ISSN 1009 – 3095 *Journal of Zhejiang University (SCIENCE)* V.2, No.3, P.294 – 297, July – Sep., 2001
http://www.chinainfo.gov.cn/periodical; http://www.zju.edu.cn;
http://lib.zju.edu.cn/English; http://www.zjupress.com

EFFECTS OF JINLU, AN ANTI-JUVENILE HORMONE ON THE GROWTH, ULTRA-STRUCTURE OF THE CORPORA ALLATA AND PROTHORACIC GLAND OF SILKWORM, *BOMBYX MORI* L*

MIAO Yun-gen(缪云根), JIANG Li-jun(江丽军), Bharathi D.†

(*Department of Sericulture & Apiculture, College of Animal science, Zhejiang University, Hangzhou 310029, China*)

Received Oct.18, 2000; revision accepted Jan.5, 2001

Abstract: The 4th instar larvae of the silkworm, *Bombyx mori* L, when treated with anti-juvenile hormone (Jinlu) had its larval period extended by 2 days and the total larval period shortened by about 4 days. The conversion ratio of tetramolters into trimolters was 100%. But anti-juvenile hormone administration to the 5th instar larvae lengthened the silkworm age by one day. When anti-juvenile hormone was administered, we could find many neurosecretory granules of the brain transferred to the cells of the corpora allata, but there was little endoplasmic reticulum. In the prothoracic gland, the micropile edge was clear and there were large nucleoli, mitochondria and endoplasmic reticulum. This study was carried out to show that anti-JH compound inhibits the secretion of Juvenile hormone in silkworm *Bombyx mori* L. The investigation revealed that the anti-juvenile hormone inhibited the secretion of corpora allata and initiated the activity of the prothoracic gland.

Key words: anti-juvenile hormone (anti-JH), endocrine gland, corpora allata and prothoracic gland, silkworm, *Bombyx mori* L

Document code: A **CLC number:** S881.2

INTRODUCTION

Insect postembryonic development consists of growth punctuated by a series of molts followed by metamorphosis. These molts and metamorphosis are initiated and coordinated by hormones (Riddiford, 1994). It is generally accepted that the interplay of ecdysteriods, a group of steroid hormones, and juvenile hormone (JH), a sesquiterpene, serves to orchestrate the progression from one developmental stage to the next, with the ecdysteroids regulation, the onset and timing of the molt and JH regulating the quality of the molt (Sehnal, 1989; Gilbert et al., 1996; Riddiford, 1994). In the 1970s, a substance that works against JH was extracted from plants and was called "anti-juvenile hormone (Anti-JH)" (Bowers et al. 1976). Up to now, more than 20 kinds of anti-JH compounds had been separated and synthesized; most of them are imidazole derivatives (Staal, 1986). Numerous studies had been conducted to clarify the development pattern of these important hormones. Intensive pioneering research showed that anti-juvenile hormones display different physiological effects when administered in different stages of insects, some of them act as inhibitor of insect juvenile hormone biosynthesis (Quistad et al., 1981). In tetra-molting silkworm, *Bombyx mori* L, the duration of the anti-juvenile hormone treated instar would would be increased, trimolters would be induced and the total larval period would be reduced when anti-juvenile hormone was used before the 4th instar (Kiguchi, et al., 1984; 1985; Lu and Li, 1987). At present, some imidazole derivatives such as KK-42, KK-84, SSP-11, SM-1 are applied in practice to induce trimolter and produce superfine cocoon silk (Akai et al. 1984, 1986, 1989; Lu et al., 1987).

In this work, we investigated the effects of

* Project supported by the Government of China and Ministry of Human Resource Development, Government of India for Bilateral exchange program.

† Post-Doctoral Scholar in College of Animal Sciences of Zhejiang University now, from Sri Padmavathi Women's University, India.

Jinlu, an anti-JH on the growth and development of silkworm and obtained enlightenment on the role of the ultra-structure changes of corpora allata and prothoracic gland in the mechanism of hormone regulation.

MATERIAL AND METHODS

Experimental animals

The larvae used were hybrid silkworm: Xinhang(Chinese) × Keming(Japanese), supplied by the Sericultural Department of Zhejiang Agricultural Bureau. The silkworms were reared on fresh mulberry leaves at 25℃, RH 75% – 80% and 12L : 12D photoperiod. Newly-ecdysed fourth and last (fifth) instar larvae were collected and used for each experiment.

