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IN VITRO JUVENILE HORMONE BIOSYNTHESIS IN ADULT VIRGIN AND MATED FEMALE BROWN-BANDED COCKROACHES, *SUPELLA LONGIPALPA*

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Abstract—The *in vitro* radiochemical assay for juvenile hormone biosynthesis by pairs of corpora allata has been employed in the adult female brown-banded cockroach, *Supella longipalpa*. [2-^{14}C]acetate and the methyl moiety of L-[methyl-^{3}H]methionine were incorporated into juvenile hormone in a 9:1 ratio. The corpora allata of 9-day mated females release juvenile hormone at a linear rate for 5 h and maximal release rates occur at L-methionine concentrations greater than 30 μM. The juvenile hormone homologue was identified as juvenile hormone-III by HPLC analysis of the radiobiosynthesized corpora allata product. The release of juvenile hormone by the corpora allata closely parallels oöcyte development in both mated and virgin females over two gonotrophic cycles. The corpora allata exhibit rapid, nonoverlapping cycles of activity. Mating further activates the corpora allata and accelerates ovulation. The depression of corpora allata activity when the basal oöcytes exceed a critical volume of 0.9 mm^3 suggests a negative feedback mechanism.

Key Word Index: Juvenile hormone, corpora allata, ovarian development, *Supella longipalpa*

INTRODUCTION

Among the 3000–4000 described species of cockroaches, three basic modes of reproduction are exhibited: oviparity, ovoviviparity and viviparity (Roth, 1970). Representatives of the family Blaberidae (e.g. *Leucophaea maderae*, *Nauphoeta cinerea* and *Diploptera punctata*) have been well studied in terms of the relationship between corpora allata activity and the reproductive cycle (Tobe and Stay, 1985). These ovoviviparous and viviparous species exhibit prolonged periods of ovarian arrest and low corpora allata activity during gestation.

Members of the remaining four families of cockroaches are all oviparous, but only one species, *Periplaneta americana* (Blattidae), has been studied in detail. In *P. americana*, there are always two vitellogenic oöcytes present in each ovariole (Roth, 1970) such that two peaks of *in vitro* juvenile hormone biosynthesis can be associated with the vitellogenic growth of each wave of oöcytes (Weaver *et al.*, 1975; Weaver and Pratt, 1977). However, this is not characteristic of all oviparous species. In most Blattellidae, only the basal oöcytes contain yolk at the time of oviposition. Recently, juvenile hormone biosynthetic rates from the imaginal moult through two successive oviposition cycles of *Blattella germanica* (Blattellidae) have been reported (Belles *et al.*, 1987; Gadot *et al.*, 1989). *Blattella germanica* carries a partially extruded oötheca for 22 days until the eggs hatch so that, like in the blaberids, a period of slow (non-vitellogenic) oöcyte growth (Roth and Stay, 1962) and corpora allata inhibition (Gadot *et al.*, 1989) between successive reproductive cycles is observed.

Supella longipalpa, like *B. germanica*, is an oviparous species with one vitellogenic oöcyte per ovariole at the time of ovulation. However, *S. longipalpa* females produce successive oöthecae at 5–7-day intervals, with embryonic development occurring in externally deposited oöthecae. The female carries the oötheca for less than 36 h. Consequently, we hypothesized that the cycle of juvenile hormone biosynthesis should closely parallel the cycle of oöcyte development; the corpora allata should undergo rapid changes in activity, with each wave of basal oöcyte maturation associated with one cycle of juvenile hormone biosynthesis.

To address the hypothesis, we employed the modified *in vitro* radiochemical assay (Feyereisen and Tobe, 1981) to quantify the rates of juvenile hormone release. This assay has been successfully used in representatives of several insect orders (Tobe and Stay, 1985) and is especially appropriate for the Dictyoptera, provided that certain parameters have been established (i.e. juvenile hormone homologue identification, linear release rates, molar incorporation ratios and optimal methionine concentrations). In this paper we demonstrate that the *in vitro* radiochemical assay is a valid procedure for the measurement of juvenile hormone release and report corpora allata activity over two gonotrophic cycles in virgin and mated *S. longipalpa* females.

MATERIALS AND METHODS

Insects

Late-instar *Supella longipalpa* nymphs were maintained at 27 ± 1°C under a 12 h light:12 h dark photo-

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periodic regime with dog chow and water provided *ad libitum*. Adult females were collected within 4 h after the imaginal moult (day 0) and maintained in groups of 20–30 insects under the same environmental conditions. Females were permitted to mate at an adult age of 8 days.

