IN VITRO INHIBITION OF JUVENILE HORMONE III BIOSYNTHESIS BY PRECOCENE II AND 3,4-DIHYDROPRECOCENE II ON BLATTELLA GERMANICA

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Abstract—In vitro incubations of corpora allata from 6-day old females of B. germanica with precocene II showed that the glands are intrinsically sensitive to the action of this allatocidin. However, irreversible cytotoxic effects on corpora allata were only obtained after 9 h of incubation at 10^-3 M. Measurement of intraglandular juvenile hormone III and methyl farnesoate contents of treated and control corpora allata showed a depletion of juvenile hormone III contents which was parallel to the inhibition of juvenile hormone release. Conversely, methyl farnesoate levels of treated glands were not different from those found in controls which suggested that a specific inhibition of the epoxidase did occur in addition to the general inactivation of the glands. Finally, results from incubations with 3,4-dihydroprecocene II showed that this compound is also a juvenile hormone inhibitor and the effects elicited are comparable with those observed for precocene II.

Key Word Index: Blatterlla germanica, Juvenile Hormone III, corpora allata, precocenes

INTRODUCTION

In a preceding paper (Bellés et al., 1985) we reported the antigonadotropic effects of topically applied precocenes on freshly ecdysed virgin females of Blattella germanica. Our results showed that these compounds inhibited oöyte growth and colleterial gland development, although these effects appeared to be reversible in most cases.

In order to assess the intrinsic sensitivity of the corpora allata of B. germanica to precocenes, we have studied the inhibitory activity of precocene II on the in vitro juvenile hormone III release using the radiochemical assay developed by Pratt and Tobe (1974) and further adapted to the case of B. germanica by Bellés et al. (1987). For this purpose, we used an experimental procedure comprising a variable period of incubation in the presence of the test compound, which is preceded and followed by fixed periods of incubation in fresh medium. This method allows the study of the inhibition of juvenile hormone release and the degree of reversibility of such effect in individual assays.

In addition, complementary experiments using 3,4-dihydroprecocene II are also reported. Although this derivative lacks the double bond which has been claimed to be crucial for precocene activity (Pratt et al., 1980; Soderlund et al., 1980), it also induced in vivo antigonadotropic effects on B. germanica (Bellés and Messeguer, 1981).

Finally, with the aim of clarifying the relationship between biosynthesis and release of juvenile hormone (see Feyereisen, 1985) for this species within our experimental conditions, juvenile hormone III and methyl farnesoate contents of control and precocene II-treated corpora allata have also been measured.

MATERIALS AND METHODS

Insects

Adults of B. germanica were reared at 26(+1)°C as described elsewhere (Bellés and Piulachs, 1983). Freshly ecdysed virgin females isolated from the colony were used at appropriate ages which were rigorously determined using the basal oöyte length as the main criterion (Bellés et al., 1987).

Compounds and analytical techniques

Chemicals and solvents were all of analytical grade and used as received. All glassware was coated with 1% of 201 silicone emulsion (Siliconas Hispania, Barcelona). Juvenile hormone III was obtained from Sigma. Methyl farnesoate, precocene II, 3,4-dihydroprecocene II were prepared in our laboratory following previously reported procedures (Liedtke and Djerassi, 1972; Camps et al., 1980 and Camps et al., 1979, respectively). [lo-'H(N)]-juvenile hormone III (11 Ci/mmol) was obtained from New England Nuclear and [methyl-3H]methionine (80-85 Ci/mmol) from Amersham.

Thin-layer chromatography analyses were performed on Merck precoated Silicagel plates (aluminum sheets, layer thickness 0.2 mm with concentrating zone). High-performance liquid chromatography (HPLC) analyses were carried out with a Waters system provided by two model 510 pumps, an automated gradient controller, a data model and

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a 481 u.v. detector. Radioactivity was measured with a LKB 1217 Rackbeta scintillation counter.

**In vitro incubation methods**

In all experiments glands from 6-day old females were used. Corpora allata-corpora cardiaca complexes were incubated in Millipore-filtered TC-199 medium (0.2 ml) containing L-methionine (0.1 mM), glutamine, Hank's salts, HEPES medium buffer (20 mM) plus Ficoll (20 mg/ml) and to which radioactive methionine (2 μCi) was added to achieve a final sp. act. of 100 μCi/mmol. Dissection and transfer of the corpora allata-corpora cardiaca complexes to the incubation medium, measurement of basal oocytes for determination of the physiological age of each specimen, incubations and radiochemical assay for juvenile hormone III quantification were performed as previously described (Bellés et al., 1987). According to the designed experimental model, after an initial 3 h period of incubation, glands were transferred to fresh medium which contained a 0.1 μl ethanol solution of the test compound at the desired concentration and incubation was pursued for 3, 6 or 9 h. Finally a post-treatment incubation was carried out by transferring the glands to fresh medium. In all cases, control incubations with medium containing 0.1 μl of ethanol were performed.

