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Fast induction of vitellogenin gene expression by juvenile hormone III in the cockroach *Blattella germanica* (L.) (Dictyoptera, Blattellidae)

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Abstract

The present paper describes the effect of juvenile hormone III (JH III) upon vitellogenin (Vg) gene expression in cardioallatectomized females of *Blattella germanica*. Northern blot analyses of time course studies showed that Vg mRNA can be detected 2 h after the treatment with 1 μg of JH III. Western blot analyses revealed that Vg protein is detectable 4 h after the same treatment. The study of the influence of the age showed that 48-h-old females seem more sensitive than 24-h-old females, whereas differences were less apparent between 48- and 72-h-old females. Dose–response studies indicated that 0.01 μg of JH III is ineffective, whereas the doses of 0.1, 1 and 10 μg induced the synthesis of Vg in a dose-dependent fashion. Finally, the administration of three successive doses, of 0.01 μg of JH III each, did not result in detectable Vg production, whereas two doses of 0.01 μg followed by one of 1μg of JH III induced a greater response than that resulting from a sole dose of 1μg of JH III, which suggests that sub-effective doses of JH III elicit a priming effect on Vg production.

Keywords: Vitellogenin; Juvenile hormone; German cockroach; *Blattella germanica*

1. Introduction

In 1983, Engelmann identified three chronological steps in the progression of the study of vitellogenesis controlled by juvenile hormone (JH): (1) studies on the hormone source, (2) studies on the process of vitellogenesis, and (3) studies on the action of JH upon vitellogenin (Vg) production at a molecular level. As predicted by Engelmann (1983), the emphasis is now placed on the latter aspect. Indeed, the study of the mode of action of JH on Vg production may be useful not only to shed light on the evolution of reproductive strategies in insects (see Bellés, 1998), but also to better understand the phenomenon of metamorphosis (the stimulating concept of 'minimal model of metamorphosis' of Kunkel, 1981).

Up to now, many contributions have described the action of JH or JH analogs upon Vg production in terms of protein, especially in locusts and cockroaches (see Wyatt and Davey, 1996), but also in lepidopterans (Cusson et al., 1994) and heteropterans (Ibáñez et al., 1987; Wang and Davey, 1993; Shinoda et al., 1996). In addition, methods to measure Vg mRNA or Vg transcription rates have extended the focus to a molecular level, especially in the locust *Locusta migratoria* (Dhadialla et al., 1987; Edwards et al., 1993; Glinka and Wyatt, 1996).

Different cues suggest that the German cockroach *Blattella germanica* (L.) (Dictyoptera, Blattellidae) could be a good candidate to study the action of JH on Vg synthesis. Early studies reported by Kunkel (1973) demonstrated that *B. germanica* is highly sensitive to the gonadotrophic effects of JH. Later, our own contributions on this species have shown that vitellogenesis and oocyte growth are JH-dependent processes (Piulachs, 1988), that JH III is the only JH homolog in the adult female (Camps et al., 1987), and that the cycle of JH III production during ovarian maturation (Bellés et al., 1987) is paralleled by ultrastructural changes in

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the corpora allata (CA) (Piulachs et al., 1989). The cycle of Vg production, in terms of protein (Kunkel, 1981; Martín et al., 1995) and Vg mRNA (Martín et al., 1998), and the production of Vg in ovariectomized females (Kunkel, 1981; Martín et al., 1996a), have also been described. Furthermore, studies on the processing of provitellogenin in the fat body (Kunkel and Nordin, 1985; Wojchowski et al., 1986), on the involvement of the mevalonate pathway in the glycosylation and export of Vg from the fat body (Martín et al., 1996b) and methods to study vitellogenesis in vitro, (Martín et al., 1996c) are also available.

The present contribution takes advantage of all this background and uses cardioallatectomized females of *B. germanica* to study the induction of Vg gene expression by exogenous JH III through different experimental combinations.