Measurement of juvenile hormone release

We employed the rapid partition radiochemical assay (Feyereisen and Tobe, 1981; Tobe and Clarke, 1985). The incubation medium consisted of calcium- and methionine-free medium 199 (GIBCO) to which Ficoll (20 mg/ml), calcium (5 mM $CaCl_2$), and L-[methyl-^{3}H]methionine (L-met; 67 μM; 7.4 GBq/mmol, New England Nuclear) had been added. Since in *S. longipalpa* the corpora allata are broadly connected to the corpora cardiaca, in all cases, the corpora allata were incubated with small portions of attached corpora cardiaca. Incubtation conditions were 3 h at 27°C with shaking, except where specifically noted. Oöcyte dimensions were measured with an ocular micrometer and volumes were calculated using the formula $V = 3.14\ (L \times W^2)/6$.

Identification of juvenile hormone-III

Four pairs of corpora allata from 9-day mated females were incubated together in ^{3}H-methionine-labelled medium for 11 h. Preparatory purification of the isooctane extract of the medium was performed using a Waters Sep-Pak Silica Cartridge (Waters Associates) and the pentane-ether eluent (1:1) was dried under nitrogen and redissolved in hexane. The sample was analyzed by high performance liquid chromatography (5 μm × 0.4 × 30 cm Diol column [Supelco], 5% diethyl ether in hexane, 1 ml/min, 0.02 AUFS 219 nm). Fractions were collected sequentially at 12-s intervals over a period of 14 min, and assayed by liquid scintillation spectrometry. The retention times of the labelled product and juvenile hormone-III standard (40 ng in hexane) were compared.

Time course of juvenile hormone release

Six pairs of high activity corpora allata from 9-day mated females were dissected and held in a drop of methionine-free medium for 30 min at room temperature. The medium was replaced with medium containing ^{3}H-L-met and individual pairs were incubated, as previously described, for 5 h. After each hour, the medium was aspirated, the tubes and corpora allata were rinsed with unlabelled medium and fresh, radiolabelled medium was added. The amount of juvenile hormone-III in the combined aspirate and washing from each tube was determined by liquid scintillation spectrometry.

[2-^{14}C]acetate and L-[methyl-^{3}H]methionine incorporation ratio

Five sets of four pairs of corpora allata were incubated for 12 h in modified medium 199 (acetate-, glucose- and methionine-free) to which L-[methyl-^{3}H]methionine (67 μM; 7.4 GBq/mmol, NEN) and [2-^{14}C]acetate (8 mM; 0.063 GBq/mmol, NEN) had been added. The medium was analysed using the isooctane radiochemical assay.

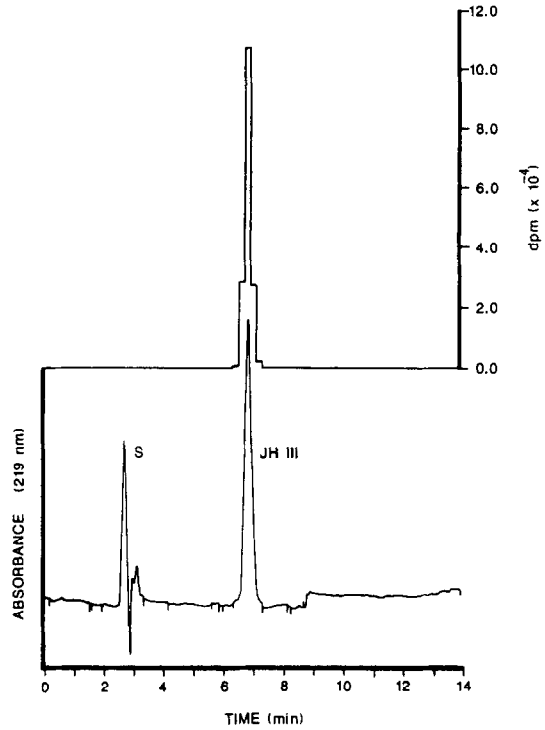

Fig. 1. High performance liquid chromatographic analyses of the *in vitro* radiobiosynthesized corpora allata product of *S. longipalpa* and the juvenile hormone-III standard. Refer to Methods for details. S identifies the solvent front.

