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Research Articles

Juvenile hormone catabolism in *Manduca sexta*: homologue selectivity of catabolism and identification of a diol-phosphate conjugate as a major end product

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Abstract. We studied time-dependent metabolism of (10*R*)-[^{3}H] juvenile hormone (JH) III and (10*R*, 11*S*)-[^{3}H]JH I injected into *Manduca sexta* larvae; the hormones are metabolized to polar metabolites, especially the JH acid-diol, and an unknown. Products were analyzed using a reversed-phase liquid chromatography assay. (10*R*)-JH III is metabolized much more rapidly than (10*R*, 11*S*)-[^{3}H]JH I, whether injected seperately or as a mixture of hormones. The unknown metabolites of JH I and JH III were identified as phosphate conjugates of JH I and JH III diol by tandem mass spectral analysis of isolated samples. The phosphate conjugate of JH I diol is the principle end product of JH I metabolism.

Key words. Juvenile hormone esterase; juvenile hormone epoxide hydrolase; juvenile hormone binding protein; reversed-phase liquid chromatography assay.

Juvenile hormones (JHs) are secreted by a pair of minute retrocerebral glands in insects, the corpora allata (CA). JHs are structurally related sesquiterpenoids that possess morphogenetic and gonadotropic activity. Based on many studies, there is a hypothesis that only insects from the order Lepidoptera, the butterflies and moths, have the ability to synthesize ethyl branched JH I, JH II, JH 0 and 4-methyl JH I[1,2]. A recent study claimed the existence of JH I in hemolymph of a hemipteran, *Riptortus clavatus*[3], but CA of this species have been found subsequently to secrete in vitro neither JH I nor JH III, but an unknown, more polar material produced also by CA of three other hemipteran species[4]. The TLC behavior of this unknown may be similar to that of JHB_3, the bisepoxide secreted by several higher depteran species[5,6]. JH III, the simplest homologue of the 6 known JHs, is the most commonly occurring form and has been isolated from insects of several orders including lepidopterans[2].

The levels of JH play a key role in the development of insects. A decrease in JH titer during the last larval stadium of lepidopteran insects results in the initiation of pupation; this decrease in JH titer is largely attributed to JH catabolizing enzymes[7]. Research done to date has shown substantial differences among various insect species regarding the role of separate enzymes in the metabolism of JH. Two hydrolytic enzymes, juvenile hormone esterase (JHE), which forms JH acid, and juvenile hormone epoxide hydrolase (JHEH), which forms JH diol, appear to be involved to various degrees in this process in the species examined[7,8]. During the last decade much attention has been given to JHE, perhaps because it is the only catabolic enzyme present in insect hemolymph[7]. Using affinity columns, JHE has been purified from a number of lepidopteran species[9], and recently its gene was cloned and sequenced from *Heliothis virescens*[10]. Comparatively few attempts have been made to characterize other pathways, including JHEH and conjugation enzymes.

Much work done to date on JH catabolism is based on in vitro studies using techniques such as thin-layer chromatography and partition assays[7,11]. Recently, we developed a new reversed-phase liquid chromatography assay for JH metabolites and used it to study catabolism of JH III in vivo in fifth stadium larvae of the tobacco hornworm, *Manduca sexta*[12]. Our results clearly show that, in addition to JHE, other enzymes play an important part in JH catabolism in vivo.

In *M. sexta*, the balance and titer of JH I, JH II, and JH III vary during development. Eggs contain the trishomosesquiterpenoids JH 0 and 4-methyl JH I, with traces of JH I[13,14]. An examination of the hemolymph titer of fifth stadium larvae showed the presence of JH I and JH II, and occasionally traces of JH III[15], while the adult hemolymph contains JH II and JH III[16]. Even though JH I and JH II predominate in vivo in larvae, in vitro studies performed with corpora allata from larvae of the same species and stage indicate that the CA actively biosynthesize and secrete three JHs: JH I, JH II, and JH III[17,18]. This discrepancy between the virtual absence of JH III in vivo and its synthesis in vitro is curious.

We now present the results of in vivo catabolism studies of JH I and JH III as well as in vitro biosynthesis

studies in fifth stadium larvae of *M. sexta*. We have identified a new major end product metabolite of JH, a diol-phosphate conjugate.

Materials and methods

Chemicals. JH I and III were obtained from Sandoz Crop Protection. Labeled (10*R*, 11*S*)-[12, 13-^{3}H]JH I (specific activity 58 Ci/mmol) and (10*R*)-[12-^{3}H]JH III (specific activity 15 Ci/mmol) were gifts from Prof. G. Prestwich and Dr. B. Latli. L-[*methyl*-^{14}C]methionine (57 mCi/mmol) was obtained from NEN/DuPont, Boston, MA, USA. Custom prepared medium 199 lacking valine, isoleucine and methionine, but containing $NaHCO_3$ was bought from GIBCO labs. The medium was supplemented with 20 g/L of Ficoll 400 and 20 mM Hepes at final pH 6.5. JH acid, JH diol and JH acid-diol were available from an earlier study[12].

