STUDIES ON THE MECHANISM OF ACTIVATION OF THE ECDYSIAL GLANDS BY CORPORA ALLATA IN BRAINLESS DIAPAUSING PUPAE OF THE CECROPIA MOTH (*HYALOPHORA CECROPIA* (L.)

A Thesis by

GEORGE W. DELEON

Submitted to Govindan Bhaskaran, Ph.D.

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Biochemistry

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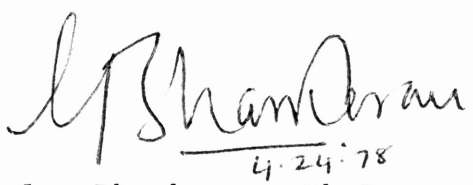

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ABSTRACT

STUDIES ON THE MECHANISM OF ACTIVATION OF THE ECDYSIAL GLANDS BY CORPORA ALLATA IN BRAINLESS DIAPAUSING PUPAE OF THE CECROPIA MOTH (*HYALOPHORA CECROPIA* (L.)

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Juvenile hormone I acid can activate the ecdysial glands thereby initiating adult development in brainless, diapausing pupae. Dauerpupae when implanted with two pairs of corpora allata from 1-3 day old adult male cecropia moths developed into pupal-adult intermediates. Injection of 100 ug of juvenile hormone I acid into dauerpupae yielded a diversity of pupal and adult development. Juvenile hormone I does not activate the ecdysial glands in dauer-pupae.

ACKNOWLEDGEMENTS

I wish to express my gratitude to the researchers and technicians at the Institute of Developmental Biology for their invaluable aid, time, and inspiration. I thank Timi and Vicki for making research a human endeavor rather than just a scientific inquiry. I thank Dr. Karl H. Dahm for supplying me with the juvenile hormone I and juvenile hormone I acid that I used in my experiments. I thank Paul Shirk for his suggestions, assistance, instruction, and his interrogations. Lastly, I have the greatest regard for my advisor, Dr. Bhaskaran, for his patient help and time while directing me through the labyrinthine interrelationships of insect endocrinology.

TABLE OF CONTENTS

PAGE

LIST OF FIGURES

Page

INTRODUCTION

A form of insect development familiar to most of us is that of the moths and butterflies. The sequence of developmental processes is called complete metamorphosis. In complete metamorphosis the adult female lays eggs which hatch into larvae. In the springtime the larvae feed, grow, and molt through five stages called instars. The larvae of the moths and butterflies are called caterpillars. After the fifth instar the larva molt into a pupa of the moths or into the chrysalis of the butterflies. In moths, coccoons usually enseath the pupae. In many lepidopteran insects such as the cecropia moth, a dormant period called diapause occurs during the pupal stage. During diapause, development is arrested and the metabolic rate is low. Physiologically, diapause is a natural and innate mechanism for survival against coldness and dryness. The cecropia moth has an obligatory diapause during winter. In the springtime the moth breaks through the remnants of its pupal cuticle and coccoon, and emerges as an adult.

The miraculous sequence of developmental processes beginning with the fertilization of the insect egg, passing through the five larval instars and the pupal stage, and finally culminating in the adult moth is hormonally controlled via the nervous and endocrine systems.

Gross dissection reveals that the endocrine system

of the cecropia moth consists of the lateral and medial neurosecretory cells in the brain and a neurohemal organ called the corpora cardiaca. Two pairs of epithelial endocrine glands are present. The corpora allata, a paired gland, lies adjacent to the corpora cardiaca. The ecdysial glands (also known as the prothoracic glands) are located in the foremost part of the thorax. (Fig. 1).

Researchers have demonstrated the endocrinological interrelationships between the brain, corpora allata, and the ecdysial glands, and have related their function with the developmental processes occurring during complete metamorphosis. Prothoracicotropic hormone (also known as brain hormone or ecdysiotropic hormone) is secreted by the neurosecretory cells in the brain, and induces the ecdysial glands to secrete ecdysone. Ecdysone, a steroid hormone, initiates molting, and therefore is frequently referred to as molting hormone. The corpora allata secrete juvenile hormone, a sesquiterpenoid derivative. In contrast to ecdysone, juvenile hormone does not initiate molting, but rather determines the character of the ensuing morphogenetic change. For example, larval epidermis exposed to ecdysone and high titers of juvenile hormone will reform larval epidermis after molting. If larval epidermis is exposed to ecdysone and trace titers of juvenile hormone , it will differentiate into pupal epidermis. Larval and pupal epidermis exposed only to ecdysone will

differentiate into adult epidermis. Hence, the developmental processes are initiated by ecdysone, whereas the transformations from larva to pupa, or from pupa to adult are dependent on the presence or absence of juvenile hormone. (Fig. 2).

