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Cyclic Volumetric Changes in Corpus Allatum Cells in Relation to Juvenile Hormone Biosynthesis During Ovarian Cycles in Cockroaches

Ann-Shyn Chiang and Coby Schal

Institute of Life Science, National Tsing-Hua University, Hsinchu, Taiwan, Republic of China (A-S.C.); and Department of Entomology, Cook College, Rutgers University, New Brunswick, New Jersey (C.S.)

Development and activity of the corpora allata (CA) were investigated in adult female *Blattella germanica* and *Supella longipalpa*. These two cockroach species differ in their reproductive modes, with relatively uninterrupted cycles of oocyte development in *S. longipalpa* and discrete patterns of oocyte development which are interrupted by pregnancy in *B. germanica*. During ovarian cycles in both cockroach species, elevated rates of juvenile hormone (JH) synthesis closely coincide with synchronous volumetric growth of the CA. Declines in CA activity before ovulation coincide with synchronous declines in the size of CA cells. However, in adult females of both species the number of CA cells remains relatively constant. Quantitative studies in normal and ovariectomized adult *B. germanica* females show that the volumetric changes in CA cells are paced and synchronized by ovarian factors. Without the ovaries, the enlargement of CA cells in newly eclosed females is slower and relatively asynchronous. Without an ootheca in ovariectomized females, the volume of CA cells fails to decline synchronously, resulting in variable but high rates of JH synthesis. The precise relationship between volume of CA cells and JH biosynthesis in oviparous and viviparous cockroaches suggests that in cockroaches, cell volume, and not CA cell number, is a better predictor of JH biosynthetic activity.

Key words: endocrine gland development, cell size, egg case, reproductive cycle

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Address reprint requests to Dr. Coby Schal, Department of Entomology, North Carolina State University, Box 7613, Raleigh, NC 27695-7613.

INTRODUCTION

In cockroaches, a cycle of JH* biosynthesis is essential for successful egg maturation and is closely paralleled by a cycle of oocyte development [1–4]. JH production increases as the oocytes grow and is suppressed just before and during ovulation and pregnancy. Cycles of JH synthesis during gonotrophic cycles are regulated by environmental cues, internal states (e.g., nutrition, mated status), and feedbacks from target tissues acting either directly on the CA or indirectly through the brain. In spite of extensive progress in studies of CA regulation in the last three decades, how the CA respond to regulatory factors that modulate the production of JH during the gonadotrophic cycle remains uncertain. It has been hypothesized that the biosynthetic activity of the CA during the gonadotrophic cycle may be regulated at several levels, including enzymatic rate-limitation as well as a cyclic increase and subsequent regression of CA cell organelles and CA cell number [2].

Histological studies have shown that a cycle of CA activity is associated with cyclic changes in the amount of cellular organelles as indicated by cyclic changes in the nucleocytoplasmic ratio in *Leucophaea maderae* [5], *Diploptera punctata* [6], and *Blattella germanica* [7]. The involvement of developmental changes in CA cells in the regulation of CA activity is further supported by quantitative studies of changes in CA cell size in three cockroach species [8]. On the other hand, the involvement of cyclic changes in the number of CA cells in the regulation of cyclic JH production in the adult female remains unsettled since histological techniques of counting cell number from CA sections have produced inconsistent results even in studies involving the CA of the same cockroach species, *L. maderae* [5,9].

Cockroaches exhibit various reproductive modes including oviparity, ovoviviparity, and viviparity [10]. Thus they offer excellent models for comparative studies on the ovarian feedback regulation of JH biosynthesis. *B. germanica* (German cockroach) and *Supella longipalpa* (brown-banded cockroach) are both oviparous blattellids, but they exhibit different oviposition behaviors. *B. germanica* females carry the ootheca externally for about 21 days until the young hatch. During this time, oocyte development is inhibited as in viviparous *D. punctata* in which the ootheca is also oviposited externally but then retracted into the brood sac. In contrast, under identical rearing conditions in our laboratory, *S. longipalpa* females deposit the ootheca within 36 h of oviposition and the new basal oocytes develop quickly after oviposition.

