Sexual differentiation of nymphal corpora allata and the effects of ovariectomy on adult gland morphometrics in *Blattella germanica*

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Summary. Changes in the number of corpus allatum (CA) cells were investigated in nymphs and in intact and ovariectomized adult female *Blattella germanica*. The CA of intact adult females exhibit cyclic changes in volume in relation to juvenile hormone (JH) synthesis, while the CA of ovariectomized females become significantly hypertrophied as a result of a gradual and continuous increase in volume that is independent of JH biosynthesis. In both intact and ovariectomized females changes in JH synthesis and CA volume are not related to total cell number which remains relatively constant. However, adult females have twice as many CA cells as do adult males as a result of a female-specific increase in total cell number late in the last nymphal instar.
Key words. Cell number; corpora allata; juvenile hormone; cockroach; ovary; sexual dimorphism; *Blattella germanica*.

Cyclic changes in corpus allatum (CA) volume are associated with oocyte growth in many insects[2,3], and in adult female cockroaches volumetric changes in the CA are related to their cycle of in vitro juvenile hormone (JH) biosynthesis[4-7]. The contribution of CA cell number to the cyclic changes in CA volume and activity has been studied extensively in cockroaches, but with little consensus among researchers. Engelmann[8] observed that activation of the CA in *Leucophaea maderae* is characterized by an increase in the amount of cytoplasm without significant changes in the number of nuclei. Using the same procedure of counting nuclei in representative sections of fixed glands, cyclic numerical changes in CA cells during the ovarian cycle were demonstrated in *L. maderae*[9] and in *Diploptera punctata*[10]. In ovariectomized adult *D. punctata*[11,12] and *L. maderae*[13], total CA cell number increases significantly while JH biosynthesis remains low in *D. punctata*[10].

Recently, we used hemocytometric counts of dissociated CA cells and total counts of cells in fresh monolayers to show that the total number of cells does not change significantly during CA activation in the first gonotrophic cycle in adult females of the cockroaches *D. punctata* and *Blattella germanica*[14]. We now report that the number of CA cells remains relatively constant through 2 gonotrophic cycles in intact *B. germanica* females and for at least 7 weeks after the imaginal molt in ovariectomized females. In contrast, total CA cell number increases significantly in nymphal cockroaches, and we show a rapid, female-specific numerical increase in CA cells late in the last nymphal stage which may account for differences in activity between adult male and female CA.

Materials and methods

Blattella germanica nymphs and adults were reared at 27 ± 1 °C under a 12 h light : 12 h dark photoperiodic regime and supplied with dog food and water ad libitum. Male and female penultimate and last instar nymphs were maintained in separate groups. Under these conditions, the penultimate nymphs take at least 8 days to molt into the last instar and at least 12 more days to reach the adult stage. Newly-eclosed adult males and females (day 0) were collected daily and maintained in separate groups[15]. Intact females were allowed to mate on day 6 and they oviposited on day 9. Mated females carry the egg case externally for approximately 20 days before the onset of the second ovarian cycle. Ovariectomy was performed early in the last instar. Ovariectomized females were presented with males for at least 8 h daily from day 3 until mating was observed; thereafter, each mated female was kept with two males[16,17]. Only females that mated before day 8 were used. The rate of JH synthesis from each CA was determined by a radiochemical assay as described previously[7]. The total number of cells per CA was counted from a monolayer of CA cells prepared by spreading enzymatically desheathed CA in a crystal violet and citric acid solution[14,18]. CA volume was determined by the formula $v = 4/3 \cdot \pi \cdot abc$, where a, b and c are the radii of the three principal axes measured with an ocular filar micrometer under a dissecting microscope.

Results

The size of CA from adult female *B. germanica* increases and decreases during two ovarian cycles in relation to oocyte maturation and in vitro JH biosynthesis[7] (fig. 1). The CA were largest on day 7 of the first ovarian cycle and on day 4 of the second cycle, their volume declined through ovulation (day 9) and pregnancy and increased again late in pregnancy. During the first two ovarian cycles, total CA cell number did not change significantly ($p > 0.05$; Duncan's multiple range test of 7 ages): each CA, regardless of its size and gonotrophic stage, contained approximately 2000 cells.