Much of the pioneering work on the role of the brain in activation of the ecdysial glands has been done by Carroll Williams from Harvard University. In an early experiment, Williams extipated the brain from a diapausing pupa, and increased the ambient temperature to terminate diapause, and thereby initiate adult development. The dauerpupae did not develop and remained permanently in diapause. In another experiment Williams implanted a brain from a developing pupa into a dauerpupa. The pupa developed concomitantly. From these experiments Williams postulated that the brain is involved in the activation of the ecdysial glands. In another experiment Williams bisected a pupa traversely. After the implantation of an active brain, no development was initiated. However, if Williams implanted an active ecdysical gland from pupa in which development had started, the abdomens developed into adult-type abdomens with normal scales and pigmentation. Subsequently, Williams took inactive ecdysial glands from diapausing pupae and implanted them into the isolated abdomens. No development occurred. However, if Williams implanted an active brain and inactive ecdysial glands into

the isolated abdomens, development followed. From these experiments, Williams concluded that the brain releases a diffuseable hormone known as brain hormone, prothoracicotropic or ecdysiotropic hormone, and that the principal mode of activating the ecdysial glands occurs via the brain hormone.

In a paper published in April of 1959, Carroll Williams reported that the corpora allata from adult cecropia moths are more active in the adult than in any other stage of its life history. Williams also reported the unexpected and perplexing observation that dauerpupae which had been implanted with corpora allata from adult cecropia moths would initiate adult development. Instead of developing normally, these pupae molted into pupal-adult intermediates The question immediately arose on how could the corpora allata which secrete juvenile hormone, the classic juvenilizing hormone responsible only for the character of the morphogenetic change, be involved in the activation of the ecdysial glands .

Williams writes in his paper,

> It happened by chance that a pair of adult corpora allata was implanted into a brainless diapausing cecropia pupa. Ten days later, the host showed the termination of diapause, and the initiation of development. This result would have been puzzling in a normal diapausing pupa; in a brainless diapausing pupa, it was incomprehensible. Even more puzzling was the character of the development which then took place. Within two weeks the brainless pupa transformed, not into a moth but

> into a bizarre creature in which large areas of pupal cuticle had been freshly formed. The animal, in short, was a mosaic of pupal and adult characteristics.

In his experiments Williams generated pupal-adult intermediates approximately in twenty-three per cent of his experimental animals. The residual pupae remained in diapause. Up to this time insect endocrinologists had tacitly assumed that corpora allata from adult cecropia moths secrete juvenile hormone exclusively. Williams concluded that a hormone from the corpora allata can activate the ecdysial glands and therefore mimic brain hormone.

In a paper published several months later in Nature, Gilbert and Schneiderman explored the possibility that juvenile hormone could stimulate the ecdysial glands. Using ether extracts from the abdomen of the male cecropia moth (these extracts contained large quantities of juvenile hormone), Gilbert and Schneiderman attempted to demonstrate whether the ether extracts would provoke molting by stimulating the brain or by stimulating the ecdysial glands directly. Three dauerpupae were injected with 200, 400, and 600 milligrams of the ether extract. The two pupae which recived the two largest injections molted into second pupae. Hence, Gilbert and Schneiderman showed that the ether extracts would in fact stimulate the ecdysial glands.

In subsequent experiments, Gilbert and Scheiderman

tried to determine whether the activating factor was juvenile hormone or some other substance. Juvenile hormone was concentrated in the ether extracts by 100 to 500 fold. These extracts when injected into brainless dauerpupae were capable of activating the ecdysial glands. In another experiment, brainless diapausing pupae were injected with large quantities of ether extract in which juvenile hormone had been removed by countercurrent distribution. There was no activation of the ecdysial glands in these pupae, and hence no development. Since the procedures which concentrated the juvenile hormone might have conceiveably concentrated another ecdysial gland activating factor, or conversely those procedures which removed juvenile hormone might have conceiveably removed another factor capable of activating the ecdysial glands, Gilbert and Scheiderman concluded that juvenile hormone or some other molecule with similar properties may trigger the ecdysial glands. In light of the observation recorded by Williams, Gilbert and Schneiderman felt that the available evidence was consistent with the conclusion that juvenile hormone itself could activate the ecdysial glands.

