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OVARIAN REGULATION OF CYCLIC CHANGES IN SIZE AND ACTIVITY OF CORPUS ALLATUM CELLS IN *BLATTELLA GERMANICA*

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Abstract—The roles of the ovary and egg case in the regulation of the development and activity of the corpora allata were studied in *Blattella germanica* adult females. The corpora allata of ovariectomized females exhibit a delayed pattern of development and activity compared with intact females. Implantation of a young ovary into ovariectomized females resulted in a faster activation of the corpora allata to the same peak juvenile hormone biosynthetic rates exhibited by intact females. Injection of 20-hydroxyecdysone caused transient declines in both the activity and the size of corpus allatum cells. Insertion of a waxed egg case into the genital atrium of ovariectomized females, with or without injection of 20-hydroxyecdysone, mimicked the 21-day pregnancy in intact females and caused corpus allatum cells to become small and inactive. The size and activity of corpus allatum cells increased again when the inserted egg case was removed manually or had been carried by the ovariectomized female for more than 3 weeks. In both intact and ovariectomized females, the activity of the corpora allata, as measured by the spontaneous and farnesoic acid-stimulated rates of juvenile hormone biosynthesis, and corpus allatum development, as reflected by the size of cells, always exhibited the same patterns and responded concurrently to the various ovarian factors. These results indicate that ovarian factors, both stimulatory and inhibitory, regulate juvenile hormone production mainly by inducing changes in the cellular machinery rather than in rate-limiting enzymes.

Key Word Index: *Blattella germanica*; corpora allata; juvenile hormone; farnesoic acid; cell size; ovariectomy; ovarian stimulation; ovarian inhibition; 20-hydroxyecdysone; egg case

INTRODUCTION

The cyclic reproductive pattern of the female German cockroach, *Blattella germanica* (L.), is controlled by the corpora allata which produce stage dependent quantities of juvenile hormone III (Belles *et al.*, 1987; Camps *et al.*, 1987; Gadot *et al.*, 1989b). During oöcyte maturation, corpus allatum cells enlarge, resulting in an increase in gland volume and in the rate of juvenile hormone synthesis (Chiang *et al.*, 1991b). The size and activity of corpus allatum cells decline prior to ovulation, remain low during pregnancy and increase again after the nymphs emerge. The cyclic pattern of juvenile hormone biosynthesis in adult females corresponds to changes in corpus allatum intracellular components (Piulachs *et al.*, 1989).

The activation of the corpora allata to normal levels is suppressed in ovariectomized females of the cockroaches *Diploptera punctata*, *Nauphoeta cinerea* and *Periplaneta americana* (Stay and Tobe, 1978; Lanzrein *et al.*, 1981; Weaver, 1981). Implantation of young ovaries into ovariectomized cockroaches restores a normal cycle of juvenile hormone biosynthesis, suggesting that a stimulatory factor from young ovaries is required for the activation of the corpora allata (Lanzrein *et al.*, 1981; Stay *et al.*, 1983). In ovariectomized *B. germanica* however, the corpora allata become active, and after a 7-day delay they attain similar rates of juvenile hormone synthesis as in intact females (Gadot *et al.*, 1991). The corpora allata then exhibit a moderate but significant decline in the rates of juvenile hormone biosynthesis followed by a second increase and a high plateau of activity for at least 21 days. The corpora allata of adult ovariectomized females increase in volume continuously to double the peak volume in intact females but the number of corpus allatum cells does not change significantly in either intact or ovariectomized *B. germanica* females (Chiang *et al.*, 1991a). Since corpus allatum cells respond readily to ovarian feedback factors, ovariectomized *B. germanica* provide an excellent experimental model for studies of the ovarian regulation of the activity and development of corpus allatum cells.

Ecdysteroids, which are synthesized by the mature ovaries in many adult insects (Hagedorn *et al.*, 1975;

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Lagueux *et al.*, 1977; Nijhout and Koeppe, 1978; Hagedorn, 1985) and are detected in the haemolymph, have been suggested to play a role in the initial decline in juvenile hormone production before ovulation (Stay *et al.*, 1980; Lanzrein *et al.*, 1981; Stay *et al.*, 1984; Weaver *et al.*, 1984; Rankin and Stay, 1985). Ecdysteroids have been shown to inhibit oöcyte growth in various insects (Engelmann, 1959a, 1971; Robbins *et al.*, 1968; Wright *et al.*, 1971; Fraenkel and Hollowell, 1979; Garcia *et al.*, 1979), and injections of ecdysteroids into adult cockroaches result in dose-dependent declines in corpus allatum activity in *Leucophaea maderae* (Engelmann, 1959a), *D. punctata* (Friedel *et al.*, 1980; Stay *et al.*, 1980) and *B. germanica* (Chiang *et al.*, 1991b).

During gestation in cockroaches mechanoreceptors in the uterus or genital atrium respond to the presence of the egg case and transmit signals via the ventral nerve cord to the brain which in turn inhibits the corpora allata (Engelmann, 1964; Roth and Stay, 1959; Roth, 1964, 1973). In *B. germanica*, insertion of a wax plug or a glass bead into the genital atrium mimics pregnancy, and their removal, transection of the ventral nerve cord or denervation of the corpora allata restore the development of succeeding basal oöcytes (Roth and Stay, 1959, 1962; Gadot *et al.*, 1991).

Here, we examine mechanisms responsible for the delayed activation, moderate decline and subsequent high plateau of corpus allatum activity in ovariectomized *B. germanica* females.