Our pivotal question is whether the CA of cockroaches that exhibit different reproductive modes and therefore different patterns of activity share a common underlying regulatory mechanism of their cyclic activity. Our data indicate that CA cells of *S. longipalpa* exhibit rapid cyclic growth and regression in relation to JH biosynthetic activity. In contrast, CA cells in *B. germanica* exhibit slow developmental cycles corresponding to the protracted ovarian cycle. Ovariectomized *B. germanica* are an ideal model in which to study the interaction among ovarian factors, JH biosynthesis, and CA cell development because JH biosyn-

*Abbreviations used: CA = corpus allatum or corpora allata; JH = juvenile hormone.

thesis remains high without apparent cycles in the absence of an ootheca [11]. Insertion and subsequent removal of an artificial egg case in ovariectomized females with active CA mimics a transient pregnancy and restores a cycle of JH biosynthesis and CA cell development. Yet, total CA cell number remains constant in both cockroach species during cycles of oocyte development, as well as in ovariectomized *B. germanica.*

MATERIALS AND METHODS

Insects

Colonies of *B. germanica* and *S. longipalpa* were maintained at 27 ± 0.3°C as described previously [12,13]. Under our rearing conditions, grouped *B. germanica* females that mated on day 6 ovulated on day 9 and nymphs hatched from the retained ootheca on day 30, while *S. longipalpa* females that mated on day 8 ovulated on day 10 and dropped the ootheca on day 11. Ovariectomy was performed early in the last instar in *B. germanica* and only females that mated within 8 days after the imaginal molt were used [11]. Artificial egg cases were inserted into the genital vestibulum as described previously [14].

CA Biosynthetic Activity

In vitro rates of JH biosynthesis were determined by a radiochemical assay modified from Pratt and Tobe [15] as described previously [13,16].

Morphometric Measurements of the CA

The volume of a fresh CA was determined by the formula $v = 4/3 \bullet \pi \bullet abc$, where a, b, and c were the radii of the three principal axes as measured with an ocular filar micrometer under a dissecting microscope.

The maximal diameter of each CA cell was measured and served as an indicator of cell size [8]. Enzymatically dissociated cells were randomly sampled in a hemocytometric grid. We have reported earlier that in *B. germanica* the size of dissociated cells does not appear to change, and they continue to synthesize JH in a cell suspension [8].

Total cell number was determined as an absolute count from a whole-mount monolayer of CA cells prepared from a single CA [8,17]. To prepare a monolayer, the CA was dissected, cleaned, and then digested with collagenase. After fixation and staining CA cells were spread into a monolayer beneath a coverslip.

RESULTS AND DISCUSSION

JH Biosynthesis

In both *B. germanica* and *S. longipalpa,* the CA exhibit cyclic patterns of JH biosynthesis in relation to age and oocyte development (Fig. 1). In the first and second ovarian cycles of both species, JH production increased as the basal oocytes matured, but declined just before and during ovulation. Between ovulation and deposition of the ootheca, CA activity was suppressed while females carried the egg case. The patterns of JH synthesis differed in the two species by the duration of suppression of CA activity during the gravid period,

