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Activity of the corpora allata of adult female Aedes aegypti: effects of mating and feeding

Yiping Li [a], Salvador Hernandez-Martinez [b], Gopalan C. Unnithan [c], René Feyereisen [d], Fernando G. Noriega [a,*]

[a] *Department of Biochemistry and Molecular Biophysics, and Center for Insect Science, University of Arizona, Tucson, AZ 85721, USA*
[b] *Centro de Investigaciones Sobre Enfermedades Infecciosas, Instituto Nacional de Salud Pública, Cuernavaca, Morelos, 62508, Mexico*
[c] *Department of Entomology, and Center for Insect Science, University of Arizona, Tucson, AZ 85721, USA*
[d] *Centre de Recherches d'Antibes, INRA, UMR 1112, France*

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Abstract

The synthesis of juvenile hormone III (JH III) by the isolated corpora allata (CA) of *Aedes aegypti* adult female was studied using an in vitro radiochemical assay. We dissected the corpora allata-corpora cardiaca (CA-CC) complex attached to a piece of aorta. The complex was left connected to the intact head capsule to facilitate the visualization and transfer of the glands. A linear increase in the cumulative amount of biosynthesized JH III was found for at least the first 6 h of incubation; approximately 45% of the synthesized JH III was present in the medium. There was a dependence of JH III synthesis on exogenous methionine supply. Using reversed phase high performance liquid chromatography two major labeled products biosynthesized by the CA were separated. They co-migrated with JH III and methyl farnesoate (MF). The identity of the biosynthesized JH III was confirmed by gas chromatography–mass spectrometry. JH III synthesis was only 2.0 fmol/pair gland/h immediately after adult emergence, but increased to 32.6 fmol/ pair gland/h 18 h later in sugar-fed females. Two days after emergence, the CA biosynthetic activity slowly started to decrease, and reached values of around 5.3 fmol/pair gland/h by one week after emergence. Synthesis of JH was similar from either sugar-fed females mated or unmated. A blood meal resulted in a decrease of JH III synthesis in CA from mated females by 12 h after feeding and from virgin females by 24 h after feeding. JH III biosynthesis remained low for at least 96 h in mated females, but was back to higher levels 72 h after feeding in virgin females. Rates of JH III biosynthesis closely reflected the hemolymph levels of JH III both after emergence and after a blood meal described by Shapiro et al. (1986). The activity of the CA in *Aedes aegypti* females seems to be regulated by developmental changes and nutritional signals, and to be independent of mating stimulus.

Keywords: *Aedes aegypti*; Juvenile hormone; Corpora allata; Mating; Blood-feeding

1. Introduction

Juvenile hormones (JH) are a class of regulatory sesquiterpenoids that control metamorphosis in immature insects and reproduction in adult insects (Gilbert et al., 2000). The corpora allata (CA), a pair of endocrine glands with nervous connections to the brain, synthesize and secrete JH. CA activity is dependent upon and modulated by both internal and environmental factors, such as developmental stage, photoperiod, diapause, feeding, nutritional status, and mating (Engelmann, 1990; Clements, 1992; Davey, 1997; Schal et al., 1997). JH levels in the yellow fever mosquito *A. aegypti* increase during the first two days after adult emergence and remain high before feeding (Shapiro et al., 1986). When a female takes a blood meal, the JH level falls rapidly during the first 3 h and reaches its lowest point 24 h after the blood meal. Forty-eight hours after the blood meal, the JH level starts to rise, and after 96 h it is equivalent to the pre-blood meal value (Shapiro et al., 1986). Although many studies have been conducted on the regulation of anautogenous mosquito reproduction by feeding and mating stimuli, less is known about the

* Corresponding author. Tel.: +1-520-621-1772; fax: +1-520-621-9288.
E-mail address: fnoriega@email.arizona.edu (F.G. Noriega).

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effect of these factors on CA activity (Clements, 1992; Raikhel et al., 2002). Biosynthesis of JH in mosquitoes was first studied in *Culex pipiens* (Readio et al., 1988, 1999). The in vitro and in vivo biosynthesis of JH in *A. aegypti* was studied by Borovsky and collaborators (Borovsky and Carlson, 1992; Borovsky et al., 1992, 1994a, 1994b); they developed an "exposed *corpora allata*" assay, i.e. the head–thorax complex was incubated in the presence of different radioactive precursors (methyl farnesoate, methionine or acetate). Using this assay they described that the CA of sugar-fed and blood-fed females synthesized JH III in vitro. Our studies complemented and extended those previously made by Borovsky and collaborators. We describe the establishment of a different type of in vitro CA preparation; fat body and other thoracic-tissues present in the "exposed CA assay" developed by Borovsky and Carlson (1992) were removed in these assays. We used this CA preparation for a systematic study of the effects of mating and blood-feeding on the activity of the CA of adult female *A. aegypti* during the first gonotrophic cycle. These studies are the basis for further research on factors regulating the synthesis of JH in mosquitoes.