Purification of JH I and JH III. The labeled (10*R*, 11*S*)-JH I and (10*R*)-JH III were purified by normal phase liquid chromatography, using a Brownlee 22 × 0.46 cm Spheri-5 column (5 µm), monitored at 220 nm, and eluted with hexane:ether (94:6, v/v). The retention times of JHs were determined with unlabeled standards. The labeled JH was then injected and a peak with appropriate retention time was collected.

Animals. M. sexta larvae were reared on an artificial diet[19] at 27 °C, 40% humidity and a 16 h light:8 h dark photoperiod. For in vivo metabolism studies, fourth stadium larvae with a clear head capsule were removed and injected within 10 min of molting to the fifth stadium. For the biosynthesis study, the CA were removed within 1 h of molting to the fifth stadium. The average weight of the newly molted fifth stadium larvae was 1.4–1.6 g.

In vivo catabolism study. Approximately 200,000 dpm of (10*R*, 11*S*)-JH I or 50,000 dpm of (10*R*)-JH III was dissolved in 1 µl acetone and injected into the dorsal prothorax of a single larva. This gave approximately 0.4 ng of JH I and JH III per insect, less than the calculated endogenous titer of JH I. After incubating at 27 °C for various time periods, the insects were homogenized in 4–5 ml of CH_3CN containing triethylamine (2 drops per 100 ml of CH_3CN) using a Polytron PT-20 (Brinkmann). The extract was evaporated and analyzed for JH metabolites on reversed-phase liquid chromatography (RPLC) as described earlier[12].

Incubation of the unknown metabolite with enzymes. The unknown metabolite of JH III was separated from other metabolites on a polymer column as described earlier[12]. Sulfatase (Sigma, type V) and glucuronidase (Sigma, type IX-A) were dissolved separately in 5 ml of 0.2 M sodium acetate buffer (pH 7.0) and the unknown fraction was incubated for 24 h at room temperature. Similarly, alkaline phosphatase (50 µg, Type 1-S, Sigma, bovine intestinal mucosa) was dissolved in 5 ml of 0.1 M sodium acetate buffer (pH 10.4) and incubated with the metabolite. At the end of the incubations, the samples were extracted with ethyl acetate (3x), and the organic fraction vacuum evaporated. The residue was dissolved in 50 µl of water and analyzed by RPLC as described by Halarnkar and Schooley[12].

Isolation and purification of the unknown metabolite. To isolate the unknown metabolite of JH III, newly molted fifth stadium larvae were each injected with a solution containing 1 µg of (10*R*, *S*)-JH III mixed with 200,000 dpm of (10*R*)-[^{3}H]JH III. The insects were incubated for 1 h, and then homogenized in 5–7 ml of CH_3CN containing triethylamine (1 drop per 100 ml of CH_3CN). The extract was vacuum evaporated and the residue redissolved in CH_3CN:water (50:50). This solution was passed through 100 mg of Vydac C_4 reversed-phase packing to remove materials which would bind to the RPLC column with this solvent. The sample was concentrated under nitrogen to about 0.3 ml and injected on RPLC along with cold standards of JH as described previously[12]. The unknown radioactive peak was collected and pooled from several animals.

For isolating the metabolite of JH I, midgut slices (~10–30 mg) from newly molted fifth stadium larvae were removed and each was incubated in 100 µl buffer containing 2 µg of (10*RS*, 11*SR*)-JH I and 200,000 dpm of (10*R*, 11*S*)-[^{3}H]JH I. After 2 h of incubation, midgut slices were removed and the medium was injected on RPLC. The unknown metabolite eluted between the acid-diol and the acid standards. Several fractions were collected between these peaks and radioactivity of aliquots measured; radioactive fractions were pooled.

To remove buffer and further purify each sample, pooled fractions from JH I or JH III incubations were injected on a Brownlee ODS column (10 × 0.46 cm) eluted with a 25 min gradient from 100% water to 100% CH_3CN. The fraction containing the radioactivity appeared to be pure. Samples obtained from several insects were pooled and the volume was reduced under a stream of nitrogen to 15 µl for mass spectrometric analysis.

Mass spectrometry. Liquid secondary ion mass spectra (LSIMS) were obtained on a Finnigan-MAT TSQ70 triple stage quadrupole, equipped with an Antek cesium ion gun. A primary beam of 8 keV nominal energy was used to bombard the sample (0.1–1.0 µg) dissolved in 1 µl of a glycerol matrix. Collision-induced dissociation mass spectra were obtained at energies of ~ ±20 eV, using argon as a collision gas at a pressure of 0.8–1.0 millitorr.

In vitro biosynthesis of JH. 5 pairs of CA from newly molted fifth stadium larvae of *M. sexta* were incubated for 4 h in 100 µl of custom prepared medium 199 as above, but containing 1 µCi of L-[*methyl*-^{14}C]methionine for 4 h. At the end of incubation, the products were extracted with isooctane and then subjected to normal phase LC to separate JH I, JH II and JH III. Peaks corresponding to cold standards were collected and radioactivity was assayed.