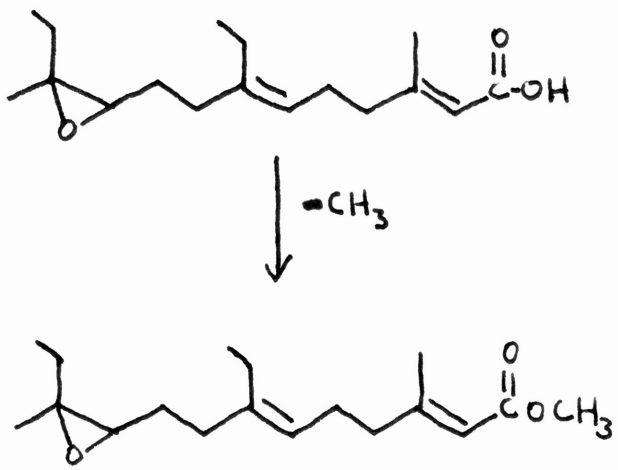
In recent years, workers at the Institute of Developmental Biology at Texas A&M University (Roller et al.) have demonstrated that in vitro corpora allata from adult male cecropia moths secrete juvenile hormone I acid almost exclusively. With respect to this fact, alternate interpretations may be proposed to explain the confounding

observations made by Williams.

Three alternatives exist:

Alternative 1:

The implanted corpora allata may in fact acquire the capability to convert juvenile hormone I acid to juvenile hormone I via methylation.



In this case an endogenous factor could induce the implanted corpora allata to methylate juvenile hormone I acid. Subsequently, juvenile hormone I activate the ecdysial glands as suggested by Gilbert and Schneiderman. Development ensues. (Fig.3).

Alternative 2:

The implanted corpora allata secrete juvenile hormone I acid continuously. A peripheral tissue conceiveably would methylate juvenile hormone I acid. Then the juvenile hormone would activate the ecdysial glands and development follows. (Fig. 4).

Alternative 3:

The implanted corpora allata secrete juvenile hormone I acid continously after implantation. Juvenile hormone I acid activates the ecdysial glands. Development of the pupa follows. (Fig . 5).

In my research I have explored these three possible alternative explanations. My experiments demonstrate that juvenile hormone I acid which has not been relegated a role in insect endocrinology may indeed have an important role. In my experiments I adduce evidence that Alternative 3 may explain the activation of ecdysial glands by the corpora allata.

MATERIALS AND METHODS

Animals:

Diapausing pupae (Hyalophora cecropia) were purchased from Lepidoptera Corporation. These pupae were kept chilled at 4 degrees Celsius for 2 to 3 months. The diapausing pupae would break diapause when the ambient temperature was raised to 25 degrees Celsius with a relative humidity of 70 to 80 per cent, and would complete adult development in two to three weeks.

Chemicals:

All reagents and solvents were reagent grade. Anhydrous ether was analytical reagent grade. Penicillin-streptomycin (1:1) was purchased from GIBCO (Grand Island, N.Y.). Weaver's saline was prepared in these laboratories. Juvenile hormone I and juvenile hormone I acid were synthesized in these laboratories by Dr. Dahm.

Preparation of juvenile hormone I for injection:

Juvenile hormone I in ether (0.729 ml of a solution with concentration of 2.06 mg/5 ml ether) was pipetted into a test tube. Olive oil (300 ml.) was added. After the solution was mixed, a slow stream of gaseous nitrogen was used to evaporate the ether. The final concentration was 300 ug juvenile hormone I/300 ul olive oil.

Preparation of juvenile hormone I acid for injection:

Pipette 2 ml of an ether solution of juvenile hormone I (3.9 mg JH I/3.9 ml ether) into a test tube. Pass a stream of nitrogen through solution to evaporate the ether. Add 100 ul of ethanol. Shake. Add 1900 ul of insect Ringer's solution. Sonicate in water bath for five minutes. Take 100 ul of the sample. Extract with ethylacetate. Evaporate the ethylacetate extract to dryness with a stream of nitrogen. Redisslove in 100 ul of ethylacetate. Spot 10, 30, and 60 ul on a chromatograph plate. Spot similiar amounts of juvenile hormone I acid from the original sample to compare. Run in a benzene solvent system containing 15% ethylacetate and 1% acetic acid.

Let the chromatograph dry. Spray with p-molydate. The final concentration is 2mg juvenile hormone I acid/ 2 ml insect Ringer with 5% ethanol.

Injection of pupae:

Narcotize with gaseous carbon dioxide. Inject dorsolaterally between the sixth and seventh abdominal segments in an anterior direction.

Ablation of brain from diapausing pupae:

Chilled diapausing pupae were first narcotized with gaseous carbon dioxide. Sterilize head area with 70% (v/v) ethanol. Remove frons to expose epidermis. Cut epidermis and retract to expose the brain. Retract the optic lobes

medially. Sever the nerve tracts running from the deutocerebrum on each side. Remove brain. Add a pinch of penicillin- streptomycin into the wound. Add Weaver's saline to replace the lost hemolymph. Spread epidermis evenly to cover operating field. Replace frons and seal with melted paraffin.