2. Materials and methods

2.1. Chemicals

(*E, E)* methyl farnesoate (MF) was purchased from Echelon (Salt Lake City, UT, USA), and JH III from Sigma (St. Louis, MO, USA) or ICN (Irvine, CA, USA). HPLC grade ethyl acetate, hexane and methanol were from Burdick and Jackson (Muskegon, MI, USA).

2.2. Insects

A. aegypti of the Rockefeller strain were reared at 28 °C and 80% relative humidity under a photoperiod cycle of 16 h light: 8 h dark. Adults were offered a cotton wool pad soaked in a 3% sucrose solution until 12–16 h before blood feeding. In this paper we will refer to the cotton wool pad sucrose-fed females as "sugar-fed". Mosquitoes were fed pig blood equilibrated to 37 °C, and 1 mM ATP was added to the blood-meal immediately before use as previously described (Noriega et al., 1999).

2.3. In vitro radiochemical assay for CA activity

For preparation of isolated CA complexes, mosquitoes were immobilized by brief exposure to ice. To facilitate dissections, legs, wings and antennae were rapidly cut off, and the anterior half of the body was pinned to a silicon dissecting dish and covered with a drop of mosquito saline-buffer (138 mM NaCl, 8.4 mM KCl, 4 mM $CaCl_2$, 2 mM $MgCl_2$, 12 mM NaH_2PO_4 and 42.5 mM sucrose). The thorax was split open and the corpora allata (CA) plus corpora cardiaca (CC) complex attached to the aorta were exposed by carefully removing the thoracic muscles, cuticle and other tissues from the neck region using razor-blade scalpel, fine forceps and scissors. The aorta and CA-CC complex, connected to the brain and head capsule were isolated. This facilitated the visualization and transfer of the complexes. In all the experiments described in this paper we used CA + CC + aorta + brain + head capsule preparations that we will refer as "CA complexes". The dissection of one CA complex was completed in about 5 min. We did not observe changes in the size of the CA gland during development.

After dissection, the CA complexes were held in tissue culture medium M-199 (Lavallette, NJ, USA) without methionine, containing 2% Ficoll 400 and 25 mM HEPES (pH 6.5). After a pre-incubation of 1–2 h to consume intraglandular methionine, the CA complexes were transferred into a carbowax-coated flat bottomed glass tube containing 100 µl of sterile tissue culture medium M-199 with 25 mM HEPES (pH 6.5) and 2% Ficoll 400 containing L-[methyl-^{3}H]methionine (specific activity 2.96–3.11 TBq/mmol; 80–84 Ci/mmol, Amersham Pharmacia, IL, USA) as described by Feyereisen and Tobe (1981) and Feyereisen (1985). The final concentration of methionine in the medium was 50 µM and the specific activity was 0.56 TBq/mmol (15 Ci/mmol). The CA complexes were incubated individually in the dark at 30 °C under continuous gentle agitation on an ADAMS™ Nutator Mixer (Becton-Dickinson, NJ, USA). Incubations were terminated by the addition of 100 µl 1% EDTA, and 100 µl methanol containing 25 µg each of cold JH III and methyl farnesoate (MF) as carriers and internal standards. Medium and gland were extracted together with 1 ml of hexane, and separated by thin-layer chromatography (TLC). After TLC separation (developed in 2:1 (v/v) hexane and ethyl acetate), the JH III band was detected under UV light, cut, put into 10 ml scintillation cocktail overnight and assayed for ^{3}H. JH III synthesized is the total amount accumulated in the medium and in the gland complex. When JH III release was studied, the cultured tissues were separated from the medium, and extracted separately. The quantity of JH produced in the experiment was calculated from the specific activity of the L-[methyl-^{3}H] methionine in the medium, with assumption of a specific incorporation ratio of 1 (non isotopic dilution).

JH degradation was checked by incubating [^{3}H]-JH III in medium in the presence or absence of CA complexes, and analyzing the recovery of labeled JH. Between 95 and 99% of the hormone was recovered intact after 4 h of incubation (results not shown).