2. Materials and methods

2.1. Insects

Adults of *B. germanica* were obtained from a colony fed on Panlab dog chow and water, and reared in complete darkness at 30±1°C and 60–70% r.h. Freshly ecdysed virgin females were isolated and used at the appropriate physiological ages, which were assessed by measuring the basal oocyte length (Bellés et al., 1987). All dissections and treatments were carried out on specimens anesthetized with CO_2.

2.2. Cardioallatectomy and juvenile hormone III treatments

Females were cardioallatectomized within 4 h after the imaginal ecdysis, by removing the cervical sclerite and dissecting out the corpora cardiaca (CC)–CA complex. Sham operations were carried out as cardioallatectomies but without removing the CC–CA complex. For replacement experiments we used JH III, which is the native JH of the adult female of *B. germanica* (Camps et al., 1987). JH III (Sigma) was dissolved in acetone (Merck) and administered in a volume of 1 μl, which was applied topically on the dorsal part of the abdomen, after cutting off the wings. Cardioallatectomized and sham operated specimens used as controls of JH III treatments were treated with 1 μl of acetone alone.

2.3. Preparation of samples for protein quantification and Western blot analysis

Abdominal fat body samples were obtained according to Martín et al. (1995). The gut, the accessory glands and the ovaries were removed, and the open abdomen was thoroughly rinsed with Ringer saline to eliminate hemolymph Vg. Then, fat body tissue was carefully dissected out, homogenized with a nylon pestle in a buffer composed of 100 mM saccharose, 40 mM K_2HPO_4, 30 mM EDTA, 50 mM KCl (pH 7.2), supplemented with 0.5 mM PMSF, 0.25% Triton X-100 and 10 mM DTT, and centrifuged for 20 min at 10,000***g***. The supernatant was collected and stored at −20°C. Fat body soluble proteins were quantified using bovine serum albumin as standard (Bradford, 1976). The Vg-vitellin antibody of *B. germanica* and the electrophoresis (SDS–PAGE) of Vg have been described in Martín et al. (1995).

2.4. RNA isolation and Northern blot analysis

Abdominal fat bodies were dissected and processed for total RNA following the phenol/chloroform method described by Chomczynski and Sacchi (1987). Total RNA (10 μg) was subjected to electrophoresis in 1.5% agarose gel containing formaldehyde and then transferred to nylon membranes (Hybond-N^+, Amersham). A partial 2,645 nucleotide sequence of the Vg cDNA of *B. germanica* (clone 16A reported by Martín et al., 1998) was used as a probe. Labeling and detection procedures were as described by Martín et al. (1998).

3. Results

3.1. Time course of vitellogenin expression

In order to determine the minimum time required for Vg transcription and translation after treatment with JH III, a time course study was carried out. For this purpose we used 48-h-old cardioallatectomized females, which were topically treated with 1 μg of JH III. Previous studies (Kunkel, 1981; see also Section 3.3) had shown that this dose elicits clear cut effects on Vg production, thus facilitating the comparison of different experimental situations. Then, the abdominal fat body of treated specimens was dissected out 2, 4, 6 and 8 h later, and Vg analysis, in terms of mRNA (Northern blot) and protein (Western blot), was performed. According to Northern blot analysis (Fig. 1), transcripts clearly appeared 4 h after the treatment, but, when the film was overexposed, they were detected as early as 2 h after the treatment. As expected, the amount of Vg mRNA increases in parallel with the time elapsed between the treatment and the analysis. Western blot analysis (Fig. 2) showed that the first signs of Vg protein did not appear until 4 h after JH III treatment. No Vg was detected when the fat bodies were dissected out 2 h after the treatment, even after extended autoradiographic exposure of the films. As in the case of mRNA, the amount of Vg protein observed increased in parallel with the time elapsed between the treatment and the analysis. Neither Vg mRNA nor Vg protein was detected in cardioallatectomized specimens