MATERIALS AND METHODS

Insects

German cockroaches were reared at $27 \pm 1°C$ under a 12 h light–12 h dark photoperiodic regime and supplied with pelleted Purina dog food No. 1780 and water *ad libitum*. Ovariectomy was performed early in the last instar and only females that mated within 8 days after the imaginal moult were used (see Gadot *et al.*, 1991), unless otherwise indicated. Individual mated females were always maintained with 2 males to avoid deleterious effects of isolation (Gadot *et al.*, 1989a) and females from day 0 to day 49 after emergence were used. Insects were always immobilized by chilling on ice before performing various treatments and antibiotics were not used.

20-Hydroxyecdysone injection and egg case insertion

Injections were made through the base of the coxa of the right metathoracic leg. Each insect received 10 μg 20-hydroxyecdysone (purity 99%, Sigma, St Louis, Mo.) in 2 μl Ringer solution containing 1% ethanol.

Egg cases were collected from gravid females and infiltrated with molten bees-wax overnight to prevent dehydration before insertion into ovariectomized females. The waxed egg case was inserted into the oöthecal chamber and attached to the terminal abdominal tergite with a small amount of bees-wax. Females that failed to retain the inserted egg case (fewer than 10%) were discarded. Sham control females were injected with 2 μl Ringer 1% ethanol solution and an egg case was inserted and immediately removed.

Ovary implantation

Young ovaries were collected from female donors one day after the imaginal moult and rinsed with *B. germanica* saline (Kurtti and Brooks, 1976). Each ovariectomized female received one ovary which was implanted into the abdomen by injection with a small amount of cockroach saline. Sham controls were implanted in the same manner with metathoracic femur muscle.

Corpus allatum volume

Pairs of corpora allata were removed from adult females of various ages and separated in cockroach saline. Corpus allatum volume was determined by the formula $v = 4/3\ \pi abc$, where a, b and c were the radii of the three principal axes measured with an ocular filar micrometer under a dissecting microscope. The two members of corpora allata pairs were either incubated separately to determine juvenile hormone release rates, or one corpus allatum was assayed and the contralateral gland used for cell size measurements.

Corpus allatum cell size

Because the right and left corpora allata of ovariectomized females are highly symmetrical in both function and development (Chiang and Schal, 1991), we correlated changes in juvenile hormone biosynthesis in one gland with cellular changes in the contralateral gland. The isolated corpus allatum was digested with 0.1% trypsin in isotonic cockroach saline and then dispersed by gentle vortexing (Chiang *et al.*, 1989). Corpus allatum cells were randomly sampled in a haemocytometric grid. Since dissociated corpus allatum cells of *B. germanica* are largely globular (Chiang *et al.*, 1990) the maximal diameter of each cell was measured with an ocular filar micrometer under a compound microscope at $\times 400$ and served as an indicator of cell size. The mean diameter of corpus allatum cells was determined from 100 measurments in at least 4 insects.

Radiochemical assay of juvenile hormone synthesis

L-[methyl-^{3}H]Methionine (specific activity of 200 mCi/mmol) was obtained from New England Nuclear, Wilmington, Del. Faresoic acid (about 70% pure, 2E, 6E isomer) was a generous gift from Dr F. C. Baker (Zoecon Corp., Palo Alto. Calif.). the spontaneous and fanesoic acid-stimulated rates of juvenile hormone synthesis were determined *in vitro* according to the methods of Tobe and Pratt (1974) and Pratt and Tobe (1974) with modifications after Gadot *et al.* (1989b). Each corpus allatum was

incubated in 50 μl of medium 199 (GIBCO; special formulation after Kikukawa *et al.*, 1987) 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 corpus allatum was transferred to fresh medium containing 100 μM farnesoic acid for an additional incubation period of 2 h. The gland was removed at the end of the incubation and radiolabelled juvenile hormone in the incubation medium was extracted with 200 μl isooctane (modified from Feyereisen and Tobe, 1981). After a 5 min centrifugation (2500 *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 2 to express the total isooctane phase. Incubations and extractions were performed in the same 0.7 ml disposable borosilicate glass culture tubes.

Changes in the size of the left colleterial gland

The left colleterial gland was dissected from each ovariectomized female that was used for a determination of the activity and development of the corpora allata. For each female the relative size of the gland was established as the mean of the maximal diameters of 3–5 distal tubules measured with an ocular filar micrometer under a dissecting microscope. This measure is highly correlated with protein content in the gland in both intact and ovariectomized females (Burns *et al.*, 1991).

RESULTS

Activity and size of corpus allatum cells in control ovariectomized females

In ovariectomized *B. germanica* adult females both spontaneous and farnesoic acid-stimulated juvenile hormone biosynthesis increased gradually after the imaginal moult. On days 15–16 both parameters reached peak rates similar to those attained by intact females 8–9 days earlier (Fig. 1). A subsequent moderate decline to an intermediate level was followed by an increase by day 28 to the previous high level where juvenile hormone biosynthesis remained through day 49. Data for days 21–49 were derived from ovariectomized females that received both a sham egg case insertion and a sham injection on day 17. In these females the mean size of corpus allatum cells remained large (12–13 μm in diameter) and comparable to the maximal mean cell diameter attained by the corpora allata in intact females (Fig. 2). Concurrently, both spontaneous and farnesoic acid-stimulated juvenile hormone biosynthesis in 21–49-day-old ovariectomized females were similar to the respective peak rates in intact females. On the other hand, the volume of the corpora allata increased continuously and they became hypertrophic between days 21 and 49 compared with the maximal corpus allatum volume in intact females (Fig. 2).