Removal of corpora allata from adult cecropia moths:

Narcotize one to three old day adult cecropia moths with gaseous carbon dioxide. Remove scales from the dorsal part of the neck. Decapitate the moth at the juncture of the neck and thorax. Mount the head on a paraffin dish by passing a needle through the esophagus so that it exits via the mouth. Incise on the lateral part of the head in an anterior direction. Retract the chitinous exoskeleton anteriorly thereby exposing the corpora allata at the base of the brain near the aorta. Remove glands with fine forceps. Keep glands in Weaver's saline until transplantation.

Implantation of corpora allata into brainless diapausing pupa:

Implant two pairs of corpora allata into fifth segment of the abdomen laterally. Add Weaver's saline to compensate for lost hemolymph. Add pinch of penicillin-steptomycin into wound. Seal incised cuticle with paraffin. Incubate the pupa at 25 degrees Celsius with a relative humidity of 70-80%.

Parabiosis of adult abdomens with brainless diapausing pupa:

Sever the male adult abdomen from the thorax. Remove the crop with forceps. Add saline into the abdominal cavity. Also add a pinch of phenylthiourea. Form a paraffin collar around the freshly cut edge of the abdomen. Cut a small opening in the thorax of a pupa. Form a paraffin collar aound this opening. Fill the body cavity of the pupa with Weaver's saline. Seal the abdomen and pupa together with melted paraffin so that the body cavities are confluent. Make sure that no air is trapped within the body cavities. Incubate the parabiosed animals at 25 degrees Celsius with a relative humidity of 70-80%.

EXPERIMENT AND RESULTS

Implantation of adult corpora allata:

Experiment #1. Two pairs of corpora allata from the male adult were implanted into brainless diapausing pupae. Twenty replicates were performed. Forty per cent of these pupae initiated adult development that yielded pupal-adult intermediates. These pupal-adult intermediates were unable to emerge from their old pupal cuticle. Pupal cuticle was located on the thorax and parts of the head. The abdomen, some of the head, and the antennae showed adult development.

Experiment #2. Two pairs of corpora allata from the female adult were implanted into brainless diapausing pupae. The corpora allata from the female adult cecropia moth secrete juvenile hormone I predominantly. In the event that juvenile hormone I were capable of activating the ecdysial glands, development could be expected. However, in the five replicates that were performed there was no devlopment.

In short, these two experiments are similar to the previous ones performed by Williams in which pupal-adult intermediates were observed. My experiments show that corpora allata from the adult male cecropia moth may in fact be involved in the initiation of development.

Parabiosis of adult male abdomen with dauerpupae:

Experiment #3. The abdomen from an adult male cecro-

pia moth (1-3 days old) was parabiosed with a brainless diapausing pupa. Of the fifteen replicates performed, no development occurred in any of the experimental animals.

In his master's thesis submitted to the Graduate College at Texas A&M University, Paul Shirk demonstrated that the accessory sex glands, located in the abdomen of the adult male cecropia moth, are repositories of juvenile hormone I. When a male abdomen is parabiosed to a pupa juvenile hormone will diffuse into the pupal body cavity via the hemolymph. If a male abdomen is parabiosed to an intact pupa and if the newly parabiosed pupa allowed to develop, a pupal-adult intermediate will form. The pupal characteristics can be attributed to the presence of juvenile hormone I. On the other hand, an unparabiosed pupa will develop into normal adult after two to three weeks once diapause is terminated. If juvenile hormone I were to activate the ecdysial glands, a parabiosed dauerpupa would be expected to develop into a pupal-adult intermediate. Since none of the parabiosed pupae developed, juvenile hormone I probably lacks the capability of activating the ecdysial glands.

Injection of juvenile hormone I and juvenile hormone I acid:

Experiment #4. Brainless diapausing pupae were injected with 10 ug of juvenile hormone I. None of the thirty animals which were injected showed any development and the pupae remained permanently in diapause.

Experiment #5. Fifteen dauerpupae were injected with 10 ug of juvenile hormone I acid and incubated at 25 degrees Celsius with a relative humidity of 70-80%. None of the animals developed.

Experiment #6. Fifteen dauerpupae were injected with 100 ug of juvenile hormone I acid. Eight of these pupae remained permanently in diapause and did not show any signs of development. The other seven initiated adult development after three to four weeks yielding two complete and normal adults. One adult managed to break through the remains of its pupal cuticle; however, it could not inflate its wings. Both adults showed correct pigmentation and scales. On the other hand, the five remaining pupae were pupal-adult intermediates. These intermediates had adult type abdomens, wings, and antennae. Parts of the upper abdominal segments and thorax were pupal predominantly. This experiment is significant because it suggests that juvenile hormone may indeed activate the ecdysial glands thereby initiating the cascade of developmental processes leading to the adult characteristics.