Ovariectomized females exhibited high but delayed CA activation (JH biosynthesis) and they experienced at least

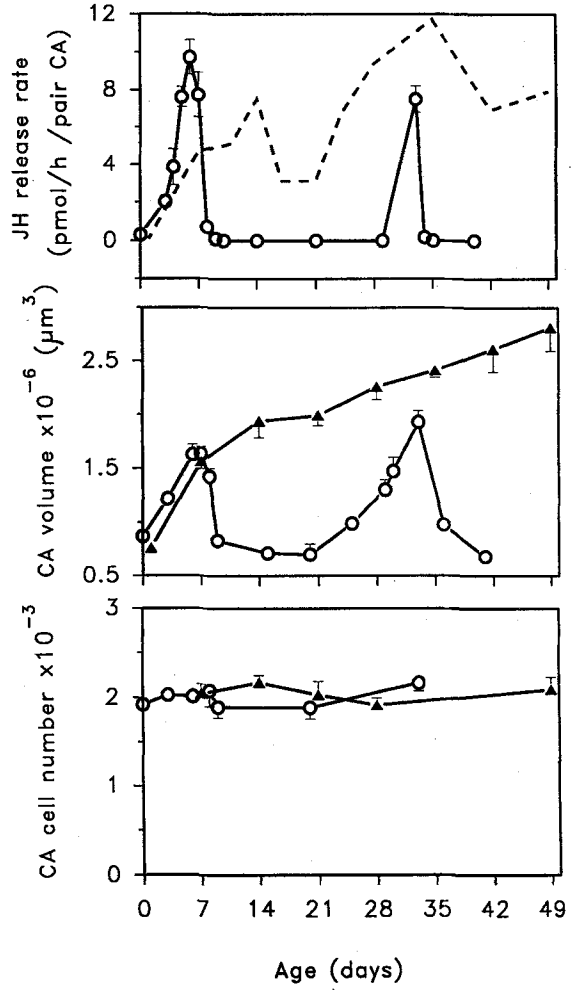

Figure 1. Juvenile hormone (JH) biosynthesis, corpus allatum (CA) volume and cell number in relation to age in intact (○) and in ovariectomized (▲) grouped adult females. Ovariectomy was performed on early last instar nymphs. The dashed line represents the values of JH biosynthesis by the CA from ovariectomized adult females published previously[17]. Each point is the mean of 6–16 measurements of CA from at least 4 insects. Vertical bars represent SEM.

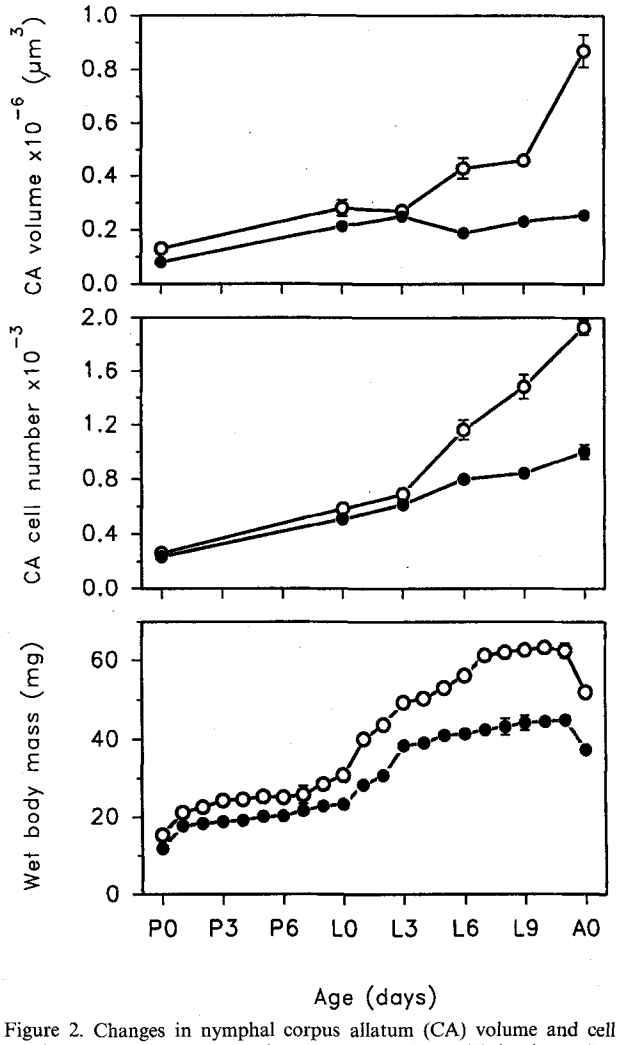

Figure 2. Changes in nymphal corpus allatum (CA) volume and cell number, and body mass in relation to age. Penultimate (P), last instar (L), and teneral adult (A) males (●) and females (○) were examined at intervals after the respective ecdyses, as indicated. Gland volume and cell number for each mean were determined from 6–8 CA from 4 insects. The values for body mass are means from 10 insects. Vertical bars represent SEM.