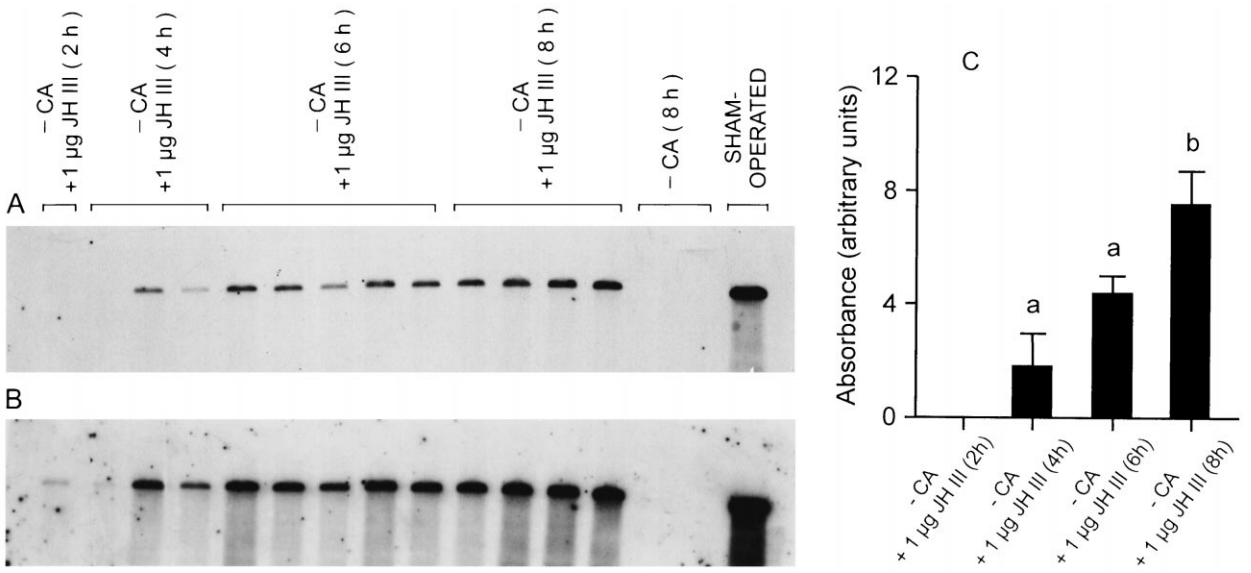

Fig. 1. (A) Northern blot of the time-course experiments of Vg expression. A dose of 1 µg of JH III was topically applied to 48-h-old cardioallatectomized (-CA) females, and the fat body was dissected out 2, 4, 6 or 8 h later. An aliquot of 10 µg of total fat body RNA was loaded per lane. (B) Overexposure of film A, in order to show that Vg mRNA can be detected 2 h after the treatment. (C) Densitometry of the bands corresponding to treated specimens in film A. Results are expressed as the mean±SEM (replicates are those in A), and different letters above the columns indicate significant differences (*t*-test, $P<0.05$).

treated with acetone alone (Figs. 1 and 2) even after extended overexposure of the films (not shown). Furthermore, Vg mRNA and protein levels in sham operated females (Figs. 1 and 2) were similar to those of intact females at the age studied (not shown).

3.2. Influence of the age

After studying the effect of time on the response of the Vg gene to JH III, we examined the influence of age on this response. For this purpose, we topically applied a dose of 1 µg of JH III to 24-, 48- or 72-h-old cardioallatectomized females, and the respective fat bodies were dissected out and analyzed by Western blot 8 h after the treatment. Results (Fig. 3) showed that, although the differences found in the three ages studied were not statistically significant, 48-h-old females appeared to be more sensitive than 24-h-old females, whereas differences were less apparent between 48- and 72-h-old females. No Vg was detected in fat bodies from cardioallatectomized specimens treated with acetone alone at any of the three ages investigated.

3.3. Effects of different doses of juvenile hormone III

The next step was to study the relationships between the dose of JH III used and the effects induced on Vg production. For these experiments we used 48-h-old cardioallatectomized females, which were topically treated with 0.01, 0.1, 1 or 10 µg of JH III. Then, the fat body was dissected out 8 h later, and Vg protein was determined by Western blot analysis. Results (Fig. 4) indicated that no Vg production occurred either in cardioallatectomized specimens treated with acetone or in those treated with 0.01 µg of JH III. No single trace of Vg was observed in the lanes corresponding to these specimens, even after overexposure of the films (not shown). However, the doses of 0.1, 1 and 10 µg of JH III induced the synthesis of Vg in a dose-dependent fashion, although the differences between results from 1 and 10 µg treatments were not statistically significant.