DISCUSSION

Two pairs of corpora allata from the male adult cecropia moth when implanted into brainless diapausing pupae initiated adult development in appoximately forty per cent of the experimental animals. Similarly, the injection of 100 ug juvenile hormone I acid initiated multifarious development yielding two complete adults and five pupal-adult intermediates. These experiments provide evidence that juvenile hormone I acid can activate the ecdysial glands in the absence of the brain.

Furthermore, the lack of development after injection of 10 ug of juvenile hormone I and after the implantation of corpora allata from the female adult cecropia moth suggests that juvenile hormone I does not activate the ecdysial glands. Since dauerpupae did not initiate development when parabiosed to the abdomen of a male adult cecropia, juvenile hormone I alone does not seem capable of mimicking brain hormone by activating the ecdysial glands.

In general, my work suggest lucidly that juvenile hormone I acid has the capability of activating the ecdysial glands and thereby potentiates brain hormone. This finding attributes a significant endocrinological role for juvenile hormone I acid. If the corpora allata can activate the ecdysial glands via juvenile hormone I

acid, conceiveably juvenile hormone I acid could be involved in the larval-pupal transistion which occurs during metamorphosis. This prospect is hinted since juvenile hormone titers go down during the fifth instar whereas the activity of the ecdysial glands increases. In the tobacco hornworm (Manduca sexta), juvenile hormone titers decrease during the fifth instar. Vince and Gilbert have shown that there is a corresponding increase in the titers of juvenile hormone esterase, the enzyme which demethylates juvenile hormone I to juvenile hormone I acid. Concomitantly, the levels of ecdysone increase as the ecdysial glands become more active.

The activity of the ecdysial glands is controlled by various hormones, in fact. Brain hormone has been considered classically the primary means by which the ecdysial gland's activity is modulated during the lifecycle of the cecropia moth. My experiments have the implication that juvenile hormone I acid functions as part of the network by which the ecdysial glands are activated and could possibly operate synergistically to control molting during complete metamorphosis. These possibilities await further verification as the interactions of the endocrine system of the lepidopteran insects are scrutinized more fully.

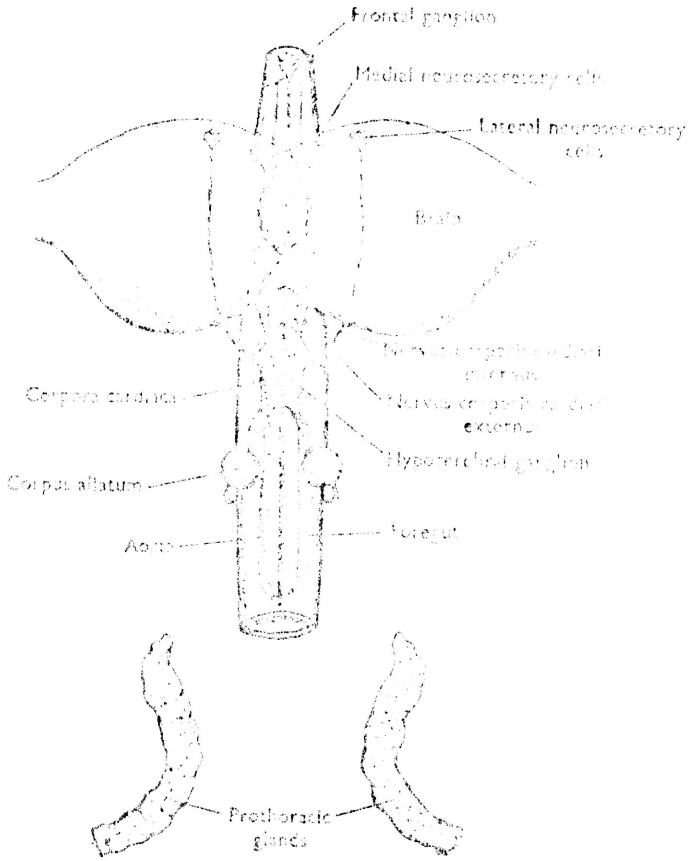


Fig. 1. The insect endocrine system and associated structures.

