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Effects of ovariectomy and mating on the activity of the corpora allata in adult female *Blattella germanica* (L.) (Dictyoptera: Blattellidae)

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Abstract. In adult female cockroaches, the ovary greatly affects the synthesis of Juvenile Hormone (JH) by the corpora allata, and in females of some cockroach species, removal of the ovaries results in a permanent depression of JH synthesis. We report that the corpora allata in ovariectomised, adult virgins of the German cockroach, *Blattella germanica* (L.), increase and then decrease in activity, as they do in intact females. Moreover, the distal tubules in the left colleterial glands of ovariectomised females accumulate abundant protein, the production of which is regulated by JH. In both ovariectomised and sham-operated females, the activity of the corpora allata more than tripled between days 1 and 4 of adulthood, during which the oöcytes of sham-operated females grew considerably in length. The corpora allata of sham-operated females produced even more JH on day 7, but very little on day 10, by which time all females had oviposited. The glands of ovariectomised females, by constrast, produced a similar amount of JH on day 7 as on day 4, but much less on day 10. Beginning on day 13, the activity of the corpora allata increased again in ovariectomised females, an increase that did not occur until day 22 in sham-operated females. Mating of ovariectomised females on day 6 resulted in a significant increase in the activity of the corpora allata by day 10. We conclude that both the ovary and mating stimulate the synthesis of JH early in the reproductive cycle, but that neither is needed for the occurrence of a complete cycle of JH synthesis.

Key words. *Blattella germanica,* colleterial glands, corpora allata, Juvenile Hormone, mating, ovariectomy, vitellogenesis.

Introduction

In adult female cockroaches, the ovary has a marked and dynamic effect on the synthesis of Juvenile Hormone (JH) by the corpora allata (Tobe & Stay, 1985; Schal *et al*., 1997). For example, the vitellogenic ovary of the blaberid *Diploptera punctata* stimulates the corpora allata to produce more hormone (Rankin & Stay, 1984), whereas the mature ovary has the opposite effect, inhibiting the activity of the glands (Stay *et al*., 1980; Rankin & Stay, 1985). These attributes of the ovary may, in fact, be common to all cockroach species, for nearly identical results were obtained in an investigation on another blaberid, *Nauphoeta cinerea* (Lanzrein *et al*., 1981).

In some cockroaches, such as *D. punctata* (Stay & Tobe, 1978; Rankin & Stay, 1983; Stay *et al*., 1983) and *N. cinerea* (Lanzrein *et al*., 1981), the corpora allata of ovariectomised females, virgin and mated alike, synthesize little JH – indeed, much less than the glands of intact, mated females. By contrast, the corpora allata of ovariectomised *Rhyparobia* (= *Leucophaea*) *maderae* apparently become at least moderately active because they hypertrophy (Scharrer & von Harnack, 1958; Scharrer, 1978) and because the fat body of ovariectomised females produces copious vitellogenin (Engelmann, 1978), whose synthesis is regulated by JH (Engelmann, 1969, 1979). Nevertheless, because JH synthesis

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itself was not measured in any of the studies on *R. maderae*, it cannot yet be categorically stated that the corpora allata in ovariectomised females produce a substantial quantity of JH.

In a recent investigation, Maestro *et al*. (1994) determined that the corpora allata of ovariectomised virgins of the German cockroach, *Blattella germanica* (L.), produced far less JH in the first 9 days of adulthood than did the glands of intact females (Gadot *et al*., 1989a,b; Maestro *et al*., 1994). The implication of this finding was that the activity of the corpora allata was similarly affected (i.e. strongly depressed) by ovariectomy in *D. punctata*, *N. cinerea* and *B. germanica*. It is therefore surprising that the quantity of vitellogenin accumulating in the haemolymph varied greatly among ovariectomised females of the three species. Whereas the vitellogenin concentrations in the haemolymph of ovariectomised *D. punctata* and *N. cinerea* never exceeded four-fold the highest levels in intact females (Lanzrein *et al*., 1981; Stay *et al*., 1983), the haemolymph vitellogenin concentration in ovariectomised *B. germanica* approached 60-fold the maximal level in normal females (Kunkel, 1981; Martín *et al*., 1996). This considerable accumulation of vitellogenin in the absence of significant synthesis of JH prompted speculation that, in *B. germanica*, factors apart from or in addition to JH regulate the increase (Martín *et al*., 1996) and subsequent decrease (Martín *et al*., 1995a, b) in the synthesis of vitellogenin during the ovarian cycle. This hypothesis, along with its supporting evidence, is clearly at odds with the long-standing and widely accepted doctrine that JH regulates vitellogenesis in cockroaches (Engelmann, 1979; Wyatt & Davey, 1996). Because of this incongruity, we have re-examined the activity of the corpora allata in ovariectomised *B. germanica*.