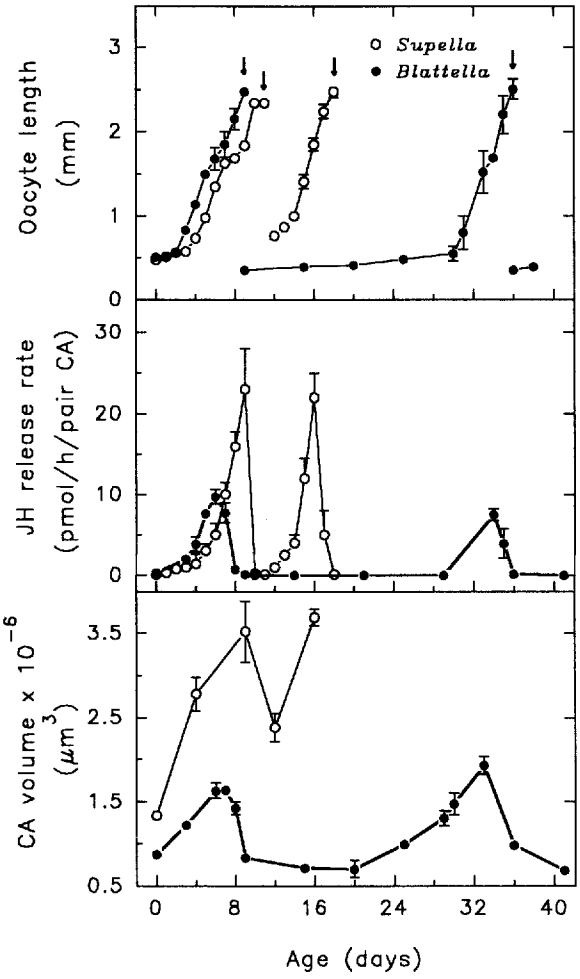

Fig. 1. Basal oocyte length, in vitro rates of JH synthesis by pairs of CA, and volumes of single CA during the first two reproductive cycles of mated *B. germanica* and *S. longipalpa* females. Arrows indicate day of oviposition. Values represent the mean ± S.E. of 5–16 individual determinations. For some means, error bars are obscured by the symbol. Data for JH release rates and CA volumes for *B. germanica* redrawn from [25] and data for JH release rates and oocyte length for *S. longipalpa* redrawn from [13].

which persisted for 21 days in *B. germanica* and for less than 2 days in *S. longipalpa*. Removing the ootheca, or transecting the ventral nerve cord in gravid *B. germanica*, results in activation of the CA [18,19]. Thus, presence of the ootheca plays an important role in arresting CA activity and oocyte development. Another difference between the two species is the maximum JH biosynthetic rates which are more than twice as high in *S. longipalpa* as in *B. germanica* during the first two ovarian cycles. The similar relationship between oocyte maturation and JH synthesis in both species, and the importance of cyclic developmental changes in *B. germanica* CA [20] suggested that despite their different reproductive patterns, both species might share common mechanisms that regulate JH synthesis.

CA Volume

Cyclic changes in CA volume during the gonadotrophic cycle have been found in all cockroaches examined to date and they usually, but not always, correlate with fluctuations in CA activity [21–25]. In both *B. germanica* and *S. longipalpa* CA volume and JH production peaked concurrently in the first and second reproductive cycles (Fig. 1). However, the two parameters did not correlate absolutely at other times. For example, in 8-day-old *B. germanica* adult females, the rate of JH biosynthesis declined to an almost undetectable level before ovulation while CA volume decreased only slightly. Also, during late pregnancy in *B. germanica,* growth of the CA preceded a subsequent elevation in JH production in the second gonotrophic cycle. Thus, one day before the young hatched (day 29), the volume of the inactive CA was as large as the volume of maximally active CA in the first ovarian cycle. In *S. longipalpa,* the CA became completely inactive after ovulation whereas gland volume decreased only to an intermediate level before increasing again in the second gonotrophic cycle (Fig. 1).

The relatively concurrent changes in JH biosynthesis and CA volume suggest that developmental changes in the CA are involved in the regulation of JH biosynthesis. However, since CA volume is not a good predictor of JH production, other, more specific cellular parameters need to be considered. Growth and atrophy of an organ can be achieved through (1) changes in the number of cells, (2) changes in the size of cells, or (3) changes in the amount of extracellular substance and intercellular spaces. The contributions of each of these parameters to changes in CA volume have been investigated extensively in insects but with little agreement among researchers [5,8,9,23]. Here, we examine the first two parameters (cell number and size) in relation to cyclic changes in CA volume and activity. Our goal was to determine which parameter correlated most closely with the cyclic activity of the CA.

