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JUVENILE HORMONE PRODUCTION AND ACCESSORY REPRODUCTIVE GLAND DEVELOPMENT DURING SEXUAL MATURATION OF MALE *BLATTELLA GERMANICA* (L.) (DICTYOPTERA, BLATTELLIDAE)

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Abstract—1. A study of the release of juvenile hormone (JH) by *in vitro*-incubated corpora allata (CA) and on the development of the accessory reproductive glands (ARG) during sexual maturation of male *Blattella germanica* has been performed.

2. JH release rates are comparable with those observed in previtellogenic females.

3. The ARG undergo a progressive increase in size and protein content during sexual maturation.

4. After CA removal, ARG protein accumulation is reduced (by 50%) in comparison with controls, and administration of exogenous JH restores normal development.

5. The involvement of JH in ARG growth is discussed.

INTRODUCTION

The accessory reproductive glands of male insects (ARG) produce proteinaceous materials associated with both protection of sperm and their transfer to the female (see Happ, 1984), and with modulatory functions on female behaviour (Leopold, 1976).

In past decades, anatomical, physiological and biochemical studies of these glands have revealed a variety of basic features. Therefore, they are now reasonably well-known from a morphological point of view (Adiyodi and Adiyodi, 1975), and at least partly characterized from a functional point of view (Chen, 1984). However, there is still little agreement concerning the involvement of the endocrine system in ARG development, and the role of juvenile hormone (JH), produced by the corpora allata (CA), remains uncertain. In most insect species ARG development during sexual maturation seems to be regulated by JH, but in some the absence of this hormone does not appear to influence ARG development (see Happ, 1984 for review).

Similar disparity is reported within the order Dictyoptera. In *Leucophaea maderae* and *Diploptera punctata* (Scharrer, 1946 and Tobe *et al.*, 1979, respectively) the ARG remain active and continue to secrete proteins after removal of the CA, while in *Periplaneta americana* the ARG are small and fail to produce their normal secretion after allatectomy (Blaine and Dixon, 1973).

The present study examines the role of JH in ARG development in adult males of the cockroach *Blattella germanica*. We have investigated the development of the ARG during sexual maturation, and the effect of allatectomy alone or followed by JH treatment, on their development. In addition, we used a radiochemical assay (Pratt and Tobe, 1974; Tobe and Pratt, 1974) adapted to *B. germanica* (Bellés *et al.*, 1987) to determine the production of JH by *in vitro*-incubated CA from males at different stages of sexual maturation.

MATERIALS AND METHODS

Insects

Colonies of *B. germanica* were reared in complete darkness at 30 ± 1°C and 60–70% r.h. (Bellés *et al.*, 1988). Freshly ecdysed males were isolated from females and maintained in these conditions until use.

Measurement of JH release by in vitro-*incubated CA*

Corpora cardiaca–corpora allata (CC–CA) complexes were incubated in 100 μl of Millipore-filtered TC-199 medium (Flow) containing L-methionine (0.1 mM), Hank's salts, Hepes buffer (20 mM) plus Ficoll (20 mg/ml), to which L-[methyl-^{3}H]methionine (Amersham) had been added to achieve a final specific activity of 7.4 GBq/mmol. Dissection and transfer of glands to the incubation medium and measurements of CA volume were performed as previously described (Bellés *et al.*, 1987). After 4 hr of incubation, the medium was subjected to extraction by addition of methanol (200 μl), 1% aqueous solution EDTA (100 μl) and chloroform (500 μl). After vigorous shaking the chloroform phase was collected and aqueous phase was again extracted (500 μl). Both chloroformic phases were joined and unlabelled JH III (Sigma) was added as standard. The chloroformic extract was then evaporated to dryness with a Univap evaporator (Uniscience). Dry extracts were redissolved in 100 μl of chloroform and applied directly to TLC plates (Merck); the solvent system was hexane:ethyl acetate (4:1). Radioactivity from fractions corresponding to the biosynthesized juvenile hormone III (JH III) was counted with a Kontron Betamatic V spectrometer.

