Turk J Zool
25 (2001) 95-103

The Effects of Precocene II on the Fine Structure of Corpus Allatum in Adult Female *Anacridium aegyptium* L. (Orthoptera, Acrididae)

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Received: 16.12.1999

Abstract: In this study, the effects of the antijuvenile hormone Precocene II on the corpus allatum of adult females of *Anacridium aegyptium* L. (Orthoptera) were investigated with electron microscopy. In the insects, topically treated with 500 µg precocene II, the corpora allata did not show marked atrophy or destruction. After 10 or 20 days of precocene II treatment, the ultrastructural changes of especially the nuclei, mitochondria, endoplasmic reticulum membranes, Golgi complexes and some other intracellular organelles in the gland cells, probably reflect a moderate sensitivity to this compound.

Key Words: Corpus allatum, Precocene II, Ultrastructure, *Anacridium aegyptium.*

Ergin Dişi *Anacridium aegyptium* L. (Orthoptera, Acrididae)'da Korpus Allatum'un İnce Yapısı Üzerine Precocene II'nin Etkisi

Özet: Bu çalışmada *Anacridium aegyptium* L. (Orthoptera)'un ergin dişilerinde, antijuvenil hormon precocene II'nin korpus allatum üzerine etkisi elektron mikroskobu ile araştırılmıştır. Topikal olarak 500 µg precocene II uygulanmış böceklerde, korpora allata belirgin bir yıkım veya küçülme göstermemiştir. Precocene II uygulamasından 10 veya 20 gün sonra, bez hücrelerinin özellikle nukleusları, mitokondrileri, endoplazmik retikulum membranları, Golgi kompleksleri ve diğer bazı hücre içi organellerindeki ultrastrüktürel değişiklikler, olasılıkla bu bileşiğe karşı hafif bir duyarlılığı yansıtmaktadır.

Anahtar Sözcükler: Korpus allatum, Precocene II, Ultrastrüktür, *Anacridium aegyptium.*

Introduction

The corpora allata of insects are endocrine glands which secrete juvenile hormones that affect the regulation of metamorphosis and the development of gonads. In many insect groups, antijuvenile hormone agents inhibiting the biosynthesis of juvenile hormones of the corpora allata and the insufficiency of these substances exert some abnormalities in certain biological phenomena, which are controlled by the juvenile hormones (1,2).

Treatment of several heteropterous insect nymphs with certain ageratochromenes, called precocenes, induces the development of precocious sterile adults (3) and these compounds also cause necrosis or atrophy in the corpora allata of heteropterous nymphs and adults (4-7) and in some instar larvae of locusta (8-12). These toxic chemicals are antiallatal and antigonadotropic in the adults of different groups of insects (4,13-18). In different insects, after observing abnormal metamorphosis and degeneration of the corpora allata and also the antigonadotropic effects of precocenes, these compounds received attention as a new group of insecticides.

The research material, *A. aegyptium*, is a locust which passes autumn and winter months in the adult diapausing phase. Although there is considerable research about the hormonal control of adult diapause (19-22), little was found on the fine structure of the fat body (23) and the ultrastructure of last larval and adult corpora allata (24) in *A. aegyptium*.

The aim of this electron microscopical work was to investigate the probable effects of precocene II on the gland cells of the corpora allata of *A. aegyptium* during the imaginal life.

Materials and Methods

Adult diapausing *A. aegyptium*, when the corpora allata gland activity was lower, were collected around Bornova (Izmir) in autumn. 500 µg precocene II (Aldrich Chem. Co. 6-7 dimethoxy 2, 2-dimethyl-3 chromene) dissolved in 20 µl pure acetone, was applied topically on the abdominal tergum after lifting the wings of each adult female. Control insects were treated with acetone or were untreated.

The precocene treated insects and the controls, 10 individuals in each group, were kept in separate cages with adult males under laboratory conditions, where the adult diapause of the females broke at a temperature of 30±1°C, relative humidity 40-50%, and with a long-day photoperiod (16:8 hours), and were fed with fresh *ligustrum vulgare* L. leaves, bran and water.

For the electron microscopy, on the 10^{th} and 20^{th} days, the corpora allata of the control insects and the precocene II treated ones were fixed in 2.5% formaldehyde glutaraldehyde (25). Post fixation was carried out in 1% osmium tetroxide (26) and dehydration was graded in ethanol and embedded in Epon 812. For light microscopy semi-thin sections were stained with toluidine blue. Thin sections were double stained with uranyl acetate and lead citrate and microphotographs were taken under the Jeol 100-C transmission electron microscope. Only the glands on the 10^{th} day were evaluated in the control group while both were taken into consideration in the treated group. Statistical analysis was made according to CD (coefficient of difference) values (27).

Results

The corpus allatum of *A. aegyptium* is a paired ovoid endocrine gland, each encapsulated with a connective tissue. This stromal sheath is either a thin basal lamina or a thick layer furnished with scattered glial cells, trachae, tracheoles and neurosecretory axons (Figure 1). The thickness of the basal lamina was measured to be 400 nm in precocene II treated insects and 800 nm in control insects.

