Abstract—Juvenile hormone III biosynthesis in vitro by the corpora allata of adult female Blattella germanica is precisely correlated with the gonotrophic cycle. Farnesoic acid-stimulated rates of juvenile hormone synthesis are always higher than the corresponding basal rates, and follow the same pattern of cyclic changes during oocyte maturation. Both basal and farnesoic acid-stimulated rates decline to undetectable levels while the female carries an ootheca and oocyte development is inhibited ('pregnancy'). This suggests that, in this species, the main control mechanism of the corpora allata operates through structural/developmental processes, affecting the maximal biochemical capacity of the glands. Morphometric data relating gland volume and the main events during oocyte development and 'pregnancy' support this conclusion. We speculate that the type of corpora allata control which is predominant in different insects might be functionally related to their ovipositional behavior.

Key Words Index: Juvenile hormone; in vitro assay; Farnesoic acid; Corpora allata regulation; Ovarian cycle; Pregnancy; Blattella germanica

INTRODUCTION

Farnesoic acid biosynthesis in the corpora allata is one of the last two steps in juvenile hormone (juvenile hormone III) biosynthesis, and is considered to be non-rate-limiting (Tobe and Pratt, 1976; Feyereisen, 1985). Isolated corpora allata, incubated in vitro, have been shown to utilize exogenous farnesoic acid with high efficiency in a number of insect species (e.g. Tobe and Pratt, 1974; Pratt et al., 1975; Weaver et al., 1980; Feyereisen et al., 1981; Gadot and Applebaum, 1986). The farnesoic acid-stimulated activity of the glands is considered to reflect their maximal biochemical capacity and, in all cases examined, was found to correlate with the size of the glands (Tobe and Pratt, 1976; Feyereisen, 1985). On the basis of their research on the locust, Schistocerca gregaria, Tobe and Pratt (1976) proposed a dual control mechanism of corpora allata activity: rapid modulation of rate-limiting step(s) provides an independent control of the basal activity of the glands, while structural and ultrastructural changes are responsible for the slower responses related to the maximal capacity of the glands. Experimental evidence on the control of corpora allata activity in Locusta migratoria was recently presented in support of this hypothesis (Gadot and Applebaum, 1986; Baehr et al., 1986; Gadot et al., 1987; Dale and Tobe, 1988; Couillard et al., 1988).

Independent control of basal activity and maximal capacity of the corpora allata was also demonstrated in the cockroach Periplaneta americana (Weaver and Pratt, 1981). However, in the cockroach Diptoptera punctata, a close relationship between these parameters suggests that the relative importance of the two control modes of the corpora allata (i.e. through the rapid and slow responses) may not be the same in different species (Feyereisen, 1985).

Blattella germanica exhibits an ovipositional behavior that is functionally intermediate between oviparity and ovoviviparity (review: Roth, 1970): unlike most oviparous cockroaches, such as P. americana, which form and deposit oothecae in rapid succession, B. germanica forms an ootheca which is extruded but not deposited. Rather, the ootheca is carried externally attached to the genital atrium and basal oocyte growth is arrested until the nymphs hatch (Roth and Stay, 1962). This incubation period is, therefore, functionally similar to pregnancy in ovoviviparous (e.g. Nauphoeta cinerea, Leucophaea maderae) and viviparous (D. punctata) cockroaches which retract the ootheca into a brood sac and incubate the embryos internally. We refer to this period in B. germanica as 'pregnancy'.

In this paper we show that the basal rate of juvenile hormone synthesis in the adult female B. germanica is dictated to a large extent by the total synthetic capacity of the corpora allata, as measured by the farnesoic acid-stimulated rate of juvenile hormone synthesis in vitro. Morphometric evidence supports the conclusion that the main control mechanism of corpora allata activity in this species operates through structural changes in the glands. By comparing several species of cockroaches and locusts, we propose a hypothesis which relates the type of corpora allata control and ovipositional behavior in the female adult insect.
MATERIALS AND METHODS

German cockroach (B. germanica) nymphs were reared at 27 °C under 12 h light-12 h dark, with dog food and water provided ad lib. Newly emerged adult females (day 0) were isolated daily and maintained under the same environmental conditions either individually or in groups, as indicated.