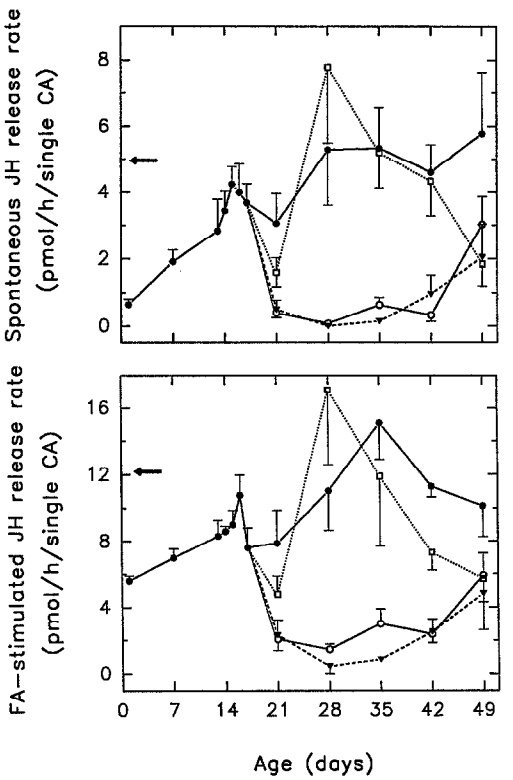

Fig. 1. Effects of 20-hydroxyecdysone injections (□), egg case implants (○) and both (▼) on the spontaneous and farnesoic acid (FA)-stimulated rates of juvenile hormone (JH) biosynthesis. Ovariectomized females that mated before day 8 were treated on day 17. Control females (●) were injected with 2 μl Ringer 1% ethanol solution and implanted with an egg case which was immediately removed. Rates of juvenile hormone biosynthesis were determined by a radiochemical assay with or without presence of farnesoic acid in the culture medium. Arrows indicate the peak spontaneous and farnesoic acid-stimulated rates of juvenile hormone synthesis respectively in intact females during the first ovarian cycle. Each point is the mean ± SEM of 4–6 single corpora allata randomly chosen from 4–6 pairs.

Ovarian inhibition of the corpora allata

In three independent experiments with ovariectomized females (Gadot *et al.*, 1991; Burns *et al.*, 1991; Fig. 1) we have confirmed a moderate decline in corpus allatum activity after peak rates of juvenile hormone synthesis were attained on days 14–16. In intact females the rates of juvenile hormone biosynthesis declined rapidly following oöcyte maturation and remained low during pregnancy (Gadot *et al.*, 1989a, b). The following experiments were designed to examine the roles of inhibitory ovarian factors in effecting the initial regressions in corpus allatum activity and development and maintaining both during pregnancy, as seen in intact females. We chose 17-day-old ovariectomized females for various treatments because in these females the corpora allata had undergone an activation comparable to that in intact females before ovulation.

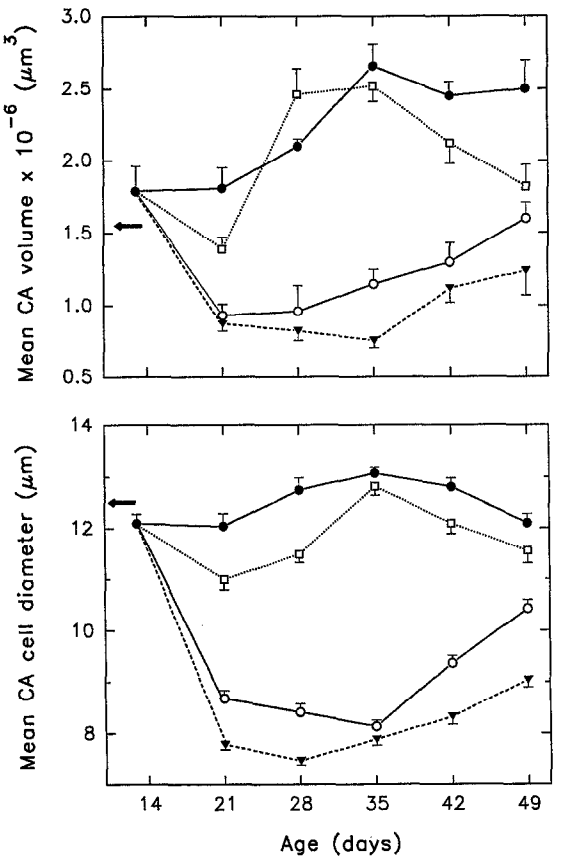

Fig. 2. Effects of 20-hydroxyecdysone injections (□), egg case implants (○) and both (▼) on corpus allatum (CA) volume and size of corpus allatum cells. Control females (●) were injected with 2 μl Ringer 1% ethanol solution and implanted with an egg case which was immediately removed. Mean corpus allatum volume was determined from both members of 4–6 pairs of corpora allata before they were used to determine cell size and gland activity. Mean cell size of each corpus allatum was derived from measurements of the maximum diameters of 100 cells from the contralateral corpus allatum of the same females used for the radiochemical assays as shown in Fig. 1. Arrows indicate the peak corpus allatum volume and the maximum mean cell diameter respectively in intact females during the first ovarian cycle. Vertical lines represent SEM.

Four days after injection of 20-hydroxyecdysone into 17-day-old ovariectomized females both spontaneous and farnesoic acid-stimulated rates of juvenile hormone synthesis declined to significantly lower levels (t-test; $P < 0.05$) than in sham treated females (Fig. 1). However, this inhibition by 20-hydroxyecdysone was only transient because by day 28, 11 days after injection, both parameters of juvenile hormone synthesis increased again to similar rates as in the controls. In contrast with sham treated controls, in which the juvenile hormone biosynthetic rates remained high after day 28, corpus allatum activity continued to decline for at least 3 weeks after day 28 and were significantly lower by day 49 in 20-hydroxyecdysone-injected ovariectomized females (Fig. 1).