2.4. Histology of CA

Corpora allata were dissected as above, but CA-CC complexes were isolated from head and aorta, fixed with methanol and stained with Giemsa. Whole mosquito samples were fixed in Carnoy's solution (60% ethanol, 30% chloroform, 10% acetic acid). The samples in the fixative were kept at room temperature for 24 h and transferred to 70% ethanol until paraffin embedding. Samples were dehydrated in progressive ethanol concentrations, infiltrated in Paraplast X-TRA (Oxford, St. Louis, MO.) overnight at 58 °C, and embedded in Paraplast X-TRA using Simport histological cassettes (Quebec, Canada). Serial sections of 6 μm of whole mosquitoes were made and mounted in glass slides precoated with 3% 3-aminopropyltriethoxysilane (Sigma, St. Louis, MO.) in acetone. Sections were stained with aqueous Harris haematoxylin and eosin, and were observed with a Nikon light microscope (Eclipse 600, Tokyo, Japan) using bright field optics.

2.5. HPLC and Mass spectral analysis of products synthesized by CA complexes

Detection of radiolabeled products was done using a Beckman Gold HPLC system model 126, with an Ultrasphere® C18 reverse-phase HPLC column (250 × 4.6 mm, 5 μm particles) and a scanning model 167 UV detector set at 214 nm. The column was eluted using a linear gradient of 40% acetonitrile to 100% acetonitrile in H_2O. The separating conditions were as described by Borovsky and Carlson (1992), with some modifications: solvent flow rate 1 ml/min; gradient: 0–5 min 40% CH_3CN, 5–45 min 40–100% CH_3CN (linear gradient), 45–50 min 100% CH_3CN, 50-55 min, 40% CH_3CN. Data from HPLC were analyzed using the Beckman System Gold software. Recoveries were between 70–98%. One ml fractions were diluted with 10 ml scintillation cocktail and analyzed for ^3H. The JH III peak from HPLC was analyzed by chemical ionization mass spectroscopy (MS) using a Finnigan-Matt ITS 40® ion trap MS interfaced to a Varian Star 3400® gas chromatograph having a cool-on-column injector as described by Teal et al. (2000). Identification of JH III was based on comparison of fragmentation patterns (60–300 amu) and retention indexes of compounds eluting during analysis of natural product samples with those of synthetic standards.

2.6. Statistical analysis

Statistical analyses of the data were performed by linear regression or *t*-test (unpaired with two-tailed), using GraphPad Prism version 3.00 for Windows, GraphPad Software (San Diego, CA, USA). The results were expressed as mean ± SEM and considered significantly different at $P < 0.05$.

3. Results

3.1. Corpora allata preparations

The corpora allata-corpora cardiaca (CA-CC) complex from 2-day-old sugar fed unmated female mosquitoes were dissected attached to a piece of aorta. The aorta-CA-CC was left connected to the intact head capsule to facilitate the visualization and transfer of the glands. The nature of the CA preparations was analyzed by light microscope examination (Fig. 1). The glands were recognized as oval bodies with a length of about 30 μm, normally attached to the tracheal commissure.

3.2. Identification of the radio labeled products synthesized by the CA

Thin layer chromatography (TLC) was routinely used for the isolation and characterization of labeled JH III. In vitro biosynthesized labeled compounds were further characterized by HPLC. We found only two major radioactive peaks; elution of the two radioactive fractions coincided exactly with the peaks for the MF and JH III internal standard (retention times: 22.4 min for JH III and 36.6 min for MF) (results not shown). Standards for JH I and JH II were also used and no evidence for the presence of JH I or JH II homologues was found in any of the samples. After HPLC purification, the JH III fraction was analyzed by GC-MS. In vitro biosynthesized JH III identity was confirmed based on the diagnostic ions of the standard JH III (m/e = 111, 125,147, 189, 217 and 235). These diagnostic ions were the same described by Teal et al. (2000).

3.3. Time course for JH III biosynthesis and relationship between JH III synthesized and released

CA complexes from 2-day-old females were incubated in medium supplemented with 50 μM L-[methyl-^3H] methionine. The amount of JH III synthesized was determined by transferring the complexes to fresh radio labeled medium at 1 h intervals. A constant rate of JH III synthesis by CA from 2-day-old females was observed, indicating that the incorporation of L-[methyl-^3H] methionine into JH III was linear for at least 6 h under these conditions (Fig. 2a). In all subsequent assays, a 4 h incubation time was chosen. When the amount of JH III was evaluated independently in the tissue and the medium, we found that approximately 44.7% (±6.3 %, mean ± s.e.m.) of the synthesized JH III was present in the medium. There was a linear relationship between the amount of JH III synthesized and the amount "released"

