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Autoinhibition of juvenile hormone production. The case of the cockroach *Blattella germanica* (L.)

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Abstract. In 6-day-old females of *Blattella germanica*, the activity of corpora allata (CA) was inhibited in vitro by juvenile hormone III (JH III). Effective doses (281.5 and 375.4 μM in the medium) were somewhat higher than (although of the same order of magnitude as) the estimated intraglandular concentration of JH III at this age, and they induced about 45% inhibition of hormonal release and a significant intraglandular accumulation of JH III and methyl farnesoate. The results suggest that autoinhibitory mechanisms operate in the CA to constrain the upper limit of JH III production at the end of the gonadotrophic cycle.
Key words. Juvenile hormone; *Blattella germanica*; methyl farnesoate; autoinhibition.

Oocyte growth in the cockroach *Blattella germanica*, as in many insects, is dependent on the production of juvenile hormone (JH) by the corpora allata (CA)[1,2]. As shown by in vitro radiochemical assay on CA from either virgin[3] or mated[4] females of this species, the profile of JH release parallels that of basal oocyte growth until oviposition. Then, during the period of ootheca transport, the CA are almost inactive and the next batch of basal oocytes remain at the previtellogenic stage.

The cyclic activity of CA suggests the mediation of precise regulatory mechanisms and, in this context, peptidic allatostatins have been identified from brain extracts of the cockroach *Diploptera punctata*[5-7]. Allatostatic factors have also been shown in *B. germanica*[8], and their identification is currently in progress in our laboratory.

In addition, regulatory feedback mechanisms involving a direct effect of JH on CA activity have also been suggested[2,9]. However, the demonstration of such mechanisms by testing in vitro seems to be elusive, since the rather high concentrations of the corresponding JH homologue(s) used in the experiments have generally been regarded as unphysiological. For example, in *Periplaneta americana*, doses up to 1 mM did not affect CA activity[10], and in *Leptinotarsa decemlineata*, concentrations up to 0.02 mM were equally ineffective[11]. Inhibitory effects were obtained on CA from *Manduca sexta* at a dose of 0.1 mM for either JH I, JH II or JH III[12], and on ring glands of *Drosophila melanogaster* at a concentration of 1 mM of JH bisepoxide (JHB_3), which caused a decrease of 'total JH production', i.e. methyl farnesoate (MF) + JH III + JHB_3[13]. Since levels of JH in the haemolymph are usually lower than the concentrations tested experimentally, these results may not be applicable to physiological conditions if they are considered in relation to the hypothesis of the occurrence of short-loop feedback mechanisms[14], by which circulating JH would inhibit CA activity.

An alternative approach is to investigate whether autoinhibitory feedback mechanisms[14] could operate inside the CA. We examined this possibility using virgin adult females of *B. germanica*, which produce JH III as the only JH homologue[15]. We incubated the CA in a medium containing JH III at a concentration somewhat higher than that estimated to occur inside untreated glands (see below) at the time when hormonal release rates are at their highest, i.e. on day 6 of imaginal life, just before oviposition[3,16,17]. The aim was to mimic in a physiological fashion the critical intraglandular concentration of JH III that would elicit the autoinhibitory response.

Materials and methods

Adults of *B. germanica* were reared in the dark at 30 ± 1 °C and 60–70% relative humidity. Freshly ecdysed virgin females were isolated from males and used when 6 days old. The physiological age was additionally assessed by measuring the basal oocyte length. Isolated CA pairs 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. For the treatments, synthetic JH III (Sigma) was directly added to the labelled medium which was then sonicated. Osmolality, both of fresh medium and of medium with JH III added, was between 333 and 335 mosmol. (measured with a Micro-Osmometer 3MO, Advanced Instruments, Inc.). To study the influence of dose, glands were incubated for 3 h in treated medium, and then JH III release was determined. Studies of reversibility were carried out by incubating the CA in a medium containing JH III (at a

concentration of 375.4 μM) for 3 h, and then the glands were transferred to fresh medium where the incubation was continued for 3 h. The JH III release was determined in the corresponding treatment and post-treatment incubation periods. Dissection and transfer of glands to the incubation medium, measurements of basal oocytes, and extraction and determination of de novo-synthesized JH III released to the medium, were performed as described elsewhere[18]. Quantification of intraglandular JH III and MF was as previously reported[16,17], with slight modifications.