3.4. Priming effect of juvenile hormone III

Finally, we examined whether low, sub-effective doses of JH III could elicit a 'priming' effect on Vg production. For this purpose, we used 48-h-old cardioallatectomized females, which were treated with three doses of 0.01 µg of JH III each, topically applied at 2 h intervals. Then, the fat body was dissected out 8 h after the last hormonal administration, and Vg protein was determined by Western blot analysis. Results (Fig. 5) showed that no Vg production was detected in these specimens, as occurred in the control cardioallatectomized specimens equivalently treated three times with acetone alone. As expected, the specimens treated the first two times with acetone and the third time with 1 µg of JH III produced detectable amounts of Vg. However, the amounts of Vg produced were significantly higher in specimens treated the first two times with 0.01 µg of JH III each, and the third time with 1 µg of JH III. The lower amounts of Vg measured in these experiments compared with the previous ones (cf Figs. 5(A) and 4(A), for example) may be attributed to the disturbing

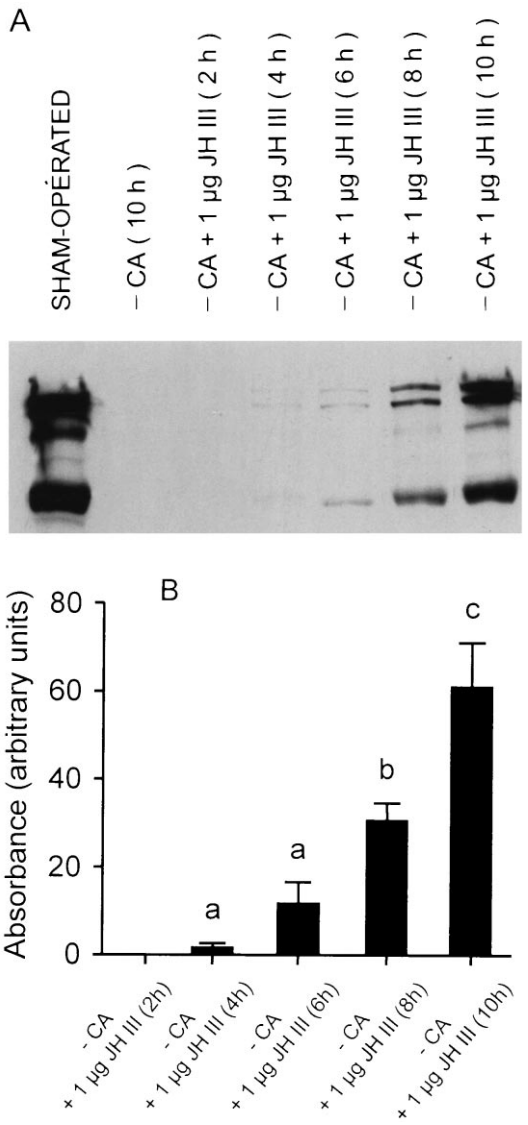

Fig. 2. (A) A representative Western blot of the time-course experiments of Vg production. A dose of 1 µg of JH III was topically applied to 48-h-old cardioallatectomized (-CA) females, and the fat body was dissected out 2, 4, 6, 8 or 10 h later. An aliquot of 80 µg of total fat body proteins was loaded per lane. (B) Densitometry of the bands corresponding to treated specimens in the blots studied. Results are expressed as the mean±SEM (n=3–4), and different letters above the columns indicate significant differences (t-test, $P<0.05$).

