Life Sciences, Vol. 25, pp. 445-450
Printed in the U.S.A.

Pergamon Press

EFFECTS OF THE ANTI HORMONE-HORMONE MIMIC ETB ON THE INDUCTION OF INSECT JUVENILE HORMONE ESTERASE IN TRICHOPLUSIA NI

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(Received in final form June 15, 1979)

Summary

Juvenile hormone (JH) esterases can be artificially induced to appear in the hemolymph of last instar larvae of the lepidopterous insect Trichoplusia ni (Noctuidae) by topical treatment with JH I, JH II, or dihomo branched juvenoids. ETB (ethyl-4-[2-(t-butylcarbonyloxy)butoxy] benzoate; ZR-2646) at high doses is a weak inducer of JH esterase (JHE). However, at doses of ETB that induce only low levels of JHE activity, ETB will block the JHE induction caused by the dihomo juvenoid epofenonane and at higher doses will reduce the induction caused by JH I or JH II. ETB is not a JHE inhibitor; rather, it appears to be acting as a JH agonist/antagonist in normal larvae and in isolated abdomens. These effects of ETB on JHE induction may illustrate a new mode of action of anti-JH's.

Insect metamorphosis is regulated, in part, by juvenile hormone (JH) (Fig. 1) (1,2), and compounds that mimic the action of JH have proven to be effective insect control agents under the right circumstances (3,4,5). Another approach for developing insect control agents based on the insect endocrine system is the disruption of JH function by prematurely lowering the JH titer or by blocking JH receptors. These anti-JH effects would result in precocious metamorphosis, which would be effective against the larval stage of pest insects. The recent reports of compounds with anti-JH activity; precocene II (6,7), A_{11} (8,9) and ETB (ZR-2646) (10,11); have confirmed the feasibility of this concept. Although the exact mode of action for the three anti-JH's is currently unknown, one of the actions of precocene II seems to be directed toward the glands which produce and release JH (corpora allata) (12,13).

Ester cleavage is the major route of degradative metabolism of JH in the Lepidoptera examined (14,15) and esterases that seem to be specific for JH (16-18) appear in the hemolymph of lepidopterans during the last larval instar at times that correlate with decreases in the hemolymph JH titer (19-22). The stimulation of these enzymes to appear during an earlier instar could prematurely degrade JH and lead to anti-JH effects. These JH esterases (JHE's) can be caused to appear in the hemolymph of last instar larvae of the cabbage looper, Trichoplusia ni, by the topical application of JH I, JH II,

0024-3205/79/050445-06$02.00/0

and dihomo juvenoids (23). This phenomenon is referred to as esterase induction. Because ETB is effective as an anti-JH (10,11), its effects on JHE induction during the last larval instar of T. ni were investigated.

Methods

Compounds. Racemic mixtures of isomerically pure juvenile hormones were used in this study and include JH I (Fig. 1, methyl[2E,6E,10cis]-10,11-epoxy-3,11-dimethyl-7-ethyl-2,6-tridecadienoate), JH II (methyl[2E,6E,10cis]-10,11-epoxy-3,7, 11-trimethyl-2,6-trimethyl-2,6-tridecadienoate) (Calbiochem), and JH III (^{3}H methyl [2E,6E]-10,11-epoxy-3,7,11-trimethyl-2,6-dodecadienoate) (18). Juvenoids used include epofenonane (Fig. 2, 1-[4'-ethylphenoxy]-3-ethyl-7-methyl-6,7-epoxynonane, Ro-10-3108) (U. Schwieter, Hoffman-LaRoche), R-20458 ([2E] 1-[4'-ethylphenoxy]-3,7-dimethyl-6,7-epoxy-2-octene) (synthesized by published procedures) and methoprene (isopropyl[2E,4E]3,7,11-trimethyl-11-methoxy-2,4-dodecadienoate) (G. Quistad, Zoëcon Corp.). Anti-JH's used were precocene II (6,7-dimethoxy-2,2-dimethyl chromene) (W. S. Bowers, Cornell University), A_{11} (methyl-6,7-dioxo-5α-podocarpa-8,11,13-triene-15-oate) and ETB (Fig. 2, G. B. Staal, Zoëcon Corp.). DFP (diisopropyl phosphofluoridate) (Sigma) is a potent inhibitor of the hemolymph esterases hydrolyzing α-naphthyl acetate but not JH while ethyl benzoate (Aldrich) is analogous to a major functionality of ETB.

Insects and JHE assay. Last instar larvae of T. ni were reared individually with a photoperiod of 14L:10D (lights on at 5AM) and separated into groups that would pupate in four (gate I) or five (gate II) days, as described previously (22). Gate I (GI) larvae were used unless otherwise noted. The anal prolegs were clipped on 3-4 larvae and the hemolymph was collected in a culture tube (6x50 mm) at 4°C. An aliquot (25 µl) of the pooled hemolymph was diluted 1:100 with pH 7.4, I=0.2 sodium phosphate buffer containing 0.01% phenylthiourea to inhibit tyrosinases. JHE activity was monitored in this diluted hemolymph using methoxy labeled JH III as substrate ($5x10^{-6}$M) (18,19). All assays were performed in a manner that approached substrate saturation and for which the rates of hydrolysis were linear during the time used for the analysis. The ability of the compounds listed above to directly inhibit JHE activity *in vitro* was determined by adding the candidate inhibitor in 1 µl of ethanol to 100 µl of diluted hemolymph and preincubating for 10 min at 30°C before adding the JH III substrate and proceeding as above. I_{50}'s for JHE inhibition (inhibitor concentration that results in 50% of the control enzyme activity) were taken from semilog plots of percent inhibition vs. inhibitor concentration.

