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Correlation Among Corpus Allatum Volume, Cell Size, and Juvenile Hormone Biosynthesis in Ovariectomized Adult *Blattella germanica*

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The corpora allata (CA) of both intact and ovariectomized *Blattella germanica* adult females exhibited a high degree of bilateral symmetry in the rate of juvenile hormone (JH) biosynthesis, the mean size of CA cells, and gland volume (81.3%, 98.3%,and 100% respectively with less than a twofold difference between the two glands in CA pairs). This permitted us to split each CA pair randomly, measure JH biosynthesis in one gland, and dissociate the other gland into a cell suspension in which the size of CA cells was measured. In ovariectomized females, changes in CA volume and the spontaneous and farnesoic acid (FA)-stimulated rates of JH biosynthesis, measured from the same glands, were well correlated ($r = 0.78$, for both correlations). Similarly, the mean volume of CA cells in one gland increased in relation to increases in both the spontaneous and FA-stimulated rates of JH biosynthesis by the contralateral member of the pair ($r = 0.83$ and $r = 0.91$, respectively). Concurrent changes in CA cell size and activity suggest that in the CA of *B. germanica* cellular growth and degradation are involved in the regulation of JH biosynthesis.

Key words: cockroach, farnesoic acid, gland volume, symmetry, ovariectomy

INTRODUCTION

Changes in CA* volume have been correlated with glandular activity in many insects; however, the rate of JH biosynthesis has been determined in only a few species [1–4]. In adult female cockroaches volumetric changes in the CA

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*Abbreviations used: CA = corpus allatum or corpora allata, FA = farnesoic acid, JH = juvenile hormone.

are related to the normal cycle of in vitro JH biosynthesis [5–7] but not under certain experimental conditions [8,9]. For example, ovariectomy results in hypertrophy of the CA in *Leucophaea maderae* [10] and *Blattella germanica* [11]. In ovariectomized *B. germanica* a moderate decline in JH synthesis after CA activation is not accompanied by regression in CA volume [9]. Conversely, the CA of ovariectomized *Diploptera punctata* [12,13] and *Nauphoeta cinerea* [14] do not undergo hypertrophy and they exhibit low activity compared with the respective peak rates of JH biosynthesis in intact adult females.

Volumetric changes in normal and hypertrophic CA have been attributed to changes in cell size and not in the number of CA cells in *B. germanica* [11]. In other cockroaches, changes in CA cell size are common but the contribution of cell number to changes in CA volume remains controversial [11,15–18]. The size of CA cells has been measured directly in glands that were dissociated into cell suspensions; such cells from *B. germanica* CA retain their capacity to synthesize JH at normal rates in vitro [18]. In three cockroach species cells from active CA were significantly larger than cells from inactive CA [18]. This correlation has also been shown in ovariectomized *B. germanica* [19]. However, because cells from the CA of several females of the same age were pooled, a direct correlation between cell size and JH biosynthesis for each individual CA is not available. This is particularly important when changes in CA activity are not synchronous among insects, as is common in ovariectomized females [9,20].

In this study the degree of symmetry between the two members of CA pairs was established and used as a tool to study the correlation between the size of CA cells and JH biosynthesis within the same females.

MATERIALS AND METHODS

Insects

German cockroaches, *B. germanica*, were reared at 27 ± 1°C under a 12 h light: 12 h dark photoperiodic regime and supplied with pelleted Purina Dog Chow #1780 and water ad libitum. Newly ecdysed adult females (day 0) were collected daily and maintained in groups [21]. Intact females in the first two ovarian cycles (from day 0 to day 49) were used and they were all mated on day 6 [9]. Ovariectomies were performed early in last-instar nymphs and adult females from day 0 to day 49 that mated within 8 days after the imaginal molt were used. Insects were always immobilized by chilling on ice before performing various treatments and antibiotics were not used.

CA Volume

Pairs of CA were removed from adult females of various ages and separated in saline [22]. CA volume was determined by the formula *B. germanica* $v = 4/3 \cdot \pi \cdot abc$, where a, b, and c are the radii of the three principal axes measured with an ocular filar micrometer under a dissecting microscope at × 40. The two CA were either incubated separately to determine JH release rates or one CA was assayed and the contralateral gland used to determine mean CA cell size.

CA Cell Size

Individual CA were digested with 0.1% trypsin in isotonic cockroach saline and then dispersed by gentle vortexing [18]. CA cells were randomly sampled

in a hemocytometric grid. Since dissociated CA cells of *B. germanica* are largely globular [26] cell volume was determined by the formula $v = 4/3 \cdot \pi \cdot r^3$, where r is one-half of the maximum diameter measured under a compound microscope at $\times 400$. Each mean cell volume was determined from 40–100 cells from a single CA.