Materials and Methods

Insects

The German cockroach colony was reared in 4-litre battery jars at 27°C under a LD 12 : 12 h photoregime and was provided with rat chow (no. 5012; Purina Mills Inc., St. Louis, Missouri) and water *ad libitum*. Newly ecdysed last instars were collected directly from the colony.

Surgeries and dissections

Females were ovariectomised within 48 h after having moulted into the last larval stadium. They were anaesthetized with carbon dioxide and immobilized with Plasticine on a paraffin block, whereupon bilateral longitudinal slits were made with fine scissors in their seventh abdominal tergites. The ovaries were removed through these slits, and when the operation was completed, the females were placed in groups of 10–20 in 150×25 mm Petri dishes containing water, rat chow and shelters constructed of filter paper. Sham-operated females were treated similarly, but their ovaries were left intact. After eclosing, females were maintained in groups of 5–20 under conditions identical to those of post-operative last instars. Except where noted, females were kept virgin.

The corpora allata were dissected from the severed heads of females that had been anaesthetized with carbon dioxide. The glands were cleaned of adhering tissues beneath cockroach saline (Kurtti & Brooks, 1976). Ovaries and left colleterial glands were also dissected under cockroach saline, and the lengths of basal oöcytes and diameters of distal tubules in the colleterial glands were measured using an ocular micrometer in a dissecting microscope.

Quantification of juvenile hormone released by the corpora allata

A rapid partition radiochemical assay (Feyereisen & Tobe, 1981) was used to quantify the Juvenile Hormone released *in vitro* by the corpora allata of sham-operated and ovariectomised females. Freshly dissected glands were transferred into 6×50 mm borosilicate culture tubes, where they were maintained at 27°C for 90 min in 100 µl of methionine-free TC199 (Specialty Media, Lavalette, New Jersey) containing 100 µM L-[^{3}H-methyl]-methionine (NEN, Wilmington, Delaware), 5 mM $CaCl_2$ and 20 mg/ml Ficoll type 400. The corpora allata were then transferred to 100 µl of radiolabelled medium, in new culture tubes, for an additional 3 h of incubation at 27°C. All glands were floated at the surface of the medium (Holbrook *et al*., 1997) and all culture tubes were rotated at 90 r.p.m. at a 16° pitch on an orbital shaker. Except where noted, the glands were discarded after 3 h and the medium in each tube was extracted with 250 µl isooctane, 100 µl of which was suspended in liquid scintillation cocktail for radiospectrometry. All spectrometric measurements were corrected by subtracting counts in blank incubations without corpora allata.

Mating

In experiments addressing the effect of mating on the activity of the corpora allata, we placed individual, 6-day-old ovariectomised or sham-operated females in 100×20 mm Petri dishes, in each of which we also placed a pair of virgin, adult males at least 10 days old. Females were then monitored for copulation at 15 min intervals for the next 5 h. After females had finished mating, which took about 90 min (Schal & Chiang, 1995), they were placed in groups of 5–20 in 150×25 mm Petri dishes containing rat chow, water and a filter paper shelter. In a preliminary experiment, 18 of 32 (56%) ovariectomised and 21 of 30 (70%) sham-operated females mated. In other experiments, we forcibly separated females from their partners within 5 min of the onset of copulation. Careful inspection of the genital atrium in each of the females showed that males did not transfer spermatophores to them within this time. Females, whose copulation had been prematurely terminated, were maintained under conditions identical to fully mated females.