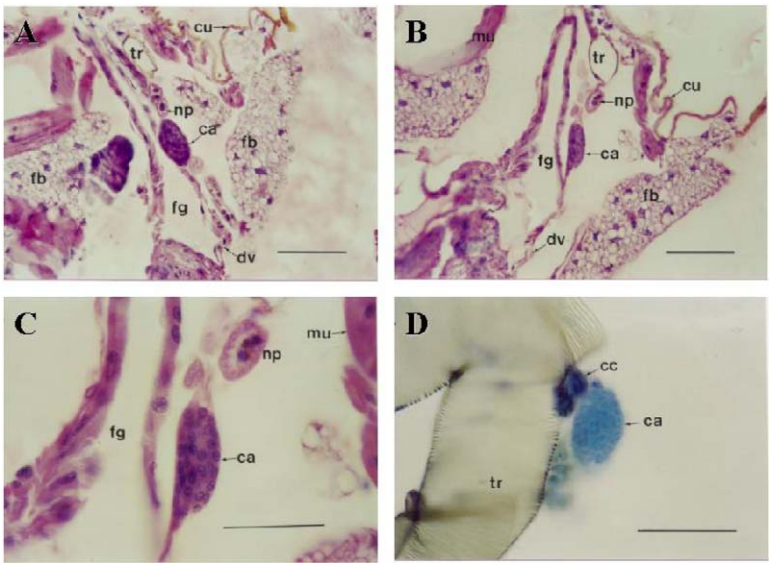

Fig. 1. Corpora allata in 2-day-old sugar fed unmated female *A. aegypti*. (A–C) Mosquitoes were fixed in Carnoy's solution and processed for paraffin histology. Sections of 6 μm were stained with hematoxylin and eosin. (D) Mosquito CA were dissected out, fixed with methanol and stained with Giemsa. ca, corpus allatum; cc, corpus cardiacum; tr, tracheal commissure; cu, cuticle; dv, dorsal vessel; fb, fat body; fg, foregut; np, nephrocyte; mu, muscle. Bar length: A, B = 50 μm; C, D = 30 μm.

or present in the medium (Fig. 2b). The mean amount of JH III synthesized by the CA of a 2-day-old sugar-fed females was similar when studies were done using individual gland (18.4 ± 2.0 fmol/pair gland/h mean ± SEM; $n = 62$) or pools of 5 glands (17.9 ± 1.4 fmol/pair of glands/h mean ± SEM; $n = 33$; $P = 0.8649$, *t*-test).

3.4. Effect of methionine concentration

CA complexes from 2-day-old females were incubated in medium with final methionine concentration adjusted between 6.25 and 200 μM. There was a dependence of JH III synthesis on exogenous methionine supply. Increases in the concentration of methionine in the incubation medium resulted in a linear increase in JH III synthesis (Fig. 3a). Preincubation of CA for 2 h in medium containing L-[methyl-^{3}H] methionine, followed by transfer into a methionine-free medium stopped JH synthesis within 90 min (Fig. 3b). Glands incubated for up to 5 h in methionine-free medium did not synthesize more JH III, showing that the intraglandular pool of methionine was used up within 90 min.

3.5. JH synthesis in sugar-fed virgin and mated females

In sugar-fed females, biosynthesis of JH III was very low (2.07 fmol/pair gland/h) during the first 2 hours after adult emergence, and showed a sharp increase 18 h after adult emergence (32.6 fmol/pair gland/h) (Fig. 4). After 2 days, CA biosynthetic activity slowly decreased, and reached values of around 5.3 fmol/pair gland/h one week after emergence. We analyzed the effect of mating on the biosynthetic activity of the CA. Males of *A. aegypti* require 24–36 h to complete their sexual maturation, and females are sexually unreceptive during the first 30–60 h (Clements, 1999); therefore we started our studies 2 days after emergence. JH III biosynthesis was similar in mated and virgin sugar-fed females (Fig. 4).

3.6. JH synthesis in blood-fed virgin and mated females

The production of JH III by the CA decreased significantly after a blood meal in both mated and virgin females (Fig. 5). JH biosynthesis dropped sharply by 12 h after a blood meal in mated females, and by 24 h after a blood meal in virgin females. JH III biosynthesis remained low for at least 96 h in mated females, but was back to higher levels 72 h after feeding in virgin females (Fig. 5).

4. Discussion

This work establishes new conditions for measuring JH synthesis in female *A. aegypti* by the short-term in vitro radiochemical assay. The CA of the mosquito is a small endocrine gland (30–40 μm); the recognition, dissection and handling of the gland are difficult. At first we dissected and incubated the glands attached to a piece of aorta. The transfer of the glands from the dissecting dish to the incubation vial was a critical step. We could not visually detect the effectiveness of this transfer or the presence of the gland in the incubation vial. Our first experiments typically consisted in the incubation of 12–

