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Effects of allatotropin and allatostatin on in vitro production of juvenile hormones by the corpora allata of virgin females of the moths of *Heliothis virescens* and *Manduca sexta*

P.E.A. Teal*

Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, 1700 SW 23rd Drive, Gainesville, FL 32604, USA

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Abstract

Retrocerebral complexes (RCs) were isolated from adult females of the moths *Heliothis virescens* and *Manduca sexta*. Different homologs of juvenile hormone (JH) produced by the isolated RCs were identified and amounts measured by capillary gas chromatography-chemical ionization (isobutane)-mass spectroscopy. Only JH I, II and III were identified. Incubation of RCs from both species in media containing acetate, but no propionate, induced production of approximately equal amounts of JH II and JH III, but the amount of JH I present was very low in all samples. Incubation of RCs with synthetic *Manduca sexta* allatotropin stimulated significant increases in production of all three homologs but increases in JH I and JH II were greater than those for JH III. The effect of allatotropin was mimicked by addition of propionate to the medium, which indicated that allatotropin increased supply of acetyl- and propionyl-CoA precursors. Incubation of tissue from *H. virescens* females during the first 24 h after eclosion with synthetic *Manduca sexta* allatostatin did not reduce production of JH. However, incubation of tissue from 3-day-old females with allatostatin significantly reduced production of JH. Similarly, incubation of tissue from *H. virescens* females during the first 24 h after eclosion with both allatotropin and allatostatin did not increase JH over the amount present in extracts from tissue incubated without the neuropeptides, indicating that allatostatin negated the action of allatotropin. Incubation of tissue from *H. virescens* females with allatostatin plus farnesol or JH III acid resulted in significant production of JH III, but neither JH I nor JH II was detected. These findings indicated that allatostatin acts prior to formation of the sesquiterpene alcohol precursors of JH. © 2002 Published by Elsevier Science Inc.

Keywords: Insect neuropeptides; Lepidoptera; Terpene biosynthesis; Endocrinology

1. Introduction

Insect juvenile hormones are critical developmental hormones that have direct effects on both larval development and adult reproductive competence. Most insect orders appear to synthesize a single JH homolog, methyl (2*E*,6*E*)-10,11-epoxy-3,7,11-trimethyl-2,6-dodecadienoate (JH III) but Lepidoptera [17] and at least some Diptera [24] synthesize additional homologs. The usual homologs produced by the corpora allata of adult female Lepidoptera include JH III, JH II (methyl (2*E*,6*E*,10*cis*)-(10*R*,11*S*)-10,11-epoxy-3,7,11-trimethyl-2,6-tridecadienoate) and JH I (methyl (2*E*,6*E*,10*cis*)-(10*R*,11*S*)-10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate) [1,7,14,19,20]. Interestingly, the corpora allata of male Lepidoptera appear to lack the methyl transferase required to produce JH and, instead produce the acid analogs of JH [2,8]. Presumably, the acids are transported to the accessory glands where they are esterified to JH [2]. JH that is present in the male accessory glands of several species of Lepidoptera is transferred to the female during mating [2,13,18], where it may induce significant physiological and behavioral changes.

Although not well documented, regulation of JH production in adult female Lepidoptera is complicated and involves endogenous neural, neuroendocrine signals and, in some cases, male produced exogenous regulators transferred to the female during mating [6,9,13,17,20,23]. Among virgin females, JH is required for vitellogenesis [6,12,15,16,21,25] and, thus, females do not become reproductively competent until JH production is stimulated. To date, only two neuropeptides that regulate JH biosynthesis in adult Lepidoptera have been identified. These were iden-

* Tel.: +1-352-374-5788; fax: +1-352-374-5707.
E-mail address: pteal@gainesville.usda.ufl.edu (P.E.A. Teal).