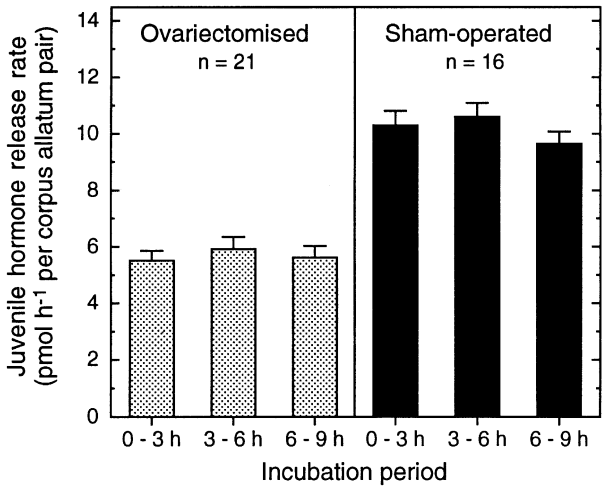

Fig. 1. Release of JH by the corpora allata of 7-day-old ovariectomised and sham-operated virgin females. The corpora allata were pre-incubated for 90 min in TC199 with 100 µM L-[^{3}H-methyl]-methionine and subsequently transferred to fresh medium. After 3 h, the glands were again transferred to new medium, and the medium in which they had been incubating was extracted to quantify the JH released by the glands. This procedure was repeated three times. Error bars are + SEM.

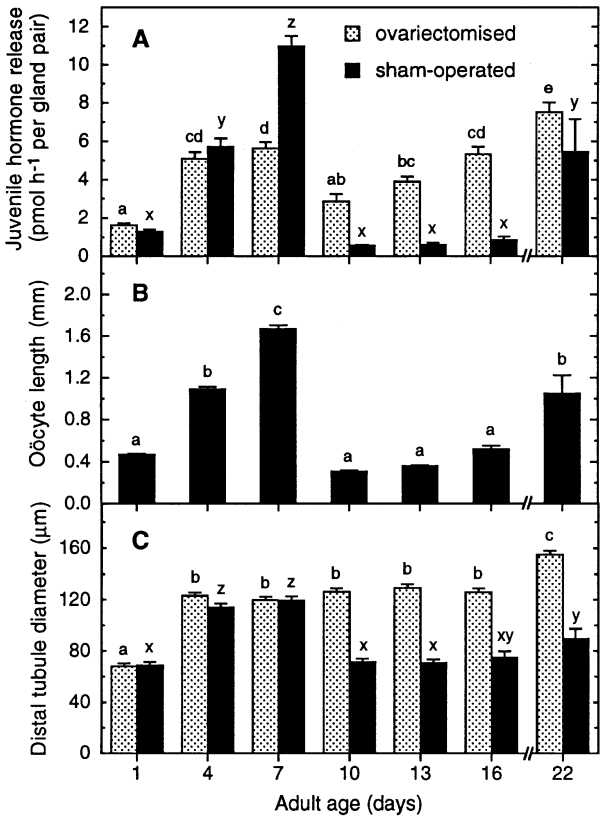

Fig. 2. Changes in (A) the mean rate of JH release by the corpora allata of ovariectomised ($n = 18$–31) and sham-operated ($n = 14$–25) virgin females, (B) the mean length of the basal oöcytes of sham-operated virgin females ($n = 12$–25) and (C) the mean diameter of the distal tubules in the left colleterial glands of ovariectomised ($n = 18$–31) and sham-operated ($n = 14$–25) virgin females. Letters denote the relationship among means for JH release rate, oöcyte length and distal tubule diameter in females with or without ovaries. Means sharing no common letters differ from each other (Tukey–Kramer test, $P < 0.05$). Error bars are + SEM.

Statistics

Analysis of variance (ANOVA) was carried out with the computer programme SuperANOVA (Abacus Concepts, Inc., Berkeley, California). A significant finding in an ANOVA was followed by the Tukey multiple comparison of means test when the sample sizes were equal and the Tukey test with the Kramer modification when the sample sizes were unequal. All *t*-tests were two-tailed and unpaired. A significance level of 0.05 was set as the decision criterion for rejecting null hypotheses. Standard error of the mean (SEM) served as the measure of variability in the data.

Results

Release of JH in vitro *by the corpora allata*

Maestro *et al.* (1994) found that the corpora allata of ovariectomised *B. germanica* increased in activity in each successive 2-h period of an 8-h incubation. Because this finding differed from an earlier one – Gadot *et al.* (1989b) reported constant release of JH by the corpora allata of normal females – we re-examined the production of JH by the glands of ovariectomised females. The corpora allata of 7-day-old females lacking ovaries did not release different amounts of JH in the three 3-h periods of a 9-h incubation (repeated measures ANOVA, $F = 2.95$, d.f. = 2, 40, $P > 0.05$; Fig. 1). The activity of the corpora allata of sham-operated females did change ($F = 5.88$, d.f. = 2, 30, $P = 0.007$), but did not increase. Instead, it declined slightly, albeit significantly (Tukey test, $P < 0.05$), to a lower level in the 6–9 h interval than in the 3–6 h one. It was, nonetheless, appropriate for us to use 3-h incubations in subsequent experiments to determine rates of JH release because the glands of all females produced JH at a constant rate for at least 6 h.

