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Developmental changes in the ability to synthesize juvenile hormone in vitro by corpora allata from the Eri silkworm, *Samia cynthia ricini* (Lepidoptera: Saturniidae)

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Key words. *Samia cynthia ricini*, developmental changes; corpora allata; juvenile hormone; radiochemical assay

Abstract. A radiochemical assay (RCA) has been used for the measurement of juvenile hormone (JH) synthesis in vitro by corpora allata (CA) from the Eri silkworm, *Samia cynthia ricini*. Using CA from newly emerged female adults for the bioassay, the most suitable incubation conditions were determined. A high rate of JH synthesis was found in medium TC199 at pH 6.5, 30°C and 4 mM Ca^{2+}. The time course of JH synthesis showed a steady decrease during the first 6 hours of incubation.

Under optimal incubation conditions, CA from the 4th and 5th larval instars, pupae and adults were used for measuring JH synthesis in vitro. The highest rates of JH synthesis were found on the 1st day of both larval stages, and then JH synthesis decreased steadily during the following two days of each instar. From the 4th day of the 5th larval instar to the 2nd day after pupation, CA could still synthesize small amounts of JH. However, from the 3rd day of the pupal stage to 12 hours before adult emergence, no JH release was observed. About 6 hours before emergence, CA of both female and male pharate adults regained the ability to synthesize JH. JH synthesis increased to a maximum shortly after emergence and then decreased again during the following two days. During this period, JH synthesis in vitro by CA from females was always higher than that of males. This is the first report on JH synthesis in vitro by CA from both female and male pharate adults and adults of a lepidopteran species, where the adults do not feed, are relatively short-lived, mate only once, and ovarian maturation and vitellogenesis are completed before emergence.

INTRODUCTION

Juvenile hormone, a unique insect sesquiterpenoid hormone, is produced by the corpora allata (CA). During insect development, juvenile hormone (JH) maintains the juvenile character. Metamorphosis to the pupa or adult form requires a reduced level or absence of JH (reviewed by Riddiford, 1994). In the adult stage, JH regulates many reproductive functions, including vitellogenesis, spermatogenesis and growth of the accessory sex gland (ASG) (reviewed by Wyatt & Davey, 1996). The rate of JH synthesis appears to be regulated by stimulating and inhibiting neuropeptides, allatotropins (AT) and allatostatins (AST) (reviewed by Stay et al., 1994; Guan, 1996). Before studying AT and AST in an insect species, it is necessary to optimize the radiochemical assay (RCA) for the measurement of JH synthesis in vitro by corpora allata (CA) (reviewed by Feyereisen, 1985).

An RCA for JH synthesis was established in 1974 (Pratt & Tobe, 1974; Tobe & Pratt, 1974). When assaying glands of a species not previously studied, an optimal culture medium should be identified. Parameters such as pH and Na^+/K^+ ratio are very important (reviewed by Feyereisen, 1985; Yagi & Tobe, 2001). In addition, Ca^{2+} can act as a second messenger controlling JH synthesis. This means that the Ca^{2+} concentration in the medium will also affect JH synthesis (reviewed by Rachinsky & Tobe, 1996). Using the suggested incubation conditions for CA of the beetle *Coccinella septempunctata* (Guan & Chen, 1986), it was found that the rate of JH synthesis in vitro by CA from newly emerged female adult Eri silkworm *Samia cynthia ricini* was extremely low. In this paper, we present the most suitable incubation conditions for CA from *S. cynthia ricini*, the time course of JH synthesis and release, as well as a dose-response curve for methionine (Met).

Eri silkworm *S. cynthia ricini*, silkworm *Bombyx mori*, and tobacco hornworm *Manduca sexta* belong to three closely related families of Lepidoptera: Saturniidae, Bombycidae, and Sphingidae, respectively. The Lepidoptera may be divided into four distinct groups based on their gonadotropic hormones and other reproductive and biological characteristics, regardless of phylogenetic relationships (reviewed by Ramaswamy et al., 1997; Table 1). The silkworms *Hyalophora cecropia, S. cynthia ricini* and *B. mori* belong to group I, and *M. sexta* belongs to group III. *B. mori* and *M. sexta* are two important model insect species for studying insect biochemistry and physiology. Developmental changes in their abilities to synthesize JH in vitro have been studied in detail. We now report on JH synthesis in vitro by CA from *S. cynthia ricini* 4th and 5th larval instars, pupae, as well as pharate adults and adults of both sexes. This study is prerequisite for the identification of AT and AS from *S. cynthia ricini*, which is the main purpose of our research project.