Waxed egg cases were inserted into the genital atria of 17-day-old ovariectomized females. Four days later, on day 21, the spontaneous and farnesoic acid-stimulated rates of juvenile hormone synthesis declined significantly to nearly undetectable levels (Fig. 1). This was a much greater decline in activity than that caused by 20-hydroxyecdysone injection and it mimicked the situation in intact gravid *B. germanica* females, in which the corpora allata are inactive for 3 weeks (Gadot *et al.*, 1989a, b). Also unlike the results with 20-hydroxyecdysone injection, the rates of juvenile hormone biosynthesis remained low for about 3 weeks but then increased significantly by day 49 while the inserted egg case was still attached.

The combination of 20-hydroxyecdysone injection and egg case insertion into 17-day-old ovariectomized females inhibited the corpora allata as did egg case insertion alone (Fig. 1). The spontaneous and farnesoic acid-stimulated rates of juvenile hormone synthesis remained low for 2–3 weeks and both increased by day 49 while the inserted egg cases were still carried by the females.

The developmental responses of the corpus allatum to 20-hydroxyecdysone injection, to egg case insertion, to both or to sham treatments correlated precisely with the respective rates of juvenile hormone biosynthesis by the contralateral gland (Fig. 2). As did juvenile hormone biosynthesis, the size of corpus allatum cells decreased transiently in response to 20-hydroxyecdysone injection. Subsequently, the size of corpus allatum cells was comparable to that seen in sham treated females. After egg case insertion, with or without 20-hydroxyecdysone injection, the mean size of corpus allatum cells decreased rapidly in 4 days from about 12 μm to less than 9 μm in diameter, remained small for 2–3 weeks and then increased gradually as did juvenile hormone biosynthesis.

Corpus allatum volume responded to these treatments in a similar manner as did mean cell diameter (Fig. 2). Hypertrophy of the corpora allata, which occurred in sham treated ovariectomized females, was prevented following insertion of an egg case into 17-day-old ovariectomized females with or without 20-hydroxyecdysone injection. Corpus allatum volume decreased within 4 days to a level similar to that seen in intact gravid females, it remained small for about 2 weeks and then increased slowly and gradually as did juvenile hormone biosynthesis (Fig. 2). Injection of 20-hydroxyecdysone alone did not prevent the hypertrophy of the corpora allata after a transient but significant decline on day 21. Subsequently, despite a dramatic decline in juvenile hormone synthesis and a continuous decline in corpus allatum volume, the gland was significantly larger on day 49 in injected females than at any stage in intact females (Figs 1 and 2).

Removal of inserted egg cases

Following removal of the inserted egg case on day 28 (11 days after insertion) both the spontaneous and

farnesoic acid-stimulated rates of juvenile hormone synthesis as well as the size of cells in the contralateral corpus allatum increased significantly within 7 days (by day 35) (Fig. 3). Unlike in old ovariectomized females in which the frequency distribution of the size of corpus allatum cells became flattened and widely dispersed and juvenile hormone biosynthesis rates became variable but high (Chiang *et al.*, 1991b), egg case insertion followed by its removal re-synchronized the development of corpus allatum cells by inducing a uniform regression and then a synchronous increase in cell size (Fig. 3). Seven days after the egg case was removed the corpora allata became significantly hypertrophic (almost double the maximal volume in intact females) while the mean cell diameter and activity were equivalent to the respective maximal values in intact females (Figs 1–3).

Effect of egg case insertion on active corpora allata

The present and previous results (Chiang *et al.*, 1989, 1991b) document a positive relationship between corpus allatum cell size and juvenile hormone synthesis. The egg case could inhibit the corpora allata either by preventing small inactive cells from growing and becoming active or by inducing a decline in the size and activity of active cells. The former effect is clear since the presence of an egg case inhibited the corpora allata for long periods of time both in intact and in ovariectomized females, and removal of the egg case removed this inhibition. To test the possibility that signals from presence of the egg case cause declines in the size and activity of active corpus allatum cells, we inserted egg cases into 9-day-old ovariectomized females in which both the size and activity of corpus allatum cells were increasing. Four days later corpus allatum activity diminished and the cell size distribution shifted to significantly smaller cells than in control females (Fig. 4). The decrease in rates of juvenile hormone synthesis was well reflected by changes in gland volume and the size of corpus allatum cells. These results indicate that the stimuli from the presence of an egg case not only prevent the growth and activation of small inactive corpus allatum cells, but also induce the decline of large active cells.

Effects of young ovary implants

To examine the stimulatory effects of young ovaries on the corpora allata we implanted an ovary or muscle tissue from 1-day-old adult female donors into 28-day-old ovariectomized females into which a waxed egg case had been implanted on day 17 and removed just before the ovary or muscle implantation. Without any tissue implantation the corpora allata exhibit significant increases in developmental parameters and activity following removal of the inserted egg case (Fig. 3). Seven days after muscle implantation, spontaneous and farnesoic acid-stimulated rates of juvenile hormone synthesis were significantly higher than in ovariectomized females from which the inserted egg case was not removed, but they were significantly lower than in females whose implanted egg cases were removed on day 28 without muscle or ovary implantation (Figs 3 and 5). This effect may be due to the trauma associated with tissue implantation. In females implanted with one ovary, both spontaneous and farnesoic acid-stimulated rates of juvenile hormone synthesis increased significantly compared with control females that received muscle implants, and the implanted basal oöcytes developed normally, averaging 1.7 ± 0.1 mm in length on day 35 (Fig. 5).