Radiochemical Assay

L-[methyl-^{3}H]Methionine (specific activity of 200 mCi/mmol) was obtained from New England Nuclear, Wilmington, DE. Farnesoic acid (about 70% pure) was a generous gift from Dr. F. C. Baker (Zoecon Corp., Palo Alto, CA). The rate of JH synthesis was determined according to the methods of Pratt and Tobe [23] with modifications after Gadot et al. [24]. Individual CA were incubated in 50 μl of medium 199 (GIBCO; special formulation after Tobe and Clarke [25]) containing 75 μM of L-[methyl-^{3}H]methionine (1.5 μCi). After a 2 h incubation with gentle shaking in the dark at 28°C the CA were transferred to fresh medium containing 100 μM FA for an additional incubation period of 2 h. The gland was removed at the end of the incubation and radiolabeled JH in the incubation medium was extracted with 200 μl isooctane. After a 5 min centrifugation (2,500*g*) a 100 μl aliquot from the isooctane phase was mixed with 5 ml Scintilene (Fisher) and assayed for radioactivity by liquid scintillation spectrometry. Radioactivity from each incubation was corrected by a blank incubation and multiplied by two to express the total isooctane phase. Incubations and extractions were performed in the same disposable borosilicate glass culture tubes (0.7 ml).

RESULTS

Symmetry of the Corpora Allata

The CA of *B. germanica* are highly symmetric both functionally and developmentally. Figure 1 shows spontaneous and FA-stimulated JH release rates by both members of 160 CA pairs from ovariectomized adult females of different ages. Although the FA-stimulated JH release rates were always higher than the corresponding spontaneous rates, the degree of symmetry between members of each CA pair was similar for both parameters; therefore, data for both parameters were pooled. The JH release rate of the more active gland of each pair was not more than twice that of the less active CA in 81.3% of the determinations and not more than threefold greater in 92.4% of the determinations. Greater glandular asymmetry was observed mainly when the JH release rates were relatively low in both members of a pair (Fig. 1), and in some CA only one member of the pair had detectable JH synthesis. When both members of the CA pair were active (greater than 2 pmol/h/single CA) the degree of glandular assymetry was not correlated with either age or the level of CA activity.

In *B. germanica*, CA volume changes cyclically in relation to JH synthesis and oocyte growth in intact females, but it increases continuously in ovariectomized females despite a moderate decline in JH synthesis; CA cell number remains constant in both groups of females [11]. To determine whether the

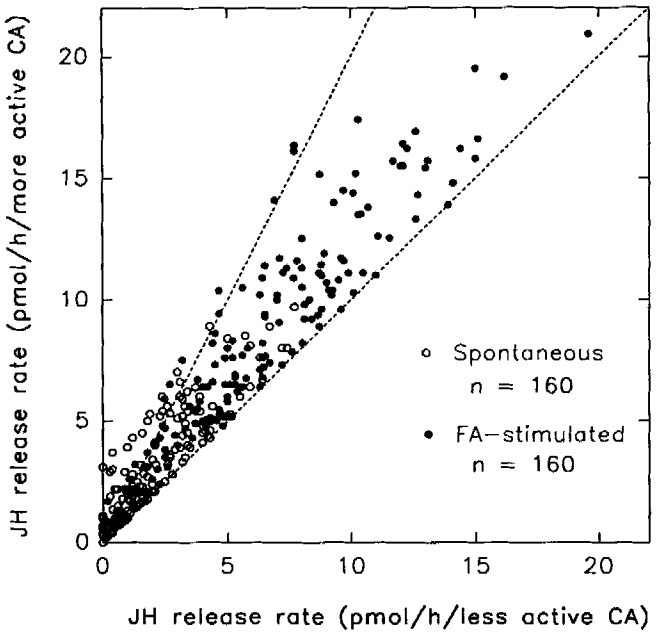

Fig. 1. Spontaneous and FA-stimulated rates of JH synthesis of one CA in relation to the respective rates of the contralateral CA from ovariectomized *B. germanica* adult females. Ovariectomy was performed on early last-instar nymphs. FA-stimulated rates of JH synthesis were determined after the spontaneous rates were obtained for the same CA from females at various ages. The area between the two dashed lines represents up to a twofold difference between the two members of the CA pairs.

CA of *B. germanica* are developmentally symmetric as they are functionally symmetric, we measured gland and cell volumes of each member of CA pairs from intact and ovariectomized females of different ages. The CA were highly symmetric with respect to both parameters (Fig. 2). In all CA pairs the volume of the larger gland was not more than twice the volume of the smaller gland, averaging 1.16 ± 0.01-fold (n = 174). In only 1.7% of the CA pairs measured, the gland with the larger mean cell volume exceeded by twofold the mean cell volume of the other CA, averaging 1.19 ± 0.03-fold (n = 65).