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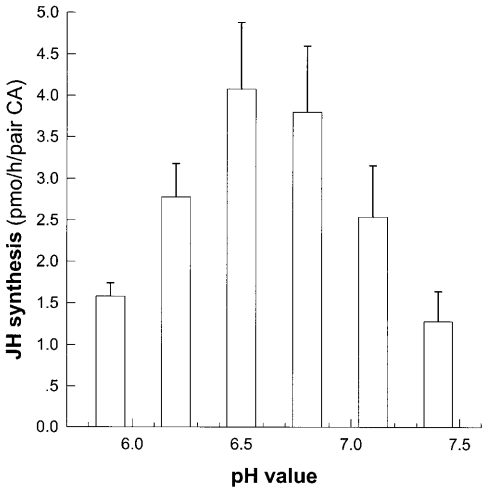

Fig. 1. JH synthesis in vitro by CA from newly emerged female adults of *S. cynthia ricini* at different pH values. Each point and bar represents the mean ± SE, n = 6. Compared with JH synthesis at pH 6.5, $P > 0.05$ at pH 6.8, $P < 0.05$ at pH 6.2 and pH 7.1, and $P < 0.01$ at pH 5.9 and pH 7.4 (t-test).

MATERIALS AND METHODS

Animals

S. cynthia ricini larvae and pupae were received from Zhengjiang, China. Larvae were reared on fresh castor-oil plant leaves at 25 ± 1°C under a 14h light (6:00–20: 00) : 10 h dark photoperiod. Developmental stages were adjusted at the time of each ecdysis and at wandering in the 5th larval instar. After pupation, the pupae were sexed, and males and females were kept separately under the above rearing conditions until emergence. Pharate adults were collected at 24, 12 and 6 hours before emergence for the CA assay. Newly emerged adults (less than 4 hours after emergence), as well as the 1- and 2-day-old adults (24 and 48 hours after emergence) were also used.

JH synthesis in vitro by CA

The suggested incubation conditions for CA of the beetle *C. septempunctata* (Guan & Chen, 1986) were at first used for incubation of CA from newly emerged female adult *S. cynthia ricini*. Corpora cardiaca-corpora allata complexes (CC-CA) were dissected under a saline solution (0.111 g/l NaCl, 3.355 g/l KCl, 0.555 g/l $CaCl_2$, and 5.083 g/l $MgCl_2$, pH 6.5), and placed into medium TC199 (Gibco). The interval between extirpation of CC-CA and the commencement of incubation in the presence of radiolabeled methionine (Met) was about 30 min. One pair of CC-CA were incubated in 100 µl medium TC199 (KangDa, with L-Glu, Hank's salts and 25 mM HEPES buffer, without $CaCl_2$ and Met, pH 7.2) containing 1 µCi ^{3}H-Met (NEN; specific activity 100 mCi/mmol; initial concentration 10 mM; final concentration 100 µM), 1.3 mM $CaCl_2$, 0.2% Ficoll (type 400, Sigma), and 0.1% bovine serum albumin (Sigma). Routinely, gland pairs were randomized into small groups and incubated in the dark for 3 hours at 30 °C with gentle agitation. Then, 300 µl isooctane was added to terminate the incubation. After vortexing and centrifugation, 200 µl supernatant was used for measuring radioactivity (DPM) in an LS6500 scintillation counter (Beckman) using a scintillation cocktail of toluene + 0.7% PPO + 0.05% POPOP.

Additionally, some material extracted with isooctane was subjected to NP-HPLC and RP-HPLC separation. Radiolabeled JH I, II and III were found, but JH II was the main JH released by CA of this insect species (Li et al., unpublished data).