Activity of the corpora allata of sham-operated and ovariectomised females

The amount of JH produced by the corpora allata changed considerably in the first 22 days of adulthood in both sham-operated (single factor ANOVA, $F = 57.45$, d.f. = 6, 125, $P < 0.001$) and ovariectomised ($F = 28.16$, d.f. = 6, 138, $P < 0.001$) females (Fig. 2A). In sham-operated females, the corpora allata released more JH ($P < 0.05$, Tukey-Kramer test) on day 4 (5.7 ± 0.44 pmol/h) than they did on day 1 (1.3 ± 0.10 pmol/h) and during this time the length of the

basal oöctyes increased ($P < 0.05$) from 0.47 ± 0.01 mm to 1.09 ± 0.03 mm (Fig. 2B). The activity of the corpora allata continued to increase until day 7 (11.0 ± 0.54 pmol JH h^{-1}), by which time the oöcytes were 1.67 ± 0.04 mm in length. All females subsequently oviposited between days 8 and 10, and on day 10, the corpora allata produced just 0.6 ± 0.04 pmol JH h^{-1}. Afterward, they released less than 1 pmol JH h^{-1} through day 16, but much more JH (5.5 ± 1.69 pmol h^{-1}) on day 22.

In ovariectomised females, as in sham-operated ones, the corpora allata more than tripled in activity between days 1 (1.6 ± 0.12 pmol JH h^{-1}) and 4 (5.1 ± 0.35 pmol JH h^{-1}), and on day 4, the glands of females with and without ovaries did not differ in the mean amount of JH they released (*t*-test, $t = 1.13$, d.f. = 28, $P = 0.27$). The corpora allata of 7-day-old ovariectomised females released only slightly more JH than those of 4-day-olds, and just half as much as the glands of 7-day-old sham-operated females. On day 10, the corpora allata of ovariectomised females produced significantly (Tukey-Kramer test, $P < 0.05$) less JH (2.9 ± 0.39 pmol h^{-1}) than they did on day 7, but more (*t*-test, $t = 6.38$, d.f. = 37, $P < 0.001$) than the glands of identically aged sham-operated females. Thereafter, the glands of females lacking ovaries increased continuously in activity through day 22, when they released JH at the highest rate of any day.

Colleterial gland development

To assess whether the results of *in vitro* assays reflected the activity of the corpora allata *in vivo*, we examined changes in the diameter of the distal tubules in the left colleterial gland (Fig. 2C). The tubules increase in diameter when they accumulate colleterial protein, the synthesis of which is regulated by JH (Burns *et al.*, 1991). In sham-operated females, the tubules increased greatly in diameter (single factor ANOVA, $F = 31.83$, d.f. = 6, 125, $P < 0.001$) from days 1–7 (Tukey-Kramer test, $P < 0.05$), while the corpora allata went from releasing little to considerable JH (Fig. 2A). The diameter of the distal tubules then decreased significantly by day 10, as a direct result of the allocation of colleterial protein to the oötheca. In ovariectomised females, the corpora allata increased in activity between days 1 and 7 (Fig. 2A), and the distal tubules likewise increased in diameter from 68 ± 2.5 µm to 120 ± 2.4 µm (single factor ANOVA, $F = 87.30$, d.f. = 6, 138, $P < 0.001$; Tukey–Kramer test, $P < 0.05$; Fig. 2C). On days 10, 13 and 16, the corpora allata produced less JH than they did on day 7 and the diameter of the distal tubules remained between 126 and 129 µm. On day 22, however, the glands increased again in activity, producing more JH than on day 7, and the distal tubules correspondingly attained their largest size (155 ± 2.9 µm).