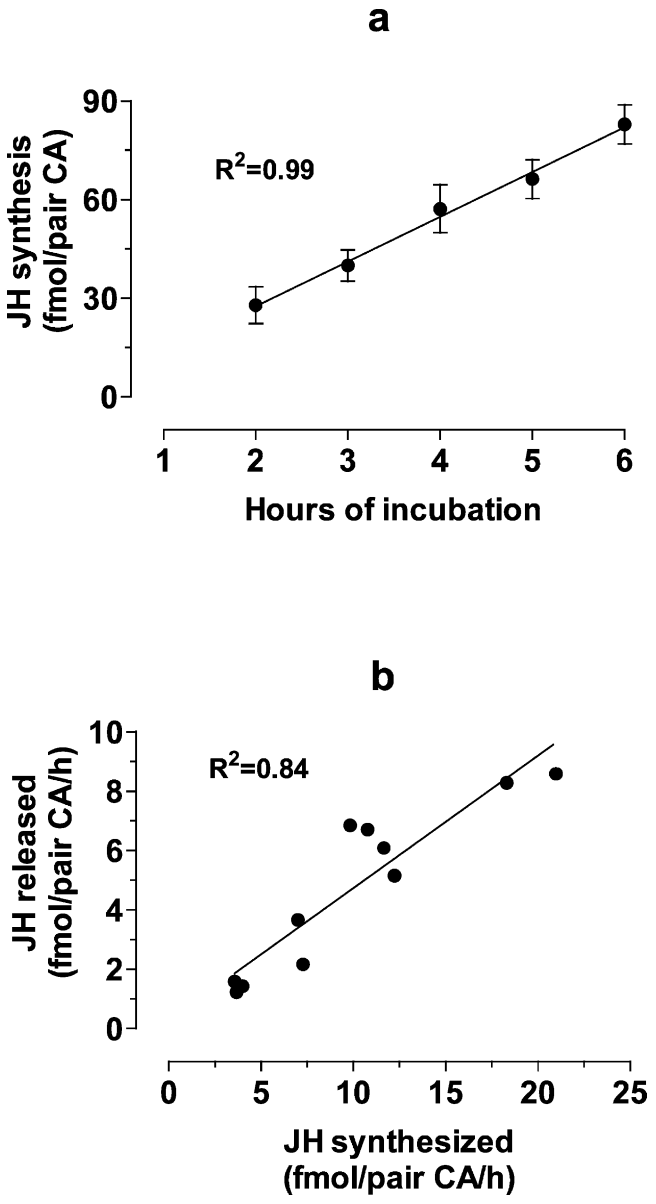

Fig. 2. Time course of cumulative JH III biosynthesis and rate of release. (a) Time course of cumulative biosynthesis of JH III by CA in vitro: the amount of JH III synthesized was determined by transferring the complexes to fresh labeled medium at 1 h intervals. (b) Relationship between the rate of JH III biosynthesis and the rate of JH III release into the medium: after 4 h of incubation, JH III was evaluated independently in the tissue and the medium. In both figures, each point (± s.e.m.) represents the mean of five incubations with single pairs of CA complexes from 2-day-old *A. aegypti* unmated sugar-fed females. The lines are linear regression lines.