Results and discussion

Recently we developed a reversed-phase liquid chromatographic (RPLC) method to study JH catabolism in vivo[12]. This method uses a polymer column and has significant advantages over methods such as TLC and partition assays as, in addition to separating all known metabolites, it allows one to work with large, impure samples. We found significant levels of JH acid-diol in *M. sexta* injected with labeled (10*R*)-JH III, as well as JH acid, JH diol, and an unknown metabolite[12] eluting between JH acid-diol **4** and JH acid **2**. We have now investigated this further. Previously, JH diol was not regarded as a detectable metabolite in lepidoptera[7,20], but recent in vitro studies have shown the existence of JHEH activity in tissues of *Galleria mellonella*[21] and *M. sexta*[22,23].

Using the RPLC assay, we performed an in vivo metabolism study of (10*R*, 11*S*)-[^{3}H]JH I and (10*R*)-[^{3}H]JH III in newly molted fifth stadium larvae of *M. sexta*. We selected this stage because the JH titer is high and JH catabolic activity is low[15]. Insects were injected with 0.4 ng of labeled JH III or JH I, and after certain time periods they were homogenized in CH_3CN. An aliquot of crude extract was analyzed admixed with non-labeled standards; peaks corresponding to standards were collected in scintillation vials and the radioactivity was assayed. Figure 1 shows an RPLC trace with histogram obtained after a 30 min incubation with JH I. Radioactivity was obtained in peaks which coeluted with JH I acid-diol and JH acid standards. Significant amounts of unknown metabolite(s) eluted between the acid-diol and the acid.

The unknown metabolite did not have different LC behavior after treatment with diazomethane, and gave varying yields of JH diol on treatment with enzymes (see below). These data suggested that the metabolite was a conjugate of JH diol.

The unknown metabolite was further purified as described in 'Materials and methods'. Figure 2A shows the negative ion LSIMS spectrum of the JH I metabolite.