During the examination of semi-thin sections stained with toluidine blue, in precocene treated *A. aegyptium*, the corpus allatum did not show any atrophy. There were no significant differences between the control and precocene treated insects after 20 days in terms of the width, length and the width-length ratio of the corpus allatum (N=10), according to the CD values (range 0.13-0.43), since these values were lower than the significant level of 1.28. In the terminal oocytes of treated *A. aegyptium*, the yolk deposition was also observed.

Corpora allata consist of mostly gland cells (Figures 1-5) and between axons of corpus allatum nerves, glial cells, trachae and tracheoles (Figures 6-9). Dark and light interdigitated gland cells are seen in both the controls (Figures 2,3,5) and precocene treated groups (Figure 6). Cells in the interior part of the corpus allatum are closely packed and narrow intercellular spaces with dark material are generally observed (Figure 2), whereas large electron lucent intercellular spaces with some substance always occurred at the peripheral region of the gland (Figure 1). Intercellular spaces between gland cells and glial cells are widened where some vesicular, fibrillar, granular or membranous structures and also many small finger-like cell processes are occasionally observed in treated *A. aegyptium* (Figures 6,9).

Both smooth and rough endoplasmic reticulum membranes are present in the gland cells of the corpus allatum (Figures 1-7,10-14). In controls smooth endoplasmic reticulum is found predominantly arranged in distinctive whorls of concentric cisternae and a dense body may be found in the central area of this assemblage (Figures 1-4), whereas in other regions of the same cell or in other cells, smooth endoplasmic reticulum is observed as small tubules or vesicles (Figures 4,6,7). Concentric cisternae of smooth endoplasmic reticulum observed in precocene-treated *A. aegyptium* are different in shape (Figures 12-14). In Figure 13, two concentric clusters are seen adjacently, and a group of mitochondria are also observed in the center of these structures (Figure 14). In some other cells concentric membranes seem to become unrolled or degenerated.

In the controls (Figures 3,4) and in the treated *A. aegyptium* (Figures 13-15) polysomes are found to be abundant in the cytoplasm, and the cisternae of rough endoplasmic reticulum are richly studded with ribosomes (Figures 3-5). However, they are distributed as either short or long coiled cisternae or dilated vacuoles (Figures 10-12,16) with fewer ribosomes in the corpus allatum of precocene treated *A. aegyptium*. Microtubules and lipid droplets are also observed in the cytoplasm of gland cells in both controls and treated insects (Figures 10,11).

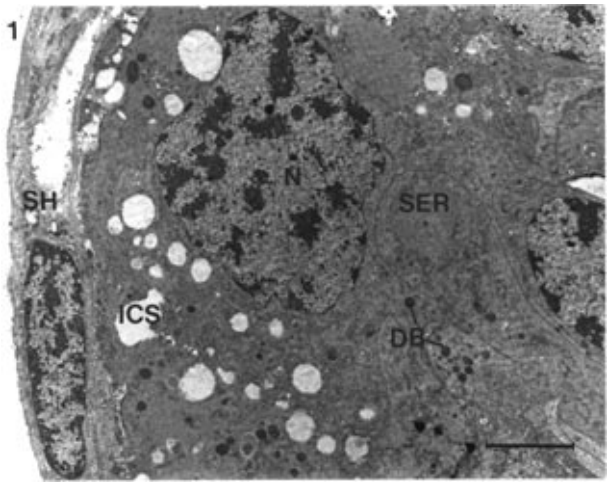

Figure 1. Peripheral area of corpus allatum showing large intercellular space (ICS). Note some invaginations of nuclear envelope (N). Dense body (DB), sheath (SH), whorls of smooth endoplasmic reticulum (SER). Bar = 4µm.

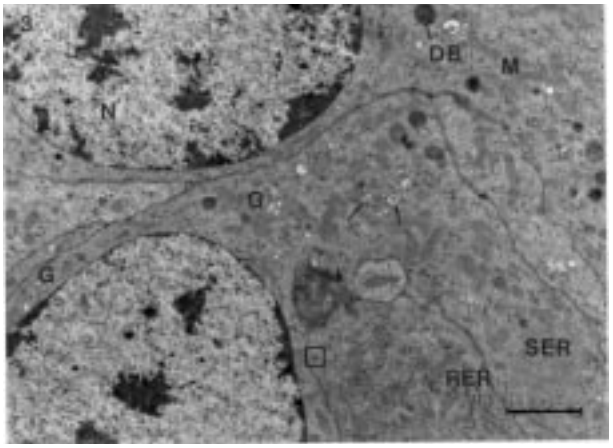

Figure 3. All mitochondria (M) appear rod-like or globular with electron lucent matrix. Dense body (DB), Golgi complex (G), light gland cell (✶), mitochondria (M), multivesicular body (➞), nucleus (N), polysomes (❑), rough endoplasmic reticulum (RER), whorls of smooth endoplasmic reticulum (SER). Bar = 2µm.