Induction and Inhibition of Induction of JHE *in vivo*. Normal fifth instar (GI) larvae have very low JHE activity on day 3 (L5D3) and were used for all induction studies (23). Compounds in 1 µl of ethanol were applied topically to the dorsum of abdominal segments 5-8 at 4 hrs after lights on (ALO). For inhibition experiments constant doses of epofenonane or JH I were co-applied with varying doses of candidate inhibitors. In each case hemolymph JHE was monitored as described above 12 hrs after topical treatment (16 hr ALO). The topical application of up to 5 µl of ethanol per larva was found to have no effect on the induction or inhibition of induction of JHE.

Results

Induction. When L5D3 larvae were treated topically with selected doses of JH I, JH II or epofenonane, induction occurred in a dose dependent manner with maximal JHE induction following 200 nmol applied per larva. High doses of ETB (>200 nmol/larvae) can induce JHE with maximal induction occurring at 470 nmol/larva. However, maximal induction caused by ETB is only 66% of the

maximal induction caused by epofenonane (Fig. 1). The other compounds listed in the methods section caused no significant ($p < 0.05$) induction at up to 200 nmol/larva.

Inhibition of Induction. To explore the possibility that ETB could act as an antagonist for JH or epofenonane-dependent JHE induction, varying doses of ETB were co-applied with a standard dose of the inducers (100 nmol/larva). ETB co-applied with epofenonane reduced the JHE detected 12 hrs later in a dose dependent manner (Fig. 2). However, when larvae were treated simultaneously with ETB and JH I, the JHE activity was not detectably reduced until a dose of 200 nmol/larva of ETB was attained (Fig. 2). Attempts to follow the JH I antagonistic activity of ETB at still higher doses were not practical due to the inherent JH-agonistic activity of ETB. Results obtained with JH II co-applied with ETB were similar to those obtained with JH I. When epofenonane and ETB were applied separately or co-applied to larval abdomens isolated by a ligation between the thorax and abdomen, the resulting JHE induction and inhibition of induction were similar to the results obtained in normal larvae. When the other compounds listed in the methods section were examined for their ability to block the induction caused by epofenonane (100 nmol/larva) following co-application of the test compound at up to 100 nmol/larva, no significant reduction in JHE induction was noted.

In vitro JHE Inhibition. The juvenoids epofenonane, R-20458, and methoprene demonstrated I_{50}'s for JHE of $>1 \cdot 10^{-4}$M (solubility limits precluded testing higher concentrations) while the I_{50}'s for all other compounds were $>1 \cdot 10^{-3}$M although at this concentration DFP caused >90% inhibition of the esterases hydrolyzing α-naphthyl acetate (19,24). Other compounds have been shown to be potent in vitro JHE inhibitors (18,24).

Discussion

In M. sexta ETB is an anti-JH at low doses and a JH mimic at high doses (10, 11), yet its mechanism of action has not been elucidated. After a critical time in T. ni, JHE can be induced by JH I, JH II, and some homo juvenoids such as epofenonane (23,24). Thus, ETB was examined for its ability to induce JHE in T. ni. ETB caused induction in a dose dependent manner, but its maximal induction was less than that caused by JH I or epofenonane (Fig. 1). Induction of JHE by ETB has also been observed in the pupae of the wax moth Galleria mellonella (25).

If ETB binds at the same abdominal receptor as JH and epofenonane, yet fails to maximally activate that receptor, then it might be expected to inhibit the induction caused by JH I or epofenonane. Such interactions are illustrated by studies with suboptimal inducers of steroid (26-30) and pharmaceutical receptors (31). As shown in Fig. 2, ETB does inhibit JHE induction and several additional experiments were designed to examine the mechanism of this inhibition.

If ETB were a direct inhibitor of JHE it would apparently block JHE induction, and such in vivo inhibition of JHE has been observed with other compounds such as phosphoramidothiolates (23,24). Neither ETB nor any of the other compounds tested caused direct in vitro inhibition of JHE at concentrations far exceeding those expected from topical application. Also, if ETB were a direct JHE inhibitor, one would not expect it to induce JHE at very high doses (Fig. 1).