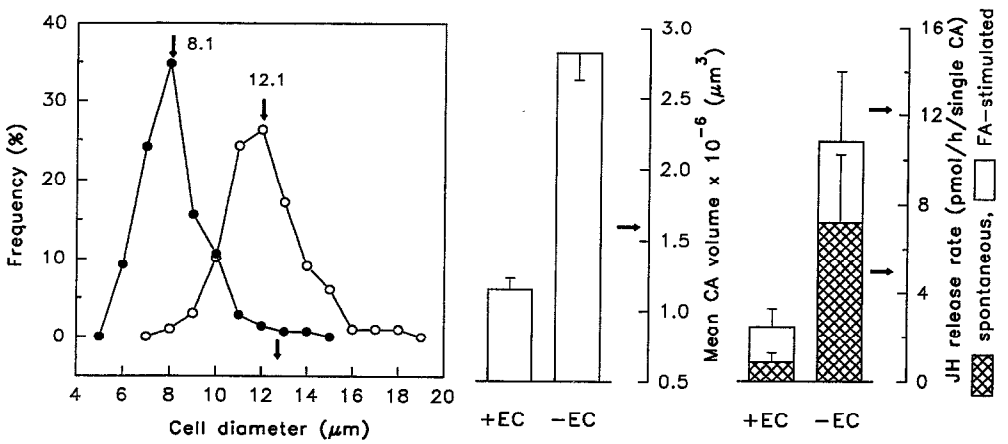

Fig. 3. Effects of egg case removal on the corpora allata. Waxed egg cases were implanted into 17-day-old ovariectomized females and removed (−EC or ○) or retained (+EC or ●) on day 28. Rates of juvenile hormone biosynthesis, corpus allatum volume and cell size were determined on day 35 by the same methods as described in Figs 1 and 2. Each frequency distribution consists of 100 corpus allatum cells from at least 4 insects. Arrows and adjacent numbers (μm) indicate the mean of the distribution. Other arrows represent the respective maximal values from intact females in the first gonotrophic cycle. Vertical lines represent SEM. All four parameters are significantly greater for the −EC treatment (*t*-test; $P < 0.05$).

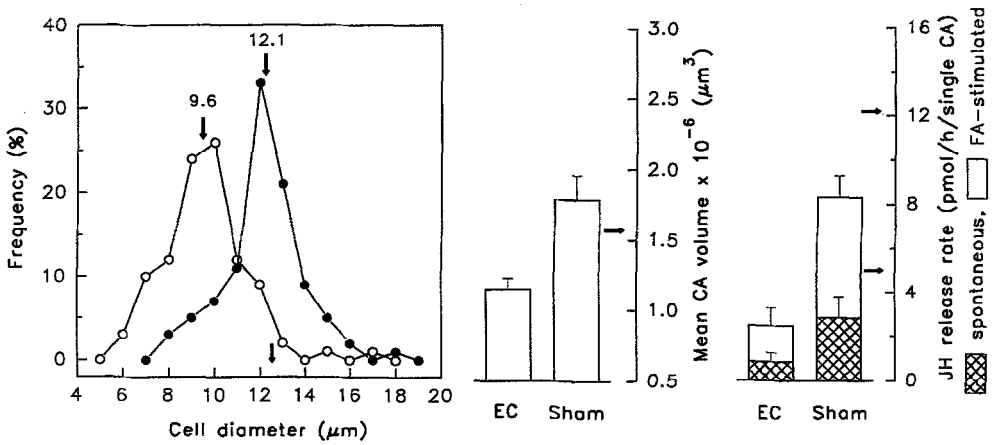

Fig. 4. Effects of egg case insertion on active corpora allata (CA). Ovariectomized mated females were implanted with waxed egg cases (EC or ○) on day 9 and examined on day 13. Sham controls (sham or ●) received an egg case which was immediately removed. Rates of juvenile hormone biosynthesis, corpus allatum volume and cell size were determined by the same methods as described in Figs 1 and 2. Each frequency distribution consists of 100 corpus allatum cells from 5 insects. Arrows and adjacent numbers (μm) indicate the mean of the distribution. Other arrows represent the respective maximum values from intact females in the first gonotrophic cycle. Vertical lines represent SEM. All four parameters are significantly greater for the sham treatment (t-test; $P < 0.05$).

Cumulative record of juvenile hormone in the haemolymph

The left colleterial gland of ovariectomized females continuously synthesize and accumulate oöthecal proteins which is reflected in a gradual increase in the size of its distal tubules; allatectomy arrests the enlargement of colleterial tubules (Burns *et al.*, 1991). Therefore, the relative exposure of ovariectomized females to juvenile hormone can be estimated from the mean diameter of the distal tubules of the colleterial glands, which can be used as basal oöcyte length is used in intact females. We used the size of the colleterial gland to ascertain whether any significant unexpected changes occurred in corpus allatum activity in the intervening periods between assays.

