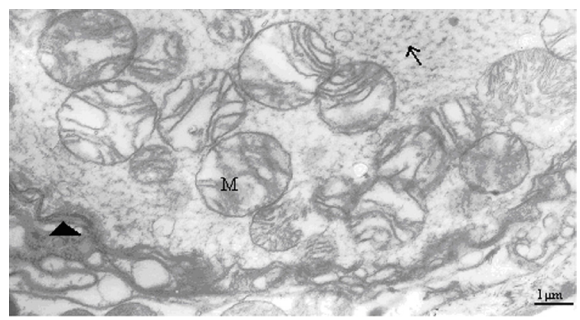

Figure 7. Electron micrograph of terminal axon of nervi corporis cardiaci revealed mitochondria (M), microtubules labelled with arrows and membrane foldings with arrowheads.

In our further studies, we consider to examine the non-studied physiological and biochemical side of CC consequently, these glands of *P. turionellae* will be learned precisely.

The cells of CC had large nuclei full of euchromatin and several granules in a variety of different sizes. Fine structure of the granules showed that these granules include both electron

dense and lucent material. The electron dense material is considered to be proteinaceous hormone. The neurosecretion material of nervi corporis cardiaci seems to be released out of the cell dropwise.

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