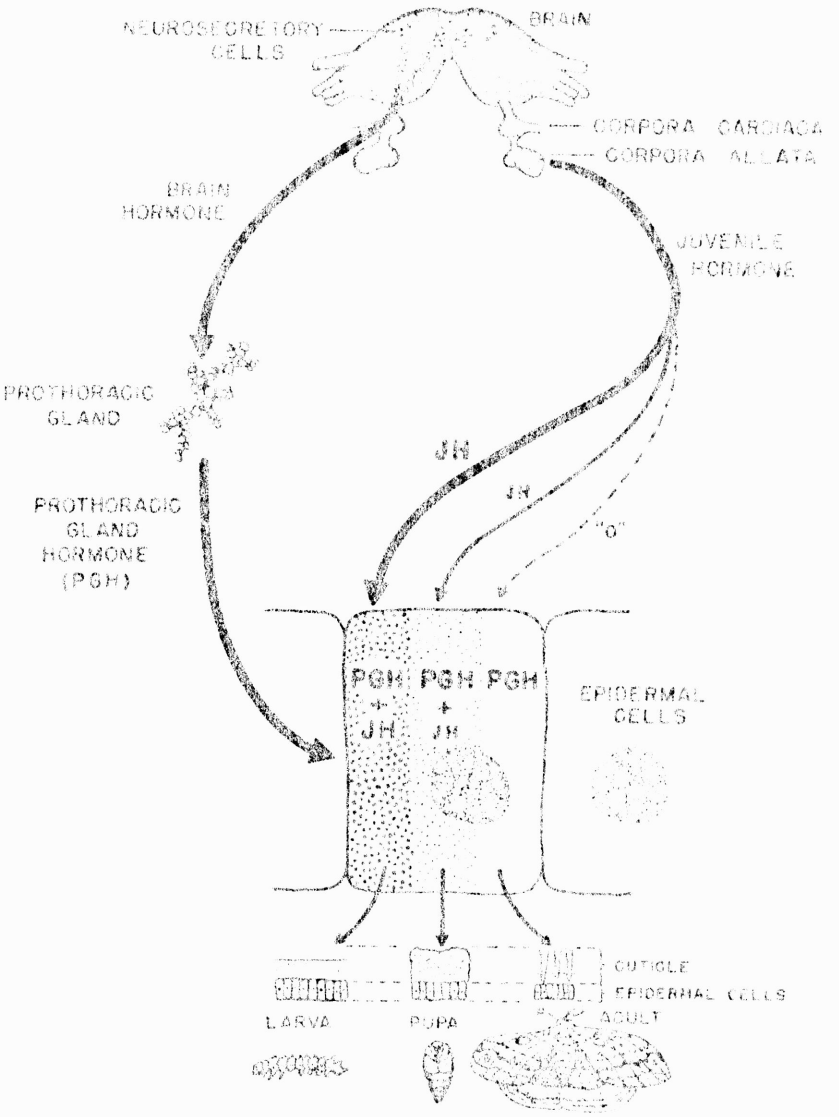


Fig. 2. Principal endocrine organs of the cecropia moth and the mode of action of their hormones.