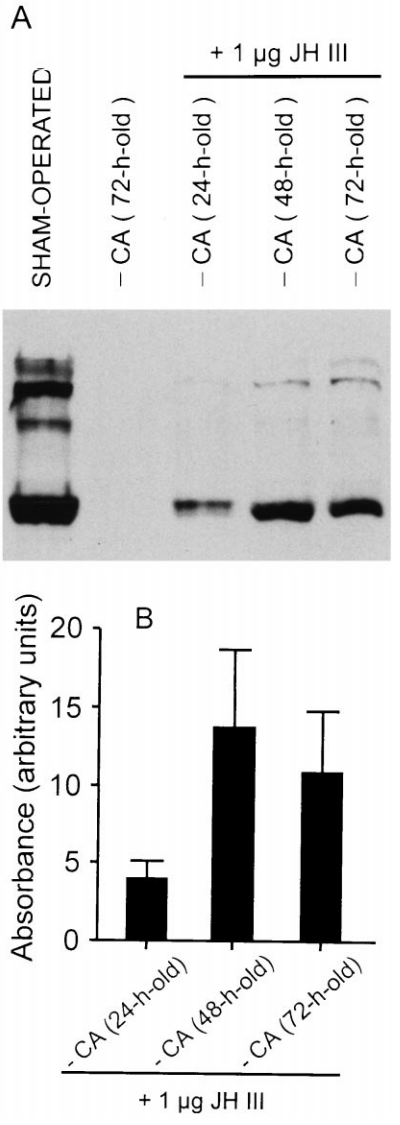

Fig. 3. (A) A representative Western blot of the experiments on the influence of age on Vg production. A dose of 1 µg of JH III was topically applied to 24-, 48- or 72-h-old cardioallatectomized (-CA) females, and the fat body was dissected out 8 h later. An aliquot of 80 µg of total fat body proteins was loaded per lane. (B) Densitometry of the bands corresponding to treated specimens in the blots studied. Results are expressed as the mean±SEM (n=3–4). No significant differences were found between the three ages studied (t-test, $P<0.05$).

effects of a triple treatment involving CO_2 anesthesia and acetone administration.

4. Discussion

A number of previous reports (for example, Don-Wheeler and Engelmann 1991, 1997 in the cockroach *Leucophaea maderae*; Chen et al., 1979; Chinzei and Wyatt, 1985 in the locust *L. migratoria*; Wang and Davey, 1993 in the blood-sucking bug *Rhodnius prolixus*; Cusson et al., 1994 in the armyworm moth *Pseudaletia unipuncta*; Shinoda et al., 1996 in the bean bug *Riptortus clavatus*) have shown that the induction of Vg production by JH or JH analog administered to specimens that have been made JH-deficient (by allatectomy or by treatment with allatocide compounds) seems to be delayed at least 24 h (Wyatt and Davey, 1996). In *L. migratoria*, experiments measuring Vg mRNA or Vg transcription rates (Dhadialla et al., 1987; Edwards et al., 1993; Glinka and Wyatt, 1996), have shown that the lag phase of ca 24 h represents a delay in the initiation of Vg transcription itself. This remarkably long lag time contrasts with the fast induction of Vg transcription that