To estimate the theoretical intraglandular concentration of JH III, CA volumes were determined using a digital system of image processing 'Microm Image processing' (Microm, Barcelona), on the basis of the formula $V = 4/3\pi abc$, where a, b and c are the radii of the three principal axes of the gland.

Results

The effects of JH III on CA activity are summarized in figure 1. As shown, JH III had no significant effect on hormonal release at concentrations of 93.8 and 187.7 μM (i.e., 25 and 50 μg/ml), whereas it elicited around 45% inhibition at concentrations of 281.5 and 375.4 μM (i.e., 75 and 100 μg/ml). Lower concentrations (0.38, 3.75 and 37.5 μM, not shown) were ineffective. The highest concentration tested (3753.8 μM) practically abolished CA activity, but this treatment caused irreversible damage to the glands, which were no longer functional when transferred to fresh medium (not shown), whereas CA treated with 375.4 μM recovered normal levels of activity when incubated again in JH III-deprived medium (fig. 2).

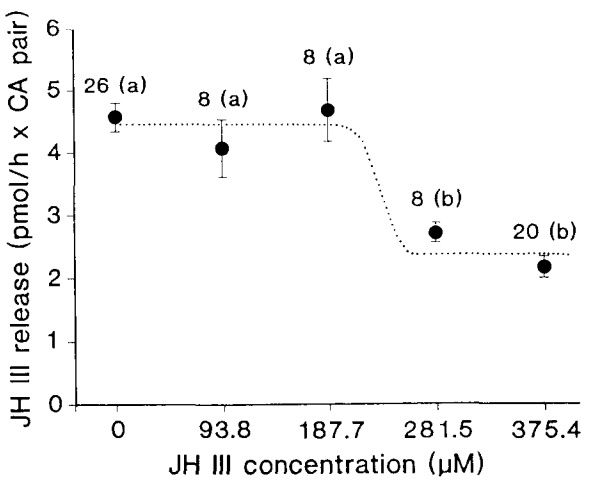

Figure 1. Dose response of juvenile hormone III (JH III) in hormonal release by corpora allata (CA) from 6-day-old virgin females of *Blattella germanica*. Each point represents the mean ± SEM. The number of determinations is indicated at each point. Different letters (a, b) indicate significant differences (Student's t-test) at $p < 0.005$, except in the case of concentration 93.8 μM with respect to that of 281.5 μM, which was $p = 0.02$.

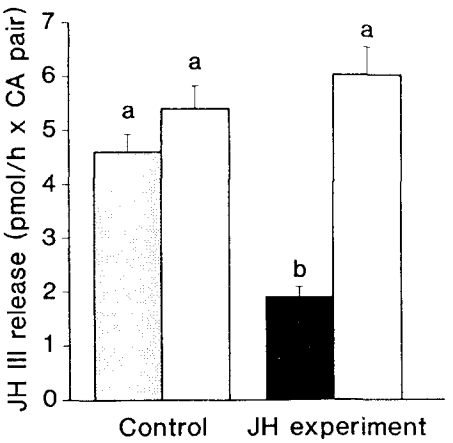

Figure 2. Reversibility of the inhibitory effects of 375.4 μM of juvenile hormone III (JH III) on hormonal release by corpora allata (CA) from 6-day-old virgin females of *Blattella germanica*. Shaded (controls) or black (JH-treated) columns represent the 3-h treatment period. White columns represent the corresponding 3-h post-treatment incubation in fresh medium. Columns represent the mean and vertical bars the SEM. The number of determinations were $n = 10$ and $n = 11$ for control and JH experiment respectively. Different letters (a, b) indicate significant differences (Student's t-test, $p < 0.0001$).

In addition, the intraglandular contents of JH III and its immediate precursor, MF, were measured in CA incubated with 375.4 μM JH III. Results (table) indicate that the levels of both JH III and MF were significantly higher in treated CA than in control glands. Relative accumulation was much more apparent in MF than in JH III, although JH III contents were also abnormally high, especially considering the low release rates in these treated CA. However, if intraglandular contents of JH III plus MF are added to absolute JH III release in the 3-h period of incubation to give 'total synthesis of JH III and MF' (table), the values for

Intraglandular contents of juvenile hormone III (JH III) and methyl farnesoate (MF), and related parameters, in corpora allata pairs of 6-day-old females of *Blattella germanica* incubated for 3 h in a normal medium (controls) or in a medium containing 375.4 μM of JH III (treated). Values (mean ± SEM) are expressed in pmol. Significant differences (Student's t-test) between treated and controls for each parameter are indicated as follows: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