Effect of mating on the activity of the corpora allata

The corpora allata of ovariectomised females that were not allowed to mate released abundant hormone, but they never produced as much JH as those of ovariectomised females that had mated (see Gadot *et al.*, 1990, 1991; Chiang *et al.*, 1991b). To determine whether mating stimulated the production of JH, we allowed ovariectomised females to mate on day 6 and measured the activity of their corpora allata 4 days later. On day 10, the glands of mated, ovariectomised females released considerably more JH (*t*-tests, $P < 0.001$; Fig. 3) than did the glands of females kept as virgins ($t = 7.14$, d.f. = 32; Fig. 2A) or allowed to copulate for less than 5 min ($t = 5.70$, d.f. = 25; Fig. 3). Moreover, the distal tubules in the left colleterial gland were significantly larger ($t = 8.36$, d.f. = 25, $P < 0.001$) in mated females than in females separated early in copulation (Fig. 3). All sham-operated females that mated on day 6 oviposited by day 10, and on day 10, their corpora allata produced little JH (0.65 ± 0.06 pmol h^{-1}) and the distal tubules in their colleterial glands were small in diameter (77.1 ± 3.4 µm).

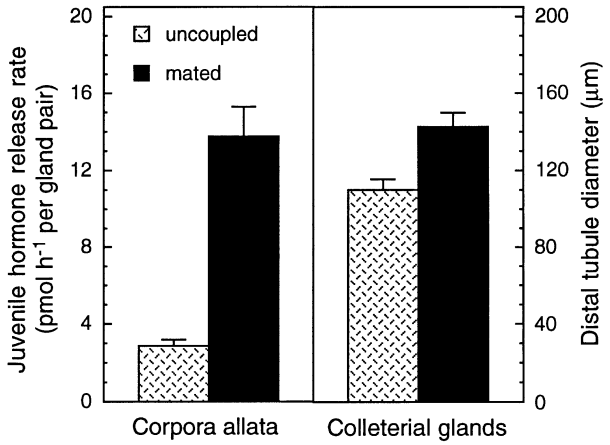

Fig. 3. Effect of mating on the activity of the corpora allata and size of the distal tubules in the left colleterial gland. Six-day-old ovariectomised females were allowed either to mate fully or to copulate for less than 5 min before being separated forcibly from their partners. The JH release rate of the corpora allata and the diameter of the distal tubules in the left colleterial gland were measured on day 10 in briefly copulated ($n = 11$) and completely mated ($n = 16$) females. Error bars represent + SEM.

Discussion

Activity of the corpora allata in vitro *and in differently aged females*

Our results prompt us to make conclusions that differ radically from those made by Maestro *et al.* (1994), who asserted that the corpora allata of unmated, ovariectomised *B. germanica* produced JH at a low, almost constant rate in the first 9 days of adulthood. By contrast, we contend that the activity of the corpora allata markedly increases and then decreases, that is, cycles in ovariectomised virgins. It is worth noting, however, that the data of Maestro *et al.* (1994) do, in fact, support our contention, for their results show a muted cycle of JH synthesis in ovariectomised females, which went

unrecognized by the investigators. Nevertheless, in spite of this accord, a prominent difference remains between our results and theirs. Namely, we do not detect a great depression of corpora allata activity in ovariectomised females. Maestro *et al*. (1994) found that the most highly active glands of ovariectomised females produced just one-sixth the JH of maximally active glands of intact females. We, however, determined that the corpora allata of 7-day-old ovariectomised females produced about half the JH of glands of identically aged, sham-operated females, glands that are very near or at their peak of activity (Schal *et al*., 1997).

Although Maestro *et al*. (1994) found large differences in the activities of the corpora allata of identically aged, intact and ovariectomised females, they concluded nonetheless that the glands of ovariectomised females could synthesize as much JH as those of sham-operated ones, but were constrained *in vivo* from doing so. The basis of this conclusion was their finding that the glands of ovariectomised females increased in activity throughout an 8-h incubation and ultimately produced JH at almost the same rate as the glands of similarly aged, intact females. Nevertheless, because Maestro *et al*. (1994) did not determine whether the glands of intact females also increased in activity in a long-term incubation, they did not truly test their hypothesis that the corpora allata of intact and ovariectomised females were biosynthetically equivalent.

The corpora allata of females that either have or do not have ovaries release JH at a near constant rate throughout a 9-h incubation (Fig. 1). This finding is clearly in discord with the results of Maestro *et al*. (1994) but in accord with those of Gadot *et al*. (1989b), who reported that the corpora allata of normal females released JH at an almost invariant rate for 8 h. We cannot, at this time, explain the increasing activity of the corpora allata reported by Maestro *et al*. (1994). It is possible, however, that these differences may be due to different methods of organ culture. Indeed, both physical and nutritional factors greatly influence how much JH the corpora allata synthesize *in vitro* (Holbrook *et al*., 1997).