Accessory gland development

The male ARG of *B. germanica* have been described in detail by Khalifa (1950) and Ballan-Dufrançais (1968). They

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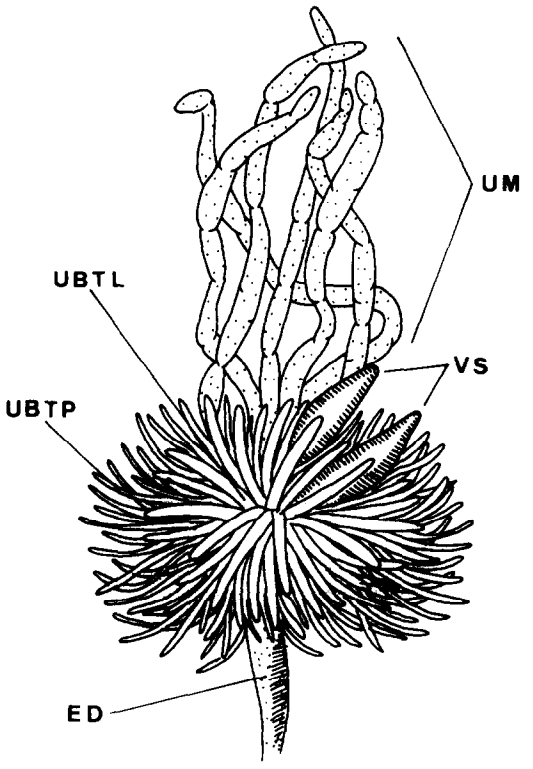

Fig. 1. Morphology of the accessory reproductive glands of a 5-day-old virgin adult male of *Blattella germanica*. ED: ejaculatory duct; UBTL: utriculi breviores translucent; UBTP: utriculi breviores transparent; UM: utriculi majores; VS: vesiculae seminales.

consist of a mass of tubules arranged in two principal groups. The first group, the utriculi majores (or uricose glands; Roth, 1967), comprises 4–6 long tubules arising from the ventral part. The other group, the utriculi breviores, forms a large mushroom-shaped body within which two different sets of tubules can be seen: a ventral group showing a translucent secretion and surrounding the vesiculae seminales, and a dorsal group formed by a great number of shorter, transparent tubules (Fig. 1). To study the normal development of ARG, adult males were dissected at 24 hr intervals from freshly ecdysed (day 0) through to day 10. Quantification of utriculi majores growth was carried out by measuring and averaging the diameter of every tubule at three different levels: near the apex, at the base and at the middle. In the case of utriculi breviores, five tubules of each type (transparent and translucent) were selected at random and their diameters were measured at the middle and averaged (Fig. 1). Total protein content of the ARG was determined according to the method described by Bradford (1976).

Allatectomies and JH administration

The corpora allata–corpora cardiaca complex was explanted as previously described (Piulachs, 1988) from freshly ecdysed adults. Sham operations were carried out by removing the cervical sclerite and adjacent main tracheae. All operations were conducted under CO_2 anaesthesia. JH III was administered in "rescue" experiments in a single dose (10 μg) just after allatectomy, or in two doses (10 μg each): one after allatectomy and the other 48 hr later. The hormone was topically applied in acetone solution (2 μl) on the dorsal part of the abdomen. Experimental effects induced by allatectomy alone or followed by JH treatment(s) were studied on day 5 of adult life.

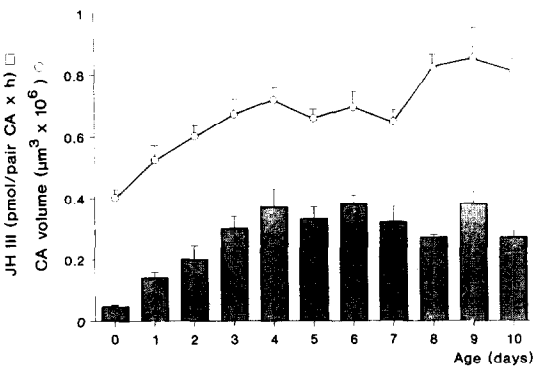

Fig. 2. Rates of release of JH III by *in vitro* incubated CA and the corresponding CA volumes in male *B. germanica* during sexual maturation. Each value represents the mean ± SEM of 8–20 replicates.

RESULTS

JH production and CA volumetric changes

The JH III release by *in vitro*-incubated CA from isolated males of *B. germanica* was investigated throughout the first 10 days of adult life. Results (Fig. 2) show that JH release increased rapidly from emergence to day 4, after which rates were maintained between 0.2 and 0.4 pmol/hr and CA pair, with minor fluctuations.

Figure 2 also shows the volumetric changes of CA during the studied period. CA volume was low just after emergence, showed a steady increase until day 4, and then seemed to stabilize until day 7 to slightly increase again towards the end of the period studied. In addition, the relationships between the volume of the CA of the same pair shows a quite high degree of asymmetry (Fig. 3), which is also apparent if the smaller CA volume is divided by the larger in the same CA pair (Fig. 3, inset).