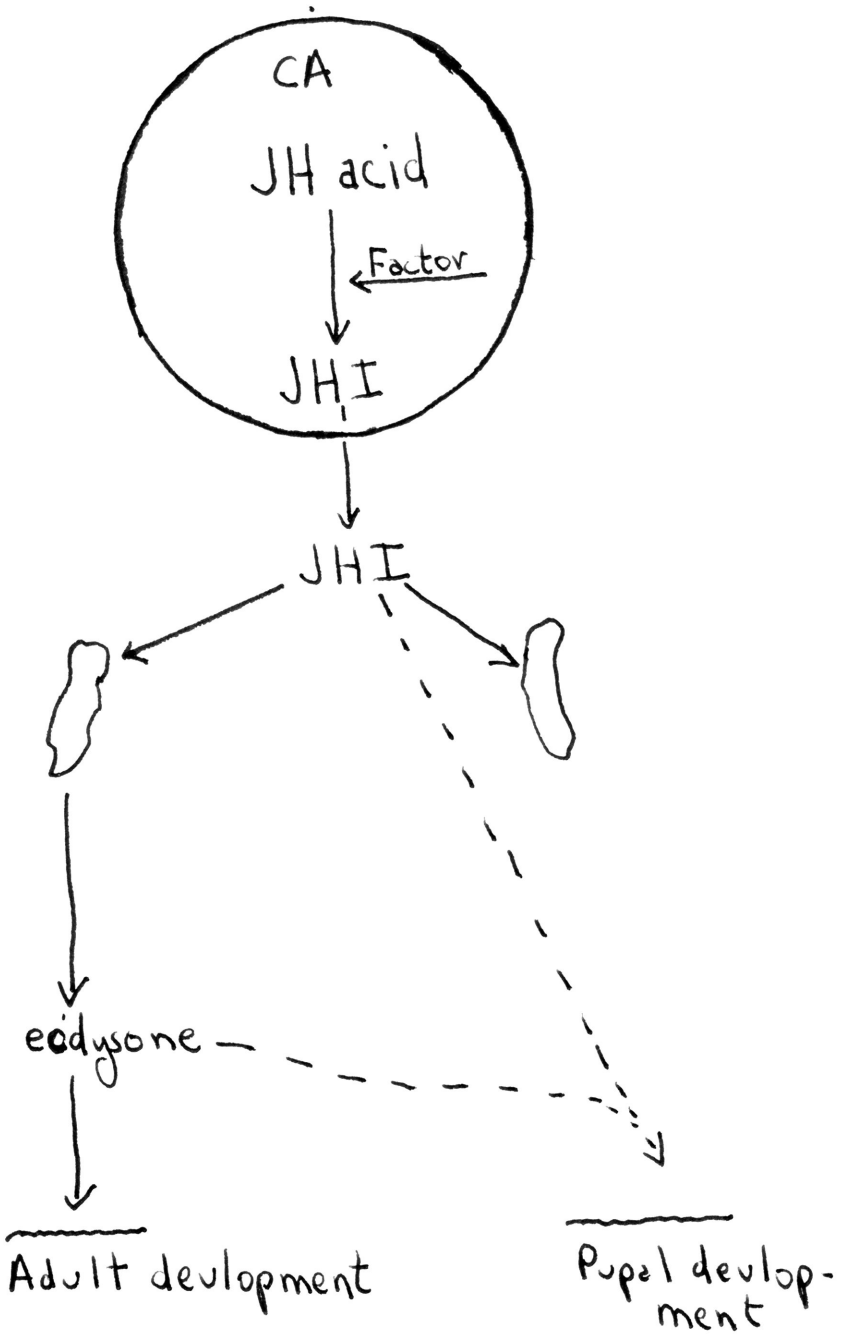


Fig. 3. Alternative 1.