Farnesoic acid (about 70% pure) was a generous gift from Dr Staal (Zoecon Corp., Palo Alto, Calif.). L-[methyl-3H]methionine (93% pure, specific activity of 200 mCi/nmol) was obtained from New England Nuclear, Wilmington, Del.

The radiochemical assay for juvenile hormone biosynthesis was adapted from Pratt and Tobe (1974) with modifications after Feyereisen and Tobe (1981) and Gadot and Applebaum (1985): corpora allata-corpora cardiaca complexes were dissected from carbon dioxide-anaesthetized females in modified methionine-free TC-199 medium (GIBCO, Grand Island, N.Y.; special formulation after Kikukawa et al., 1987) and transferred to 100 µl of the same medium containing 100 µM of L-[methyl-3H]methionine (2 µCi) in tissue-culture-treated disposable Cell Wells multidishes (Corning, N.Y.). The glands were incubated with gentle shaking in the dark, at 28 °C for 2 h, and then were transferred to fresh medium containing 100 µM farnesoic acid for an additional incubation period of 2 h. The medium from each incubation was collected into 1.5 ml Eppendorf tubes and extracted with 200 µl of isooctane. The glands were removed and extracted separately.

De novo synthesis of juvenile hormone, from either endogenous or exogenous farnesoic acid, was assayed from an aliquot of the isooctane phase by liquid scintillation spectrometry, and corrected by a blank incubation. Thin-layer chromatography confirmed that more than 85% of the radioactivity in the isooctane, which was not attributed to methionine (i.e. after blank subtraction), corresponded to juvenile hormone III. The remaining radioactivity (about 15%) found in the isooctane phase consisted of a tailing of more polar unidentified substances. It is unknown whether this radioactivity corresponds to other metabolic byproducts or to impurities from methionine which are more soluble in isooctane. The glands were removed and extracted separately.

Results

Parameters of juvenile hormone synthesis in vitro

Juvenile hormone release rate is an accurate measure for juvenile hormone biosynthesis rate in vitro for both basali and farnesoic acid-stimulated synthesis (r = 0.975, n = 28; Fig. 1), and the two terms will be used interchangeably throughout this paper. Juvenile hormone synthesis rate is unaffected by the presence of the corpora cardiaca (t-test, P > 0.05; Table 1) and since corpora allata-corpora cardiaca complexes are easier to dissect, they were used in all physiological studies which follow.

Table 1. The effect of coincubation of corpora allata with corpora cardiaca on juvenile hormone biosynthesis in vitro

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Juvenile hormone synthesis (pmol·h·pair glands·⁻¹)</th>
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<tbody>
<tr>
<td>Low-activity glands†</td>
<td>1.4 ± 0.2 (5)</td>
</tr>
<tr>
<td>High-activity glands†</td>
<td>10.5 ± 1.4 (9)</td>
</tr>
<tr>
<td>Pair of corpora allata</td>
<td>1.5 ± 0.3 (6)</td>
</tr>
<tr>
<td>Corpora allata-corpora cardiaca complexes</td>
<td>7.8 ± 0.9 (8)</td>
</tr>
</tbody>
</table>

*Mean ± SEM from pairs of corpora allata incubated for 2 h either alone or as intact complexes with corpora cardiaca.
†Number of replicates indicated in parentheses.
‡Glands obtained from 3-day isolated females.
§Glands obtained from 5-day grouped females.

Fig. 1. Relationship between juvenile hormone synthesis and release by corpora allata incubated with (C) and without (O) farnesoic acid (100 µM). Each point represents an individual assay.

Linear rates of juvenile hormone synthesis were demonstrated in individual corpora allata-corpora cardiaca complexes through at least 8 h in vitro (Fig. 2). Dose-response effects of methionine and farnesoic acid concentrations on juvenile hormone synthesis were demonstrated with high-activity corpora allata (Figs 3 and 4).

In vitro rates of juvenile hormone synthesis during the gonotrophic cycle

The farnesoic acid-stimulated rate of juvenile hormone synthesis is highly correlated to the basal rate of synthesis [r = 0.93, n = 127, and y = 2.56x + 3.35 is the regression of farnesoic acid-stimulated rate (y) on the basal rate (x)]. Both rates exhibit similar cyclic patterns during at least the first two ovarian cycles (Fig. 5). Both basal rates and farnesoic acid-stimulated rates of synthesis are undetectable in mid-'pregnancy' (day 25). The farnesoic acid-stimulated activity of the corpora allata increases before the termination of 'pregnancy' (Fig. 5).