RESULTS

Identification of juvenile hormone-III

The juvenile hormone-III standard and the biosynthesized product had identical retention times in high performance liquid chromatographic analysis (Fig. 1). Neither juvenile hormone-I nor juvenile hormone-II, which have shorter retention times, were detected in the incubation medium. Because no other labelled product was detected, we conclude that the sole juvenile hormone biosynthesized by the corpora allata of adult female *S. longipala* is juvenile hormone-III.

Methionine concentration and time course of juvenile hormone release

Individual pairs of high-activity corpora allata from 9-day mated females were incubated in medium 199 with concentrations of L-[methyl-^{3}H]methionine ranging from 2 to 160 μM. The corpora allata of *S. longipalpa* were efficient scavengers of L-met: at concentrations as low as 2 μM L-met, the corpora allata synthesized and released measurable quantities of juvenile hormone-III (Fig. 2). The relationship between juvenile hormone release rates and L-met concentration was nearly linear between 2 μM and 20 μM L-met; at higher L-met concentrations (30–160 μM) maximal rates were observed.

To determine accurately rates of juvenile hormone release, the corpora allata must exhibit linear activity over the entire 3 h incubation period. Figure 3 demonstrates that rates of juvenile hormone release were virtually constant over the 5 h incubation.

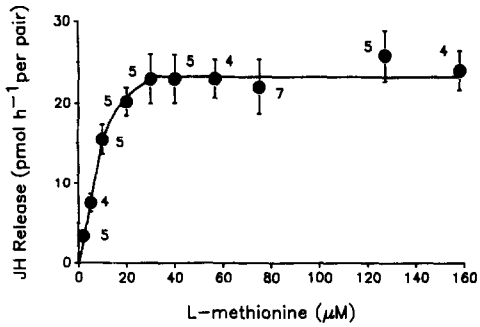

Fig. 2. Dose-response relationship for juvenile hormone release from pairs of corpora allata of 9-day mated females as a function of L-[methyl-^{3}H]methionine concentration in the incubation medium. Values are means ± SEM for the number of individual determinations shown beside each data point.

[2-^{14}C]acetate and L-[methyl-^{3}H]methionine incorporation ratio

In *Diploptera punctata*, 9 acetate units from [2-^{14}C]acetate are incorporated into the juvenile hormone-III carbon skeleton (Feyereisen *et al.*, 1984) so that a 1:9 ^{3}H:^{14}C ratio would be expected if one methyl group from L-met is incorporated into each juvenile hormone-III molecule. From the rapid partition radiochemical assay, a ratio of 1.08:9 was obtained, confirming stoichiometry of incorporation.

Juvenile hormone release during successive gonotrophic cycles

Corpora allata from newly-ecdysed adult females, as well as from any stage of the first two reproductive cycles, release measurable quantities of juvenile hormone *in vitro*. The mean release rates vary from 0.2 to 22.0 pmol h^{-1} per pair. Corpora allata activity is closely correlated with growth of the basal oöcytes up to an adult age of 8 days in virgin females [Fig. 4(A)]. As reported for other species (Tobe and Stay, 1985), mating enhances corpora allata activity *in vitro* in *S. longipalpa* [Fig. 4(B)]. However, virgin females attain juvenile hormone release rates and basal oöcyte volumes comparable to those of mated females. The distinction between mated and virgin females lies in the timing and duration of corpora allata activity and growth of the basal oöcytes (Fig. 4). Rates of juvenile hormone synthesis declined rapidly in both virgin and mated females after their basal oöcytes exceeded a volume of 0.9 mm^3 (Fig. 5). In mated females, this occurred between 24 and 48 h after mating.

