Archives of Insect Biochemistry and Physiology 2:397–404 (1985)

Critical Roles for Juvenile Hormone and Its Esterase$^+$ in the Prepupa of *Trichoplusia ni*

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In the caterpillar *Trichoplusia ni* (Lepidoptera: Noctuidae) it has been demonstrated by allatectomy that the appearance of juvenile hormone during the prepupal stage is crucial for the successful larval-pupal ecdysis of most larvae. Application of juvenile hormone or juvenile hormone esterase inhibitors at key times disrupted normal development as well. Thus the subsequent disappearance of juvenile hormone is regulated by degradation by juvenile hormone esterase in addition to a hypothetical reduction in biosynthesis. This reduction in juvenile hormone titer in the prepupa is just as critical for normal development as was its previous appearance. These observations on the critical role of juvenile hormone in the prepupa are in contrast to observations in some other species. For instance, in the case of *Manduca sexta* (Lepidoptera: Sphingidae), juvenile hormone is considered only supplementary to the action of prothoracicotropic hormone in the postwandering stage and primarily is required for normal pupal development. It thus appears that even within the Lepidoptera the role of juvenile hormone in prepupal development can vary dramatically.

Key words: ***Trichoplusia ni*, allatectomy, juvenile hormone, juvenile hormone esterase, esterase inhibition**

INTRODUCTION

Insects go through a sequence of endocrinological events resulting in metamorphosis from the immature to the adult state. In the holometabolous insect orders, juvenile hormone declines in the last larval stadium, so that

Acknowledgments: This work was supported by grant R01 ES02710-05; B.D.H. was supported by Research Career Development Award ES 00107-05, both from the National Institutes of Environmental Health Sciences, as well as the Herman Frasch Foundation. We express our appreciation to Dr. Davy Jones for assistance with these experiments and to him, Dr. Douglas Dahlman, and Dr. Thomas Sparks for helpful comments on the manuscript.

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Received July 23, 1984; accepted April 8, 1985.

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the subsequent release of ecdysone, a steroid hormone, can commit the larva to molt to a pupa [1,2]. In the early last larval stadium of Lepidoptera, the hemolymph JH* titer declines as the feeding stage terminates [3–5]. This decline in JH allows the release of ecdysone and results in wandering behavior and formation of the prepupa [2,6,7]. However, it is not until a second period of ecdysone release in the prepupa that the actual molt to the pupa takes place. In all of the Lepidoptera examined a prepupal burst of JH also occurs, but, as discussed below, the role of this JH burst has not been firmly established. Also most of the evidence presented on JH regulation emphasizes synthesis [1–3,6,8–11], and little in vivo evidence exists for the importance of degradation [12].

Although reports indicate that prepupal JH plays a supplementary role in stimulating the prothoracic glands [2,13–16], most available evidence still suggests that a brain neurohormone (PTTH) is the hormone absolutely required for the stimulation of the glands. Some larvae apparently deprived even of brain PTTH ultimately will pupate but only after long delays. The larvae of most species studied that are deprived of JH during the prepupal stage successfully ecdyse to the pupa either on time or after short delays [2], and the only demonstrated need for JH in vivo in the late last stadium is to suppress certain adult characters in the pupa [17].

Our studies were designed to evaluate the role of JH and its esterase during the prepupal stage of the important pest family Noctuidae. This report further demonstrates that in the cabbage looper (*Trichoplusia ni* [Hübner]) the presence of JH is crucial for successful larval-pupal ecdysis. Second, it is shown that the subsequent disappearance of the prepupal JH is critical and is regulated by increasing the rate of degradation in addition to the more conventional hypothesis of regulation of biosynthesis [1,2,8,9,11]. This appearance and subsequent disappearance of JH must occur during a period of less than 12 h for normal development to proceed.

MATERIALS AND METHODS

Insects

Larvae of *T. ni* were raised at 28°C, 14:10 LD (day begins at lights on) as described elsewhere [18]. Once wandering behavior had begun, selected larvae were staged according to various developmental markers [19].

Chemicals

Juvenile hormone I (methyl [2*E*,6*E*,10-*cis*]-10,11-epoxy-3,11-dimethyl-7-ethyl-2,6-tridecadienoate) was obtained from Calbiochem-Behring (La Jolla, CA). EPPAT was obtained from Dr. Roy Fukuto (UC Riverside). The JH analog Ro 10-3108 was obtained from Hoffman-LaRoche.

*Abbreviations: CA, corpora allata; EPPAT, O-ethyl-S-phenyl-phosphoramidothiolate; JH, juvenile hormone; JHE, juvenile hormone esterase; PTTH, prothoracicotropic hormone; Ro 10-3108, epofenonane.