Using the length of the basal oocytes as a correlate of juvenile hormone synthesis in vitro, no significant differences in the rates of juvenile hormone synthesis are evident between the first two gonotrophic cycles (t-test for each oocyte length, P < 0.05, Fig. 6). The rate of juvenile hormone synthesis peaks in females with basal oocyte lengths in the range of 1.4–2.2 mm, and declines just before ovulation, when oocytes are larger than 2.3 mm (Fig. 6). During 'pregnancy' (about 22 days in our colony), both juvenile hormone synthesis and maturation of basal oocytes are inhibited.
Fig. 2. Time-course of juvenile hormone release in vitro. Each point represents the cumulative juvenile hormone released by an individual pair of corpora allata. The glands were transferred to fresh media for each successive period.

The cyclic changes in the volume of the corpora allata correspond to the cyclic changes in their activity (Table 2). The volume of the glands increases 2-fold during oocyte development and decreases again at ovulation. The corpora allata are smallest in mid-'pregnancy', when both basal and farnesoic acid-stimulated activity are undetectable (Table 2).

**DISCUSSION**

Juvenile hormone III was found to be the only juvenile hormone homologue in *B. germanica* adult females, in both *in vivo* and *in vitro* studies (Camps *et al.*, 1987; Belles *et al.*, 1987). Stoichiometry of the *in vitro* incorporation of the radiolabelled methyl group from methionine into juvenile hormone was also demonstrated in this species (Belles *et al.*, 1987). We examined several other parameters of the *in vitro* system in order to verify its adequacy as a tool for physiological investigations on the regulation of the corpora allata in this insect. All of these parameters (Table 1, Figs 1–4) are in agreement with similar studies in other cockroach species (reviews: Tobe and Stay, 1985; Feyereisen, 1985). Although our rates of juvenile hormone synthesis are quite moderate in comparison to rates reported for other insect systems, they are much higher than reported for *B. germanica* by Belles *et al.* (1987), who used somewhat different incubation conditions and employed different methods for the purification and assay of the radiolabelled juvenile hormone. Thus, until a reliable method of determining the *in situ* rates of juvenile hormone synthesis is developed, all comparisons among *in vitro* studies must be made in relative terms, while absolute values are viewed only as approximations.

A precise relation between juvenile hormone synthesis *in vitro* and the gonotrophic cycle was shown: rates of juvenile hormone synthesis increase during vitellogenesis, peak in late vitellogenesis and decline sharply before ovulation (Figs 5, 6). During 'pregnancy' the basal activity of the corpora allata is undetectable, but resumes after hatching of the nymphs. Volumetric changes in the corpora allata follow a similar cyclic pattern (Table 2) as was also shown by Belles *et al.* (1987). In adult females reared in isolation and mated on day 8, the second oocyte maturation cycle is shorter than the first, but the patterns of corpora allata activity, relative to maturation of the oocytes, are identical in both cycles (Figs 5, 6). External stimuli, such as from mating and grouping, which modulate the activity pattern of the corpora allata, may confound the relationship

Fig. 3. Dose–response relationship for juvenile hormone release *in vitro* as a function of L-methionine concentration. Each point is the mean ± SEM of 3–4 glands from 5–6-day grouped virgin females.

Fig. 4. Dose–response relationship for juvenile hormone release *in vitro* as a function of farnesoic acid concentration. Each point is the mean ± SEM of 3–7 glands from 5–6-day grouped virgin females.

Fig. 5. *In vitro* rates of juvenile hormone release during two successive gonotrophic cycles. Each point represents the mean ± SEM of the basal (○) or farnesoic acid-stimulated (●) rates from 4–20 assays. Day 0 represents either the day of adult emergence, or the day of hatching of the nymphs (females enter second gonotrophic cycle). Isolated females were allowed to mate on day 8 of the first gonotrophic cycle, and ovulation occurred on day 12, on the average.
levels and different modes of regulation (i.e. inhibitory and excitatory mechanisms). Tobe (1980) associates the degree of regulation of the corpora allata with the reproductive mode of the female, predicting that insects which show periods of ovarian 'quiescence' will possess a more precise regulatory mechanism of the corpora allata to insure the inactivation of the glands during these periods.