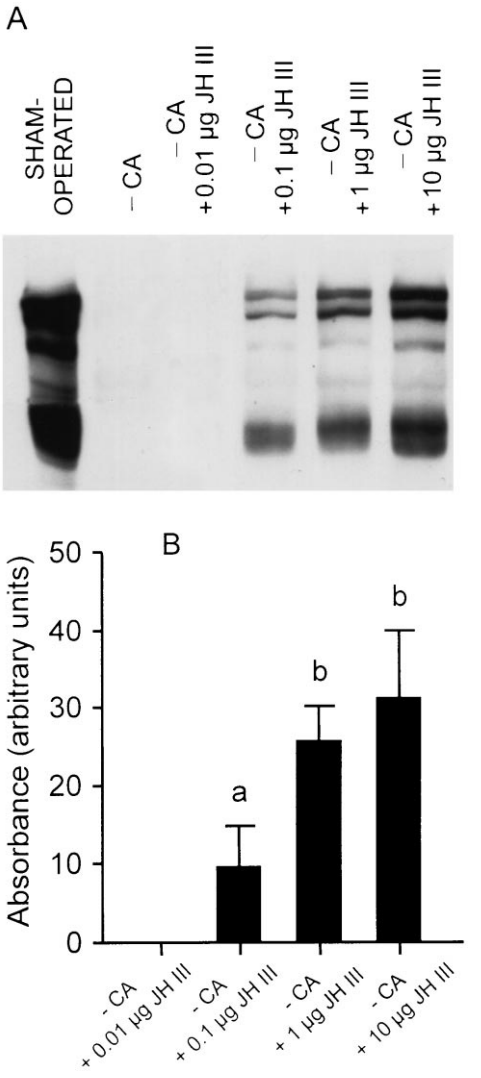

Fig. 4. (A) A representative Western blot of the dose–response experiments of Vg production. A dose of 0.01, 0.1, 1 or 10 µg of JH III was topically applied to 48-h-old cardioallatectomized (-CA) females, and the fat body was dissected out 8 h later. An aliquot of 80 µg of total fat body proteins was loaded per lane. (B) Densitometry of the bands corresponding to treated specimens in the blots studied. Results are expressed as the mean±SEM (n=5–6), and different letters above the columns indicate significant differences (t-test, $P<0.05$).

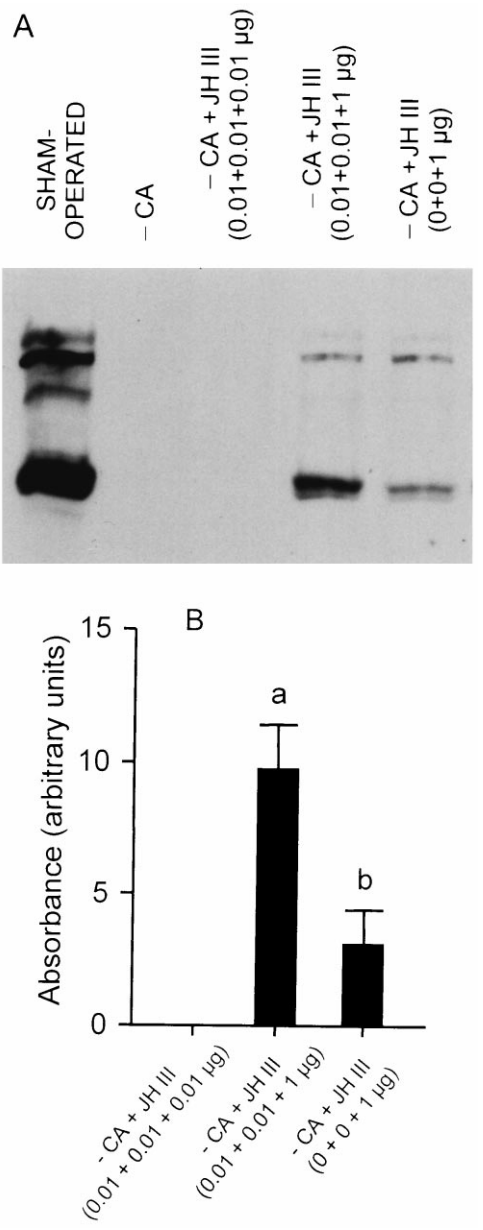

Fig. 5. (A) A representative Western blot of the priming effect experiments on Vg production. Three successive treatments at 2 h intervals were topically applied to 48-h-old cardioallatectomized (-CA) females, and the fat body was dissected out 8 h after the last administration. Treatments were 0.01 µg JH III×3; 0.01 µg JH III×2+1 µg JH III; and 0 (acetone alone) µg JH III×2+1 µg JH III. An aliquot of 80 µg of total fat body proteins was loaded per lane. (B) Densitometry of the bands corresponding to treated specimens in the blots studied. Results are expressed as the mean ± SEM (n=4–5), and different letters above the columns indicate significant differences (t-test, $P<0.05$).

we have observed in *B. germanica*, where Vg mRNA can be detected as early as 2 h after the administration of JH III to cardioallatectomized females.