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tified from the tobacco hornworm moth (*Manduca sexta*) (THW) and are an allatotropin (Manse-AT, Gly-Phe-Lys-Asn-Val-Glu-Met-Met-Thr-Ala-Arg-Gly-Phe-NH_2) [10] and an allatostatin (Manse-AST, *p*Glu-Val-Arg-Phe-Arg-Gln-Cys-Tyr-Phe-Asn-Pro-Ile-Ser-Cys-Phe-COOH) [11]. Although both neuropeptides have significant influence over JH biosynthesis by adult THW females surprisingly little work has been conducted on the effects of these neuropeptides. Studies by Kataoka et al. [10] demonstrated that the C-terminus of Manse-AT must be amidated and that truncated fragments having as few as the eight C-terminal amino acids were as effective in inducing JH biosynthesis as the intact peptide. Their work also showed that Manse-AT was not effective in stimulating JH biosynthesis in TBW larvae or pupae and adult females of *Tenebrio molitor, Schistocerca nitens,* or *Periplaneta americana,* but induced JH biosynthesis in adult females of the tobacco budworm moth, *Heliothis virescens* (TBW). Unni et al. [23] showed that analogs formed by substitution of Met by Nle at positions six and seven and acetylation of the N-terminus of the C-terminal nine amino acid fragment of Manse-AT had significant allatotropic activity. Their results suggested that Manse-AT stimulates a rate limiting enzyme in the biosynthesis of farnesoate but does not affect methylation or epoxidation of the acid [23]. Manse-AST is active in both TBW adults and larvae and in adult TBW females, but is inactive in adults of *T. molitor, P. americana and Melanoplus sanguinipes* [11]. Additionally, the results suggested that Manse-AST acts prior to the conversion of farnesoic acid to JH [11]. Although both Manse-AT and Manse-AST affect JH biosynthesis in adult THW and TBW females, no detailed studies have been conducted on how the effects are manifest. The following reports the results of studies in which the retrocerebral complexes (corpora cardiaca plus corpora allata) (RC) of adult females were incubated with either Manse-AT or Manse-AST or both. Biosynthesis of JH I, II and III in the presence of these neuropeptides was compared to those from unstimulated retrocerebral complexes.

2. Method

2.1. Chemicals

Capillary GC-MS grade ethyl acetate, hexane and methanol were from Burdick and Jackson and 18 megohm water was obtained from a Milli Q UVplus® water purification system. Tissue culture medium 199 containing Hank's salts and glutamine was obtained from Gibco. Synthetic Manse-AT was purchased from Sigma and Manse-AST was custom synthesized at the Interdisciplinary Center for Biotechnology Research, Protein Core Facility (University of Florida) using methods described elsewhere [11]. Both peptides were purified by reversed phase liquid chromatography as described elsewhere [4] and assessed to be ≥97% pure by analytical reversed phase liquid chromatography, mass spectroscopy and amino acid analysis.

(E,E)-3,7,11-trimethyl,2,6,10-dodecatrien-1-ol acetate (farnesyl acetate, FA) and (E,E)-farnesol were purchased from Aldrich. Synthetic JH I, II and III were a gifts from D. A. Schooley (University of Nevada, Reno, NV). These synthetics were purified by liquid chromatography using a Rheodyne 7125® injector, a Kratos Spectraflow 400® pump and Waters 410® differential refractometer using an Adsorbosil® silica column (250 × 4.6 mm, 5 μm particles) eluted with 5% ethyl acetate in hexane (flow = 1.5 ml/min). Mass spectral analysis of purified sesquiterpenes indicated that all were at least 98% pure and that they did not contain any of the other JH homologs. JH III acid was synthesized by saponification of JH III. JH III, dissolved in methanol, was added drop-wise to an equal volume of 2 M KOH and the mixture was stirred overnight at 25°C. The reaction mixture was neutralized and extracted with ethyl ether. JH III acid was purified by liquid chromatography under gradient conditions using an Adsorbosil® C18 column (250 × 4.6 mm, 5 μm particles) and detection with a Kratos Spectra Flow 757® variable wavelength detector set at 210 nm. The column was eluted using a linear gradient of 30% acetonitrile to 70% acetonitrile in H_2O over 40 min at 1 ml/min using a Kratos Spectra Flow 430® gradient former. Under these conditions JH III acid eluted at 24 min and JH III at 33 min. The fraction containing JH III acid was diluted by addition of an equal volume of H_2O and extracted with an equal volume of hexane, to remove apolar contaminants, prior to extraction three times with equal volumes of dichloromethane to extract the JH III acid. Analysis of the dichloromethane fraction by GC-Mass spectroscopy indicated that the JH III acid was free of JH III. An aliquot of the acid was dissolved in ethanol and derivatized to the methyl ester by addition of an equal volume of hexanes containing 2 M trimethylsilyldiazomethane (Aldrich) and stirring for 1 h. Analysis of the derivatized sample indicated that 98% of the acid had been esterified to JH III.