CA Cell Number

Our results of total cell counts from whole-mount CA monolayers showed that the number of CA cells remained relatively constant in both *B. germanica* and *S. longipalpa* adult females throughout the first and second ovarian cycles (Fig. 2). Each corpus allatum contained approximately 2,000 cells in normal *B. germanica* adult females and approximately 3,300 cells in *S. longipalpa.* Although declines in CA cell number are evident around ovulation and oviposition in both species, these are not significant changes. Also, unlike *D. punctata,* in which total cell number increases after ovariectomy [26], the total CA cell number in ovariectomized *B. germanica* remained unchanged (Fig. 2). In our observations of hundreds of CA monolayers of intact and ovariectomized *B. germanica* and *S. longipalpa* adult females we have not seen any mitotic nuclei. By contrast, mitosis of CA cells is frequently observed in nymphal cockroaches and can be arrested with colchicine (unpublished observation). These results indicate that in *B. germanica* and *S. longipalpa* the cyclic changes in CA activity and volume in adult females do not involve significant changes in total CA cell number.

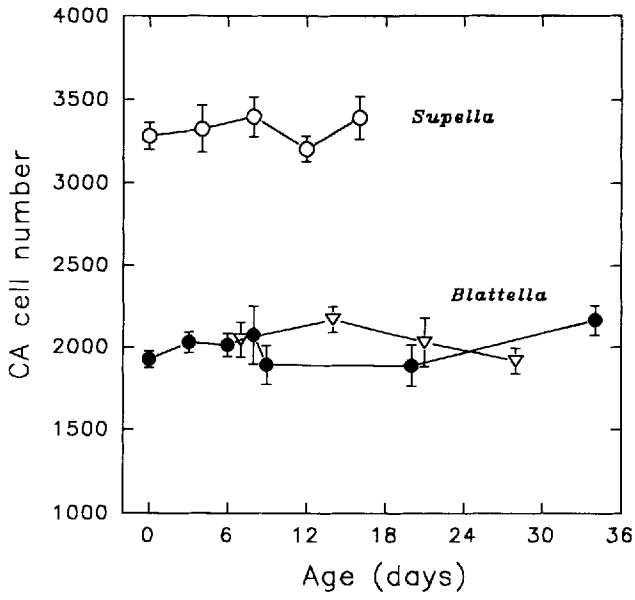

Fig. 2. Absolute count of cell number per single CA in intact (triangles) and ovariectomized (closed circles) adult *B. germanica* and intact *S. longipalpa* females (open circles) during the first two reproductive cycles. Means represent 4–16 individual determinations. Vertical bars represent S.E. Data for *B. germanica* redrawn from [25].

However, the total number of CA cells appears to play a major role in setting the maximum rate of JH production in each species. Peak rates of JH synthesis during the first ovarian cycle were approximately 100, 20, and 10 pmol/h/pair CA in *Diploptera punctata* (12,000 cells/single CA), *S. longipalpa* (3,300 cells/single CA), and *B. germanica* (2,000 cells/single CA), respectively. Hence, by having more functional units (cells), the CA of *D. punctata* and *S. longipalpa* may have higher capacities to synthesize JH than the CA of *B. germanica*. Interestingly, if similar fractions of the CA cell populations contribute to JH biosynthesis in vitro in all three species, then it appears that JH production per cell might be surprisingly similar in these species whose CA activity is widely different. To obtain more precise correlations between cell number and CA capacity to synthesize JH, one must determine the optimal conditions for maximal JH synthesis in vivo and in vitro in each species or in the CA of the same species with different total CA cell number.

Cell Size

CA cells of adult *B. germanica* and *S. longipalpa* females undergo cyclic volumetric changes in relation to JH synthesis during the first and second ovarian cycles (Figs. 3, 4). In *B. germanica*, the mean size of CA cells and the rate of JH synthesis increased simultaneously during oocyte maturation and peaked on day 7 and day 34 in the first and second ovarian cycles, respectively (Fig. 3). Both parameters declined dramatically just before and during ovulation (days 8–9 and 35–36). In gravid females, CA cells remained small and JH synthesis