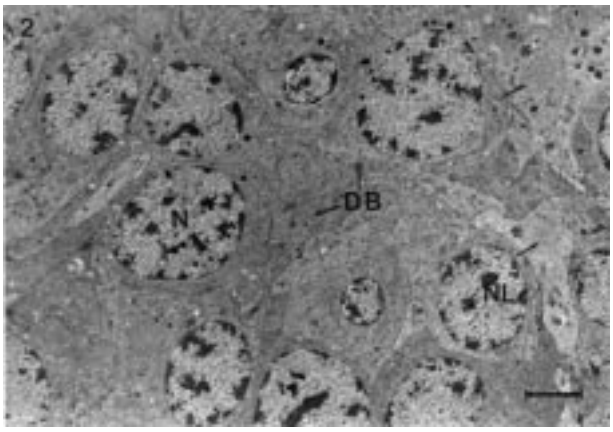

Figure 2. Internal area of corpus allatum showing dark and light (✶) interdigitated gland cells with narrow intercellular spaces. Dense body (DB), nucleus (N), nucleolus (NL), whorls of endoplasmic reticulum (➞) Bar = 4µm.

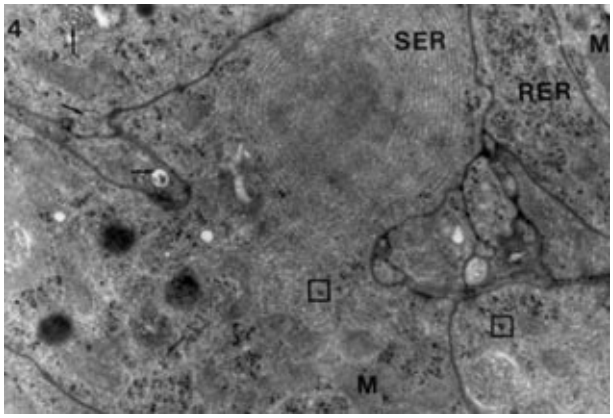

Figure 4. Appearance of whorls of concentric cisternae of smooth endoplasmic reticulum (SER), note rough endoplasmic reticulum (RER) with attachment of numerous ribosomes. Mitochondria (M), multivesicular body (➞), nucleus (N), polysomes (❑). Bar = 1µm

The nuclei with a prominent nucleolus are generally round or ovoid but in the peripheral cells they are mostly convoluted (Figures 1-3,7,12). In the controls nuclear envelopes are mostly regular (Figures 2,3,5) but in some treated cells the nuclei have slight invaginations (Figure 12) or slight large perinuclear spaces (Figure 10).

Golgi complexes generally consist of the stack of saccules associated with groups of small vesicles or vacuoles in both controls and treated *A. aegyptium* (Figures 3,5,6,10,13,14,17). The Golgi vesicles of treated *A. aegyptium* filled with slightly dense material are occasionally observed (Figure 17). Their vacuoles appear swollen and membranes are quite irregular (Figures 6,10). Multivesicular bodies are frequently observed in control *A. aegyptium* (Figure 4).

Although there is no difference in the appearance or in the number of lysosome- like dense bodies (Figures 1-3,5,12,17) in some treated cells they are drawn together with some other organelles such as free ribosomes or mitochondria, which may reflect the initiation of the

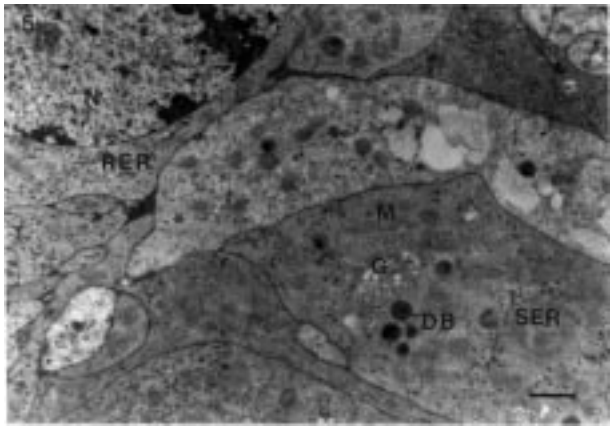

Figure 5. Golgi complex (G) with some dense bodies (DB) nearby. Light gland cell (✶), mitochondria (M), nucleus (N), smooth endoplasmic reticulum (SER). Bar = 1µm.

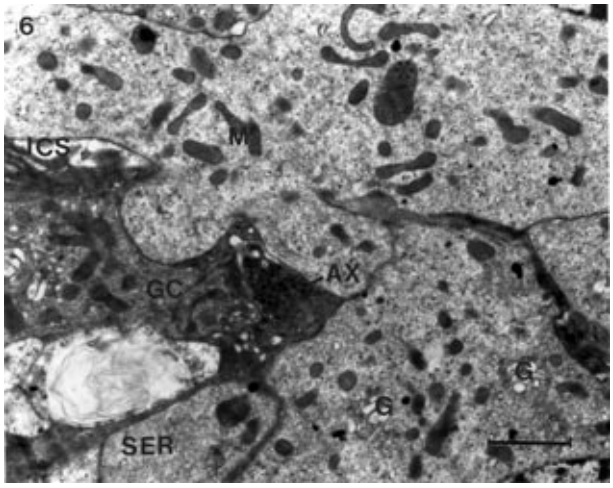

Figure 6. Parts of light gland cells and glial cell (GC). Compare dark mitochondria (M) and Golgi complex (G) with swollen cisternae and vacuoles to control (Figure 3). Neurosecretory axon (AX), large intercellular space (ICS) with few cell processes, smooth endoplasmic reticulum vesicles (SER), giant mitochondrium (✶). Bar = 2µm.