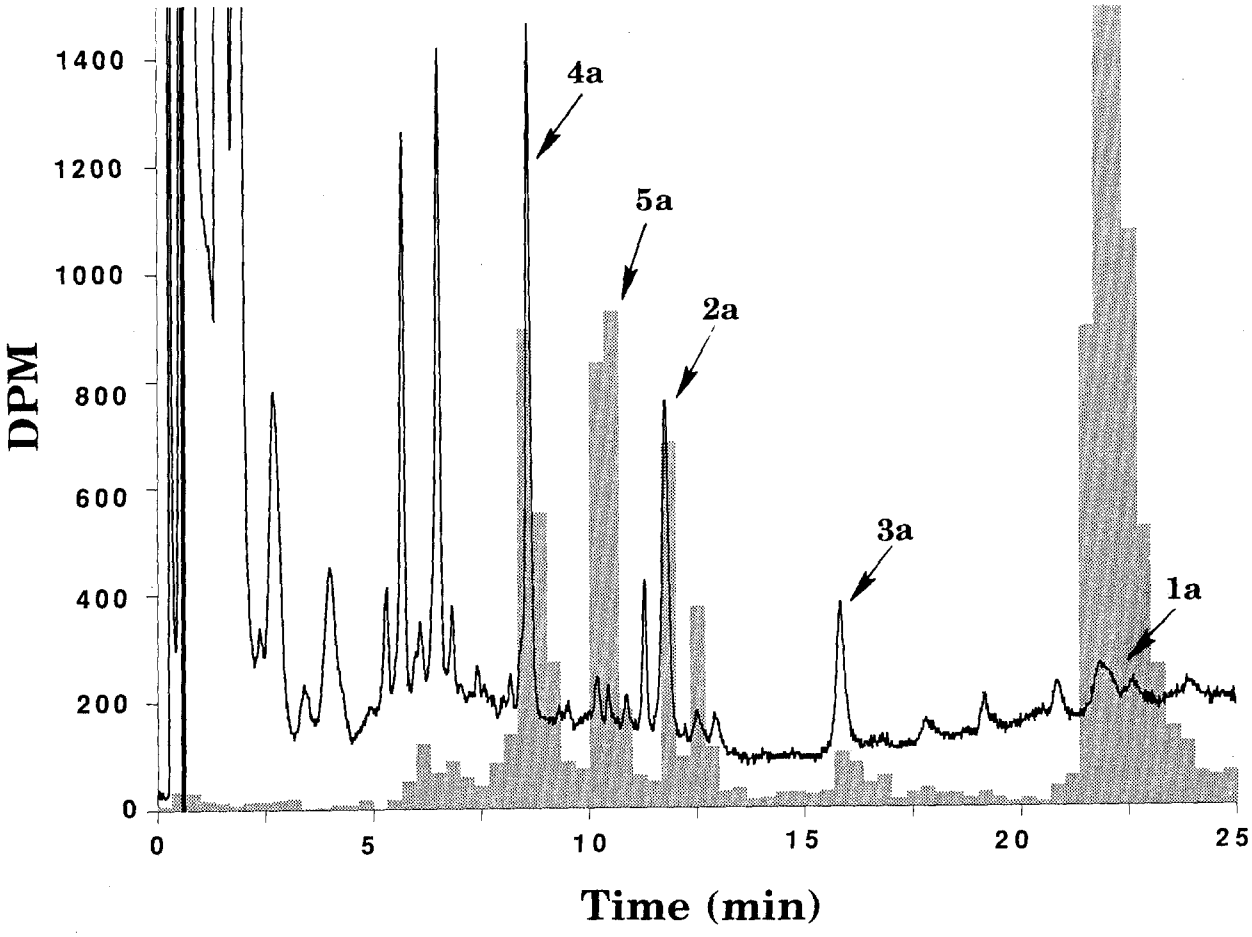

Figure 1. Radio-HPLC trace of (10*R*, 11*S*)-[^{3}H]JH I and its metabolites. A newly molted fifth stadium larva of *M. sexta* was injected with the labeled substrate, the insect incubated for 30 min, and metabolites extracted and analyzed by RPLC as described under 'Materials and methods'. The shaded portion represents the radioactivity, whereas the solid line is the absorbance at 245 nm. **1a**, JH I; **2a**, JH I acid; **3a**, JH I diol; **4a**, JH I acid-diol; **5a**, JH I diol-phosphate.

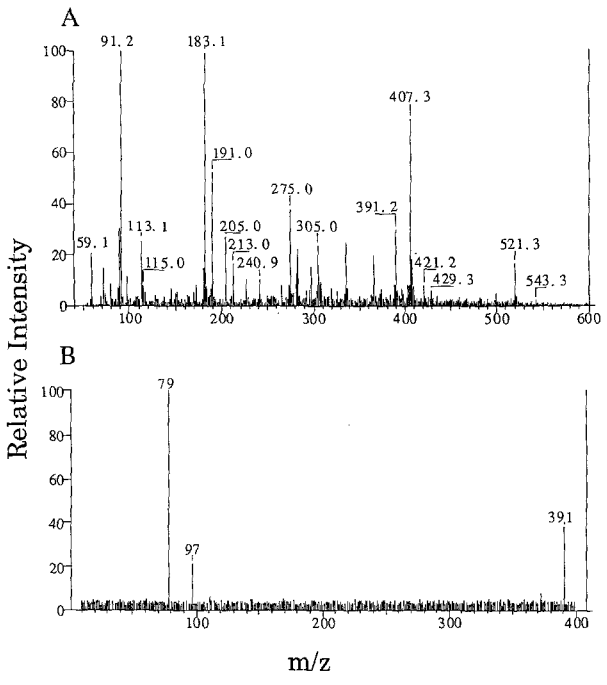

Figure 2. Mass spectrometric analysis of the 'JH I metabolite'. Figure 2A shows the negative ion LSIMS spectrum. The anion at m/z 391 corresponds to a molecular weight of 392, indicating the presence of diol conjugate of JH I. Figure 2B shows the MS-MS for m/z 391. Ions at m/z 79 (PO_3^-) and 97 ($H_2PO_4^-$) indicate the presence of a phosphate ester.

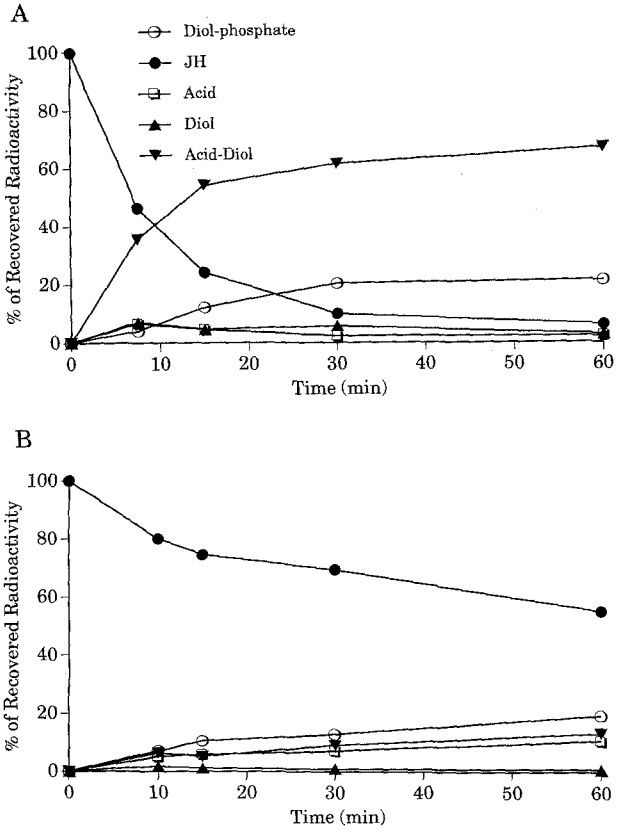

Figure 3. Time-dependent metabolism of radiolabeled (10*R*)-JH III (fig. 3A) and (10*R*, 11*S*)-JH I (fig. 3B) in fifth stadium larvae of *M. sexta*. Insects were injected with the radioactive substrate and incubated for the given time periods, and the metabolites extracted and analyzed by RPLC as described under 'Materials and methods'. Each time point is the mean of two insects.