	Controls (n = 12)	Treated (n = 11)
Total release of JH III in 3 h	17.22 ± 1.41	11.20 ± 1.20**
Intraglandular contents of JH III	0.176 ± 0.047	0.324 ± 0.041*
Intraglandular contents of MF	0.026 ± 0.008	0.219 ± 0.046***
Total synthesis of JH III (contents + release in 3 h)	17.40 ± 1.44	11.52 ± 1.22**
Total synthesis of JH III and MF (contents + release in 3 h)	17.42 ± 1.45	11.74 ± 1.24**

treated CA are still significantly lower than those in control glands. Thus the low release rates measured in treated CA cannot be explained simply as an inhibition of MF epoxidation plus an impairment of hormonal release.

Discussion

The results show that the biosynthetic activity of CA from *B. germanica* is inhibited by JH III, their own end-product. About 45% inhibition was induced by incubating the CA in a medium containing 281.5 or 375.4 μM of JH III, whereas the concentration of 187.7 μM was ineffective (fig. 1). In addition, intraglandular accumulation of MF and JH III was observed in CA incubated with 375.4 μM of JH III (table).

The haemolymph concentration of JH III in 6-day-old virgin females of *B. germanica* is around 8 ng/ml[15] (i.e., 0.03 μM), which is much lower than the concentrations used in the experiments described here. Therefore, the results do not seem to support the hypothesis that CA is inhibited by circulating JH III.

Alternately, if we consider the possibility that autoinhibitory feedback mechanisms operate inside the CA (even inside the allatal cells or in particular subcellular compartments), then we can measure the volume of a CA pair in control 6-day-old females (2.83 ± 0.19 nl, $n = 10$), and their JH III content (0.176 ± 0.047 pmol, see table), and estimate the theoretical intraglandular concentration of JH III. In this case the estimated concentration is ca. 60 μM, which is relatively close to those used in the experiments, and even higher concentrations could be expected in particular gland compartments.

The concentration of JH III in treated glands is more difficult to estimate, since we do not know the amounts of exogenous JH III which penetrated the CA from the medium. In any case, the intraglandular concentration of JH III synthesized de novo in these treated glands was approximately 100 μM (JH III contents: 0.324 ± 0.04 pmol, see table). This concentration is of the same order of magnitude as that used in the experiment (375.4 μM), and close to the threshold concentration of exogenous JH III required to inhibit CA biosynthetic activity (between 187.7 and 281.5 μM; fig. 1).

Taking the above considerations into account, our results can be interpreted as follows: 1) The incubation of CA from 6-day-old females of *B. germanica* in a medium containing JH III at a concentration some 3-fold higher than that occurring inside untreated glands partially impairs hormonal release, resulting in abnormally high levels of JH III inside the CA (endogenous hormone which accumulated plus possible exogenous JH III, which was not measured). 2) Intraglandular accumulation of MF in these CA suggests that JH III may inhibit the epoxidation of this late precursor. 3) Since total sythesis of JH III and MF are still lower in treated CA than in controls, (intraglandular) JH III or MF (or both) may additionally inhibit early steps of the biosynthetic pathway.

In untreated CA, intraglandular JH III contents correlate with hormonal release rates[9,17,18]. Thus, in physiological conditions, intraglandular autoinhibition could operate when JH III release rates are the highest. In *B. germanica* the highest rates of JH III release occur on day 6, just before the end of the gonadotrophic cycle[3]. It is plausible that this inhibitory mechanism would determine the upper limit of JH III production at the end of the gonadotrophic cycle, just before the allatostatic events which lead to almost total inactivation of CA during the period of ootheca transport.

The critical intraglandular concentration required to elicit the autoinhibitory response may depend on the species-specific maximal rates of JH production (and, thus, maximal JH contents in the CA) reached by a given species. Thus, species with higher rates of synthesis would need higher critical intraglandular concentrations to give that response. This approach could help to elucidate the physiological meaning of the apparently unphysiological or negative results obtained by other authors on other species (see introduction).

To conclude, regulatory mechanisms of JH production could be comparable in complexity to those found in similar systems in vertebrates. The case of control of cholesterol synthesis by multivalent feedback regulation of HMG CoA reductase[19,20] is especially suggestive in this regard..

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