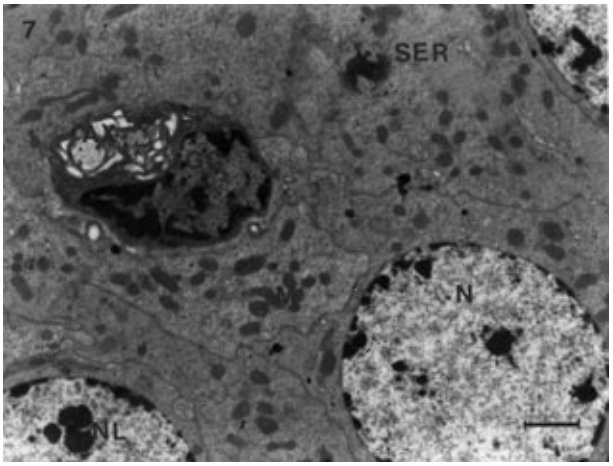

Figure 7. Parts of gland cells. Note dark mitochondria (M) with indistinguishable cristae. Neurosecretory axon (AX), glial cell (GC), nucleus (N), nucleolus (NL), smooth endoplasmic reticulum (SER). Bar = 2µm.

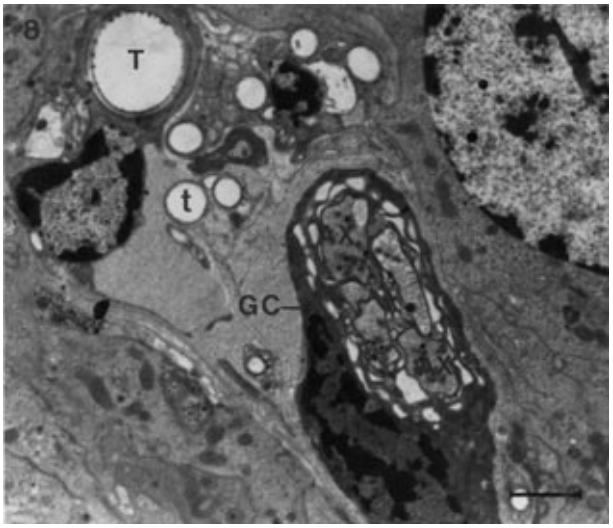

Figure 8. Glial cell (GC) foldings surrounding axons (AX) profiles with neurosecretory granules. Trachae (T), tracheoles (t). Bar = 2µm.

cytolysosomal organization (Figures 13-15). The mitochondria which are enclosed by an isolation membrane are occasionally observed in some cells (Figure 10). In Figure 11 the long coiled endoplasmic reticulum encloses a few mitochondria and a lipid droplet is seen.

The mitochondria are round, ovoid or slender in shape with lamellar or tubular cristae and electron lucent matrix in the control *A. aegyptium* (Figures 3-5), although they differ in morphology from those of treated ones (Figures 6,7,10-12,14-17). The mitochondria with electron dense matrix in some treated insects seem to be strikingly affected by the treatment of precocene II. The pleomorphic mitochondria are mostly observed in some gland cells. As well as the basic types, dumbbell-shaped, cup-shaped and ring-shaped mitochondria are frequently distributed in the cytoplasm (Figures 10,11,16,17). This rich mitochondrial pleomorphism is not observed in the controls. Giant mitochondria (Figure 6) and some others with swollen mitochondria are seldom observed (Figure 15). The outer membranes and cristae, in some dense

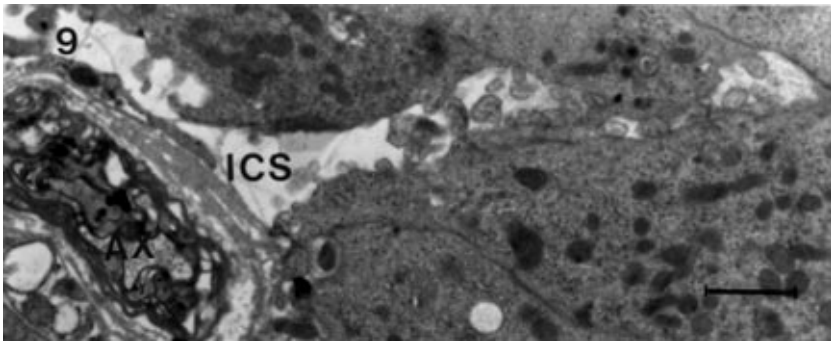

Figure 9. Large intercellular space (ICS) with small finger-like projections are noted. Axon (AX). Bar = 2µm

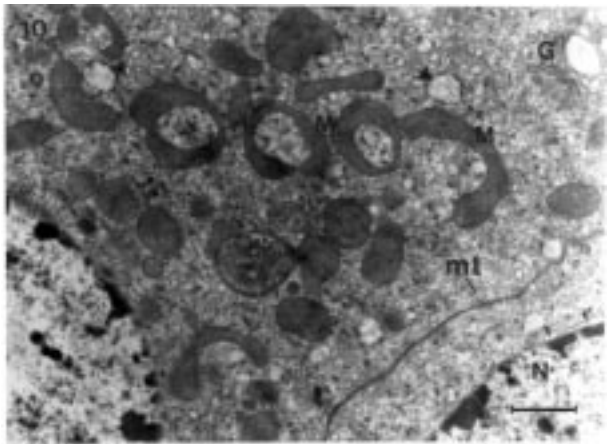

Figure 10. Part of gland cells showing mitochondrial pleomorphism. Most mitochondria (M) showing ring-like, cup-like or dumbbell-like appearance. Note slightly large perinuclear spaces (➤), microtubules (mt), a mitochondrium surrounded by a distinct membrane (✶), dilated cisternae of rough endoplasmic reticulum (▶), Golgi complex (G), Nucleus (N). Bar = 1µm.