Experimental Treatments

The first experimental approach tested the role of JH in the prepupa. Larvae were allatectomized at selected times during the day of wandering (LD03) (see Fig. 1). In some tests, the removed CA were replaced immediately; other allatectomized larvae were treated topically with 100 nmol of JH I immediately after allatectomy. The experimental larvae were then observed for 48 h for successful ecdysis to the pupa. Larvae that pupated did so within 48 h.

To evaluate the role of JH degradation, wandering larvae and prepupae were treated topically with EPPAT, Ro 10-3108, or JH I four times at 6 h

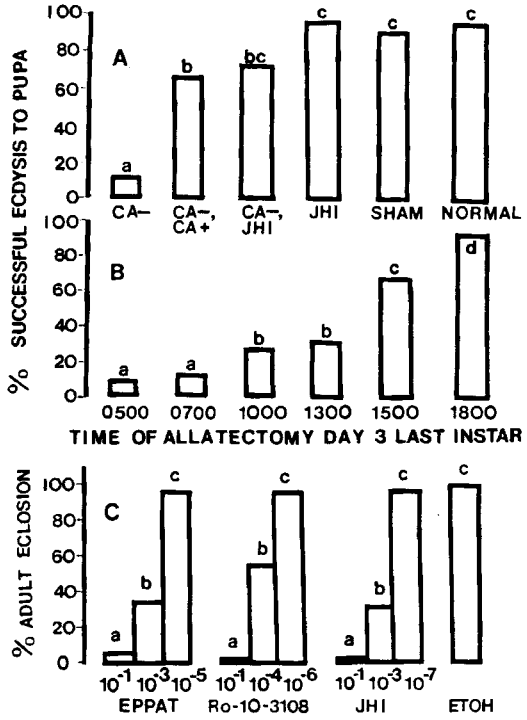

Fig. 1. Effect of allatectomy and reimplantation of corpora allata (A); time of allatectomy (B); and treatment with a JHE inhibitor (EPPAT), a JH mimic (Ro-10-3108), or juvenile hormone (JH I) (C) on the successful pupation or adult eclosion of *T. ni*. Each bar represents data from 12–27 larvae. Different letters indicate that the values were significantly different from each other at $P < 0.05$ by the χ^2 test. In A, allatectomies (CA –) were performed at 0700 h of day 3 of the last larval stadium (day begins with lights on). In B, allatectomies were performed at the times indicated during day 3 (day of wandering) of the last larval stadium of *T. ni*. The frequency of successful pupation by allatectomized larvae tended to increase with time (χ^2, $P < 0.05$) to such an extent that there were significant differences among most treatment groups. Pupation in sham-operated controls was not significantly different (χ^2, $P < 0.05$) from successful pupation in normal larvae until 1500 and 1800 h, when sham operation prevented molting in 10–15% of the larvae. In C, the bars represent the percentage of larvae to eclose to apparently normal adults. Larvae were treated four times at 6 h intervals beginning at 1100 h on the day of wandering with 1 μl of ethanol containing the indicated molarity of compound. There was no significant difference between normal and ethanol treated larvae.

intervals beginning at 1100 h on the day of wandering and observed for abnormal molting or abnormal pupal development. Finally, larvae were treated with various doses of EPPAT, Ro 10-3108, or JH I and observed through adult eclosion.

RESULTS

Importance of Appearance of Prepupal JH

Allatectomy of cocoon-spinning, last instar larvae usually prevents successful larval-pupal ecdysis, which normally occurs late the following day (Fig. 1A). The development of most of these allatectomized larvae is arrested at the early prepupal developmental marker of ocellar pigment retraction [19], which is far in advance of ecdysis (Fig. 2A). A smaller percentage of the larvae reach ecdysis but they still are unable to shed the old cuticle (Fig. 2B). Allatectomy and reimplantation of corpora allata in larvae on day 3 of the last stadium usually permits successful ecdysis (Fig. 1A), whereas allatectomy and replacement of the missing JH by application of exogeneous JH also induces completion of ecdysis (Fig. 1A). Previous studies established mid-to-late day 3 as a period of secretion of prepupal JH [19,20]. Allatectomy at progressively earlier time points during this interval of JH release results in fewer and fewer larvae achieving larval-pupal ecdysis (Fig. 1B). Shortly after 1300 h on day 3, sufficient JH has been released to enable 50% of the prepupae to complete development. The above series of experiments demonstrates that the CA are required organs in the prepupa and that JH is not simply supplementary but is indeed a vital hormone for most larvae to complete prepupal development and ecdysis.