**Quantification of juvenile hormone III and methyl farnesoate intraglandular contents**

Corpora allata-corpora cardiaca complexes from 6-day old females were used. Corpora allata-corpora cardiaca complexes were collected and radioactivity measured with the scintillation counter. The limit of detection for standard radioactive juvenile hormone III was estimated in 0.03 pmol.

**RESULTS**

**Effect of precocene II on the juvenile hormone III release**

The inhibitory effects of precocene II on the in vitro juvenile hormone III release are summarized in Table 1. Comparison of the values obtained at the three different incubation periods (3, 6 or 9 h) assayed with the same concentration of precocene II (10^{-3} M) indicates that the degree of inhibition depends on time of incubation. Nevertheless, the results of the post-treatment period showed that the glands incubated for 3 or 6 h with 10^{-3} M of precocene II were able to recover almost completely their normal biosynthetic capacity. Conversely, the inhibitory effects induced after 9 h of incubation appeared to be irreversible as inferred from the comparison of the release values measured in the 12-24 h period of this.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pmol HJ III/period × pair CA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>19.5 ± 1.44</td>
</tr>
<tr>
<td>0.05% Ethanol</td>
<td>19.5 ± 1.44</td>
</tr>
<tr>
<td>(3 x 3 h)</td>
<td>19.5 ± 1.44</td>
</tr>
<tr>
<td>10^{-3} M P II</td>
<td>19.5 ± 1.44</td>
</tr>
<tr>
<td>(3 x 3 h)</td>
<td>19.5 ± 1.44</td>
</tr>
<tr>
<td>10^{-3} M P II</td>
<td>19.5 ± 1.44</td>
</tr>
<tr>
<td>(2 x 3 h)</td>
<td>19.5 ± 1.44</td>
</tr>
<tr>
<td>10^{-3} M P II</td>
<td>19.5 ± 1.44</td>
</tr>
<tr>
<td>(3 x 3 h)</td>
<td>19.5 ± 1.44</td>
</tr>
<tr>
<td>10^{-3} M PII</td>
<td>19.5 ± 1.44</td>
</tr>
<tr>
<td>(3 x 3 h)</td>
<td>19.5 ± 1.44</td>
</tr>
<tr>
<td>10^{-3} M DHP II</td>
<td>19.5 ± 1.44</td>
</tr>
<tr>
<td>(3 x 3 h)</td>
<td>19.5 ± 1.44</td>
</tr>
<tr>
<td>10^{-3} M DHP II</td>
<td>19.5 ± 1.44</td>
</tr>
<tr>
<td>(3 x 3 h)</td>
<td>19.5 ± 1.44</td>
</tr>
</tbody>
</table>

Absolute values for each period are given as X ± SEM and those corresponding to the incubation periods with the test compound are indicated in boldface. Results of the t-test (from the comparison of the values resulting after the treatment with those obtained in the pre-treatment period of the same experiment) are summarized with the following subindexes: 0 (N.S.); 1 (P < 0.05); 2 (P < 0.01); 3 (P < 0.001). BO: Length (mm) of basal oocyte of the corpora allata donors.
Juvenile hormone inhibition by precocenes

precocene-treated corpora allata and intraglandular contents of juvenile hormone III are clearly lower in the case of precocene II-treated corpora allata. Conversely, the intraglandular contents of juvenile hormone III and methylfarnesoate are shown in Table 2. Concerning the pre-treatment period it can be concluded that at 10^-3 M and 70% of inhibition as average) and partially reversible inhibitory activity. The incomplete recovery of the biosynthetic capacity for the glands incubated for 6 h, whereas the recovery was partial and quite variable for those incubated with ethanol.

Effect of 3,4-dihydroprecocene II on the juvenile hormone III release

The results obtained on the comparison of the results obtained with precocene II and 3,4-dihydroprecocene II, it can be concluded that at 10^-3 M and 5 h of incubation, precocene II shows a higher inhibitory activity (64% and 70% of inhibition as average, respectively) than 3,4-dihydroprecocene II for 6 h of incubation (58%, of inhibition as average). The use of the same dose (10^-3 M, 3 x 3 h) suggests the possible induction of juvenile hormone III synthesis (i.e. intraglandular content plus released hormone, see Tobe and Stay, 1977), the same linear correlation was observed either in controls or in precocene II incubated corpora allata (Fig. 1). This result indicates that precocene treatment did not affect the juvenile hormone release mechanisms. Moreover, the overall values can be fitted into the function y = 0.914x + 0.008 (r = 0.998), in which the ordinate at the origin is close to 0, thus suggesting that there is not intraglandular accumulation of juvenile hormone.