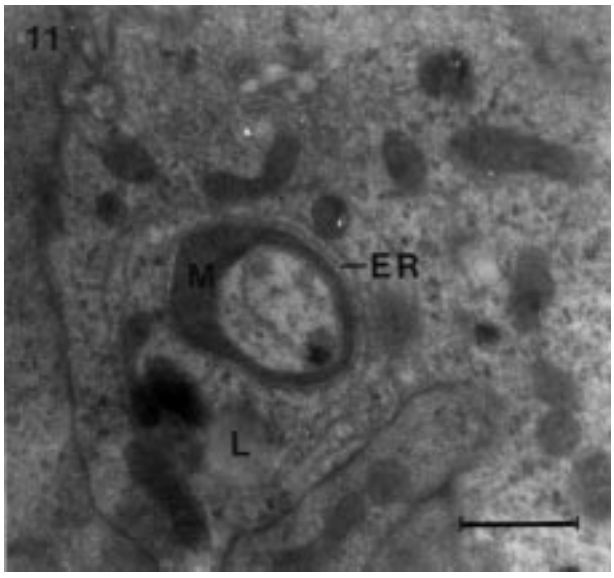

Figure 11. A long coiled endoplasmic reticulum (ER) encloses some mitochondria (M) and a lipid droplet (L). Bar = 1µm.

mitochondria, may not be observed easily and in some cases, these can not be distinguished from lysosome- like dense bodies (Figures 7,16).

Discussion

The present electron microscopical study describes for the first time in adult female *A. aegyptium* the effects of precocene II on the corpus allatum. It was demonstrated that the precocene II treated corpus allatum of adult female *A. aegyptium* did not show intensive cell destruction or atrophy like in adult *D. Punctata* (14) after in vivo treatment with precocene II. Nevertheless, finding CD values less than 1.28 also showed that precocene II did not inhibit the volume

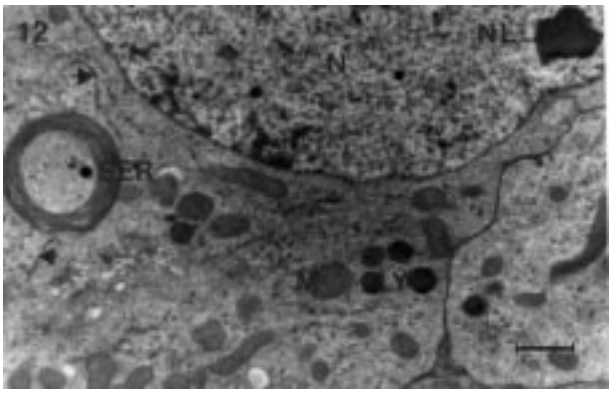

Figure 12. Part of gland cells showing abundance of dark mitochondria (M) rod or round in shape. Note the paucity of ribosomes on the rough endoplasmic reticulum (▶). Lysosome-like dense body (LY), nucleus (N), nucleolus (NL). Bar = 1µm.

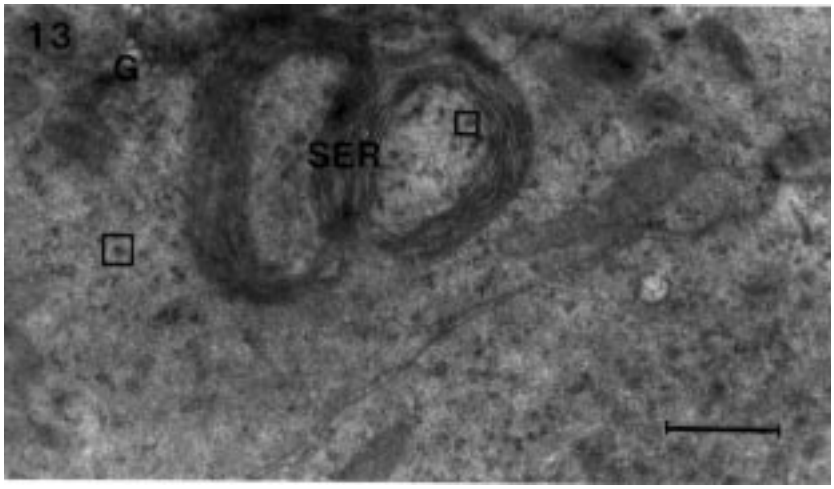

Figure 13. Two concentric cisternae of smooth endoplasmic reticulum (SER) are adjacent. Note the abundance of polysomes (❑), Golgi complex (G). Bar = 1µm