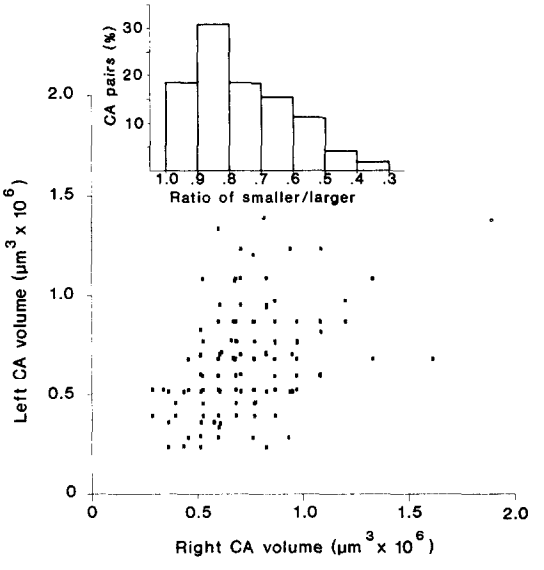

Fig. 3. Relationship between the volume of both CA of the same pair in adult male *B. germanica*. The inset shows the percentage of CA pairs ($N = 169$) falling in each ratio (smaller/larger) category.

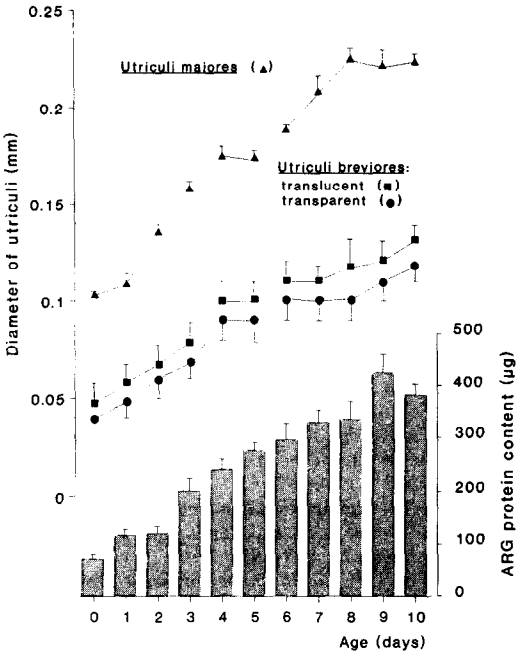

Fig. 4. Accessory reproductive gland development in male *Blattella germanica*. Each value represents the mean ± SEM of 8–20 specimens.

ARG development

The increase in diameter of the utriculi majores and of both types of utriculi breviores, transparent and translucent, is shown in Fig. 4. In all cases, the diameter showed a steady increase until day 4, stabilized on day 5 and then increased again until the end of the period studied. Figure 4 also shows the dynamics of growth in terms of total protein content of the whole gland. Protein content showed rather low values until day 2, but on day 3 it underwent a significant increase which continued until the end of the period studied.

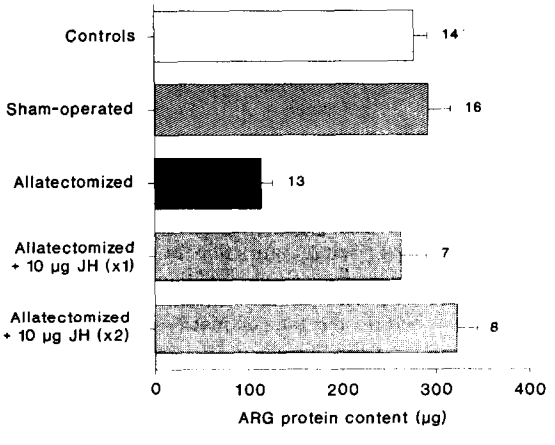

Fig. 5. Effect of allatectomy alone or followed by JH III treatment(s) on total protein content (±SEM) in the ARG of *B. germanica*. Allatectomies and JH treatments were carried out on freshly ecdysed specimens and the effects were checked on day 5. Significant differences (*t*-test) with respect to controls were only found in allatectomized specimens ($P < 0.0001$).