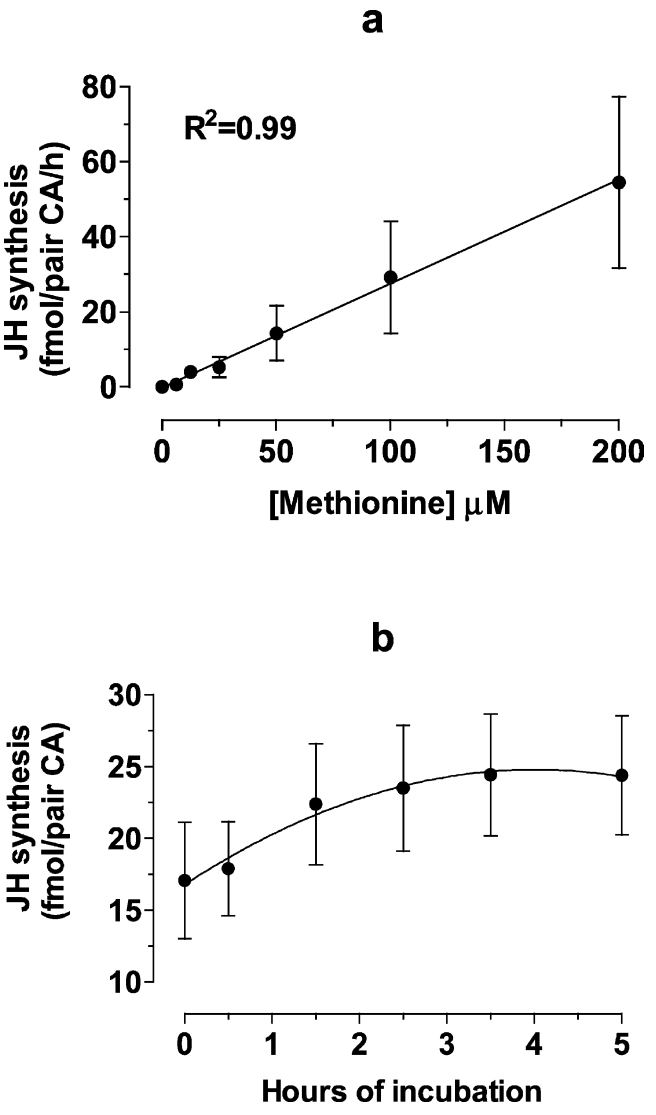

Fig. 3. Dependence of JH III biosynthesis on exogenous methionine. Effect of L-methionine concentration on the rate of JH III biosynthesis in vitro (a): Influence of precursor (L-methionine) supply for JH III biosynthesis in vitro. The line is a linear regression. (b): CA were preincubated in medium containing 50 μM [methyl-^{3}H] methionine (final activity 15 Ci mmol^{-1}) for 2 h, then transferred to a methionine-free medium and the amount of cumulative JH III synthesized was measured after 0.5, 1.5, 2.5, 3.5, 5 h. Each point (± s.e.m.) represents the mean of five incubations with single pairs of CA complexes from 2-day-old unmated females.

18 individual glands. We noticed that 60–70% of the assays resulted in values of 10,000–20,000 dpm of JH III, while others glands produced background values of 100 dpm. The conclusion was that we were loosing some of the glands during the transfer, or some of the glands were damaged or could attach to the vial wall during the incubation; either way, these glands were lost. After several experiments, we resolved to modify the protocol. We left a piece of aorta-CA attached to the intact head capsule to facilitate the visualization and transfer of the glands. This modification completely changed the conditions of the assay. Dissections were easier and faster (about 5 min/gland), and the variability among samples diminished. We considered the use of single glands or pools of several glands. Using individual glands there was a degree of variability that generated some errors if the samples were small, but with sample sizes over 5–10 glands the results were similar using either approach.

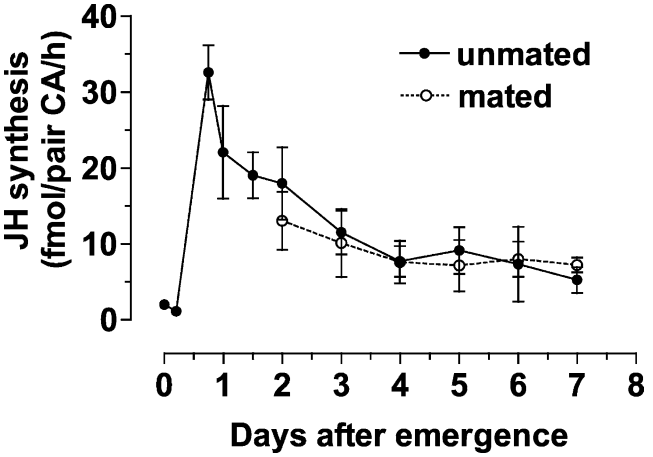

Fig. 4. Biosynthesis of JH III in vitro in sugar-fed females. CA complexes from sugar-fed females were dissected at different times after emergence and incubated for 4 h. Time-0 data point is for females within 1 h of emergence. (●): Unmated females. (○): Mated females. Each data point (± s.e.m.) represents the mean of 10–25 independent determinations of individual CA complex.

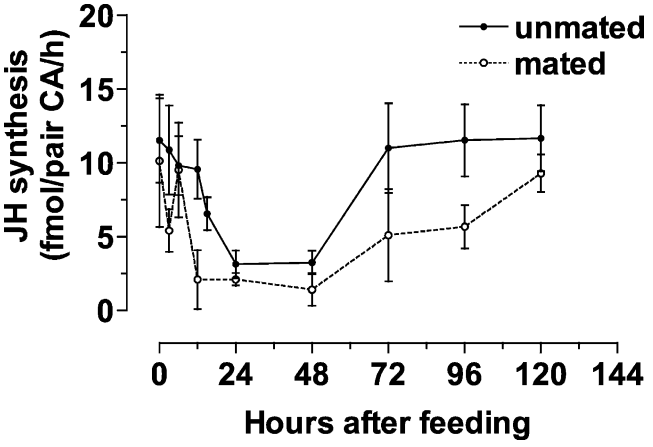

Fig. 5. Biosynthesis of JH III in vitro in female *A. aegypti* after a blood meal. Three day old females were blood-fed. CA were dissected at different times after feeding and incubated for 4 h. Time-0 data point is for the 3 day old sugar-fed females. (●): Unmated females. (○): Mated females. Each data point (± s.e.m.) represents the mean of 10–25 independent determinations of individual CA complexes.