Importance of Disappearance of Prepupal JH

Additional evidence indicates that the disappearance of prepupal JH is as mandatory and critical as was its brief appearance. Application of JH or a JH mimic to prepupae caused either an inability to shed the cuticle completely during ecdysis (Fig. 2C) or a failure of adult moths to emerge from the pupae. Dissections of these pupae reveal that they have undergone a pupal-pupal molt (Fig. 2F) or a molt to a nonviable pupal-adult intermediate. These results suggest that if the prepupal JH is not eliminated shortly after it has appeared normal metamorphosis cannot proceed. Previous work on *T. ni* has shown that there appears in the prepupal hemolymph a degradatory JH selective esterase within a matter of hours after the appearance of prepupal JH [12,19–21]. This enzyme converts the JH methyl ester to JH acid.

It has been demonstrated that EPPAT is a potent inhibitor of JHE in vivo and in vitro. Like the application of JH or juvenoids, it can disrupt normal development in the prepupa of *T. ni* [12]. Thus appplication of EPPAT prior to the JHE peak results again in blockage of successful larval-pupal ecdysis, pupal-pupal molts, or molts to nonviable pupal-adult intermediates apparently identical to those obtained with JH applications (Fig. 2C–F). Similar data also were obtained with 1,1,1-trifluoro-3-thiophenylpropan-2-one, which is a JHE inhibitor in a different chemical class than EPPAT [22]. The results of these experiments further demonstrate that prevention of natural degra-

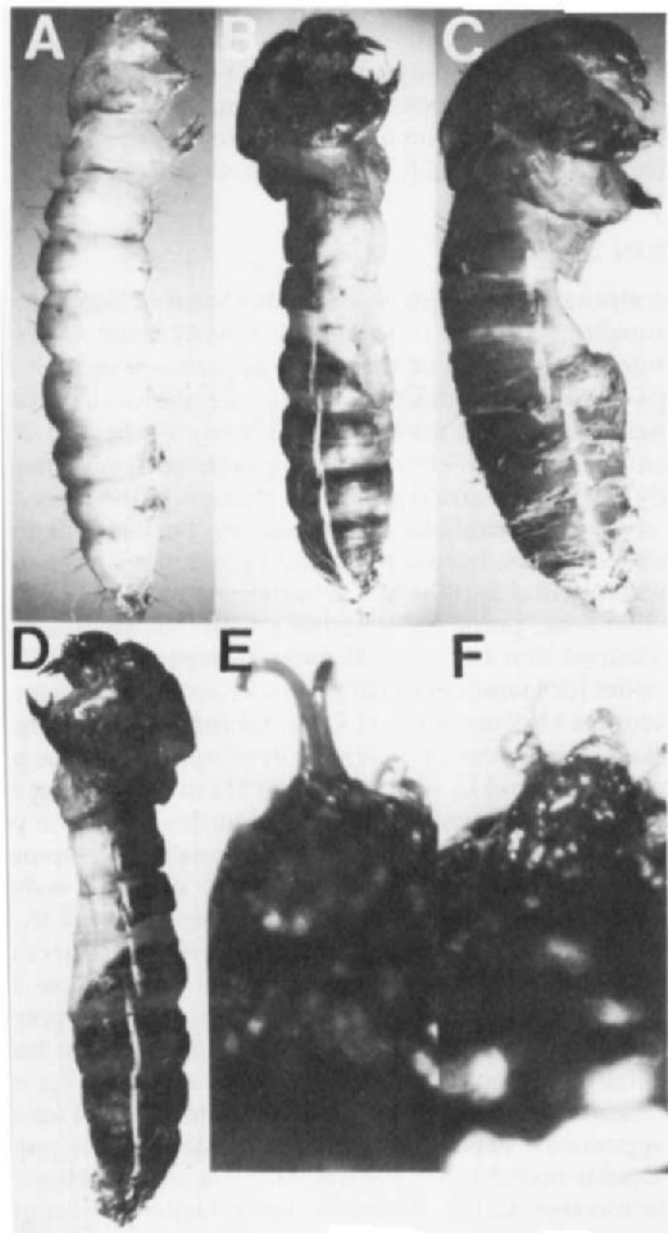

Fig. 2. Effect of disturbances in modulation of JH titer during prepupal development. In A and B, larvae were allatectomized at 0700 h on day 3 of the last larval stadium, resulting in a low JH titer. Development was permanently arrested at an early prepupal morphological marker corresponding to normal larvae at 0400 h on day 4 (A) or more rarely at a marker corresponding to 1100 h on day 4 (B), when ecdysis was attained but the larva was unable to shed its cuticle. Subsequent application of JH to the larvae shown in A could result in apparently normal pupation. When a high JH titer was maintained by application of 100 nmol of JH I (C) or EPPAT (D), as discussed in Figure 1C, larvae similarly were unable to shed their cuticle. F illustrates a pupal-pupal molt resulting from the application of 1 nmol of EPPAT to prepupae. Apparently identical second pupae also are obtained by application of JH to prepupae. The pupal cuticle has been removed to demonstrate the second pupal cuticle and caudal hooks (these structures are absent in the adult). E illustrates the abdominal tip and hooks of a normal pupa.

dation of JH by JHE will block metamorphosis and that a hypothetical decrease in the rate of synthesis of JH alone is not sufficient for rapid clearance of JH from the prepupa. These results with the JHE inhibitors effectively control for the possibility that pupal-pupal molts following JH application or CA implantation techniques previously used by many workers are an artifact of an unnaturally high, exogeneous source of JH.