one abbreviated cycle (fig. 1). Their CA increased in volume continuously for at least 7 weeks after the imaginal molt and attained twice the maximal volume of the CA of intact females. However, the total number of cells in these hypertrophied CA did not change significantly over time and was not different from that in the CA of intact females ($p > 0.05$; Duncan's multiple range test of 12 ages).

Extensive somatic growth occurred in the last two nymphal instars in both males and females, with large gains in wet body mass soon after each molt (fig. 2). Although female nymphs were significantly heavier than male nymphs, the number of CA cells was not significantly different in the two sexes until day 3 of the last instar ($p > 0.05$; t-test for each age). Thereafter, the rate of increase in CA cell number remained constant in male nymphs, but doubled in female nymphs. Thus, in males, CA cell number doubled during the last instar, while in females CA cells quadrupled in number during this period, resulting in CA of newly-ecdysed adult females with twice as many cells as CA of newly-ecdysed adult males. In both sexes the changes in nymphal CA volume corresponded to the changes in total cell number.

Discussion

Tobe et al.[12] proposed that cell proliferation is required for the CA to attain their maximal JH synthetic activity, and that factors influencing cell proliferation may be involved in the regulation of JH biosynthesis in *D. punctata*. However, the CA of adult *B. germanica* females undergo volumetric and biosynthetic cycles in relation to oocyte maturation[6,7] without significant changes in the total number of cells. The CA of ovariectomized *B. ger-*

manica females attain similar peak rates of JH biosynthesis as do the CA of intact females[16, 17] (fig. 1) and they become significantly hypertrophied, but here too, the total number of CA cells remains constant. These results clearly argue against changes in the total number of CA cells having a significant role in the regulation of JH biosynthesis in *B. germanica*. Furthermore, they suggest that the ovaries do not regulate the total number of CA cells in the adult cockroach.

Sexual dimorphism in JH synthesis by the CA is common in insects[19], but it is not clear when and how this divergence occurs. In adult *D. punctata*, the CA of males have fewer cells and a lower capacity for JH synthesis than female CA[20]. In *B. germanica*, these intrinsic differences appear to arise during CA development late in the last nymphal instar when developmental patterns of male and female nymphs diverge. During nymphal development the increase in CA volume and proliferation of CA cells are thought to be associated mainly with somatic growth[19]. However, in female *B. germanica* the number of CA cells increases most rapidly late in the last instar while the greatest gain in body mass occurs earlier in the instar. The female-specific increases in CA volume and cell number are therefore only partly related to somatic growth in the nymph, and appear to be independent of JH biosynthesis by the CA.

We conclude that, in *B. germanica*, the activity of the CA is independent of changes in its total number of cells. It is unlikely that CA cell proliferation plays a major role in regulating JH biosynthesis in either the last instar or the adult, since at both stages the changes in activity are not associated with changes in cell number. However, a significant female-specific cell proliferation in the last instar may be a prerequisite for the higher activity of the CA in the adult female than in the adult male.

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Cyclic and linear vasopressin V_1 and V_1/V_2 antagonists containing arginine in the 4-position

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Summary. Substitution of arginine for glutamine in the 4-position of a vasopressin V_1 antagonist has been reported to turn it into an agonist. We resynthesized this 4-arginine analog and synthesized additional cyclic and linear vasopressin antagonists containing a 4-arginine. The presence of a 4-arginine in the resynthesized and new analogs had relatively minor effects on their antivasopressin V_1 and V_2 antagonistic potencies.
Key words. Vasopressin antagonists; V_1-antagonists; V_2-antagonists; neurohypophysial hormones; arginine.

Several amino acids occupy the 4-position in vertebrate neurohypophysial hormones. All known tetrapod neurohypophysial hormones contain 4-glutamine in this position while neurohypophysial peptides containing 4-serine or 4-asparagine also appear among fishes. 4-Arginine has been suggested as a hypothetical evolutionary intermediate that might explain how peptides containing 4-glutamine, 4-serine and 4-asparagine could have been derived