The sensitivity of the assay allowed us to select the usage of single glands as the standard procedure.

The radiochemical assay showed a good linearity of JH synthesis up to 6 h of incubation. The characterization of the biosynthesized JH III was done by HPLC and GC-MS. Biosynthesis of JH III bisepoxide (JHB_3) by the CA and accessory gland of male *A. aegypti* has been reported (Borovsky et al., 1994a). We did not observe the presence of JHB_3 in our samples, JHB_3 standards were not available, but under the HPLC conditions used in our studies, JHB_3 should have eluted before JH III, due to the decreased lipophilicity caused by the addition of a second epoxide group (Richard et al., 1989; Borovsky et al., 1992).

We evaluated the influence of culture conditions on CA activity; as expected, there was a stringent dependence on exogenous methionine for JH III synthesis. Increases in the concentration of methionine in the incubation medium resulted in a linear increase in JH III synthesis. The pool of methionine and S-adenosylmethionine was exhausted within 90 min of transfer of the CA into a methionine-free medium; similar rapid consumption of small CA methionine pools have been described for *Schistocerca gregaria* (Tobe and Pratt, 1974) and *Diploptera punctata* (Feyereisen et al., 1984).

Borovsky and Carlson (1992) described that 78% of the newly synthesized JH III was associated with the tissue and not found in the medium. We have found that about 45% of the synthesized JH III was released into the medium; this disparity is most likely the result of differences in the CA incubations, as well as the result of some of the "released" JH sticking to non-CA tissues; in the case of Borovsky's preparations, they included even more non-CA tissues, therefore it should be expected more adsorption of JH to them. The rates of synthesis that we observed for CA dissected before and after feeding were similar to those previously reported for *A. aegypti* (Borovsky and Carlson, 1992) and *C. pipiens* (Readio et al., 1988).

The analysis of CA activity results suggest that JH titer in mosquito is essentially determined by the rate of synthesis; developmental changes on the activity of the CA correlated well with the changes of JH levels previously described (Shapiro et al., 1986). The CA is active before feeding and inactive a few hours after a blood meal; similar results were previously described in *A. aegypti* (Borovsky and Carlson, 1992; Borovsky et al., 1992), and in *Culex pipiens* (Readio et al., 1988, 1999).

We observed that JH III synthesis was only 2.0 fmol/pair gland/h immediately after adult emergence, but increased to 32.6 fmol 18 h later and remained elevated during the following 24 h. After 2 days, CA biosynthetic activity slowly decreased, and reached values of around 5.3 fmol/CA/h one week after emergence. The high levels of JH synthesis during the first 3 days after emergence are essential for the post-emergence reproductive maturation of *Aedes aegypti* females (Klowden, 1997). A juvenile hormone regulated phase of previtellogenic ovarian development has been described in mosquitoes (Feinsod and Spielman, 1980b; Klowden, 1997); post-emergence follicular growth of mosquitoes has been proposed as a reliable estimate for JH activity (Feinsod and Spielman, 1980a; Hagedorn et al., 1977). Primary follicles lengthen more than 2-fold during the first 2 days post-ecdysis, and allatectomy within 1 h of emergence prevents this ovarian development in *A. aegypti* (Lea, 1963).

Nutritional stimuli appear to strongly regulate the activity of CA in newly emerged mosquito females (Foster, 1995; Rossignol et al., 1981; Feinsod and Spiel-

man, 1980a). Nutrients provided by sugar meals are important for ovary development during the first 3 days after emergence; follicles from females raised on an inadequate larval nutrition requires a sugar meal to develop their ovaries; however follicles from females raised on a well-nourished larval nutrition develop their ovaries in the absence of a sugar meal. Relative absence of JH in nutrient-deprived mosquitoes is suggested by the finding that application of exogenous JH stimulates previtellogenic follicular growth in starved adult mosquitoes from crowded cultures. Since previtellogenic follicles developed when mosquitoes were provided carbohydrate food or in mosquitoes reared in non-crowded cultures, it was concluded that activity of the corpora allata is promoted by feeding or by presence of nutrient stores (Feinsod and Spielman, 1980a).

Decapitation within 1 h of emergence also prevents ovarian development in *A. aegypti* (Feinsod and Spielman, 1980b). Factors from the head might be essential for the normal increase of CA activity observed during the first 18 h after emergence; one of these factors appear to be *A. aegypti* allatotropin (Aedes-AT) (Veenstra and Costes, 1999); we have described elsewhere that incubation of CA complexes from newly emerged females with *Aedes*-AT plus farnesoic acid (FA) resulted in very high production of JH III, while FA or *Aedes*-AT alone have no effect (Li et al., 2003). The nutrient stores accumulated during the pre-imaginal stages are likely to control the activity of the CA by means of the head via release of allatotropin.