The left colleterial glands of intact and ovariectomized females attained similar sizes in the first 7 days (Fig. 6). In ovariectomized females the left

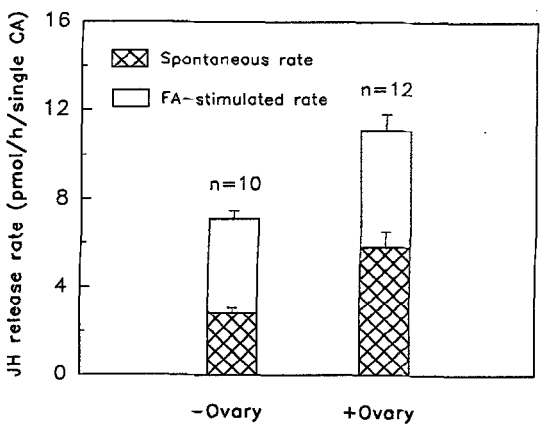

Fig. 5. Stimulatory effects of young ovaries on spontaneous and farnesoic acid-stimulated rates of juvenile hormone biosynthesis. Young ovaries (+ovary) or metathoracic femur muscle (−ovary) derived from 1-day-old adult females were implanted into 28-day-old ovariectomized females in which an egg case had been implanted on day 17 and removed on day 28. Rates of juvenile hormone synthesis from each corpus allatum were assayed separately by the radiochemical assay 7 days after ovary implantations. Vertical lines represent SEM. Both parameters are significantly greater in the +ovary treatment (t-tests; $P < 0.05$).

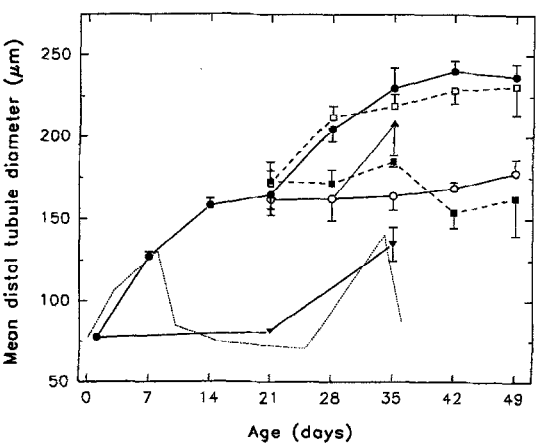

Fig. 6. Mean diameters of the distal tubules of the left colleterial gland in ovariectomized females. Treatments of 20-hydroxyecdysone injections (□), egg case implants (○), both (■) and sham controls (●) were the same as described in Fig. 1. The triangle (▲) represents the mean distal tubule diameter of ovariectomized females in which an egg case had been inserted on day 17 and removed on day 28. The inverted triangle (▼) represents data from ovariectomized virgin females that carried the inserted egg cases since day 1. Data were collected from the same females used to study the activity and morphometric changes of the corpora allata in the previous figures. Dotted line shows cyclic changes in the mean diameter of the distal tubules of the left colleterial gland in intact females during the first two ovarian cycles (Burns *et al.*, 1991). Vertical lines represent SEM.

colleterial gland continued to increase in size after day 7 and in females that were sham treated on day 17 the mean distal tubule diameter increased continuously to day 49. The size of the distal tubules was arrested by the insertion of an egg case on day 17 and they remained unchanged while the rates of juvenile hormone biosynthesis remained low. Removal of the inserted egg case on day 28 resulted in increases in both the mean distal tubule diameter and juvenile hormone synthesis within 7 days (Figs 4 and 6). The cumulative increase in the diameter of the distal tubules of the colleterial gland was comparable in ovariectomized females injected with 20-hydroxyecdysone and in control females (Fig. 6). However, in ovariectomized females that received an egg case and 20-hydroxyecdysone injection on day 17, distal tubule growth was arrested until day 35. Subsequent shrinkage of the distal tubules was largely due to changes in the colleterial proteins that were accompanied by discolouration and hardening.

Following insertion of an egg case into 1-day-old ovariectomized females the left colleterial gland remained small until day 21 (Fig. 6). By day 35 however, their tubule diameter increased while females were still carrying the inserted egg cases and the corpus allatum became significantly hypertrophic ($2.03 \times 10^6 \pm 0.17 \times 10^6$ μm, $n = 12$); the spontaneous and the farnesoic acid-stimulated rates of juvenile hormone biosynthesis were still significantly lower than the maximal activity in intact females (1.53 ± 0.32 and 5.16 ± 0.69 pmol/h/corpus allatum, $n = 12$, respectively; t-test, $P < 0.05$).

DISCUSSION

Our results show that ovarian factors are not obligatory for changes in the activity and development of corpus allatum cells in adult *B. germanica* females. However, stimulatory and inhibitory ovarian factors modulate and impose cyclicity on these changes. Furthermore, the spontaneous and farnesoic acid-stimulated juvenile hormone synthesis rates and the size of corpus allatum cells always respond concurrently to the various regulatory factors.

Stimulation from the young ovary

Although the presence of young ovaries was required for the corpora allata to reach normal peak activity in *D. punctata* and *N. cinerea*, Stay *et al.* (1983) predicted that in *L. maderae* and *B. germanica* the corpora allata of ovariectomized females might exhibit high activity since the haemolymph vitellogenin titres were much higher than in intact females. This was confirmed by our earlier results, showing that the corpora allata in *B. germanica* did not require the presence of young ovaries to reach normal peak activity (Gadot *et al.*, 1991). However, factors from young ovaries clearly accelerate the development and activity of the corpora allata in ovariectomized females which normally exhibit delayed activation (Figs 1 and 5). Furthermore, the corpora allata were able to maintain high activity for a long period of time in old ovariectomized females without any stimulatory ovarian factors (Fig. 1). The pattern of juvenile hormone biosynthesis was similar to that observed by Gadot *et al.* (1991).