The corpora allata of ovariectomised females never produce as much JH as those of sham-operated and intact ones (Gadot *et al*., 1989a), but the glands of all females increase and decrease in activity concurrently in the first 10 days of adulthood. The corpora allata of ovariectomised females therefore retain not only a cycle of activity, but a temporally correct one as well, something that has not been described in any other cockroach species. In ovariectomised (and mated) *D. punctata* and *N. cinerea*, for example, the corpora allata show neither the significant increase nor rapid decrease in activity typical of the glands of normal, mated females (Stay & Tobe, 1978; Lanzrein *et al*., 1981). In addition, although the corpora allata of ovariectomised *P. americana* increase and decrease in activity, they do so over a much longer time – a span of about five normal reproductive cycles – than the glands of normal females (Weaver, 1981).

Although the corpora allata of ovariectomised German cockroaches retain cyclic activity, the ovary still appears to have a great effect on the synthesis of JH in this species. The ovary in *B. germanica*, as in *D. punctata* (Rankin & Stay, 1984) and *N. cinerea* (Lanzrein *et al*., 1981), appears necessary for the corpora allata to attain a high level of activity, for the most highly active glands of ovariectomised females produced just half the JH of peak-active glands of sham-operated ones (Fig. 2A). And in addition, the ovary appears necessary to bring about, in full, the substantial decrease in JH synthesis that immediately precedes oviposition. Compelling evidence for this was provided in our present study (Fig. 2A) and by Gadot *et al*. (1990, 1991), who found that the corpora allata of ovariectomised females released increasing amounts of JH through day 14 of adulthood, while the glands of intact females usually declined in activity by day 8. It has been suggested that the ovaries of the German cockroach release a factor into the haemolymph – perhaps ecdysteroids (Chiang *et al*., 1991a, b; Romañá *et al*., 1995) – that inhibit the synthesis of JH, but non-ovarian factors are also known to influence the activity of the corpora allata. The egg case, for example, inhibits the production of JH, as is evidenced by the sharp drop in gland activity that follows the insertion of an artificial egg case into the genital vestibulum of an ovariectomised female (Chiang *et al*., 1991b).

After an initial cycle of JH synthesis, the corpora allata of both sham-operated and ovariectomised females increased again in activity within the first 22 days of adulthood, but the increase occurred earlier in females without ovaries (Fig. 2A). The glands of these females began producing more JH on day 13, and this upward trend continued through at least day 22. The glands of sham-operated females, by contrast, did not increase in activity until day 22. We suspect that the later re-activation of the corpora allata in sham-operated females is a direct result of these females carrying an infertile egg case for several days after oviposition. Indeed, the presence of an egg case in the genital vestibulum inhibits the activity of the corpora allata not only in ovariectomised females (Chiang *et al*., 1991a,b) but intact ones as well (Roth & Stay, 1962). It therefore seems reasonable that the rapid re-activation of the corpora allata in ovariectomised females is attributable to the absence of an inhibitory egg case, although it may also be fostered by an incomplete suppression of gland activity after the first peak of JH synthesis.

Mating

We have now ascertained that mating stimulates the production of JH in females lacking ovaries. This result is at odds with our previous investigation: Gadot *et al*. (1991) reported that the corpora allata of unmated and mated, ovariectomised females produced similar amounts of JH. It is conceivable, however, that Gadot *et al*. (1991) did not detect a mating effect on JH synthesis because the activity of the corpora allata was measured 8 days after females had mated. In our current report, we measured corpora allata activity just 4 days after mating, and the substantially greater synthesis of JH by the glands of mated females leaves little doubt of a mating effect on the corpora allata. Moreover, it appears that complete copulation, and probably spermatophore transfer, are

needed to stimulate the activity of the corpora allata because copulation for less than 5 min did not lead to an increase in the production of JH.