Effects of allatectomy

To study the possible role of JH on ARG growth, males were allatectomized at emergence and glands were dissected on day 5 to measure the total protein content. The results (Fig. 5) revealed that accumulation of ARG proteins was significantly reduced (by 50%) in comparison with untreated or sham-operated controls. However, a single dose of 10 µg of JH III (the natural JH of *B. germanica* adult females; Camps *et al.*, 1987) practically restored normal development, and allatectomized specimens treated with two doses of 10 µg showed a tendency to have higher levels of proteins than control specimens (Fig. 5).

DISCUSSION

The first conclusion that can be stated concerning JH production in adult male *B. germanica* is that the hormonal release rates are remarkably low. The maximal rates (*ca* 0.5 pmol/hr and CA pair) are clearly lower than those measured in females within the first gonadotrophic cycle (up to 3 pmol/hr and CA pair, Bellés *et al.*, 1987). This kind of sexual dimorphism has also been observed in *D. punctata*, although in this species the differences between the two sexes are much more remarkable (a ratio of *ca* 1:10, comparing JH biosynthesis in males and females; Tobe *et al.*, 1979). In *P. americana*, however, it seems that both males and females produce similar levels of JH (Weaver *et al.*, 1990). Furthermore, on the basis of histophysiological and biometrical studies, it has been observed that in *L. maderae* (Scharrer and von Harnack, 1958), *Blabera fusca* (Brousse-Gaury and Cassier, 1975) and *Blatta orientalis* (García-Alonso, Piulachs and Bellés; unpublished results), CA activity or CA volume is higher in females than in males.

Considering the phylogenetic relationships between the Blaberidae *D. punctata* (a highly apomorphic viviparous species), the Blattellidae *B. germanica* (a moderately apomorphic pseudoviviparous species) and the Blattidae *P. americana* (a plesiomorphic oviparous species) (see McKittrick, 1964), it appears that the differences in JH production between the two sexes increase with increasing levels of modification in their morphology and reproductive biology.

In addition, the relationships between both members of a CA pair shows quite a high degree of asymmetry (Fig. 3), which is also usual in females of the same species (Bellés and Piulachs, 1983). Conversely, in *D. punctata* the two CA of the same specimen seems much more symmetrical: the 50% of the CA pairs studied by Szibbo and Tobe (1981) fell into the ratio (smaller/larger) of 0.9–1.0. Similarly to JH release, the CA volume in males of *B. germanica* never reaches the maximal values observed in females. Indeed, they are equivalent to that measured in pre-vitellogenic or post-vitellogenic (period of ootheca transport) females (Bellés and Piulachs, 1983), thus with the CA in a basal activity stage or almost inactive (Bellés *et al.*, 1987).

The growth profile of ARG observed in *B. germanica* adopts an almost logarithmic form, which is also the case in *P. americana* (Blaine and Dixon, 1973) and *D. punctata* (Tobe *et al.*, 1979). Furthermore,

in *B. germanica* the progressive increase in JH production observed in the first days of adult life parallels the increase in protein content in the ARG, which already suggests the hypothesis of a functional link between JH and ARG development. The results afforded by the experiments of allatectomy are also in agreement with this hypothesis, since after CA removal, accumulation of ARG secretions is significantly reduced in comparison with controls, and the administration of exogenous JH restores normal ARG development.

Scharrer and von Harnack (1958), on the basis of histophysiological investigations carried out in *L. maderae*, suggested that the weak and more or less continuous activity of the male CA could be related to the sustained control of metabolic events. In addition, numerous studies attest to the fact that allatectomy induces fat body hypertrophy (Engelmann, 1970), which suggests that JH is involved in a mobilization of reserves.

Therefore, it is reasonable to hypothesize that JH may have a direct (e.g. by stimulating synthesis of proteins in the ARG) or indirect (e.g. by stimulating precursor synthesis in the fat body) influence on protein accumulation in the ARG of *B. germanica*. This hypothesis could be extended to *P. americana*, since this species also seems to show a partial dependence on intact CA for synthesis of ARG secretion (Blaine and Dixon, 1973).

In contrast, in *L. maderae* and *D. punctata* (Scharrer, 1946 and Tobe *et al.*, 1979, respectively), the CA seem not to be strictly necessary for growth and functioning of ARG. However, and at least in the latter species, in which quantitative measurements of ARG growth were made, slight but statistically significant differences were observed between sham-operated and allatectomized specimens.

In summary, the differences between species seem to be quantitative rather than qualitative, and are more likely to be species-specific. Data available on the protein composition of ARG secretions suggest a very marked species-specificity (Chen, 1984). Then, the differences observed on the effect of allatectomy in the cockroaches mentioned above may be simply due to differences in biochemical properties and protein patterns of the ARG of these species.

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