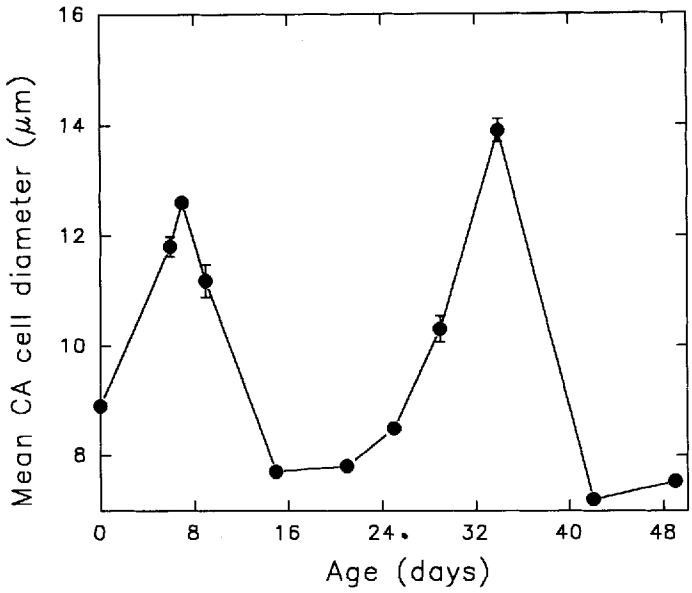

Fig. 3. Mean size of CA cells in adult *B. germanica* females during the first and second reproductive cycles. Each determination is the mean of 300–400 cells from at least 4 different insects. Vertical bars represent S.E. For some means, error bars are obscured by the symbol. Data for days 0, 7, 15, 21, 29, 34, 42, and 49 represent means from Figure 1 in [20].

was undetectable throughout most of the gestation period. Several days before the ootheca hatched, mean cell size increased significantly while the rate of JH biosynthesis was still undetectable (Fig. 3).

Ultrastructural evidence [7,27–29], together with our results, indicates that such volumetric changes in CA cells are a reflection of cyclic changes in the amount of cellular components required for JH synthesis and not of cycles of shrinkage and swelling due to osmotic changes. This developmental regulation of JH biosynthesis requires cyclic prolfieration and lysis of cellular organelles. To conserve cell energy, it is reasonable to suppose that this type of regulatory mechanism might occur only in insects with a long gestation period which requires long-term arrestment of CA activity. We therefore chose *S. longipalpa*, which exhibits more continuous JH cycles with very short periods of CA inactivity, to examine this hypothesis.

Contrary to our hypothesis, we found similar developmental cycles in CA cells of *S. longipalpa* during the first two ovarian cycles (Fig. 4). CA cells exhibited synchronous changes in size in relation to cycles of JH biosynthesis. In newly-eclosed adult females, the CA were inactive (Fig. 1) and CA cells were small, averaging 9.2 ± 0.1 μm in diameter (Fig. 4). As the CA became active in the first (days 0 to 9) and second (days 12 to 16) ovarian cycles (Fig. 1), CA cells grew synchronously, averaging 14.6 ± 0.1 μm and 14.7 ± 0.2 μm, respectively (Fig. 4). Mean cell size and the rate of JH biosynthesis declined simultaneously before and during ovulation and increased after females deposited their oothecae. However, the transient decrease in cell size was less synchronous among females. Our data