Anti-juvenile hormone analogue treatment

One gram of anti-JH(Jinlu) was dissolved in water to make 250 mg/L solution. For the first group, 250 mg/L AJH solution was sprayed on mulberry leaves and fed to silkworms at the rate of 4μg/larva during 0 – 48 hours of fourth instar. For the second group, AJH solution was sprayed on mulberry leaves and fed to silkworms from 72 – 96 hours of fifth instar. The third group was kept as control without any treatment.

The larvae were dissected in the fourth instar and the fifth instar respectively. Each experiment was performed in triplicate.

Electron microscopic study

For electron microscopy, the corpora allata and prothoracic gland of the silkworm were dissected from the anti-juvenile hormone-treated larvae in cold physiological saline solution. The electron microscopic observation followed the method described by Sato(1968) and Kiguchi (1984).

RESULTS

The growth and development of silkworm larvae treated with anti-juvenile hormone

When the anti-juvenile compound (Jinlu) was given 0 – 72 hours after the 3rd ecdysis (4th instar), the 4th instar duration was extended to equal the 5th instar duration, and about 100% of trimolters were induced. Due to lack of the 5th instar, the larval duration was reduced by about 4 days. On the other hand, administration anti-JH in middle or late 5th instar prolonged the larval duration by 1 day and increased the weight of matured silkworm larvae.

Fig. 1 shows the silkworm larva duration when administered with AJH on day 2 of the fourth instar and day 4 of the fifth instar.

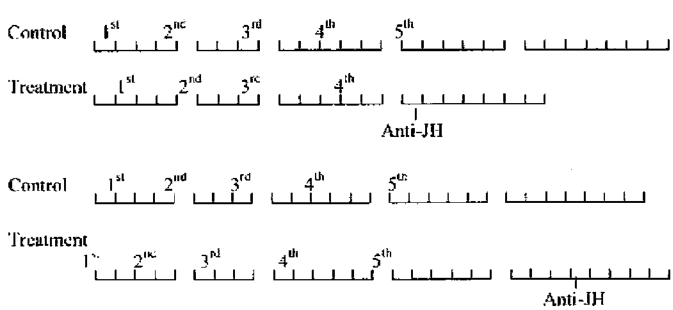

Fig. 1 Silkworm larval duration with and without AJH treatment

Effect of anti-JH (Jinlu) on the ultra-structural changes of prothoracic gland

After administration of Jinlu in 0 – 72 hours of the 4th instar, large nucleus and chromatin masses were seen in the prothoracic gland. Chromatin masses shown by arrow in plates 1 and 3 were developed like a tree. On the edge of prothoracic gland, are clear microroads(plates 2 and 4) and slit-like vacuoles in the cytoplasm. The prothoracic gland is in active biosynthesis (experimental). But in the control, the nucleus of the prothoracic gland cell is irregular in shape (Fig.2: 1 – 4).

The ultra-structural change of the prothoracic gland of larvae treated with anti-JH in the early