The spectrum includes signals derived from the glycerol matrix and its sodium adducts (ie, m/z 91, 113, 183, 205, 275, etc), as well as 3 products of M_r 392, 408, and 522. The ion at m/z 391 represents the parent anion and corresponds to a molecular weight of 392, indicating the presence of a sulfate or phosphate conjugate of JH I diol. Analysis of m/z 391 and 521 by MS-MS showed in both instances the presence of product ions at m/z 79 (PO_3^-) and 97 ($H_2PO_4^-$) (fig. 2B). These two ions indicate the presence of a phosphate ester. A sulfate ester standard (estradiol sulfate) showed m/z 80 (SO_3^-) and 97 (HSO_4^-) under these conditions. The material at m/z 407 does not appear to be related to a JH-type structure, nor does the phosphate ester at m/z 521. The unknown metabolite of JH III was also identified as a phosphate conjugate of diol by MS-MS (data not shown). MS analysis of JH-10, 11-diol[24] and JH-10-hydroxy, 11-methoxy[25] derivatives is dominated by α-cleavage of the C-10, 11 bond. In the negative ion MS of JH I diol phosphate (fig. 2A), there is an anion at m/z 71 corresponding to the α-cleavage product $CH_3CO^- = CHCH_3$. The complementary ion at 320 is not observed, but loss of PO_3^- from this ion accounts for the anion observed at m/z 241. Thus, these data are strongly suggestive that the phosphate ester is bound to the secondary hydroxyl (C-10). This appears to be the first physico-chemical identification of any JH conjugate; additionally, we demonstrate that the phosphate conjugate of JH diol is a major end product catabolite in *M. sexta* (fig. 3B).

Several studies have indicated the formation of conjugation products of JH metabolites in insects[20,26–29]. All these studies were based on enzymatic hydrolysis by sulfatases and/or glucuronidases. Many of these observations may be misleading since enzymes such as sulfatases may contain multiple hydrolytic activities. In initial studies we observed the formation of JH III diol when its phosphate conjugate was incubated with sulfatase. However, less than 3% of the conjugate was hydrolyzed. Incubation with alkaline phosphatase resulted in hydrolysis of more than 70% of the conjugate to JH III diol.

Figure 3B shows the results of a time-dependent catabolism study of radiolabeled (10*R*, 11*S*)-[^{3}H]JH I. Even after 60 min incubation, more than 50% of the recovered radioactivity was obtained in the JH I peak, about 10% was found in the JH I acid-diol peak, and about 15% in the JH I diol-phosphate peak. The total recovery of injected radioactivity varied from 80–95%. The catabolism of (10*R*)-[^{3}H]JH III was much faster than that of JH I, but showed a profile of products

similar to those from JH I. Within 30 min, more than 90% of the labeled (10*R*)-JH III was converted into polar products (fig. 3A). The major catabolite formed was JH III acid-diol rather than JH III diol-phosphate, whereas for JH I the major metabolite was JH I diol-phosphate. In both cases there was a steady increase with time in the formation of the conjugate; the amount of the diol conjugate of JH I was similar to the diol conjugate of JH III after 1 h.

For JH III, the radioactivity in the acid and the diol peaks remained low, whereas the activity in JH acid-diol increased rapidly. This indicates catabolism of JH III to acid and diol by JHE and JHEH, respectively; these metabolites are then rapidly converted to acid-diol, presumably by the same enzymes. However, for JH I the formation of the diol conjugate was somewhat greater than formation of the acid-diol, and significantly greater than for the acid and the diol. As JHEH is required for formation of both the diol-phosphate and acid-diol, epoxide hydration appears quantitatively more important in JH I catabolism than ester hydrolysis at this stage of development.

We co-injected labeled JH I and JH III in larvae to rule out the possibility of inter-animal variation or other circumstances affecting our conclusions. Such a study is possible only with the high resolution RPLC assay. We found (data not shown) that when both JHs are present, JH III is still rapidly catabolized in comparison to JH I, indicating that JH catabolic enzymes are more active towards JH III even in the presence of JH I.

We could not detect the formation of JH acid-diol conjugate in our studies. It is possible that the phosphotransferase enzyme is specific for JH diol and does not act on JH acid-diol. However, more studies are needed to confirm this. This presents an interesting parallel to the literature on ecdysteroid metabolism: at least 10 phosphate conjugates of ecdysteroid secondary or primary alcohols are known, but no phosphate conjugates of ecdysonic acids are known[30].

Even though studies on JH catabolism in the last two decades have led to a considerable understanding of the enzymes involved, a complete picture of how different enzymes interact is still lacking. Most information on JH catabolism is based on in vitro studies, and these studies have focused mainly on one enzyme: JHE. Very few in vivo studies, which take into account all possible pathways of JH catabolism, have been performed[20,31]. Based on assay of JHE in hemolymph, a dogma has arisen that a pulse of JHE in the hemolymph of lepidopteran larvae causes the decrease in JH titer, which in turn presumably initiates pupation[7]. However, recent observations by Baker et al.[15] and Zimowska et al.[32] indicate that in two lepidopteran species, JHE may not be important for the decline of the JH levels. Their results clearly show that in *M. sexta* and *Spodoptera littoralis*, JH titer in the hemolymph falls to undetectable levels before JHE begins to rise in the blood. Moreover, Bhaskaran's group showed that CA from fifth stadium larvae of *M. sexta* on day 4 stop producing JH and instead secrete JH acid, which is then converted into JH in imaginal discs by a methyltransferase enzyme[18]. Our in vivo catabolic results (fig. 3) clearly show that in newly molted fifth stadium larvae of *M. sexta*, JH acid-diol and JH diol-phosphate are the major end products of both JH I and JH III, thus indicating an important role for JHEH in addition to JHE.