Interestingly, the 4 h lag observed in our experiments measuring Vg protein is relatively close to the 6 h lag described in ootheca-transporting females, or to the 16 h lag found in last-instar female nymphs (Kunkel, 1981), after injecting 1 µg of JH III. In comparison with our experiments, the clearly longer lag observed in nymphs could be related to phenomena of fat body priming and capacitation (see below).

The study on the influence of age indicated that the effects of the same dose of JH III (1 µg) are more effective in 48- or 72-h-old females than in 24-h-old females, in all cases cardioallatectomized within the first 5 h of adult life. A number of authors, working on different insects (della-Cioppa and Engelmann, 1980, in *L. maderae*; Couble et al., 1979; Nair et al., 1981 in *L. migratoria*; Raikhel and Lea, 1990 in the yellow fever mosquito *Aedes aegypti*), have reported that fat body cells increase in ploidy and undergo a remarkable pro-

liferation of the rough endoplasmic reticulum after the adult molt, and that these processes, in addition to being a prerequisite for Vg synthesis, are JH-dependent. In our case, however, it seems that some improvement of the biosynthetic capabilities of the fat body occurs in the first 48 h of adult life in the virtual absence of JH. The fact that females were cardioallatectomized within the first 5 h after the imaginal molt leave open the possibility that some amount of JH III may have been released to the hemolymph before dissecting out the CA. However, this amount should be below 0.5 pmol, given that the average rate of JH III synthesis in a freshly ecdysed specimen is 0.08 pmol of JH III per h and per pair of CA (Maestro et al., 1994), which do not appear to be sufficient to elicit a priming effect (in the sense of Wyatt and Davey, 1996) in the fat body cells.

Concerning the dose–response relationships, doses of 0.1, 1 and 10 μg of JH III induced the synthesis of Vg in a dose-dependent manner, and a dose as low as 0.1 μg of JH III was enough to induce clearly detectable amounts of Vg. This dose is in the same order of magnitude as those reported by Kunkel in 1973 (0.06 μg of JH III and 0.08 μg of JH I) as the minimal doses necessary to elicit a gonadotrophic response in *B. germanica*. Doses in the same order of magnitude (between 0.05 and 1 μg) were sufficient to induce Vg production in CA-deprived females of *R. prolixus* (Wang and Davey, 1993) or *P. unipuncta* (Cusson et al., 1994), or in diapausing (JH defficient) females of *R. clavatus* (Shinoda et al., 1996). These results contrast with the low sensitivity of *L. migratoria*, in which doses up to 100 μg of JH III gave no detectable Vg production in CA-deprived females, thus leading the authors (Wyatt et al., 1996) to use lower doses (10 μg) of pyriproxyfen, a potent JH analog. In *L. maderae*, Don-Wheeler and Engelmann (1991, 1997) tested doses between 0.5 and 400 μg of methoprene, another potent JH analog, to study Vg production not only in females, but also in males and last-instar female nymphs, where the highest doses had to be used.

Finally, our experiments using sub-effective doses of JH III show that the administration of three successive doses, of 0.01 μg of JH III each, did not result in any detectable Vg production, whereas two doses of 0.01 μg followed by one of 1μg of JH III induced a response which was greater than that resulting from a sole dose of 1μg of JH III. This effect can be defined as priming, in the sense of Wyatt and Davey (1996) (see also Chinzei and Wyatt, 1985; Wyatt et al., 1996), in which previous exposure of a target tissue to a sub-effective dose of an effector hormone enhances the effect of a subsequent exposure to the same hormone. Vitellogenic tissues and hormones afford good examples of this, and have led to the provocative concept of 'vitellogenin gene memory' (Edinger et al., 1997). The provocation, however, is terminological rather than conceptual, given that the 'memory' of the Vg gene may be simply explained under the hypothesis that priming would involve the synthesis and storage of transcription factors for Vg that could then facilitate Vg transcription in successive esposures to the hormone (see also Wyatt et al., 1996). In any case, it is still an open issue, and transcription of Vg following the action of JH III in the fat body of *B. germanica* may be a suitable system for testing the above hypothesis.

Acknowledgements

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