In order to obtain the optimal rate of JH synthesis and release, the incubation conditions in medium TC199 (pH value, temperature, Ca^{2+} concentration, Na^+/K^+) were modified as follows: pH values of 5.9, 6.2, 6.5, 6.8, 7.1, and 7.4 at 4 mM or 1.3 mM Ca^{2+}concentration; temperature at 20, 23, 25, 28, and 30°C at pH 6.5 and 4 mM Ca^{2+} concentration; Ca^{2+} concentration of 0, 0.5, 1, 2, 4, and 8 mM at pH 6.5 and 30°C. Under the most suitable conditions derived from the above experiments, three experimental groups were tested: 1, addition of 10 µl of 0.9% NaCl; 2, incubation for 3 hours in light; 3, incubation of single CA instead of the CC-CA.

Under the most suitable incubation conditions, the time course of JH synthesis was followed over 6 hours of incubation with different glands for each period, and a dose-response curve for Met was established at concentrations of 2.5, 5, 10, 20, 50, 100, and 200 µM.

Bioassay for developmental changes

CC-CA were dissected from both 4th and 5th instar larvae and from pupae in daily intervals at 9:00. CC-CA were also isolated from the 4th instar larvae and during the first 3 days of the 5th larval stages at PM 21:00. All pupae and adults were sexed. CC-CA were dissected from pharate adults at 24, 12 and 6 hours before emergence, from newly emerged adults, as well as from 1- and 2-day-old adults of both sexes.

RESULTS

Improvement of incubation conditions in medium TC199

Effect of pH on JH synthesis

In all of the following experiments except those of "Developmental changes in JH synthesis", the assayed glands were from newly emerged female adults.

The influence of extra-glandular pH on the rate of JH synthesis was investigated by modifying the pH of medium TC199 over the range of 5.9–7.4 at 4 mM Ca^{2+}. JH synthesis showed an optimum at pH 6.5, which corresponds to the pH of *S. cynthia ricini* hemolymph. Below or above pH 6.5, the rates of JH synthesis were drastically reduced (Fig. 1). At all pH values tested, rates of JH synthesis at 1.3 mM Ca^{2+} were less than half of that at 4 mM Ca^{2+} (data not shown). A pH of 6.5 was used in subsequent assays.

Effect of temperature on JH synthesis

Temperature has a great influence on the rate of JH synthesis over the range of 20–30°C. At 20°C, the rate of JH synthesis was as high as at 30°C, whereas JH synthesis was much lower at 23–25°C (Fig. 2). Incubation at 30°C was used in subsequent assays.

Effect of Ca^{2+} concentration on JH synthesis

The Ca^{2+} concentration in Medium TC199 affected the rate of JH synthesis (Fig. 3). Without Ca^{2+} in the medium, the rate of JH synthesis was very low. At least some of the CC-CA did not synthesize JH at all. In general, higher Ca^{2+} concentrations resulted in higher rates of JH synthesis over the range of 0–8 mM Ca^{2+}. Between 0 and 1 mM Ca^{2+}, the rate increase was linear. At 4-8 mM Ca^{2+} in the medium, the rate of JH synthesis was about 10 times that without Ca^{2+}. Four mM Ca^{2+} was used in subsequent experiments.

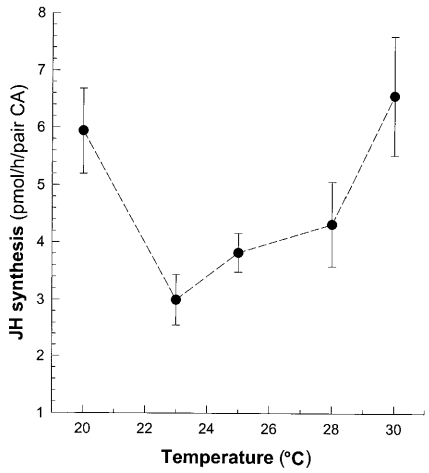

Fig. 2. JH synthesis in vitro by CA from newly emerged female adults of *S. cynthia ricini* at different temperatures. Each point and bar represents the mean ± SE, n = 6. Compared with JH synthesis at 30°C, P > 0.05 at 20°C, P < 0.01 at 25 and 28°C, and P < 0.001 at 23°C (t-test).