Affinity purified JHEs from two lepidopteran species, the cabbage looper *Trichoplusia ni*[33] and *M. sexta*[34], have been studied for their substrate specificity towards JH I, JH II and JH III. These studies indicate that JH I is hydrolyzed a little faster than JH III by the purified enzymes from both species (~1.5 x for *T. ni*[35], ~2 x for *M. sexta*[33]). This agrees with earlier results from a study of JHE from *M. sexta* purified using chromatographic procedures, which showed that JHE hydrolyses JH I at about twice the rate of JH III[36]. In vitro studies[22] on JHEH from *M. sexta* eggs showed that JHEH hy-

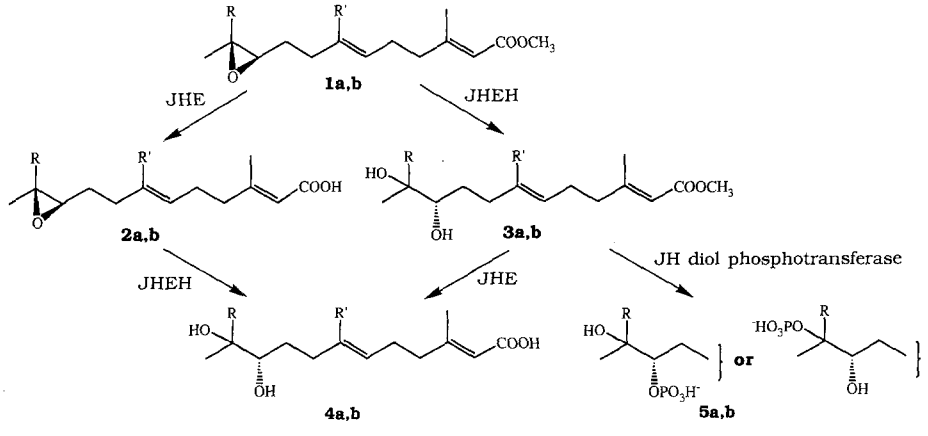

Figure 4. A revised version of the classical pathway for the catabolism of JH. **1**, JH; **2**, JH acid; **3**, JH diol; **4**, JH acid-diol; **5**, JH diol-phosphate for R = R' = CH_3CH_2, **a**, (10*R*, 11*S*)-JH I; for R = R' = CH_3, **b** (10*R*)-JH III.

drolyzed JH III about 6 times faster than JH I; a substrate specificity opposite to that of JHE. However, natural enantiomers of JH I and JH III were not used in any of these studies, rather, commercially available racemic radiolabeled JH was used[22,33,35,36]. In our study we used the natural enantiomers (10*R*, 11*S*)-JH I and (10*R*)-JH III. For in vivo studies it is important to use these natural enantiomers since several studies[37] have indicated that hemolymph JH binding proteins (JHBPs) play a crucial role in protecting endogenous JH from degradation. JHBPs differ in their binding affinities towards different JH homologues and enantiomers. JHBPs purified from *M. sexta* are both enantioselective and homologue-selective in their binding interactions with JHs. Using partially purified JHBP from *M. sexta*, Schooley et al. showed the order of binding of JH III isomers to JHBP was $10R > 10R, S \gg 10S$[38]. Peterson et al.[39] showed that purified JHBP from *M. sexta* has a relative affinity for racemic JH III of 0.29 competed against racemic JH I (1.0). Prestwich et al. further demonstrated that the relative binding affinity of the purified JHBP of *M. sexta* for natural (10*R*, 11*S*)-JH I is about 3 times higher than the unnatural (10*S*, 11*R*)-JH I, but about 19 times higher than the affinity for racemic JH III (the natural isomer was not tested[40]). Thus, in doing in vivo studies the complex interactions of binding proteins and hydrolytic enzymes may affect results depending on whether the labeled JH is racemic or the natural enantiomer.

To study ratios of JHs biosynthesized in vitro, CA of freshly molted fifth stadium larvae were incubated with labeled methionine in medium 199. The methyl group from L-[*methyl*-^{14}C]mentionine incorporates stoichiometrically into the carboxymethyl moiety of JHs, which allows determination of rates of JH biosynthesis[41]. The results in the table show the formation of JH I, II and III with biosynthetic rates of 0.012, 0.33 and 0.064 pmol/h/CA, respectively.