DISCUSSION

The critical time element involved in this form of regulation via degradation is demonstrated by the fact that JHE activity must appear within hours of the JH burst. Prevention of this rapid appearance of high JHE activity in the prepupa disrupts metamorphosis. This situation is in striking contrast to that of vertebrates such as mice in which many studies [23–25] and reviews [26] have emphasized that changes in the titers of developmental hormones occur slowly and are governed largely by changes in the rates of biosynthesis. In fact, in vivo demonstrations of the need for hormone degradation by use of inhibitors that block hormone-degrading enzymes have not represented common experimental approaches in vertebrate endocrinology.

Prior to this time, proposed schemes on the nature of larval-pupal transformation claimed that either 1) JH must disappear during the last stadium (classical model for Hemimetabola) [27] or 2) as in the popular *Manduca sexta* model system for Holometabola [1,2,11], JH reappears during the last instar prepupal stage to suppress precocious development in the pupa of certain adult characters [17] and to supplement PTTH in stimulating the prothoracic glands to produce ecdysone [13–16]. Without this ecdysone peak, prepupal development will not occur and the larvae remain developmentally stationary at a point in development far in advance of pupal ecdysis [2,6]. This prepupal burst of JH does not appear to be so essential in the species of Lepidoptera most commonly used as model insects. Allatectomized *Bombyx mori* will still undergo prepupal development and ecdyse to pupae [28]. Likewise, allatectomized *Hyalophora cecropia* will undergo prepupal development and molt to pupae [9]. Although these pupae might have some adult characters, the allatectomy experiments in *H. cecropia* offer no evidence to support the current model of the necessity of prepupal JH for stimulating the ecdysone appearance needed to push the larvae towards pupation. Finally, the most popular model lepidopteran, *M. sexta*, also reaches the pupal molt when allatectomized [2,17]. Although some larvae are caught in the molt primarily in the head region, the fact that they have reached the pupal molt at all indicates an important distinction from *T. ni*. The primary role of the prepupal peak of JH in *M. sexta* thus seems to be in promoting normal pupal-adult development rather than having a profound effect on the pupation process itself. Although differences in response to allatectomy after attainment of the pupal molt might indicate some species-specific differences in JH effects at that stage, the important point in the context of larval development up to ecdysis is that the above species do not require JH for development of the prepupa to proceed.

Results using *T. ni* are in contrast to most previous studies with other lepidopterous species. In *T. ni* the appearance of JH in the prepupa is crucial for prepupal development to take place. Without JH, *T. ni* larvae remain developmentally stationary as very young prepupae. Because the effects of allatectomy in *T. ni* are dramatic and quantitative, it is a valuable system for investigating the role of JH in prepupal development.

This critical requirement of JH for development and ecdysis might extend to other families as well. For instance, allatectomizing second instar larvae of *Philosamia cynthia* results in the development of tiny prepupae (G.B. Staal, personal communication). This effect could be due to the tissues of young caterpillars not being competent to respond to ecdysteroids in the absence of JH. However, the observations are remarkably similar to those in *T. ni* in which competence is not in question. The present study of *T. ni* also suggests that a new component to the model should be added: the necessity of prepupal JHE for clearing late prepupal hemolymph of JH. The common occurrence of a peak of prepupal JHE in Lepidoptera [29] supports this *T. ni* model for the critical role of prepupal JHE.

The present study demonstrates that the brief appearance and subsequent rapid disappearance of prepupal JH allows only a small margin of error in regulation of this important hormone in last stadium larvae. This delicate balance involved in the timing of JH secretion and degradation dictates that, at least in *T. ni*, the prepupa is a far more critical point of endocrine regulation than was previously suspected. These data again demonstrate the need to duplicate a physiological observation in a variety of insect species. Simply with regard to the effect of allatectomy in the prepupa, the Lepidoptera can be divided into insects such as *B. mori*, which pupate normally, *M. sexta*, which can develop precocious adult structures, and *P. cynthia* and *T. ni*, which remain as prepupae. Such knowledge also could be exploited in the development of new insect control strategies or in the development of rapid anti-JH bioassays [30].

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