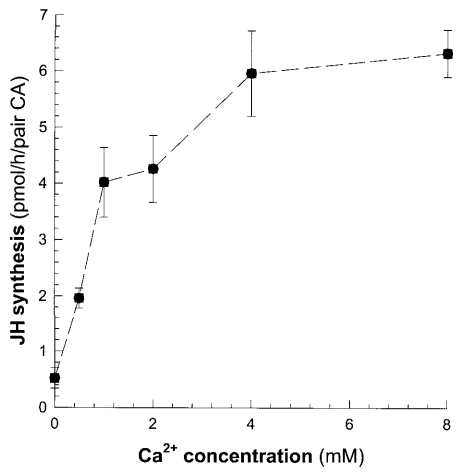

Fig. 3. JH synthesis in vitro by CA from newly emerged female adults of *S. cynthia ricini* at different Ca^{2+} concentrations. Each point and bar represents the mean ± SE, n = 6. Compared with JH synthesis at 8 mM Ca^{2+}, P > 0.05 at 4 mM Ca^{2+}, P < 0.01 at 1 mM and 2 mM Ca^{2+}, and P < 0.001 at 0 and 0.5 mM Ca^{2+} (t-test).

Additional factors affecting JH synthesis

When 10 µl of 0.9% NaCl was added to the incubation medium to raise the Na^+/K^+ ratio, no difference in the rate of JH synthesis between the experimental and the control group was found. Single CA carefully dissected from CC-CA showed similar rates of JH synthesis as the intact complex. In subsequent experiments, CC-CA were incubated. Incubation of CC-CA in light resulted in a rate of JH synthesis which was only half of that in the dark (data not shown).

Time course of JH synthesis

Under the most suitable incubation conditions, a time course of JH synthesis was determined for the first 6 hours of incubation. Fig. 4 represents JH synthesis on a cumulative basis. We found that JH synthesis decreased during the six hours of incubation. The rate of JH synthesis was highest during the 1st hour, but nearly no JH was released after 4 hours of incubation. CA were incubated for 3 hours in subsequent experiments.

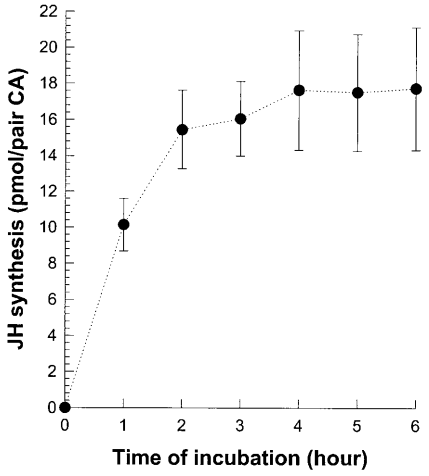

Fig. 4. JH synthesis in vitro by CA from newly emerged female adults of *S. cynthia ricini* during 6 hours of incubation. Each point and bar represents the mean ± SE, n = 6.

Dose-response curve for Met

The influence of Met on the rate of JH synthesis was investigated by incubating glands in medium TC199 containing various concentrations of ^{3}H-Met over the range of 2.5 to 200 µM. Fig. 5 shows that the highest rates of JH synthesis were obtained at Met concentrations ranging from 50 to 200 µM. Between 2.5 and 20 µM Met, the rates of JH synthesis increased linearly. 50 µM Met was used in subsequent experiments.

Developmental changes in JH synthesis

Changes in JH synthesis in the 4th larval instar

The 4th larval instar lasts for 3 days. CC-CA were extirpated and incubated at AM 9:00. JH synthesis in vitro by CA from newly ecdysed (the 1st day) larva of this instar was high (0.74 pmol/h/pair CA), then decreased steadily over the 2nd (0.48 pmol/h/pair CA) and 3rd (0.28 pmol/h/pair CA) days (Fig. 6).

JH synthesis was lower at 21:00 than at 9:00 AM of the 1st and 2nd day, respectively (data not shown). Therefore, from the 1st day to the 3rd day, JH synthesis decreased continuously, and did not show a change correlated with the time of day. However, at 21:00 PM of the 3rd day, when the 4th larval instar was preparing for ecdysis, JH synthesis was much higher (0.64 pmol/h/pair CA) than at 9:00 AM of the same day.

Changes in JH synthesis in the 5th larval instar

There are 11 days in the 5th larval instar, and wandering starts at day 7. Newly ecdysed (the 1st day) 5th larval instar showed the highest JH synthesis in vitro (1.18 pmol/h/pair CA), and JH synthesis decreased dramatically during the next 3 days (Fig. 6). From the 4th day to the 7th day (wandering), JH synthesis decreased only slightly and reached the lowest value at day 7. A drop during the last two days of the 5th larval stadium was followed by a small increase in JH synthesis at day 9.