**Farnesoic acid stimulation**

Farnesoic acid-stimulated rates of juvenile hormone synthesis in *B. germanica* indicates that the activity of the non rate-limiting enzymes, which dictates the maximal capacity of the glands, is highly correlated to the activity of the rate-limiting enzymes, which dictate the basal rate of juvenile hormone synthesis. The cyclic changes in the farnesoic acid-stimulated rates during the gonotrophic cycle (Fig. 5) and in relation to oocyte maturation (Fig. 6) are accompanied by a similar pattern of changes in corpora allata volume (Table 2). Scanning electron microscopy shows that the basal lamina of the small inactive glands from females in 'mid-pregnancy' is different from that of the small inactive glands from newly emerged females (data not shown). Large invaginations of the basal lamina in the first case are probably indicative of cell degeneration inside the glands. We suggest that during pregnancy a process of cell death and regrowth takes place, and inhibition of activity at this stage is due to the overall low biochemical capacity of the degenerated cells. Further experiments to test this hypothesis are now in progress.

Table 2. Corpus allatum volume in relation to the gonotrophic cycle

<table>
<thead>
<tr>
<th>Gland volume (10^6 µm^3)</th>
<th>Newly emerged</th>
<th>Peak activity</th>
<th>At ovulation</th>
<th>Mid-'pregnancy' 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.87 ± 0.06a (8)</td>
<td>1.63 ± 0.09b (16)</td>
<td>0.83 ± 0.02a (8)</td>
<td>0.63 ± 0.11a (8)</td>
</tr>
</tbody>
</table>

*Means ± SEM followed by the same letter are not significantly different (Duncan's multiple range test, P < 0.05). Number of replicates indicated in parentheses.

In orthopteran species (L. migratoria, S. gregaria and *Melanoplus sanguinipes*), the relation between the *in vitro* rates of juvenile hormone synthesis and oocyte development is much less precise (review: Feyereisen, 1985), although a general trend does exist, at least in *L. migratoria* (Gadot and Applebaum, 1985, 1986). Tobe (1980) suggested that the orthopteran species lack a precise regulatory mechanism of juvenile hormone synthesis because they rely on only one mode of regulation (i.e. excitatory mechanism), while the cockroach species have a more complex mechanism, which involves several
Juvenile hormone biosynthesis

but may also operate independently, as suggested by the hypertrophy of low-activity corpora allata in ovarioctomized females of *D. punctata* and *N. cinerea* (Tober et al., 1984; Lanzrein et al., 1981). Independent activation of rate-limiting steps may be superimposed on these cyclic developmental changes, as indicated by the following examples: during peak activity of corpora allata in *D. punctata*, basal rates increase relatively more than farnesoid acid-stimulated rates of juvenile hormone synthesis (Feyereisen et al., 1981). Using brain extracts of adult female *D. punctata*, rapid and reversible inhibition of juvenile hormone synthesis was demonstrated in vitro; corpora allata with low spontaneous rates of synthesis showed higher sensitivity to the allatostatic factor (Rankin and Stay, 1987). Lastly, following unilateral allatectomy, compensation in juvenile hormone synthesis by the remaining corpus allatum is also indicative of independent activation of specific enzymes, since these elevated rates are not matched by increases in gland volume or cell number (Szibbo and Tober, 1981).

It is tempting to speculate that in species which require rigorous control of juvenile hormone levels, especially during a protracted pregnancy involving arrest of oocyte maturation, the preferred mechanism of "restraining" the corpora allata will rely on structural/developmental processes, which affect the whole biochemical machinery, as well as the synthesis of specific key enzymes. Conversely, in species which develop basal oocytes in rapid succession, or even overlap oocyte maturation cycles, as in the oviparous *P. americana* and the locusts, the faster and more flexible control through rate-limiting enzymes will be preferred, although not exclusively. Before it is generalized to other insects, the proposed functional relation between the type of control of the corpora allata and the mode of ovipositional behavior can best be evaluated through comparative studies with many insect species.

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