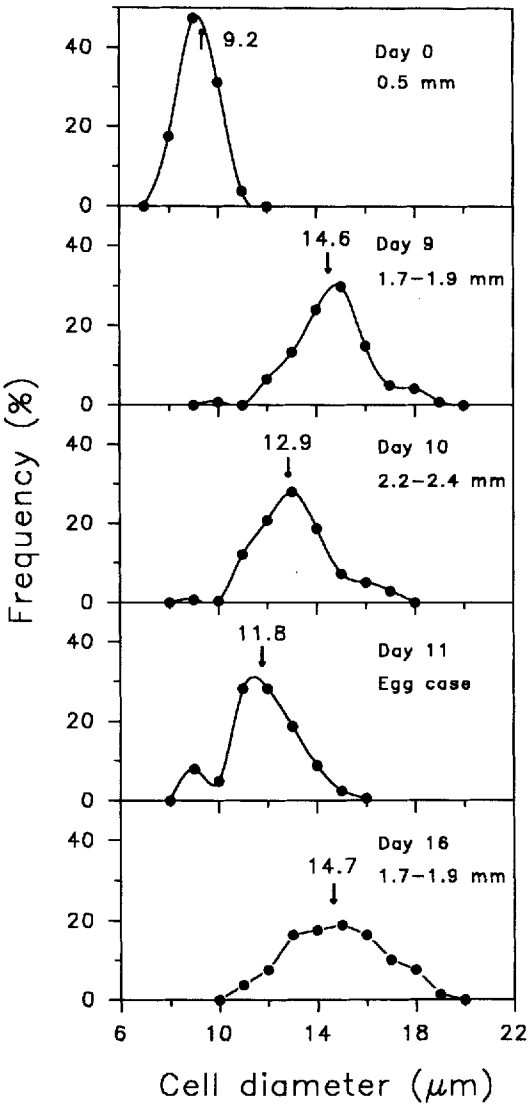

Fig. 4. Frequency distributions of CA cell sizes in *S. longipalpa* during the first and second reproductive cycles. Age and oocyte length of the insects are indicated. For day 11, we used only females that still carried their oothecae. Each frequency distribution consists of 100 CA cells from at least 4 insects. Arrows and adjacent numbers (μm) indicate the means of the distributions.

indicate that cyclic growth and regression of CA cells play a major role in the regulation of cyclic JH production during cockroach ovarian cycles.

Developmental regulation occurred in cockroaches with either discrete or more continuous cycles of JH synthesis. Yet in both species CA cells grew before the increase in JH synthesis and decreased in volume after the onset of a decline in JH biosynthesis. This suggests that other regulatory mechanisms might be involved in short-term and rapid modulation of CA activity. The allatostatins,

which inhibit the CA reversibly in vitro and are maximally effective on CA that exhibit declines in activity just before ovulation [30], are likely responsible for the declines in JH synthesis that precede changes in CA cell size.

Changes in CA Development and Activity in Ovariectomized *B. germanica*

We were interested in delineating the relationship between activity of the CA and growth and atrophy of CA cells in the absence of ovarian factors which are involved in CA activation, inhibition, and arrestment during pregnancy. The regulatory and pacemaker roles of the ovaries were examined in *B. germanica* by monitoring changes in CA cell size and JH production in ovariectomized females and after adding ovarian factors to such females. It has been demonstrated in several cockroaches that CA activity can be stimulated by presence of a young ovary and inhibited by presence of a mature ovary or ootheca [11,18,31–34]. Without any ovarian factors in adult *B. germanica* females, both size of CA cells and JH synthesis showed a delayed increase compared to normal females (Fig. 5). However, the peak levels for both parameters were similar to those in intact females [20] (Figs. 1,3,5). Gadot et al. [11] further showed that transection of the nerves between the CA and the brain accelerated CA activation in ovariectomized females, suggesting that inhibitory signals from the brain slowed CA activation in the absence of young ovaries. In older ovariectomized females, CA cell development became asynchronous and cell size ranged over a broad range [20]. However, mean cell size remained high, resulting in variable but high rates of JH synthesis (Fig. 5). These CA cells can be developmentally resynchronized by manipulating the signals from an artificial egg case.

Insertion of an artificial egg case into 17-day-old ovariectomized adult females with large CA cells resulted in a synchronous regression in CA cell size within 4 days and in low rates of JH synthesis (Fig. 5). Continued presence of this egg case mimicked pregnancy: CA cells were arrested as small cells with low JH synthesis for about 3 weeks. Subsequently, CA cells resumed relatively asynchronous and slow growth even in the presence of the artificial egg case, suggesting that either mechanoreceptors in the genital vestibulum became adapted or fatigued [see also 19], or that a clock in the central nervous system or the CA had restarted. Removal of the artificial egg case 11 days after its insertion resulted in a rapid and synchronous growth in cell size and a sharp increase in JH synthesis within 7 days even in the absence of young ovaries. Thus, the ootheca can re-synchronize the development of CA cells by inducing a uniform regression and then, upon its removal, a synchronous increase in cell size. This synchronous change in size of CA cells restored a complete JH cycle similar to that in intact females [14].