In adult female cockroaches, the activity of the corpora allata is influenced by a variety of factors, including mating, feeding and social interaction (Feyereisen, 1985; Schal *et al*., 1997; Holbrook *et al*., 2000). Among these, however, mating is unique in that it has been shown to stimulate corpora allata activity and oöcyte maturation in all cockroach species studied to date. Nevertheless, in *B. germanica*, the stimulatory effect of mating is rendered nearly undetectable when females are reared in groups (Gadot *et al*., 1989a). Our present results indicate that the ovary further obscures the stimulatory effect of mating. The corpora allata of mated, ovariectomised females produced nearly five-fold more JH than the glands of unmated, ovariectomised ones (Fig. 3), but the disparity is far smaller – much less than two-fold – in females that have ovaries (Gadot *et al*., 1989a). These results are consistent with a model stating that a number of internal and environmental stimuli interact to exert a graded lifting of allatostatic inhibition on JH synthesis in the German cockroach (Schal *et al*., 1997).

Vitellogenesis in ovariectomised females

The role of JH in regulating vitellogenesis has been well documented in cockroaches (Engelmann, 1979; Wyatt & Davey, 1996). An increase and subsequent decrease in the synthesis of JH during a reproductive cycle typically correspond with like changes in the synthesis of vitellogenin. Moreover, vitellogenin synthesis is eliminated following surgical allatectomy (Engelmann & Penney, 1966; Bell, 1969) but restored after allatectomised females are implanted with a corpus allatum (Bell, 1969; Engelmann, 1969) or dosed with JH (Engelmann, 1971; Bühlmann, 1976) or JH analogue (Engelmann, 1969). Greatly at odds with these results, Martín *et al*. (1995a) reported that the fat body of ovariectomised virgins of *B. germanica* produced abundant vitellogenin, even though the corpora allata in these females did not synthesize a substantial quantity of JH (Maestro *et al*., 1994). They suggested therefore that JH was not essential, but that other factors probably were, to induce vitellogenesis in the German cockroach (Martín *et al*., 1995a, b, 1996). Two lines of evidence argue against the verity of such speculation. First, allatectomy eliminates oöcyte maturation and, thus, oviposition in *B. germanica* (Roth & Stay, 1962; Schal *et al*., 1990), and second, administration of a JH analogue by itself to allatectomised females restores near normal oöcyte development (Schal *et al*., 1990).

A cursory analysis of our results, along with those of Martín *et al*. (1996), might lead one to conclude that JH and vitellogenin synthesis are at least partially uncoupled in the German cockroach. Indeed, ovariectomised females produce abundant vitellogenin (Martín *et al*., 1996) without their corpora allata becoming as active as those of normal or sham-operated females (see Fig. 2A, day 7). Nevertheless, convincing evidence exists that the level of activity reached by the corpora allata in ovariectomised females is sufficient to stimulate the synthesis of substantial vitellogenin. In normal females, the fat body usually produces large amounts of vitellogenin before day 4 (Martín *et al*., 1995a, b), and this was also the case in sham-operated females in our current study because the oöcytes of these females grew considerably in length by the fourth day of adulthood (Fig. 2B). Because the corpora allata of sham-operated and ovariectomised females were equally active on day 4 (Fig. 2A), it is reasonable to presume that the corpora allata of ovariectomised females, like those of sham-operated ones, were synthesizing enough JH to induce the production of prodigious vitellogenin. Our finding that considerable protein accumulates, by day 4, in the distal tubules of the left colleterial glands of ovariectomised females supports this presumption. The synthesis of colleterial protein is regulated by JH, and the diameter of the distal tubules, which comprise much of the gland, is directly related to the amount of protein within the tubules. Distal tubule diameter is therefore a good index of the past exposure of colleterial glands to JH (Burns *et al*., 1991). The distal tubules were equal in diameter on day 4 in sham-operated and ovariectomised females, indicating that the titres of JH had been similar in the females before this time.

Our current results indicate that the ovary has a dynamic effect on the activity of the corpora allata, stimulating and inhibiting the synthesis of JH at different phases of the reproductive cycle. Just how the ovary does this merits further investigation and the search for ovarian factors inhibiting the synthesis of JH in *B. germanica* is well underway (Chiang *et al*., 1991a, b; Romañá *et al*., 1995). However, as yet, little effort has been made to identify ovarian factors fostering the production of JH. Likewise, little can be said of how mating stimulates the activity of the corpora allata in *B. germanica*, although transection of the ventral nerve cord is known to eliminate mating's stimulatory effect (Schal *et al*., 1997). What is obvious though, is that ovariectomised females will be ideal subjects in investigations on mating effects on JH synthesis because the difference in activity of the corpora allata between unmated and mated females is far greater in females without ovaries than in those with them.

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