Correlation Between Morphometric Parameters and JH Synthetic Rates in Ovariectomized Females

Figure 3 shows direct correlations between CA volume and spontaneous as well as FA-stimulated JH synthesis rates from the same CA in ovariectomized females ($r = 0.78$, $n = 96$ for both correlations, Pearson correlations). CA from ovariectomized females from day 0 to day 49 were used.

Figure 4 shows the relation between mean CA cell volume in one CA and JH synthesis by the contralateral CA of the same ovariectomized female. Both spontaneous and FA-stimulated rates of JH synthesis were highly correlated with mean cell volume ($r = 0.83$ and $r = 0.91$ respectively, $n = 36$). All correlations in Figures 3 and 4 are highly significant ($P < 0.001$).

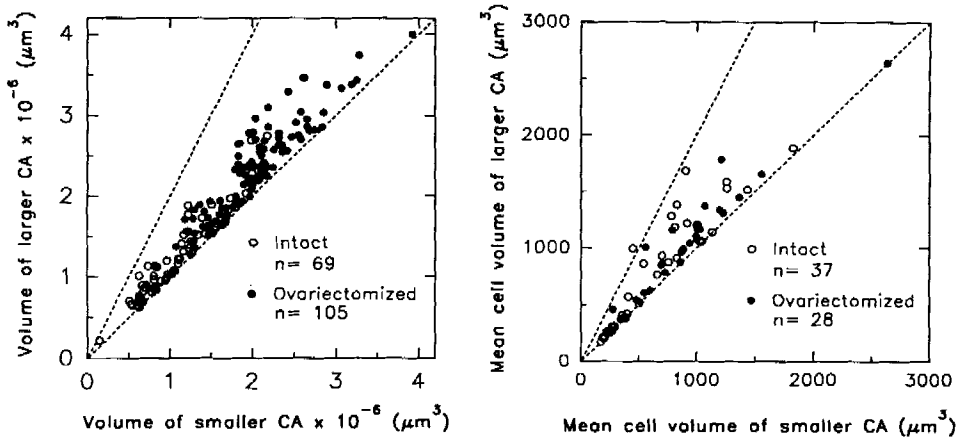

Fig. 2. Symmetry of CA volumes and mean cell volumes between glands within CA pairs from intact and ovariectomized *B. germanica* adult females at various ages. Each point represents two determinations from an individual animal. Each mean cell volume was determined from 40–100 cells from a single CA. The area between the two dashed lines represents up to a twofold difference between the two members of the CA pairs.

DISCUSSION

The right and left CA have been shown to synthesize JH in a symmetric manner with less than a twofold difference in 72% and 90% of the pairs assayed in vitro in *Periplaneta americana* and *D. punctata* respectively [17,27]. In contrast, in *Schistocerca gregaria* the rate of JH biosynthesis was highly asymmetric with 35% of the CA pairs assayed exhibiting greater than a tenfold difference between the right and left CA [28]. High degrees of asymmetry were also found in other insect species, indicating that the degree of symmetry should be examined for each species [3], especially when each member of the pair is used in different assays or when one serves as the control for an in vitro treatment of the other.

Our results showing symmetry in function and volume between the two CA in *B. germanica* are in agreement with findings in other cockroaches. Furthermore, we show that FA-stimulated rates of JH biosynthesis are as symmetric in the two members of CA pairs as the corresponding spontaneous rates regardless of the physiological ages and conditions of the insects. Developmental symmetry is also demonstrated at the cellular level by comparing the mean cell volume of the right and left CA.

The rate of JH synthesis may be regulated by rapid changes in rate-limiting steps or slowly through developmental mechanisms which may be reflected in changes in cell number, cell size, and cellular organelles [3,31]. In the presence of exogenous FA, CA activity in vitro is a measure of their competence to esterify and epoxidize FA to JH III and it has been considered to reflect the maximal biochemical capacity of the CA [29,30]. In ovariectomized *B. germanica*, the FA-stimulated rates of JH synthesis are highly correlated with the corresponding spontaneous rates ($r = 0.92$, $n = 96$) as we have reported previously in intact females ($r = 0.93$, $n = 127$) [9]. Because both parameters exhibit sim-