CONCLUSION

Using *B. germanica* and *S. longipalpa*, we demonstrated that the developmental regulation of JH biosynthesis occurred in cockroaches with either long or short periods of arrestment in CA activity following ovulation. Our results indicate that cyclic growth and atrophy of CA cells play a major role in the regulation of JH biosynthesis during reproductive cycles in female cockroaches. These

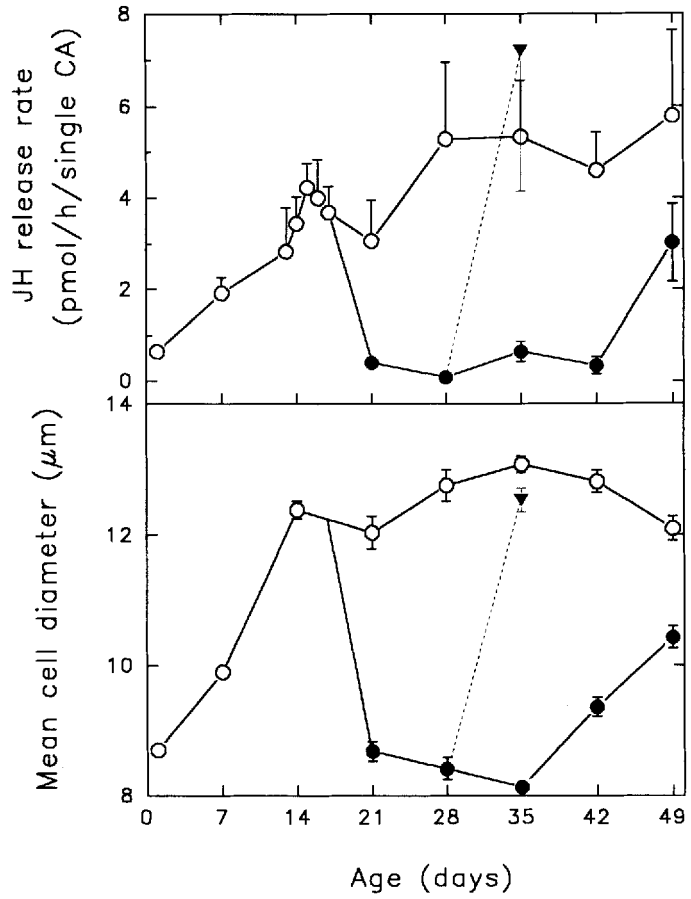

Fig. 5. Ovarian effects on CA activity and development of CA cells. Females were ovariectomized early in the last nymphal stadium. The two members of each CA pair were separated and randomly assigned for JH synthesis or cell size determinations. Artificial egg cases were inserted into the genital atria of 17-day-old ovariectomized females (closed circles). Control ovariectomized females (open circles) received an artificial egg case which was immediately removed. In some females, the inserted egg case was removed on day 28 and CA cell size and activity were measured 7 days later (triangles). For some means, error bars are obscured by the symbol. Redrawn from [14].

volumetric changes in CA cells are synchronized and paced by ovarian factors, resulting in a precise correlation between JH cycles and oocyte development. While the rate of JH biosynthesis is determined by the mean CA cell size, cyclicity of JH production is dependent upon the degree of developmental synchrony of CA cells. Changes in total CA cell number are not involved in the regulation of JH biosynthetic cycles in the adult female, but they appear to be important in the determination of the maximum capacity of the gland to produce JH. Thus, species with more CA cells would produce more JH if the normal peak rate of JH synthesis per cell were the same in all species. Since cell number does not change significantly in adult *B. germanica* or *S. longipalpa*, we hypothesize that experimental manipulations that might result in compensa-

tory increases in JH synthesis (e.g., unilateral allatectomy) would result in further increases in CA cell size.

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