These results are in reasonable agreement with previous reports which show the formation of all three JHs by larval *M. sexta* CA in vitro[17,18], although the relative ratios secreted differ somewhat. Baker et al.[15] observed that relative rates of JHs secreted by CA of fifth stadium larvae were JH II > JH I > JH III which is in accord with Bhaskaran's results[18]. Dahm et al. reported higher formation of JH II than JH III, but they could not detect JH I[42]. Using RIA assays for JHs secreted in vitro by CA, Granger et al. have reported higher levels of JH III vs JH I[43,44]. However, they could not determine levels of JH II due to the lack of a suitable antibody, and their antibodies read the acid metabolite in addition to JH. This metabolite is not detected with the radiochemical assay. Some of the variation in results may be due to the use of different incubation media, pH, or other conditions[44]. Also, the levels of JHs produced by individual glands may vary. Recently Bhaskaran's group studied the amounts of different JHs produced by individual glands; their results indicate that CA may biosynthesize JHs in pulses[45]. However, an additional complicating factor is that JH esterase has been shown to be secreted by *M. sexta* CA in vitro[46], affecting the products of the radiochemical assay. Although different ratios of JH secreted by the CA are reported in all these studies, JH III was always detected. In marked contrast, Baker et al.[15] detected only trace levels of JH III in the hemolymph of fifth stadium larvae of *M. sexta* using a highly specific GC/MS assay. The dominant JHs present were JH I and JH II, and their acid metabolites.

Our results clearly indicate that there are marked differences in rate of metabolism between (10*R*, 11*S*)-JH I and (10*R*)-JH III in vivo. The lower affinity of JH-specific binding protein for JH III will result in greater exposure of JH III to catabolic enzymes than JH I. Taken together with our results, this may be crucial in explaining why JH III is usually undetectable in larval *M. sexta* in vivo[15], whereas CA can biosynthesize JH III in vitro. Several studies have indicated that JHE is more active on JH I than JH III in vitro[33,35,36], and one in vitro study has shown that JHEH is more active on JH III than JH I[22]. It is therefore difficult to assess how the inverse homologue selectivity of these enzymes in vitro would contribute to significant in vivo differences in rate of metabolism for the different JHs.

In conclusion, we studied the catabolism of natural enantiomers of JHs in vivo and demonstrated specific metabolic differences for different types of JHs. Using MS-MS analysis, we firmly established the structure of a phosphate conjugate of JH diol. To our knowledge this is the first identification of any JH conjugate.

Synthesis of JH I, JH II and JH III by the fifth stadium larval CA in vitro[a]

Juvenile hormone	Rate ± S.D. (pmol/hour/CA)[b]
JH I	0.012 ± 0.005
JH II	0.332 ± 0.127
JH III	0.064 ± 0.036

[a]In each assay, 5 CA pairs from newly molted fifth stadium larvae were incubated in medium 199 containing 175 µM L-[*methyl*-^{14}C] methionine for 4 h at 27 °C.
[b]Rates are the mean of 6 separate assays.