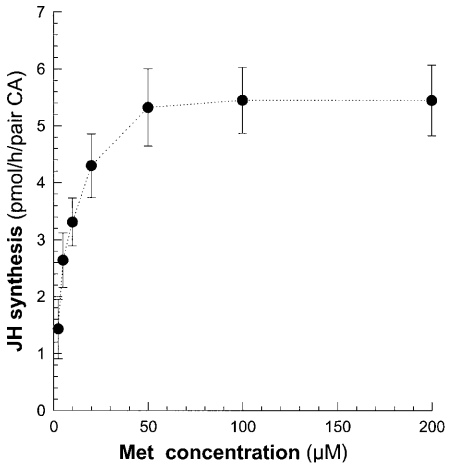

Fig. 5. JH synthesis in vitro by CA from newly emerged female adults of *S. cynthia ricini* at different Met concentrations. Each point and bar represents the mean±SE, n=6. Compared with JH synthesis at 50 μM Met, $P > 0.05$ at 100 μM and 200 μM Met, $P < 0.05$ at 20 μM Met, $P < 0.01$ at 10 μM Met, and $P < 0.001$ at 2.5 μM Met (t-test).

From the 4th to the 11th day of this instar, JH synthesis varied between 0.11 and 0.20 pmol/h/pair CA.

As in the 4th larval instar, JH synthesis in vitro by CA did not show a change correlated with the time of day during the first 4 days of the 5th larval instar. JH synthesis and release at 21:00 was always lower than at 9:00 of the same day.

Changes in JH synthesis in pupa

There are about 16 days in the pupal stage. CA still synthesized a very small amount of JH (0.07 pmol/h/pair CA) during the first 2 days of the pupal stage (Fig. 6), but no JH synthesis was observed from day 3 to day 6/7 of this stadium. It is very difficult to dissect intact CC-CA between day 7 and day 12 of the pupal stage. CA did not synthesize JH from day 12 until 12 hours before emergence. Six hours before emergence, CA from both female and male adults regained their ability to synthesize JH (Fig. 7).

Changes in JH synthesis in pharate adult and adult females and males

Pharate adults and adults were sexed and kept separately to prevent copulation. At 24 hours before emergence, a few black spots appear at the stethidium of the pupa (pharate adult), but the body is still hard. At 12 hours before emergence, many more black patterns form and the body begins to soften. CA of both female and male pharate adults at 24 to 12 hours before emergence did not synthesize JH. At 6 hours before emergence, all the stethidium of the pharate adult has blackened, some brown patterns also appear at the abdomen, and the body has become completely soft. At this time, CA from both female and male pharate adults regained their ability to synthesize JH. Female pharate adults synthesized 4.82 pmol/h/pair CA, males only 0.10 pmol/h/pair CA. Once CA regained the ability to synthesize JH, JH synthesis increased and reached a maximum at the time of emergence (0–4 hours after emergence). JH synthesis of young female adults was 6.56 pmol/h/pair CA and males 1.27 pmol/h/pair CA. During the following two days, JH synthesis of both females and males decreased steadily and rapidly. At 24 and 48 hours after emergence, JH synthesis was 3.44 and1.19 pmol/h/pair CA in females, and 0.32 and 0 pmol/h/pair CA in males, respectively (Fig. 7).