DISCUSSION

From the comparison of the results obtained with the incubations with precocene II and 3,4-dihydroprecocene II, it can be concluded that at 10^-3 M and 3 h of incubation, precocene II shows a higher inhibitory activity on juvenile hormone III release (64% and 70% of inhibition as average, respectively) than 3,4-dihydroprecocene II for 6 h of incubation (58%, of inhibition as average). The use of the same dose (10^-3 M, 3 x 3 h) suggests the possible induction of cytotoxic effects on the corpora allata in a similar way to precocene II.

Table 2. In vitro juvenile hormone (JH) III release by corpora allata (CA) from 6-day old females of Blattella germanica incubated with precocene II (P II) for 3 h and intraglandular contents of juvenile hormone III (JH III) and methyl farnesoate (MF)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>BO*</th>
<th>JH III release (pmol/h x pair CA)</th>
<th>JH III MF (pmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10^-3 M P II</td>
<td>8</td>
<td>1.81 ± 0.04</td>
<td>2.61 ± 0.33</td>
<td>0.92 ± 0.13</td>
</tr>
<tr>
<td>0.05% Ethanol</td>
<td>8</td>
<td>1.77 ± 0.05</td>
<td>2.45 ± 0.25</td>
<td>2.42 ± 0.27</td>
</tr>
</tbody>
</table>

*BO: Length (mm) of basal oocyte of the corpora allata donors. All values are expressed as x ± SEM and those corresponding to juvenile hormone release during the incubation period with precocene or with ethanol are indicated in boldface.
manner to that elicited by precocene II on *B. germanica* (Piulachs and Bellès, 1985) and other Dipteroptera, like *Diploptera punctata* (Feyereisen et al., 1981; see also Bowers, 1985, for review). In this context, preliminary observations on the ultrastructure of the glands used in the present experiments seem to confirm this cytotoxic action (Piulachs, Cassier and Bellès, unpublished).

The question arises whether 3,4-dihydroprecocene II could be bioactivated within the corpora allata to give a cytotoxic intermediate following a similar pattern to that postulated for precocenes. In this sense, results from different metabolism studies (Pratt et al., 1980; Soderlund et al., 1980; Hamnett and Pratt, 1983) have suggested that corpora allata monoxygenases convert precocenes into a reactive intermediate, generally formulated as a 3,4-epoxide, which would be the responsible for the observed cytotoxic effects.

In the case of 3,4-dihydroprecocene II, two different hypotheses might be anticipated. First, that bioactivation of 3,4-dihydroprecocene II would transform this compound into precocene II which would then exert the expected cytotoxic activity. However, this hypothesis is not plausible since desaturase activity has never been reported in the corpora allata.

The other alternative for explaining the cytotoxicity of 3,4-dihydroprecocene II takes into account the direct bioactivation of the compound by the same monoxygenase system of corpora allata. In fact, 3,4-dihydroprecocene II contains an activated benzylic position which can be oxidized (e.g. hydroxylated). Dehydration of this 3,4-dihydroprecocene II hydroxylated intermediate into precocene II and further recycle of the bioactivation process of precocene II appears unfeasible. Therefore, we consider that the cytotoxic effect could be initiated by radical species involved in the above oxidation. In addition, it is also possible that a process derived from second phase metabolism (see Caldwell, 1980), could give rise to intermediates capable of suffering covalent binding with nucleophile sites of macromolecules, analogously to the pathway postulated for bioactivated precocenes.

On the other hand, results on intraglandular contents of juvenile hormone III indicated that there is not hormonal accumulation neither in corpora allata nor in corpora allata incubated with precocene II. Depletion of juvenile hormone contents in treated corpora allata parallels the inhibitory effect measured as juvenile hormone release. Conversely, methyl farnesoate contents seem to be unaffected by precocene II. These facts suggest a possible inhibitory action of precocene II on juvenile hormone biosynthesis at level of the methyl farnesoate epoxidation. Since precocene II is presumed to undergo biotransformation into reactive intermediates—the putative allatocidins—through the action of the same corpora allata monoxygenase which epoxidizes methyl farnesoate to give juvenile hormone III, a competitive action of precocene II for these enzymes could be postulated which would result in a diminution in terms of juvenile hormone synthesis and a maintenance of methyl farnesoate levels, as observed in our experiments.

As a more general conclusion of the results herein described, a dual action of precocenes in corpora allata of *B. germanica* adult females could be inferred. First it can be postulated that, possibly as a consequence of some sort of bioactivation, precocenes exert an inhibition of juvenile hormone biosynthesis. However, this inhibition only leads to irreversible effects under very severe treatments (i.e. incubations at 10^{-7} M during 9 h). Concomitantly, it seems that a specific inhibition of the epoxidation of methyl farnesoate does also take place, analogously to those observed for conventional inhibitors of this biosynthetic step (Staal, 1986). This mechanism of action would be reversible and could be induced and evidenced through short-term incubations in the presence of precocenes. On the other hand, this specific inhibition would lead to the accumulation of intraglandular methyl farnesoate, with an overall effect which would counterbalance the expected depletion for this compound produced by the prime inhibition mechanism.

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REFERENCES