If ETB were acting as an inhibitor of JHE biosynthesis or release at a later regulatory stage than JH or epofenonane action, then ETB should inhibit equally the induction caused by these compounds. Fig. 2 clearly demonstrates

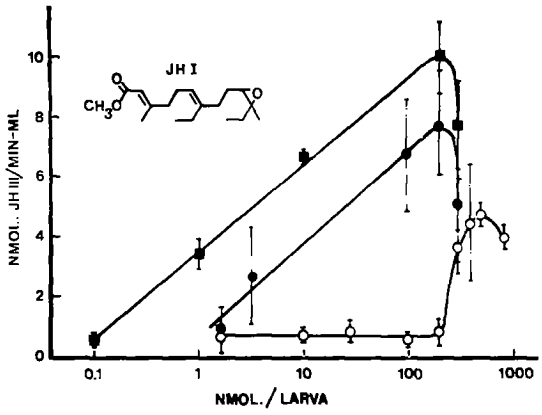

FIG. 1

JH esterase activity induced in L5D3 larvae in response to selected doses of JH I (■), epofenonane (●), and ETB (O) applied separately. JH II is slightly less active than JH I. JH esterase activity is expressed as nmol JH III hydrolyzed/ minute/ml of hemolymph. All assays were done in triplicate and the standard deviations are from studies performed on at least 3 separate occasions.

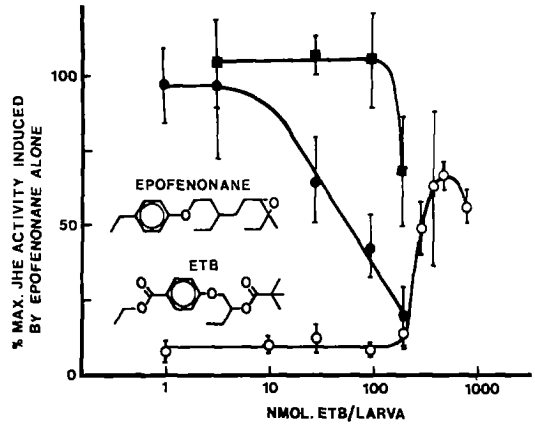

FIG. 2

JH esterase activity in L5D3 larvae treated with JH I or epofenonane (100 nmol/ larva) and varying doses of ETB compared to larvae treated only with epofenonane (100 nmol/larva). JH I and ETB (■); epofenonane and ETB (●), ETB alone (O). With 30 nmol of ETB/larva the induction following application of JH I or JH II (100 nmol) is not significantly reduced while the reduction following epofenonane application is significant at $p < 0.05$.

that ETB is a significantly more potent inhibitor of induction caused by epofenonane than induction following JH I or JH II treatment. Thus, ETB does not appear to effect JHE biosynthesis or release.

It has been previously demonstrated that JHE induction by JH or epofenonane can occur in isolated abdomens (23,24). If ETB were acting through a head or thoracic structure to inhibit JHE induction in the abdomen, then its effect should change dramatically in isolated abdomens. However, there is no significant difference in the inhibition of induction caused by ETB in isolated abdomens indicating that it, like JH and epofenonane, acts directly on some abdominal receptor, presumably in the fatbody (25,32). This JH receptor, possibly a high affinity binding site (11), appears superficially to be similar to steroid receptors (28).

Information to date on ETB is thus consistent with it acting as a partial agonist/antagonist at JH receptor(s). The lack of activity associated with the apparent binding of ETB suggests that the receptor may have to undergo a transitional change to become active as is thought to occur with many steroid receptors (26-30). Assuming that there is only a single type of binding site on the receptor (i.e., the simplest case), and taking into account that ETB can block the JHE induction by epofenonane at lower doses than that of JH I or JH II, then the relative affinity of the receptor for these compounds appears to be JH I and JH II >ETB $\cong$ epofenonane. At least on T. ni, the other anti-JH's tested (A_{11} and precocene) appear to act by separate mechanisms because they are not direct JHE inhibitors, JHE inducers, or inhibitors of JHE induction. These compounds also show no in vivo anti-JH activity in T. ni.

The anti-JH and JH mimicking activities of ETB are difficult to separate in vivo, and occur over a relatively narrow dose range. The inhibition of JH dependent JHE induction in the late last instar larvae of T. ni is also apparently not in the causal chain leading to precocious development following ETB treatment of early instar larvae; however, the assay presented here clearly distinguishes between the JH agonist and JH antagonist properties of ETB. The in vivo symptoms observed following ETB treatment may be due to ETB's agonist/antagonist action at JH receptors other than those involved in JHE regulation. If the hypothesis of ETB action presented here remains valid after further experimentation, such JH agonist/antagonists are likely to be as useful to insect endocrinologists as steroid agonist/antagonists have been to vertebrate endocrinologists.

ETB does not appear to be a practical insect control agent, but the results of this study do demonstrate a possible mode of action for an anti-JH compound and lend support to the feasibility of developing anti-JH's for insect control. The T. ni assay used here has proven useful in screening compounds for their apparent JH agonist/antagonist activity, and thus this and similar assays may speed the search for effective anti-juvenile hormones.

Acknowledgement

This work was supported, in part, by Grant No. 5 R01 ES01260-02 from the National Institutes of Health. B. D. Hammock was supported by a NIEHS Research Career Development Award, No. K04 ES00046-01. The authors thank S. O. Levinson for a critical review of the manuscript and A. Sparks for preparing the figures.

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