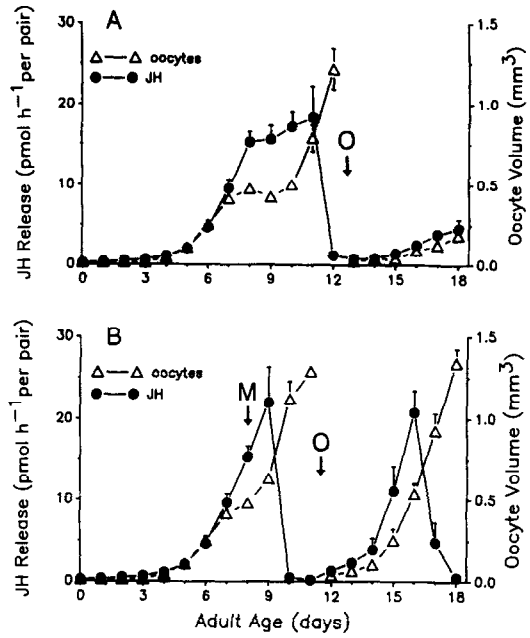

Fig. 4. *In vitro* rates of release of radiolabelled juvenile hormone by pairs of copora allata, and basal oöcyte volumes during the first 2 reproductive cycles of (A) virgin and (B) mated *S. longipalpa* females. Values represent the mean ± SEM of 5–12 individual determinations. Day of mating (M) and day of oviposition (O) are shown by arrows.

DISCUSSION

Parameters of in vitro corpora allata activity

Tobe and Stay (1985) caution that several parameters of the *in vitro* radiochemical assay must be established before the technique can be employed with confidence. These parameters include juvenile hormone homologue identification, observation of linear release rates throughout the incubation period, measurement of the stoichiometry of incorporation

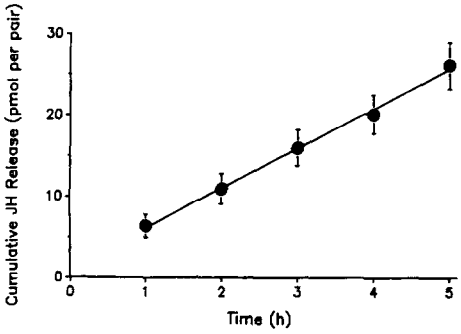

Fig. 3. Cumulative release of radiolabelled juvenile hormone from individual pairs of corpora allata from day-9 mated female *S. longipalpa* incubated *in vitro* in modified 199. Each value represents the mean ± SEM of individual determinations on six separate pairs of corpora allata.

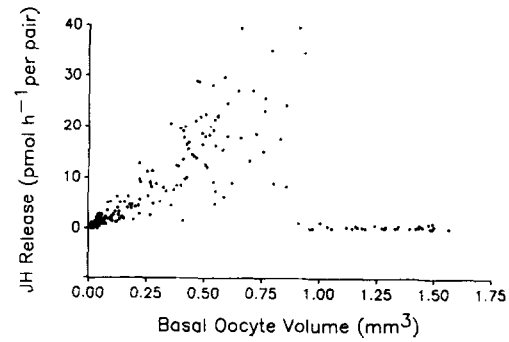

Fig. 5. Relationship between *in vitro* rates of juvenile hormone release by individual pairs of corpora allata from *S. longipalpa* and basal oöcyte volumes. All determinations ($N = 219$) from virgin and mated females over two gonotrophic cycles are included.

of L-[methyl-^{3}H]methionine and characterization of optimal L-[methyl-^{3}H]methionine concentrations. In the present study, we have met these criteria.

The identification of juvenile hormone-III as the sole homologue produced by the corpora allata of adult female *S. longipalpa* (Fig. 1) supports the contention that juvenile hormone-III predominates as the homologue synthesized by the corpora allata of adult insects (deKort and Granger, 1981). In glucose-free medium, a theoretical maximum of 9 mol of acetate are incorporated for every 1 mol of juvenile hormone-III synthesized (Feyereisen *et al.*, 1984), such that the ratio of ^{3}H-methionine to ^{14}C-acetate incorporation should be 1:9. We observed a ^{3}H:^{14}C ratio of 1.08:9, demonstrating that the molar incorporation ratio of the methyl ester function to the juvenile hormone-III product is 1:1.

Optimal L-met concentrations ($>30\ \mu$M) (Fig. 2) and linear rates of release over a 5-h incubation period (Fig. 3) have been demonstrated. Preliminary studies (unpublished) reveal that only small quantities of juvenile hormone are stored in the corpora allata, suggesting that the quantity released into the medium serves as an accurate measure of the overall biosynthetic rate. Thus, the *in vitro* radiochemical assay for juvenile hormone release is a viable method in the case of corpora allata of *S. longipalpa*.