Effects of the quantity of nutrient stores on CA activity have been described in insects; starvation has diverse effects on JH levels in different insects depending on their developmental stage. Starvation causes a decline in JH levels in adult female *Schistocerca gregaria* (Tobe and Chapman, 1979) and *Leptinotarsa decemlineata* (Khan et al., 1982). On the other hand, starvation increases JH levels in *Manduca sexta* last instar larvae (Bhaskaran and Jones, 1980). The allatotropin gene of *M. sexta* is expressed as three mRNAs that differ from each other by alternative splicing; the level of one of these mRNAs (RNA-3) is specifically increased in the nerve cord of *M. sexta* larvae that were starved, the increase in RNA-3 levels may be part of the complex response of larvae to nutrient deprivation (Lee and Horodyski, 2002).

We hypothesize that high nutrient stores carried over from the larval stages are essential for the activation of the CA during the first 3 days after emergence; the decrease in these nutrient stores might cause the decrease in CA activity we described in older sugar fed females. Besides nutrients, other factors might play a role on the regulation of CA activity in sugar-fed females; based on ablation and transplantation experiments, it has been proposed that factors from ovaries in the previtellogenic state of arrest may inhibit CA activity in *A. aegypti* (Rossignol et al., 1981).

Several JH-dependent events in mosquitoes, such as previtellogenic growth of the ovaries (Dhadialla and Raikhel, 1994; Klowden, 1997; Raikhel et al., 2002), changes in the levels of developmentally regulated JH-dependent genes (Noriega et al., 1997; Edgar et al., 2000) are expressed independently of the mating stimulus. The effect of mating on CA activity is therefore different in *A. aegypti* when compared with some other insects. In cockroaches, such as *Leucophaea maderae*, CA activity is low for at least 7 days post-eclosion in virgin females, but JH III synthesis increases more than 20-fold after mating in adult female (Aclé et al., 1990). It has been postulated that mating increases egg production by altering the degree of inhibition of the CA by the brain, consequently increasing JH secretion (Davey, 1997). Starvation reduces the rate of synthesis of JH to very low levels in adult female *L. maderae* (Aclé et al., 1990); both feeding and mating are necessary for the expression of a normal cycle of JH III synthesis in this cockroach. Adult females of the German cockroach *Blattella germanica* have clearly-defined feeding cycles related to oogenesis; intake of high-quality food, mating, social interactions and the presence of vitellogenic ovaries all promote JH synthesis. On the contrary, starvation, deficient diets, enforced virginity, isolation, and a pre- or post-vitellogenic ovary cause the CA to produce less JH (Davey, 1997; Schal et al., 1997; Osorio et al., 1998). In *M. sexta*, CA continues to release JH for only two days in virgin females (Ramaswamy et al., 1997); during mating, the male may trigger a neural/humoral response in the female, thus stimulating synthesis/release of JH or inhibiting JH degradation (Ramaswamy et al., 1997). Mating has no effect on ovarian maturation in adult females of the firebug, *Pyrrhocoris apterus* (Davey, 1997; Hodková et al., 2001).

We have also confirmed that JH synthesis was significantly reduced in glands dissected 24 after a blood meal; however, unlike the pattern described for changes of JH titer (Shapiro et al., 1986), we did not detect a sharp decrease in CA activity within a few hours after a blood meal; indicating that the fast decrease in JH titer is not due only to an effect on synthesis and release by CA, but occurs in conjunction with changes on JH turnover. JH III biosynthesis remained low for at least 96 h in mated females, but was back to higher levels 72 h after feeding in virgin females. Different feedbacks from the developing ovaries of virgin and mated females, or factors supplied by the male may contribute to the extension of the low CA activity in mated females. Borovsky et al. (1992) described an increase in the rate of JH synthesis immediately following a blood meal, reaching a peak 5 h after feeding, and declining by 10 h. We have not observed a similar phenomenon, although we

detected a high variability in the CA activity within the first 6 h after feeding.

In summary, we believe our preparation is suitable to study CA activity; fat body and other thoracic-tissues present in the "exposed CA assay" developed by Borovsky and Carlson (1992) were removed in these assays, most likely reducing JH esterase activity. The assay is reliable and very sensitive to the precursor supply, and rates of JH III biosynthesis closely reflected the hemolymph levels of JH both after emergence and after a blood meal. The activity of the CA in *A. aegypti* females seems to be regulated by developmental changes and nutritional signals, and be independent of mating stimulus.

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