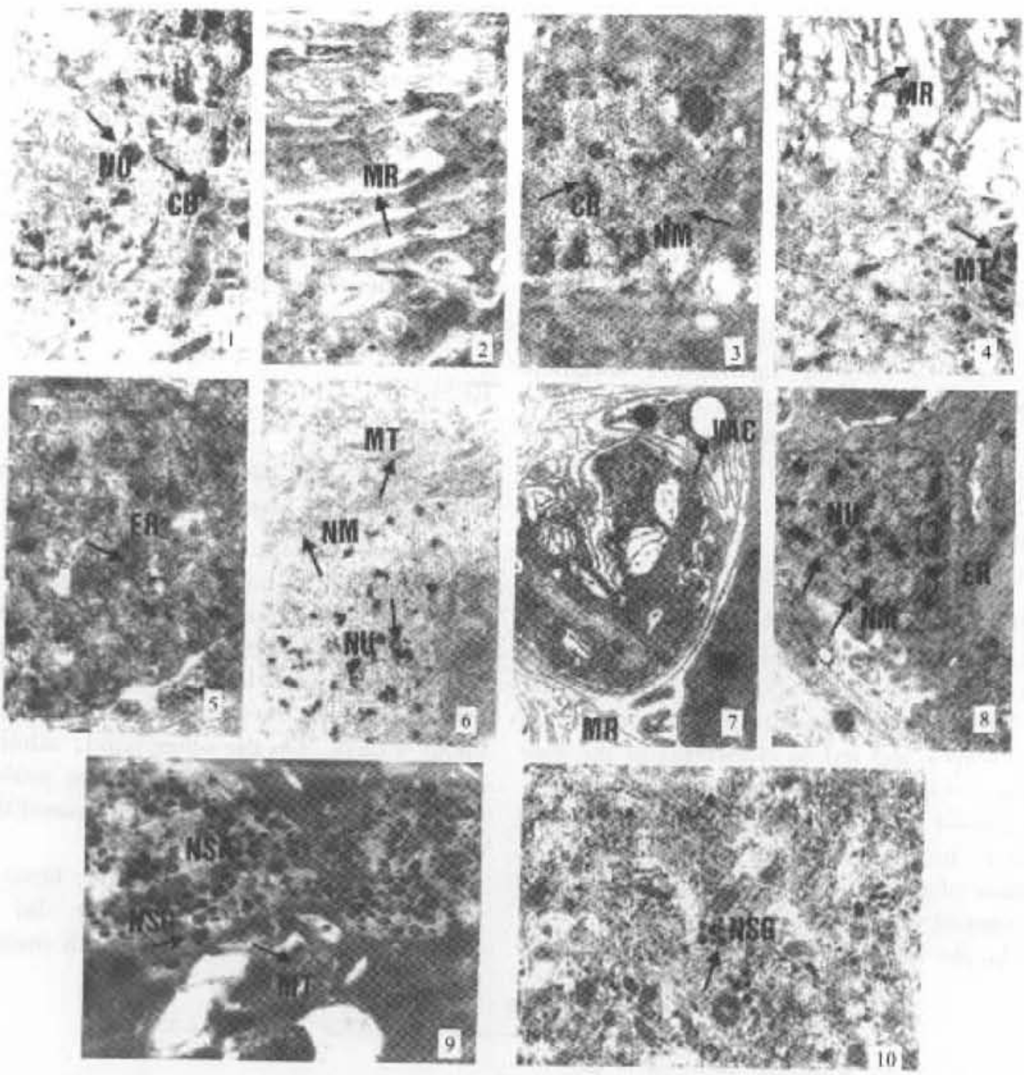

Fig.2 Ultra-structural changes of the prothoracic gland and the corpora allata treated with anti-JH

NU: Nucleus; CB: Chromatin body; MR: Microroad; NM: Nuclear membrane; MT: Mitochondria;
VAC: Vacuoles; ER: Endoplasmic reticulum; NSG: Neurosecretory granules; NSA: Neurosecretory axons

Plates 1 – 4: Prothoracic gland(PG) treated with anti-JH in early of the 4th instar(× 7500). 1. Nucleus of PG; 2. Microroad near the edge of PG; 3, 4. Control

Plates 5 – 8: Prothoracic gland(PG) treated with anti-JH in early of the 5th instar. 5. Endoplasmic reticulum(× 12000); 6. Nucleus and mitochondria(× 6000); 7, 8. Control

Plates 9 – 10: Corpora allata treated with anti-JH in early of the 4th instar(× 12000). 9. Corpora allata treated with anti-JH; 10. Control

5th instar(0 – 72 hours) is shown in Fig.2:5 – 8.

Effect of anti-JH on the ultra-structural changes of corpora allata

Observation of 4th instar larvae 0 – 72 hours after administration of anti-JH analogue (Jinlu) to them showed large numbers of neurosecretory granules transferred from the brain and deposited in the corpora allata. There was a large nucleus and mitochondria but no endoplasmic reticulum (ER) in the corpora allata(Fig.2:9 – 10). This result suggests the nuerosecretory granules from the brain inhibited the biosynthesis activity of the corpora allata.

DISCUSSION

The development and metamorphosis of insects are controlled by the endocrine system, especially the corpora allata and prothoracic gland.

Juvenile hormone secreted in the corpora allata holds in check the larval duration, inhibits the development of adult organs, and prevents metamorphosis; but the molting hormone secreted in the prothoracic gland initiates the formation of the new body wall and causes ecdysis. The nature of the larval ecdysis or metamorphosis is decided by the quantity of juvenile hormone.

Researchers found that the anti-JH activity causes the silkworm, *Bombyx mori* to develop premature larvae (Bowers, et al., 1976). When anti-JH analogues were used on 2nd, 3rd, or early 4th instars, they induced premature metamorphosis. The times of molting were reduced and duration of larval stage was shortened (Lu et al., 1987). Up to now, most of the anti-JH analogues are imidazole substances and some of these have been used in practical production.