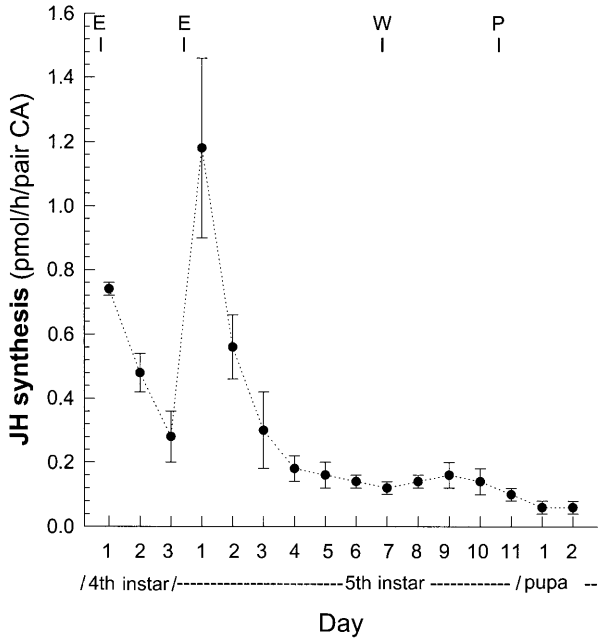

Fig. 6. JH synthesis in vitro by CA from 4th and 5th instar larvae and early pupae. Each point and bar represents the mean ± SE, n = 6. Compared with JH synthesis at the 9th day of the 5th instar ranging from the 4th day of the 5th instar to the 2nd day of the pupa stage, $P > 0.05$ at the 4th, 5th,6th, 8th, and 10th day, $P < 0.05$ at the 7th and 11th day of the 5th instar, and $P < 0.01$ at the first 2 days of the pupa stage (t-test). E – ecdysis; W – wandering; P – pupation.

In general, CA of both female and male pharate adults regained their ability to synthesize JH at approximately 6 hours before emergence, and JH synthesis increased to a maximum at the time of emergence, but later decreased. At this time, CA of female pharate adults and adults synthesized much more JH than did those of male pharate adults and adults.

DISCUSSION

The development of an RCA for measuring JH synthesis in vitro by CA has greatly facilitated research in various areas of JH biochemistry and physiology. In this assay, medium TC199 was first chosen as an optimal medium for the desert locust *Schistocerca gregaria* (Tobe & Pratt, 1974), then used for other insect species (Guan & Chen, 1986, Weaver et al., 1980), including Lepidoptera, *M. sexta* (Kramer & Law, 1980) and the corn earworm, *Heliothis armigera* (Guan et al., 1995). In this study, we used medium TC199 for the RCA in *S. cynthia ricini*. In general, pH value, Na^+/K^+ ratio and Ca^{2+} concentration are considered to be the most important parameters of the

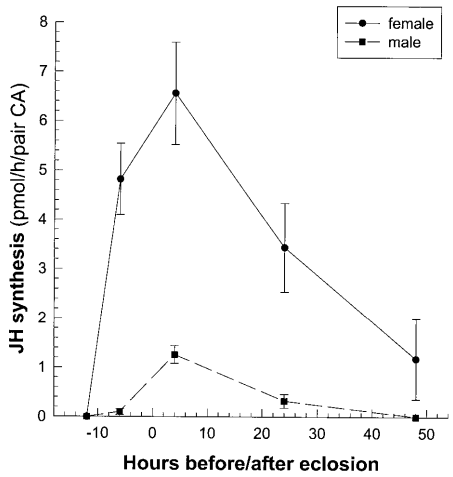

Fig. 7. JH synthesis in vitro by CA of pharate adults and 1- and 2-day-old adults of both sexes. Each point and bar represents the mean ± SE, n = 6. In both females and males, compared with JH synthesis at the time of emergence, $P < 0.01$ at all other times. Between females and males, $P < 0.01$ at all stages except 12 hours before emergence (t-test).