It is important to note however that the present assays were conducted in glass tubes and yielded higher rates than in Gadot *et al.* (1991), and that we express synthesis per corpus allatum rather than per pair of glands. Radiochemical assays of corpora allata from normal *B. germancia* females with oöcytes 1.6–1.9 mm in length performed in glass tubes yielded twice as much *de novo* synthesized juvenile hormone (10.12 ± 0.79 pmol/h/pair corpora allata, $n = 9$) as in polystyrene multiwell culture plates (4.5 ± 0.36 pmol/h/pair corpora allata, $n = 5$). Replacing the corpora allata with a known dose of synthetic [^{14}C] juvenile hormone III in the radiochemical assay, we recovered only 14.6% of the radioactivity from the culture plates but 88.9% from glass tubes (unpublished data). These data support previous evidence that adsorption and/or absorption may play a significant role in reducing the recovery of [^{3}H] labelled juvenile hormone from plastic dishes (Giese *et al.*, 1977).

Inhibition from the mature ovary

The mature ovaries in many insects synthesize ecdysteroids and exert inhibition upon the corpora allata (Tobe and Stay, 1985). However, it has been shown in several cockroach species that after reaching maximal rates, juvenile hormone synthesis declined in the absence of mature ovaries (Stay *et al.*, 1980; Lanzrein *et al.*, 1981; Rankin and Stay, 1985). In the absence of inhibitory factors from mature ovaries the rate of juvenile hormone synthesis in *B. germanica* declined moderately after the first peak (Fig. 1). This decline could be prevented in ovariectomized females whose corpora allata had been denervated from the brain while denervation of the corpora allata in otherwise intact females with ovaries did not prevent the decline (Gadot *et al.*, 1991).

Ecdysteroids from the prothoracic gland, which degenerates shortly after the imaginal moult, might be responsible for this moderate decline in corpora allata activity. Prothoracic gland ecdysteroids might act by programming the brain prior to the activation of the corpora allata to effect the later decline in juvenile hormone synthesis. According to this hypothesis removal of the ovarian source of ecdysteroids would not interfere with the first decline in juvenile hormone synthesis, which was due to prothoracic gland ecdysteroids, but would prevent any subsequent decline. Our results appear to support this prediction (Fig. 1). This hypothesis was further tested by monitoring the long term effects of 20-hydroxyecdysone injection on the corpora allata in

ovariectomized females. Our results again support the hypothesis by showing that injection of 20-hydroxyecdysone on day 17, coincidentally with the moderate decline in juvenile hormone synthesis, caused a second decline in corpus allatum activity through day 49. This delayed decline in activity presumably occurred in the absence of any inhibitory factors since the corpora allata in the control females retained high activity during this period. It thus appears that 20-hydroxyecdysone might preprogramme the corpora allata to undergo an endogenous cycle of activity. However, further experiments, such as injecting 20-hydroxyecdysone into ovariectomized and corpus allatum-denervated females, and removal of the prothoracic glands are required to test this hypothesis.

An alternative hypothesis is that in the absence of ovarian stimulatory or inhibitory factors, a second decline in corpus allatum activity was not apparent because its cells had become asynchronous (see Chiang *et al.*, 1991b). Injection of 20-hydroxyecdysone would provide a developmental synchronizer to corpus allatum cells and result in their cyclic increase and decline in activity as seen in Fig. 1. This hypothesis is also supported by the fact that egg case insertion and its subsequent removal were able to induce a synchronous decline followed by a uniform increase in size of corpus allatum cells which were accompanied by changes in gland activity (Fig. 3).

Inhibition from the egg case

During pregnancy in *B. germanica*, corpus allatum cells are uniformly small and they exhibit low rates of juvenile hormone biosynthesis (Chiang *et al.*, 1991b). In old ovariectomized females, the frequency distribution of corpus allatum cell sizes remains unimodal but becomes flattened and dispersed with hypertrophic as well as very small cells within the same gland (Chiang *et al.*, 1991b). The absence of signals from the ovary and egg case presumably results in asynchronous cells and variable but high rates of hormone synthesis without any apparent cycle. The insertion of a waxed egg case into ovariectomized females induced a sharp reduction in the size of corpus allatum cells, resulting in a synchronous population of small inactive cells as in intact females during ovulation and pregnancy (Fig. 4; Chiang *et al.*, 1991b). It has been suggested, based on presence of ecdysteroids in the haemolymph of gravid *N. cinerea* females (Imboden *et al.*, 1978), that ovarian ecdysteroids might play major roles in suppressing the activity of the corpora allata while cockroaches are carrying developing embryos (Hagedorn, 1985). From our study it is clear that the suppression of corpus allatum activity during the mimicked pregnancy in *B. germanica* is not due to either an embryonic source or a maternal ovarian source of ecdysteroids.

The activity of the corpora allata and their mean cell size increased rapidly when the inserted egg case was removed (Fig. 3) or slowly when the inserted egg case was carried for more than 3 weeks (Figs 1 and 2). The 3-week inhibition of the corpora allata of ovariectomized females by the inserted egg case is of a similar duration to pregnancy in intact females. It is not clear why this suppression does not extend beyond 3 weeks in *B. germanica*. The long-term effects of the implanted artificial egg case in ovariectomized females exclude the possibility of the involvement of either maternal or embryonic developing oöcytes. It has been hypothesized that mechanoreceptors in the genital atrium of *B. germanica* might become adapted or fatigued from the constant stimuli from the egg case (Roth and Stay, 1962). Alternatively, a timing mechanism in either the brain or the corpora allata may act together with the egg case to regulate the growth and activation of corpus allatum cells.