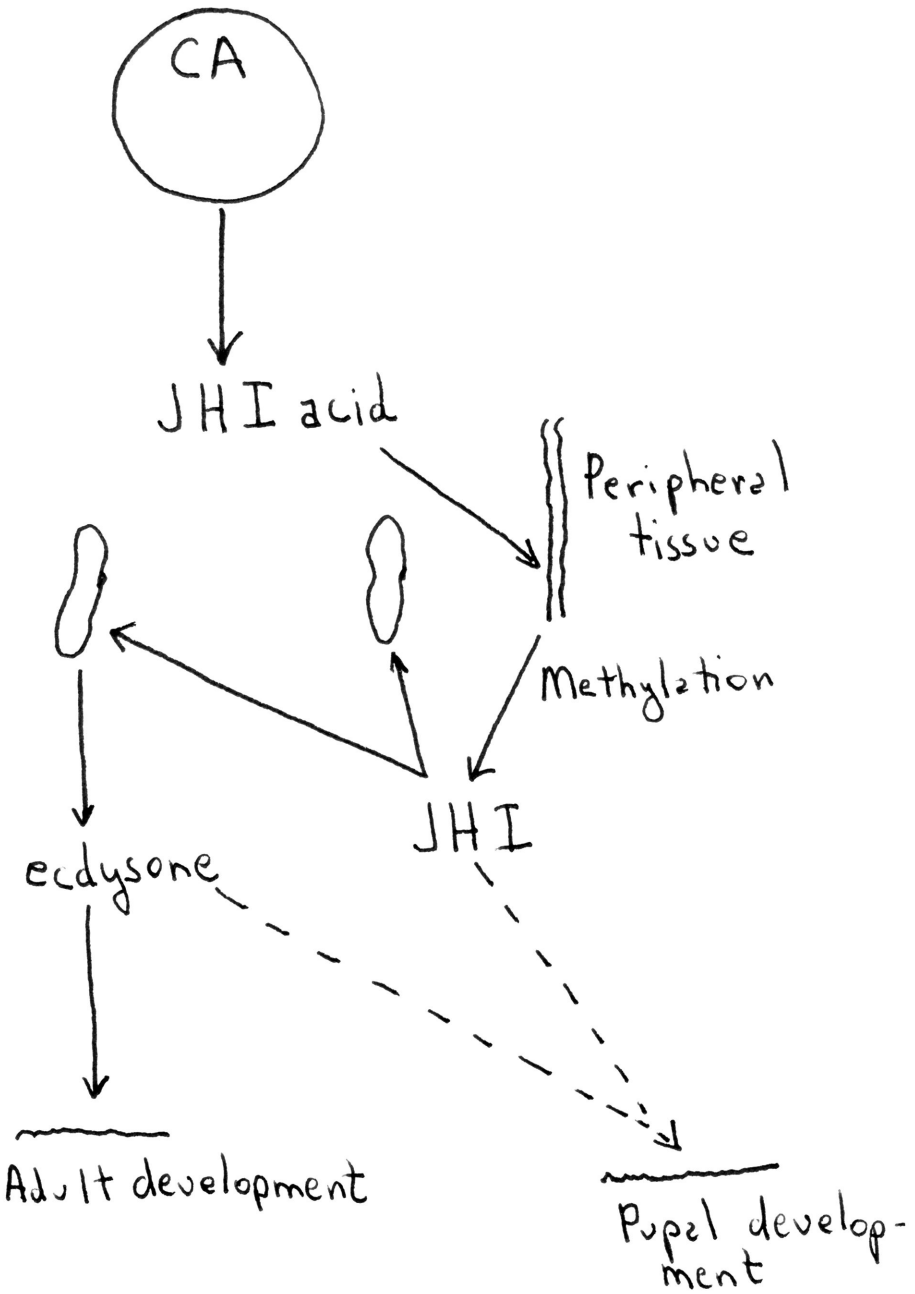


Fig. 4. Alternative 2.

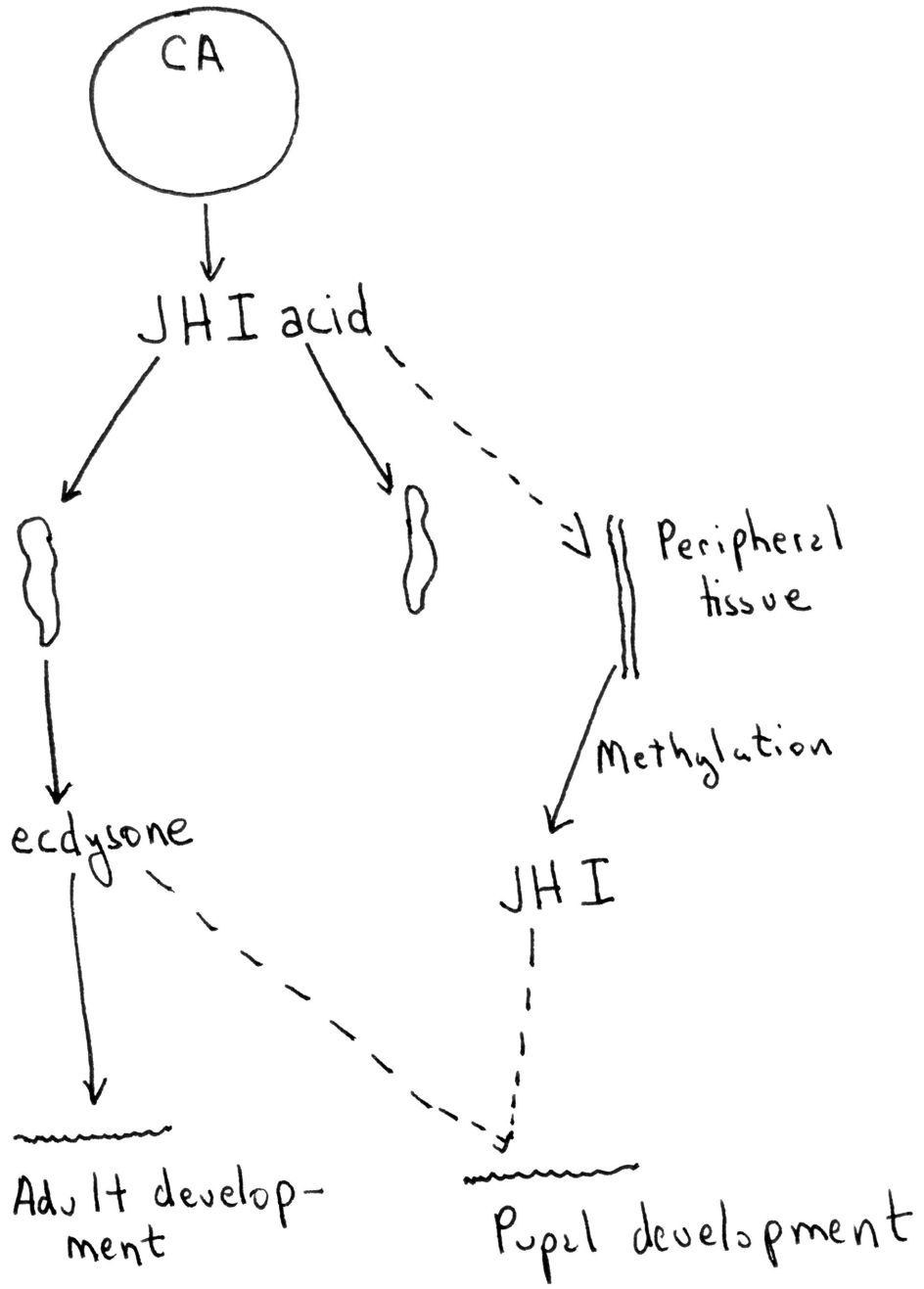


Fig. 5. Alternative 3.

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