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1 Schooley, D. A., Baker, F. C., Tsai, L. W., Miller, C. A., and Jamieson, G. C., in: Biosynthesis, Metabolism and Mode of Action of Invertebrate Hormones, p. 373. Eds J. A. Hoffmann, and M. Porchet. Springer-Verlag, Heidelberg 1984.
2 Schooley, D. A., and Baker, F. C., in: Comprehensive Insect Physiology, Biochemistry and Pharmacology, p. 363. Eds G. A. Kerkut and L. I. Gilbert. Pergamon Press, Oxford 1985.
3 Numata, H., Numata, A., Takahashi, C., Nakagawa, Y., Iwatani, K., Takahashi, S., Miura, K., and Chinzei, Y., Experientia *48* (1992) 606.
4 Kotaki, T., Appl. Ent. Zool. *28* (1993) 242.
5 Richard, D. S., Applebaum, S. W., Sliter, T. J., Baker, F. C., Schooley, D. A., Reuter, C. C., Henrich, V. C., and Gilbert, L. I., Proc. natl Acad. Sci. USA *86* (1989) 1421.
6 Lefevere, K. S., Lacey, M. J., Smith, P. H., and Roberts, B., Insect Biochem. molec. Biol. *23* (1993) 713.
7 Hammock, B. D., in: Comprehensive Insect Physiology, Biochemistry and Pharmacology, p. 431. Eds G. A. Kerkut and L. I. Gilbert. Pergamon Press, Oxford 1985.
8 Hammock, B. D., and Quistad, G. B., in: Progress in Pesticide Biochemistry, p. 1 Eds D. H. Hutson, and T. R. Roberts, John Wiley & Sons, New York 1981.
9 Hammock, B. D., Abdel-Aal, Y. A. I., Hanzlik, T. N., Croston, G. E., and Roe, M. R., in: Molecular Entomology, p. 315. Ed J. H. Law. Alan R. Liss, Inc., New York 1987.
10 Hanzlik, T. N., Abdel-Aal, Y. A. I., Harshman, L. G., and Hammock, B. D., J. biol. Chem. *264* (1989) 12419.
11 Share, M. R., and Roe, M. R., Analyt. Biochem. *169* (1988) 81.
12 Halarnkar, P. P., and Schooley, D. A., Analyt. Biochem. *188* (1990) 394.
13 Bergot, B. J., Jamieson, G. C., Ratcliff, M. A., and Schooley, D. A., Science *210* (1980) 336.
14 Bergot, B. J., Baker, F. C., Cerf, D. C., Jamieson, G., and Schooley, D. A., in: Juvenile Hormone Biochemistry, p. 33. Eds G. E. Pratt and G. T. Brooks. Elsevier, Amsterdam 1981.
15 Baker, F. C., Tsai, L. W., Reuter, C. C., and Schooley, D. A., Insect Biochem. *17* (1987) 989.
16 Schooley, D. A., Judy, K. J., Bergot, B. J., Hall, M. S., and Jennings, R. C., in: The Juvenile Hormones, p. 101. Ed L. I. Gilbert. Plenum Press, New York 1976.
17 Brindle, P. A., Schooley, D. A., Tsai, L. W., and Baker, F. C., J. biol. Chem. *263* (1988) 10653.
18 Bhaskaran, G., Sparagana, S. P., Barrera, P., and Dahm, K. H., Arch. Insect Biochem. Physiol. *3* (1986) 321.
19 Yamamoto, R. T., J. econ. Ent. *62* (1969) 1427.
20 Slade, M., and Zibitt, C. H., in: Insect Juvenile Hormones: Chemistry and Action, p. 155. Eds J. J. Menn and M. Beroza. Academic Press, New York 1972.
21 Wisniewski, J. R., Rudnicka, M., and Kochman, M., Insect Biochem. *16* (1986) 843.
22 Share, M. R., Venkatesh, K., Jesudason, P., and Roe, R. M., Arch. Insect Biochem. Physiol. *8* (1988) 173.
23 Jesudason, P., Anspaugh, D. D., and Roe, R. M., Arch. Insect Biochem. Physiol. *20* (1992) 87.
24 Nakanishi, K., Schooley, D. A., Koreeda, M., and Dillon, J., J. Chem. Soc., Chem. Commun. (1971) 1235.
25 Bergot, B. J., Ratcliff, M., and Schooley, D. A., J. Chromatogr. *204* (1981) 231.
26 White, A. F., Life Sci. *11* (1972) 201.
27 Yang, R. S. H., Pelliccia, J. G., and Wilkinson, C. F., Biochem. J. *136* (1973) 817.
28 Yang, R. S. H., and Wilkinson, C. F., Comp. Biochem. Physiol. *46B* (1973) 717.
29 Slade, M., and Wilkinson, C. F., Comp. Biochem. Physiol. *49B* (1974) 99.
30 Rees, H. H., in: Ecdysone – From Chemistry to Mode of Action, p. 28. Ed J. Koolman. Thieme, Stuttgart 1989.
31 Ajami, A. M., and Riddiford, L. M., J. Insect Physiol. *19* (1973) 635.
32 Zimowska, G., Rembold, H., and Bayer, G., Arch. Insect Biochem. Physiol. *12* (1989) 1.
33 Hanzlik, T. N., and Hammock, B. D., J. biol. Chem. *262* (1987) 13584.
34 Venkatesh, K., Abdel-Aal, Y. A. I., Armstrong, F. B., and Roe, M. R., J. biol. Chem. *265* (1990) 21727.
35 Jesudason, P., Venkatesh, K., and Roe, R. M., Insect Biochem. *20* (1990) 593.
36 Coudron, T. A., Dunn, P. E., Seballos, H. L., Wharen, R. E., Sanburg, L. L., and Law, J. H., Insect Biochem. *11* (1981) 453.
37 Goodman, W. G., and Chang, E. S., in: Comprehensive Insect Physiology, Biochemistry and Pharmacology, p. 491. Eds G. A. Kerkut and L. I. Gilbert. Pergamon, Oxford 1985.
38 Schooley, D. A., Bergot, B. J., Goodman, W., and Gilbert, L. I., Biochem. biophys. Res. Commun. *81* (1978) 743.
39 Peterson, R. C., Reich, M. F., Dunn, P. C., Law, J. H., and Katzenellenbogen, J. A., Biochemistry *16* (1977) 2305.
40 Prestwich, G. D., Robles, S., Wawrzenczyk, C., and Bühler, A., Insect Biochem. *17* (1987) 551.
41 Judy, K. J., Schooley, D. A., Dunham, L. L., Hall, M. S., Bergot, B. J., and Siddall, J. B., Proc. natl Acad. Sci. USA *70* (1973) 1509.
42 Dahm, K. H., Bhaskaran, G., Peter, M. G., Shirk, P. D., Seshan, K. R., and Röller, H., in: The Juvenile Hormones, p. 19. Ed L. I. Gilbert. Plenum Press, New York 1976.
43 Granger, N. A., Niemiec, S. M., Gilbert, L. I., and Bollenbacher, W. E., J. Insect Physiol. *28* (1982) 385.
44 Janzen, W. P., Menold, M., and Granger, N. A., Physiol. Ent. *16* (1991) 283.
45 Unni, B. G., Bhaskaran, G., Dahm, K. H., and Hayes, T. K., Arch. Insect Biochem. Physiol. *17* (1991) 129.
46 Sparks, T. C., Allen, L. G., Schneider, F., and Granger, N. A., Arch. Insect Biochem. Physiol. *11* (1989) 93.