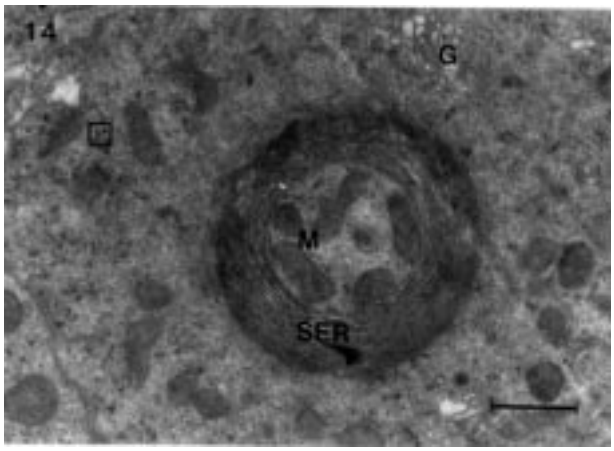

Figure 14. A group of mitochondria (M) surrounded by whorls of smooth endoplasmic reticulum (SER). Note all mitochondria (M) are mostly rod-shaped or globula with distinct cristae. Golgi complex (G), polysomes (❑). Bar = 1µm.

increase of the corpora allata in treated *A. aegyptium*. In contrast, it was reported that in adult *O. fasciatus* (4,5,6), 4th instar nymphs of *L. migratoria* (8,9) and *S. gregaria* (11), precocene II causes cellular degradation and atrophy in the corpora allata. Masner et al. (7) pointed out that the corpus allatum of 5th instar larvae of *O. fasciatus* was not affected by precocene II. Miall and Mordue (10) reported a juvenile effect of precocene II instead of an antijuvenile effect in 5th instar nymphs of *L. migratoria*. Schooneveld and Orshan (12) also showed degenerative effects of precocene in 4th instar nymphs of *L. migratoria*, whereas recovery or regeneration was seen in 5th instar nymphs. These different results can be interpreted as showing different reactions of the corpus allatum to precocene in adults or instars of the same species (28).

Some interpretive ultrastructural studies might be summarized as below. Dark and light gland cells of the

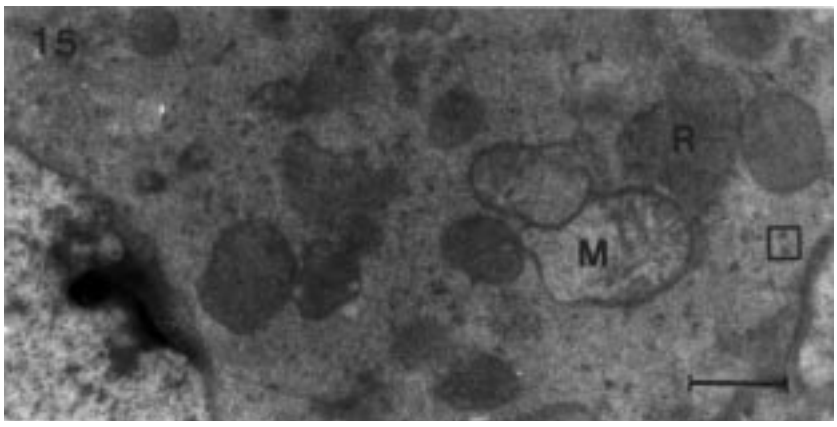

Figure 15. Swollen mitochondria (M) and free ribosomes (R) accumulation. Polysomes (❑). Bar = 1µm.

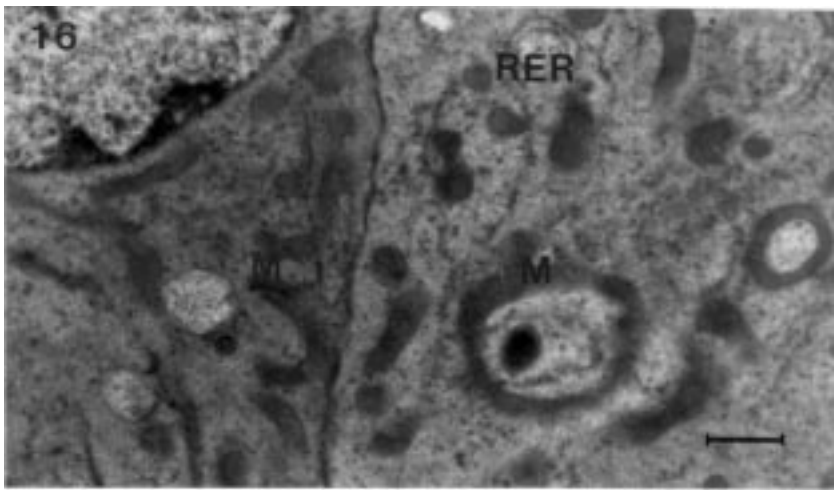

Figure 16. Appearance of long coiled cisternae of rough endoplasmic reticulum (RER) attachment of few ribosomes. Most mitochondria (M) was noted with indistinct outer membrane and cristae. Bar = 1µm.