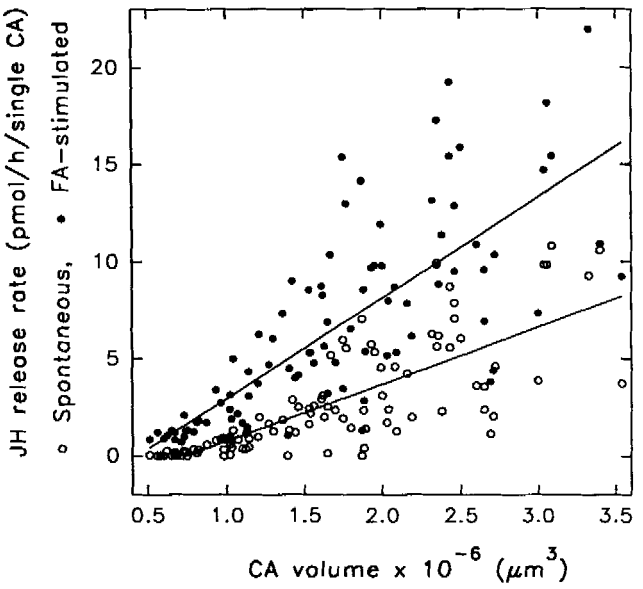

Fig. 3. Direct correlation between CA volume and spontaneous and FA-stimulated JH release rates in ovariectomized *B. germanica* adult females at various ages. All three parameters were determined from the same CA, so each point represents a single CA. The lines represent linear regressions for each parameter.

ilar patterns under various physiological conditions [9,24] it appears that CA activity in *B. germanica* is regulated by developmental mechanisms to a greater extent than by biochemical rate-limitation before the last two steps in the JH biosynthetic pathway.

CA volume has been widely used as an indicator of the developmental status of the gland but shown to not always correlate with CA activity in some insects [1]. In intact *B. germanica* females the size of the CA changes cyclically in relation to the cycles of JH biosynthesis. In ovariectomized females, CA volume increases continuously and does not reflect a significant decline in JH biosynthesis that occurs after CA activation [9,11]. In the present study, however, females of different ages were used and females at this physiological stage represented a minor fraction of the total sample (12 of 96 determinations for each correlation in Fig. 3).

The transient decline in JH biosynthesis in ovariectomized females, as well as other changes in activity in both intact and ovariectomized females, was always reflected by changes in mean CA cell size [19]. Ultrastructural studies show that in cockroaches changes in CA volume during the ovarian cycle are accompanied by changes in both intracellular components and intercellular spaces [32,33]. In our experiments, large vacuoles were often found in the CA of intact females during mid-pregnancy and in the hypertrophic CA of old ovariectomized females (unpublished observations). These data suggest that CA volume does not necessarily reflect the total volume of all cells. While the correlation between CA volume and activity is generally high but related to specific physiological stages, cell volume appears to correlate with CA activity

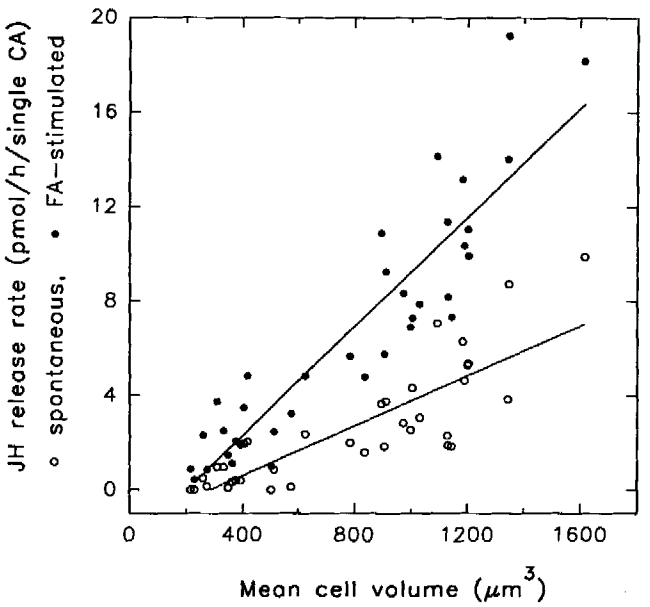

Fig. 4. Correlation between mean CA cell volume and spontaneous and FA-stimulated JH release rate in ovariectomized *B. germanica* adult females at various ages. The two members of each CA pair were separated and randomly assigned for JH release rate or cell size determinations. Each point represents the mean cell volume of 40–100 cells from one dissociated gland and the corresponding spontaneous or FA-stimulated rate of JH synthesis of the intact contralateral gland. The lines represent linear regressions for each parameter.

at all physiological stages examined. This may account for the greater correlation between JH biosynthesis and cell volume than with gland volume.

We have shown an increase in the rate of JH biosynthesis of one CA in relation to the mean cell volume of the contralateral CA in ovariectomized *B. germanica* females (Fig. 4). Furthermore, our results suggest that in *B. germanica* the size of CA cells is a reliable parameter reflecting the developmental status of the CA. Since changes in CA cell size occurred concurrently with their activity, we hypothesize that in *B. germanica* cellular growth and degradation of the CA are involved in the regulation of JH biosynthesis.

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