In this study, administration of anti-JH (Jinlu) in the 4th instar of silkworm *Bombyx mori* delayed the 4th instar stage that just like the 5th instar duration of the control, but reduced the times of molting and induced 100% trimolters. When treated with anti-JH in the 5th instar, the larval stage was delayed for about 1 day.

Prothoracicotropic hormone (PTTH, a kind of brain hormone) is produced by neurosecretory cells and transported to the corpora allata, along with the neurosecretory cell axons and at appropriate times in the developmental cycles, is released into the haemolymph to activate the prothoracic glands and initiate the synthesis of ecdysone (Williams, 1986). The ultra-structural changes of the corpora allata and prothoracic gland treated with anti-JH indicated that the corpora allata has deposited great number of the neurosecretory granules (brain hormone) but that the gland itself has no endoplasmic reticulum(ER). On the other hand, a clear micropile layer and large nucleus, large quantity of chromosomes, mitochondria, endoplasmic reticulum (ER) were seen in the prothoracic gland after administration of anti-JH in the early of 4th or 5th instar. These results revealed that anti-juvenile hormone inhibits the corpora allata secretions and initiates the activity of the prothoracic glands.

ACKNOWLEDGEMENTS

The first author is very thankful to Prof. Lu Xuefang and Prof. Xu Junliang of Zhejiang University, for their kind supervision.

References

Akai, H., Kimura, K., Kiuchi, et al., 1984. Effects of anti-juvenoid treatment on cocoon and cocoon filaments in *Bombyx mori*. *J. Seric. Sci. Jpn.*, **53**(6):545 – 546.

Akai, H., Kiuchi, M., Kimura, K., 1986. Effects of anti-JH treatment on the size and fine structure of the cocoon filament of *Bombyx mori*. *J. Seric. Sci. Jpn.*, **55**(**5**):388 – 396.

Akai H., 1989. Supresive effects of an imidazole derivative KK – 42 on JH levels in haemolymph of *Bombyx mori*. *J. Seric. Sci. Jpn.*, **58**(1):73 – 74.

Bowers W. S., Ohta, T., Cleere, J. S., et al., 1976. Discovery of insect anti-juvenile hormone in plants. *Science*, **193**(4253):542 – 547.

Gibert LI, Rybczynski R, Tobe S., 1996. Endocrine cascade in insect metamorphosis. *In*: Gilbert LI, Tata J., Atkison P. (eds): Metamorphosis: Post-Embryonic Reprogramming of Gene Expression in Amphibian and Insect Cells. Academic Press, San Diego, p.59 – 107.

Kiguchi, K., Mori, T., Akai, H., 1984. Effects of anti-juvenile hormone ETB on the development and metamorphosis of the silkworm *Bombyx mori*. *J. Insect Physiol.*, **30**(6):499 – 506.

Kiuchi, M., Kimura, K., Akai, H., 1985. Induction of trimolters from a tetramolter strain of *Bombyx mori* by anti-juvenile hormone treatment. *J. Seric. Sci. Jpn.*, **54**(**1**):77 – 81.

Lu Xuefang, Li Rongqi, 1987. Study on the trimolter induction of SM – 1 to produce superior silk filament. *J. of Sericultural Science*, **13**(2):71 – 75.

Miao Yungen, Yu Shengwen, Ren Xiaomi, 1996. Mechanism of anti-JH and molting hormone to silkworm *Bombyx mori*. *J. of Zhejiang Agricultural University*, **22**(4):345 – 348(in Chinese, with English abstract).

Quistad, G. B., Cerf, D. C., Schooley, D. A., Staal, G. B., 1981. Fluoromevalonate acts as an inhibitor of insect juvenile hormone biosynthesis. *Nature*, **289**(5794):176 – 77.

Riddiford LM, 1994. Cellular and molecular actions of juvenile hormone: generalconsiderations and premetamorphic actions. *Adv Insect Physiol.*, **24**:213 – 274.

Sehnal F., 1989. Hormone role of ecdysteroids in insect larvae and during metamorphosis. *In*: Koolman J. (ed), Ecdysone: From Chemistry to Mode of Action, Georg Thieme Stuttgart, p.271 – 278.

Sato, T., 1968. A modified method for lead staining of thin sections. *J. Electron Micr.*, **17**:157 – 159.

Staal G. B., 1986. Anti-juvenile hormone agents. *Ann. Rev. Entomol.*, **31**:391 – 429.

Williams C. M., 1986. The present status of the brain hormone. *In*: Beament J. W. L. And Treherne J. E. (ed), Insect and physiology, Oviver & Boyd, Edinburgh and London, p.133 – 139.