Hypertrophy of the corpora allata

Ovariectomy results in hypertrophy of the corpus allatum in many insects (Cassier, 1979). The hypertrophic response has been attributed to increases in cell number and cytoplasmic content in *L. maderae* (Scharrer and von Harnack, 1961) but juvenile hormone synthesis has not been measured directly in this species. In ovariectomized *D. punctata* and *N. cinerea* the corpora allata do not exhibit hypertrophy (Engelmann, 1959b; Wilhelm and Luscher, 1974) and their juvenile hormone biosynthetic activity remains suppressed (Stay and Tobe, 1978; Lanzrein *et al.*, 1981). Nevertheless, Tobe *et al.* (1984) reported a significant 50% increase in cell number in the relatively inactive glands of ovariectomized *D. punctata*. Conversely, in ovariectomized *B. germanica* hypertrophy of the corpora allata is a direct consequence of an increase in the frequency of hypertrophic cells (i.e. giant cells) rather than changes in the total number of cells (Chiang *et al.*, 1991a, b). The disparity in the roles attributed to cell proliferation in the latter two species is likely due to methodological differences. We have shown previously that the observed increase in corpus allatum cell number in relation to elevated gland activity in intact *D. punctata* (Szibbo and Tobe, 1981) was likely due to methodological inaccuracies and could not be shown with our dissociation procedures.

Our results also show that hypertrophic corpora allata are functionally not hyperactive; their rates of juvenile hormone synthesis *in vitro* are comparable to those of intact females (Fig. 1). Similarly, after the injection of 20-hydroxyecdysone, or removal of the implanted egg case, the corpora allata rapidly became hypertrophied (Figs 2 and 3). Within only 1 week of the removal of an implanted egg case the corpora allata almost tripled in volume to double their maximal volume in intact females (Fig. 3). However, the mean size of corpus allatum cells was comparable to that in intact females. This suggests that hypertrophy of the corpora allata in ovariectomized females is associated in part with changes in intercellular spaces of the extracellular matrix, while hypertrophy of

corpus allatum cells is mainly due to lack of inhibitory signals from the ovaries and the egg case.

Rate limitation and developmental regulation

It has been hypothesized that the rate of juvenile hormone biosynthesis can be regulated by quantitative changes in either the rate-limiting enzymes or the cellular machinery required for hormone synthesis (Feyereisen, 1985; Tobe and Stay, 1985). We have shown previously that the corpora allata of adult *B. germanica* females exhibit cycles of juvenile hormone biosynthetic competence in relation to the gonotrophic cycle. The small inactive corpus allatum cells from gravid females are unable to synthesize detectable amounts of juvenile hormone in the presence of farnesoic acid, a late precursor in the juvenile hormone III biosynthetic pathway (Gadot *et al.*, 1989b; Chiang *et al.*, 1991b). Similarly, in ovariectomized females, presence of farnesoic acid elevates the rates of juvenile hormone synthesis but does not completely bypass the inhibitory effects from 20-hydroxyecdysone injection or egg case insertion (Fig. 1). In intact and in ovariectomized females, both the spontaneous and the farnesoic acid-stimulated juvenile hormone synthetic rates, as well as the size of corpus allatum cells, always exhibit the same pattern and they respond concurrently to the various ovarian factors. These data suggest that in *B. germanica* rates of juvenile hormone synthesis during ovarian cycles are mainly regulated through cyclic development of cellular machinery in the corpora allata. The absolute rates can be modified by rate-limiting enzymes without changing the cyclic pattern.

Cumulative record of juvenile hormone in the haemolymph

The combination of the radiochemical assay of one corpus allatum and morphometric studies of the dissociated contralateral gland allowed us to monitor changes in activity and size of corpus allatum cells in the same female in response to various ovarian factors. However, both are relatively instantaneous measures that provide little infomation about the prior activity of the corpora allata. The mean distal tubule diameter of the left colleterial gland from the same ovariectomized female was used as an independent measure of the cumulative exposure of this target tissue to juvenile hormone (Burns *et al.*, 1991).

It is known that corpora allata activity can change within a few days or even hours. Because we monitored the development and activity of corpora allata on a weekly basis and corpora allata activity of ovariectomized females are variable, it was important to confirm that unexpected changes in activity did not occur within each 7-day interval between assays. Our results indicate that the mean distal tubule diameter of the left colleterial gland in ovariectomized females can be used as a cumulative record of juvenile hormone in the haemolymph much as basal oöcyte length is used in intact females. For example, the corpora allata of 42-day-old ovariectomized females that carried a waxed egg case since day 17 exhibited low rates of juvenile hormone biosynthesis, while an increase in juvenile hormone synthesis on day 49 was accompanied by an enlargement of the colleterial gland. The similar size of the left colleterial gland on day 21 and 3 weeks later confirmed that the corpora allata remained inactive during this period.

Conclusion

We conclude that the delayed development and activation of the corpora allata in ovariectomized *B. germanica* females were due to the absence of young ovaries. The transient decline after the first peak of activity may be due to programming by non-ovarian ecdysteroids, while the later plateau in both activity and development of the corpora allata is due to lack of inhibition from mature ovaries and the egg case. Our results indicate that both stimulatory and inhibitory ovarian factors regulate juvenile hormone production mainly by inducing changes in the cellular machinery rather than in rate-limiting enzymes. We hypothesize that young ovaries, mature ovaries and the egg case jointly act as a pacemaker that synchronizes the endogenous development of corpus allatum cells, resulting in cyclic production of juvenile hormone in intact *B. germanica* adult females.

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