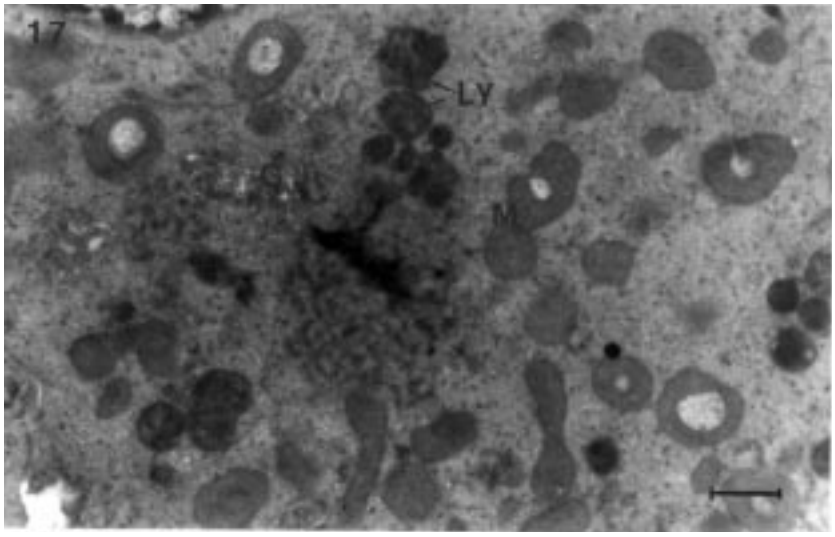

Figure 17. Appearance of pleomorphic mitochondria (M). Golgi complexes (G) have abundance of small vesicles. Lysosome-like dense bodies (LY). Bar = 1µm.

corpus allatum were reported in *C. erythrocephala* (29), *O. fasciatus* (5,6,30) as well as in the present investigation. These dark cells of *O. fasciatus* were considered inactive cells (30), active cells (6) or degenerated necrotic cells (5).

In precocene II treated *L. migratoria*, Schooneveld (8,9) showed electron dense cells with irregular nuclear and cellular boundries, little cytoplasm and strongly increased extracellular spaces. Then cells were phagocytosed by hemocytes. But phagocytosis was not seen in *A. aegyptium*. In *O. fasciatus*, Unnithan et al. (5) observed segregation of various cytoplasmic organelles, autophagic vacuoles and residual bodies, pleomorphic mitochondria and signs of disintegration in the corpus allatum of topically or contact treated precocene II bugs. Autophagic vacuoles were not observed extensively in *A. aegyptium*. Feyerisen et al. (14) pointed out disorganization within the intracellular membranes, swelling and irregularity of Golgi membranes, clumping and irregularity and smooth endoplasmic reticulum after in vivo or in vitro treatment in *D. punctata*. All these ultrastructural findings (5,8,9,14) showed some similarity to those of *A. aegyptium*.

The predominant effect of precocene II was the presence of pleomorphic mitochondria with electron dense matrix, indistinguishable outer membrane and cristae in *A. aegyptium*, which showed a great resemblence to those of (5). Girardie and Granier (24) expressed no organelles of the cells of corpora allata in *A. aegyptium* undergoing any changes with the maturation of the ovaries in contrast to *L. migratoria*, (31) which had pleomorphic mitochondria. Besides these, Lubzens et al.

(32) observed that the vitellogenesis was not prevented but inactivated the corpora allata after precocene II treatment of vitellogenic females of *L. migratoria*. Similar observations were pointed out in *A. aegyptium* with yolk deposited terminal oocytes in the present study.

Consequently, all these ultrastructural changes which were observed in some gland cells of the corpora allata of adult female *A. aegyptium* after treatment with precocene II on day 10 or 20, may show moderate sensitivity to this chemical. However, the present study must be supported by radiochemical techniques with some other electron microscopical studies in the near future.

Acknowledgements

I would like to thank Dr. B. Falakalı and Dr. M. K. Atatür for their helpful suggestions during the course of the improvement of the English manuscript. I am also grateful to Dr. H. Karakişi for technical help during the E. M. studies. This research was supported by the Research Foundation of Ege University.