Juvenile hormone biosynthesis during the gonotrophic cycle

In *S. longipalpa*, each wave of maturing oöcytes is associated with a single cycle of juvenile hormone release (Fig. 4) and the cycles of corpora allata activity *in vitro* are precisely related to basal oöcyte development (Figs 4, 5). Initially, juvenile hormone rates of release are low and oöcyte growth is slow. With increasing rates of juvenile hormone release, a corresponding increase in basal oöcyte growth is observed. Then, at a critical oöcyte volume (0.9 mm^3, oöcyte length of 2.1 mm) in both virgin and mated females, *in vitro* corpora allata activity declines sharply, followed by ovulation (Fig. 5). This suggests that ovarian feedback inhibition is involved in the decline of juvenile hormone biosynthesis.

Rates of both juvenile hormone release and basal oöcyte growth increase after mating in *S. longipalpa*, an observation which supports a previous report that mating stimulates the rate of oöcyte development and shortens the preovipositional period (Adiyodi and Adiyodi, 1974). Stimulation of the corpora allata (*in vitro* assay) and/or accelerated oöcyte maturation following mating have been demonstrated in *L. maderae* (Englemann, 1960; Koeppe *et al.*, 1980), *N. cinerea* (Roth, 1964; Lanzrein *et al.*, 1981), *D. punctata* (Roth and Stay, 1961; Stay and Tobe, 1977), and in *P. americana* (Weaver and Pratt, 1977). As in *S. longipalpa*, mating is not essential in *P. americana* for the onset of vitellogenesis and the corresponding higher rates of juvenile hormone biosynthesis (Weaver and Pratt, 1977).

In vitro juvenile hormone release rates and reproductive modes in cockroaches

The relative shape of corpora allata activity cycles in the oviparous *P. americana* is not typical of all oviparous cockroaches that produce oöthecae in rapid succession. *Periplaneta americana* (Blattidae) has 2 vitellogenic oöcytes per ovariole (Roth, 1970), and therefore a second peak of corpora allata activity (related to vitellogenin uptake in the penultimate oöcytes) is observed before oviposition of the basal oöcytes (Weaver and Pratt, 1977). Each vitellogenic oöcyte is thus subject to two overlapping cycles of juvenile hormone biosynthesis (Weaver *et al.*, 1975; Weaver and Pratt, 1977). However, unlike the blattids, most blattellids (e.g. *S. longipalpa*) possess a static ovariole system (review: Huebner, 1983) in which only one vitellogenic oöcyte is present in each ovariole (Roth, 1970). Consequently, as reported here, each wave of vitellogenic oöcytes is associated with a single peak of corpora allata activity and cycles of corpora allata activity are clearly separated.

Tobe and Stay (1977) noted that the cycles of juvenile hormone biosynthesis should be clearly distinct in the Blaberidae (as compared to the Blattidae), in which the events of vitellogenesis and oviposition are widely separated by protracted periods of gestation. Indeed, precise relation between corpora allata activity *in vitro* and the gonotrophic cycle was reported for the blaberid cockroaches *D. punctata*, *L. maderae* and *N. cinerea* (Tobe and Stay, 1985). However, a clear separation of cycles is observed in *S. longipalpa* (Blattellidae) (Fig. 4), which lacks such a period of pregnancy. Hence, the shape and frequency of corpora allata activity cycles in the three cockroach families studied to date (i.e. Blaberidae, Blattidae, Blattellidae) are dependent upon two phenomena: (1) the occurrence of intraovariole inhibition (morphological definition; review: Huebner, 1983), and (2) the mode of oviposition and subsequent embryogenesis.

In species lacking intraovariole inhibition, where more than one vitellogenic oöcyte may occur per ovariole (all Blattidae; some Blattellidae), each vitellogenic oöcyte is subject to two or more peaks of juvenile hormone biosynthesis, such that cycles of corpora allata activity overlap (e.g. *P. americana*). In contrast, species which exhibit intraovariole inhibition (most Blattellidae and all Blaberidae) have one vitellogenic oöcyte per ovariole such that each wave of basal oöcyte maturation is associated with a single peak of juvenile hormone release (e.g. *S. longipalpa*).

The shape and frequency of the corpora allata activity cycles are also affected by the mode of oviposition and embryogenesis. Successive cycles of corpora allata activity will be further interrupted in those species in which eggs develop internally (Blaberidae) (Tobe and Stay, 1977) or in externally-carried oöthecae (some Blattellidae) (Gadot *et al.*, 1989). Finally, in species which deposit the oöthecae soon after ovulation (all Blattidae, most Blattellidae), lack of inhibition by the oötheca should result in rapid cycles of juvenile hormone biosynthesis.

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