medium (reviewed by Feyereisen, 1985; Yagi & Tobe, 2001). The pH values of 7.2 and 6.5, the same pH as in the hemolymph, were used in *Tenebrio molitor* (Weaver et al., 1980) and *M. sexta* (Kramer & Law, 1980), respectively. It is not surprising that the most suitable pH in *S. cynthia ricini* was 6.5, also the same pH as found in the hemolymph. However, it appears that the Na^+/K^+ ratio does not have a great influence on JH synthesis in this insect species. It has been reported that Ca^{2+} can function as a second messenger in regulating JH synthesis (reviewed by Rachinsky & Tobe, 1996). When CA from *S. gregaria* (Thompson & Tobe, 1986), *M. sexta* (Allen et al., 1992), and cockroach *Diploptera punctata* (Kikukawa et al., 1987) were incubated in the absence of Ca^{2+}, little or no JH was synthesized. In *D. punctata*, JH synthesis correlated with Ca^{2+} concentration in a linear manner and was highest at 3–5 mM Ca^{2+} (Kikukawa et al., 1987). A similar dose-response curve of Ca^{2+} concentration was found in this study for *S. cynthia ricini*. Few data are available on the influence of temperature on JH synthesis. In most studies, 25°C or 30°C was chosen for the bioassay. However, in *S. cynthia ricini*, the rate of JH synthesis varied greatly over the temperature range of 20–30°C. Both pupae and adults were kept at 25°C, but the rates of JH synthesis were lowest at this temperature. It is difficult to understand why JH synthesis was lowest at the rearing temperature of the animals. In most insect species, including *M. sexta* (Kramer & Law, 1980) and *C. septempunctata* (Guan & Chen, 1986), JH synthesis was rather constant for several hours of incubation. However, a quite different time course of JH synthesis was seen in *S. cynthia ricini*. CA from this species synthesized JH in vitro for only about 3–4 hours. A similar time course was observed in *T. molitor* (Weaver et al., 1980), although the decrease in JH synthesis was not so rapid. It is possible that there are not enough precursors in the CA. In summary, high rates of JH synthesis were found at pH 6.5, 30°C, a Ca^{2+}concentration of 4 mM, and a methionine concentration of 50 μM.

S. cynthia ricini, B. mori and *M. sexta* belong to three closely related families of Lepidoptera (Table 1). The developmental changes in JH synthesis of these three insect species are compared below. Both similarities and differences have been found. No great differences in the developmental changes of JH hemolymph titers were observed between *M. sexta* and *B. mori* larvae. In *M. sexta*, high JH hemolymph titers were found at the beginning of the 4th (Hidayat & Goodman, 1994) and 5th larval instar (Baker et al., 1987). Another JH peak appeared at day 6 or 7 of the last larval stadium, 1 or 2 days after wandering (Baker et al., 1987). Similar developmental changes of JH hemolymph titers were seen in *B. mori*. In *B. mori*, however, JH hemolymph titers changed with photoperiod. At the end of each larval instar, JH titer started to increase concomitantly with ecdysis, which occurred during the photophase, and the peak titer was found at the end of the same photophase (Niimi & Sakurai, 1997).

In *M. sexta* 5th larval instar, developmental changes in JH synthesis in vitro did not completely correspond to the changes in JH hemolymph titers. High rates of JH synthesis in vitro were observed during the first two days of the 5th larva instar. However, the CA did not synthesize any JH at the 4th and 5th day of this instar (Kramer & Law, 1980). The reason why CA cannot synthesize JH at this time is the absence of methyltransferase activity, thus JH acid cannot be converted to JH. It was discussed that JH and JH acid (in males) were synthesized by imaginal discs during the period of wandering in this insect species (Sparagana et al., 1984; 1985). In *B. mori*, rates of JH synthesis by CA in vitro were similar to those in *M. sexta* (Niimi & Sakurai, 1997). However, it is still unclear whether imaginal discs in *B. mori* can synthesize JH and JH acid.

TABLE 1. Aspects of reproductive biology of four groups of Lepidoptera.

Type	Species	Egg development stage	Gonadotropic hormone	Adult feeding	Mating times
I	*Bombyx mori*	Larva	Ecd	No	Single
	Hyalophora cecropia	Larva/pupa	None	No	Single
	Samia cynthia ricini	Larva/pupa	None	No	Single
II	*Plodia interpunctella*	Pupa	Declining Ecd	No	Single
III	*Manduca sexta*	Pupa/adult	JH (chorionation only)	Yes	Several
IV	*Helicoverpa zea*	Adult	JH	Yes	Several
	Heliothis virescens	Adult	JH	Yes	Several

After Ramaswamy et al., 1997. Ecd = ecdysteroids.

In summary, a peak of JH synthesis or JH hemolymph titer occurs at the beginning of each larval instar in all three insect species. In *B. mori*, there are circadian changes in JH synthesis and JH hemolymph titer, but no changes in JH to photoperiod were observed in *S. cynthia ricini* and *M. sexta*. Similar developmental changes in JH synthesis were found in the 5th larval instar of both *S. cynthia ricini* and *B. mori*. Besides the peak at the beginning of this instar, there is a small peak of JH synthesis just after wandering. However, in *S. cynthia ricini* this peak is much smaller than in *B. mori*, and is not significant. In *M. sexta*, CA cannot synthesize JH from day 4 of this stadium to the day of pupation. From these results, it seemed worthwhile to investigate the developmental changes in JH hemolymph titers of *S. cynthia ricini*. We have developed both NP-HPLC and RP-HPLC to detect the nature of JH released by CA in vitro, and found that JH I, II and III were released into the medium and JH II was the main JH (unpublished data). Similar results were obtained in parasitized and non-parasitized *Heliothis virescens* (Li and Pennacchio, in press), where the same HPLC separation methods were applied.