References

1. Bowers, W.S., Endocrinology of Insects, R.G.H. Downer and H. Laufer., Liss. New York, 1983, 517-523.
2. Bowers, W.S., Comprehensive Insect Physiology Biochemistry and Pharmacology, G.A. Kerkut and L.I. Gilbert., Vol. 8, Pergamon Press, 1985, 551-564.
3. Bowers, W.S., Discovery of Insect Antiallatotropins. In the Juvenile Hormones. Edited by L.I. Gilbert; Plenum Press, New York, 1976, 394-408.
4. Bowers, W.S., Martinez-Pardo, R.M., Antiallatotropins: Inhibition of corpus allatum development. Science., 197: 1369-1371, 1977.
5. Unnithan, G.C., Nair, K.K., Bowers, W.S., Precocene-induced degeneration of the corpus allatum of adult females of the bug *Oncopeltus fasciatus*. J. Insect Physiol., 23: 1081-1094, 1977.
6. Liechty, L., Sedlak, B.J., The ultrastructure of precocene-induced effects on the corpora allata of the adult milkweed bug, *Oncopeltus fasciatus*. Gen. Comp. Endocrinol., 36: 433-436, 1978.
7. Masner, P., Bowers, W.S., Kälin, M., Mühle, T., Effect of precocene II on the endocrine regulation of development and reproduction in the bug, *Oncopeltus fasciatus*. Gen. Comp. Endocrinol., 37: 156-166, 1979.
8. Schooneveld, H., Effects of precocene II on normal and denervated allata in 4th instar nymphs of *Locusta migratoria*. VIII. ISCE, Amsterdam., 112, 1978.
9. Schooneveld, H., Precocene-induced collapse and resorption of corpora allata in nymphs of *Locusta migratoria*. Experientia., 35: 363-364, 1979.
10. Miall, R.C., Mordue, W., Precocene II has juvenile hormone effect in 5th instar *Locusta migratoria*. J. Insect. Physiol., 26: 361-364, 1980.
11. Unnithan, G.C., Nair, K.K., Syed, A.B., Precocene-induced metamorphosis in the desert Locust *Schistocerca gregaria*. Experientia., 36: 135-136, 1980.
12. Schooneveld, H., Orshan, L., Analysis of precocene effects on fifth instar larvae of *Locusta migratoria*. Gen. Comp. Endocrinol., 46: 378, 1982.
13. Bowers, W.S., Aldrich, J.R., In vivo inactivation of denervated corpora allata by precocene II in the bug, *Oncopeltus fasciatus*. Experientia., 36: 362-364, 1980.
14. Feyerisen, R., Johnson, G., Koener, J., Stay, B., Tobe, S.S., Precocene as pro-allatocidins in adult female *Diploptera punctata*: A functional and ultrastructural study. J. Insect. Physiol., 27: 855-868, 1981.
15. Prat, G.E., Pener M.P, Precocene sensitivity of corpora allata in adult female *Locusta migratoria* after electrocoagulation on the pars intercerebralis neurosecretory cells. J. Insect. Physiol., 29: 33-39, 1983.
16. Bitsch, C., Bitsch, J., Antigonadotropic and antiallatal effects of precocene II in the firebrat, *Thermobio domestica* (Thysanura, Lepismatidae). J. Insect. Physiol., 30: 463-470, 1984.
17. Bitsch, C., Delbecque, J.P., Mathelin, J, Bitsch, J., Effects of precocene on intermolt length and ecdysteroid titers in relation to ovarian maturation in *Thermobia domestica* (Thysanura: Lepismatidae). J. Insect Physiol., 32: 535-541, 1986.
18. Hardie, J., Morphogenetic effects of precocenes on three aphid species. J. Insect Physiol., 32: 813-818, 1986.
19. Geldiay, S., *Anacridium aegyptium* L. (Orthoptera: Acrididae)' da fotoperiyodun imaginal diyapoz üzerine etkisi. Ege Üniv. Fen Fak. İlmi Rap. Ser., No. 40, 1966.
20. Geldiay, S. Hormonal control of adult reproductive diapause in the Egyptian Grasshopper, *Anacridium aegyptium* L. J. Endocrinol., 37: 63-71, 1967.
21. Geldiay, S., Photoperiodic control of neurosecretory cells in the brain of the Egyptian grasshopper *Anacridium aegyptium* L. Gen. Comp. Endocrinol., 14: 35-42, 1970.
22. Girardie, A., Granier, S., Système endocrine et physiologie de la diapause imaginal chez le criquet Egyptien *Anacridium aegyptium*. J. Insect. Physiol., 19: 2341-2358, 1973.
23. Ergen, G., Light and electron microscopic investigations of the fat body of adult female of diapausing and non-diapausing *Anacridium aegyptium* L. (Orthoptera). J. Fac. Scien. Ege Univ., Ser B, 12: 1-8, 1990.

24. Girardie, A., Granier, S., Ultrastructure des corps allates d'*Anacridium aegyptium* (Insecte Orthoptère) a l'lavant-dernier stade larvaire et durant la vie imaginale. Arch. Anat. Microsc. Morphol. Exp., 63: 251-268, 1974.

25. Karnovsky, M.J., A formaldehyde-glutaraldehyde fixative of high osmolaritiy for use in electron microscopy. J. Cell. Biol., 27: 137A-138A, 1965.

26. Millonig, G., Advantages of a phosphate buffer for O5O4 solution in fixation. J. Appl. Physiol., 32: 1637, 1961.

27. Mayr, E., Principles of systematic zoology, McGraw-Hill Inc., New York, 428 p, 1969

28. Staal, G.B., Antijuvenile hormone agents. Ann. Rev. Entomol., 31: 391-429, 1986.

29. Thomsen, E., Thomsen, M., Fine structure of the corpus allatum of the female blow-fly *Calliphora erythrocephala*. Z. Zellforsch., 110: 40-60, 1970.

30. Dorn, A., Electron microscopic study on the larval and adult corpus allatum of Oncopeltus fasciatus Dallas. (Insecta, Heteroptera). Z. Zellforsch., 145: 447-458, 1973.

31. Fain-Maurel, M.A., Cassier, P., Pleomorphism mitochondrial dans les corpora allata de *Locusta migratoria migratorioides* (R. Et F.) au cours de la vie imaginale. Z. Zellforsch., 102:543-553, 1969

32. Lubzens, E., Moshitzky, P., Applebaum, S.W., Active vitellogenesis in precocene-treated Locusta migratoria. Gen. Comp. Endocrinol., 43: 178-183, 1981