CA from *M. sexta* female adults synthesized JH in high amounts, but CA from male adults did not (Kramer & Law, 1980). Further studies showed that there was a reactivation mechanism of CA in pharate adult *M. sexta*. About 18 hours before emergence, CA from female pharate adults were reactivated and began to synthesize JH until several days into the adult stage. CA from male pharate adults at the same time did not synthesize JH, but rather JH acid. This situation was maintained until several days after emergence. The reason why CA cannot synthesize JH in male pharate adults and adults is that again no methyltransferase activity is present, which converts JH acid to JH (Hebda et al., 1994).

In general, CA cannot synthesize JH in both female and male pharate adults and adults of the Lepidopteran group I, including *B. mori* and *H. cecropia* (reviewed by Ramaswamy et al., 1997). CA from adult Saturniidae *H. cecropia* did not synthesize JH (reviewed by Ramaswamy et al., 1997), although a great amount of JH-like substance was extracted from its abdomen (Kramer & Law, 1980). How can CA from pharate adults and adults of *S. cynthia ricini* synthesize JH? It is possible that a similar reactivation mechanism of CA exists in *S. cynthia ricini* pharate adults as in *M. sexta*. Nevertheless, several differences in JH synthesis in vitro were seen between these two species. The greatest difference was that CA from male pharate adults and adults of *S. cynthia ricini* synthesized small amounts of JH, but CA from *M. sexta* did not. Another difference was that the ability to synthesize JH was maintained for a much shorter time, and changes were more rapid in *S. cynthia ricini* females than in *M. sexta*. In fact, it is very difficult to distinguish CA between 12 and 6 hours before emergence by their outward appearances, each gland is large and transparent. In *S. cynthia ricini*, Mas-AT and AT factor cannot reactivate non-activated CA at 24 hours before emergence to synthesize JH at all, but can partially induce CA at 12 hours before emergence to synthesize a small amount of JH (0.1–0.4 pmol/h/pair CA, unpublished data). So it seems that Mas-AT and AT factor are involved in CA reactivation in this insect speices. However, in *M. sexta*, Mas-AT did not induce JH production in inactive female CA and did not increase methyltransferase activity at all, and it was concluded that reactivation of CA was a process distinct from stimulation of JH production (Hebda et al., 1994). It is very interesting that such a great difference exists between these two insect species. But why does JH synthesis by CA change so quickly? There is no doubt that some stimulating and inhibiting neuropeptides exist which regulate JH synthesis in *S. cynthia ricini*. AT and AS factors have been found in brain extracts during the process of purification (Li et al., in press).

In the early 1960s, a great amount of a JH-like substance was extracted from the abdomens of male adult *S. cynthia ricini* in our laboratory. Injection of a JH-like substance to early pupa (not longer than 48 hours after pupation) resulted in a second pupation and pupa-adult metamorphosis occurred later (Cao et al., 1963). We are now investigating if accessory genital gland (ASG) in male *S. cynthia ricini* can synthesize JH, or it can only convert JH acid to JH, and the main location of the source of JH in the abdomen of male adult *S. cynthia ricini*.

In general, it is believed that CA isolated from both female and male pharate adults and adults of Lepidoptera group I animals (where the adults do not feed and are relatively short-lived, mate only once, and ovarian maturation and vitellogenesis are completed before emergence) cannot synthesize JH (reviewed by Ramaswamy et al., 1997). The results of our studies on pharate adults and adults of *S. cynthia ricini* contradict the above conclusion. Therefore, how JH functions might be worthy of further studies in both female and male pharate adults and adults of *S. cynthia ricini*. It is possible that JH may trigger oviposition in females and stimulate copulation in males. We are now using classical physiological techniques, such as allatectomy, ASGectomy, and JH or JH analog treatment to test these hypotheses.

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