2.2. Insects

Female TBW moths were obtained as pupae from a colony maintained at the Center for Medical, Agricultural and Veterinary Entomology (USDA-ARS), Gainesville, FL and female THW were obtained from the Division of Neurobiology, University of Arizona, Tucson, AZ as late stage pupae. Adults, transferred to cages upon eclosion and before the wings had expanded, were provided with a 5% sucrose solution soaked onto commercial cotton balls and were held under a normal photoperiod of 12:12 (L:D) h at 26 ± 2° and 60 ± 5% relative humidity until use. The RC, containing the corpora allata and corpora cardiaca, of females of differing ages were dissected from the head under tissue culture medium 199. No attempt was made to separate the corpora allata from the corpora cardiaca so as to minimize tissue damage. RCs were placed in 30 μl of medium 199 contain-

ing 2% Ficoll 400, 72 mg/ml $CaCl_2$ and 0.6 mM sodium acetate (NaOAc) with or without 0.6 mM sodium propionate (NaOPr) in a conical amber vial [22] and incubated for various time periods. For most experiments RCs from THW females were incubated for the first 4 h in media alone and subsequently transferred to new media containing various substrates and/or neuropeptides for an additional 4 h. Thus, paired comparisons were obtained. The size and rates of JH biosynthesis by RCs of TBW females precluded transfer to different media. Therefore, for these experiments some RCs were incubated for 24 h in media containing precursors and/or neuropeptides while others were incubated in just media at the same time. When RCs were incubated with neuropeptides the incubation medium was supplemented by addition of Manse-AT or Manse-AST or both to yield final concentrations of each peptide of 10 nM. In some instances tissues were incubated in media containing 10 nM Manse-AST and 0.1 μM of either farnesol or JH III acid. In these cases farnesol, or JH III acid was first dissolved in a solution of medium 199 containing 10% acetone and appropriate amounts of this solution were added to the incubation medium prior to adding tissues. Incubations were stopped by addition of 50 μl each of first methanol and then hexane containing 10 pg/ul FA as internal standard. Samples were vortexed at 3200 rpm for 2 min, centrifuged at 18000$\times$ g for 5 min, to break the emulsion, and the organic layer removed. The aqueous layer was extracted two additional times with 50 μl of hexane. The organic fractions were combined in a clean vial and concentrated under N_2 to ca. 50 μl prior to mass spectral analysis.

2.3. Mass spectral analysis of JH

Extracts of incubations of RCs were analyzed by chemical ionization mass spectroscopy (MS) using a Finnigan-Matt ITS 40® ion trap mass spectrometer (MS) interfaced to a Varian Star 3400® gas chromatograph having a cool-on-column injector as described elsewhere [22]. The analytical column, a 30 m $\times$ 0.25 mm (id) DB5-MS® (0.1 μm film thickness) (J&W) was interfaced to a 10 m $\times$ 0.25 mm (id) uncoated, deactivated fused silica retention gap and a 10 cm $\times$ 0.5 mm (id) length of uncoated, deactivated fused silica in the injector. Conditions of chromatography were: initial injector temperature = 40°C for 30 sec; injector temperature increased at 170°C/min to 270°C; initial column temperature = 40°C for 5 min; column temperature increased at 5°C/min to 210°C; He carrier gas linear flow velocity = 24 cm/sec; GC-MS transfer line temperature = 230°C. Under these conditions farnesyl acetate eluted at 32.3, JH III at 33.8, JH II at 35.4, and JH I at 37.3 min, respectively. The MS was operated in the chemical ionization (CI) mode using isobutane as reagent gas (partial pressure = 2.6×10^{-3} pa, ionization time = 1.5 millisec, reagent reaction time = 12 sec) under the following conditions: multiplier voltage = 1900 volts; manifold temperature = 130°C; emission current = 16 μamps; mass acquisition range = 60–350 amu; scan rate = 1 sec; scan mode = chemical ionization; isobutane reagent gas. Identification of JH homologs was based on comparison of fragmentation patterns (60–300 amu) and retention indexes of compounds eluting during analysis of natural product samples with those of synthetic standards. Quantification of amounts of JH homologs was based on ion intensities of six diagnostic ions for each homolog (JH I—m/e = 263, 245, 217, 161, 153, 111: JH II—m/e = 249, 231, 203, 147, 139, 111: JH III—m/e = 235, 217, 189, 147, 125, 111) and was accomplished as described by Teal et al. [22].

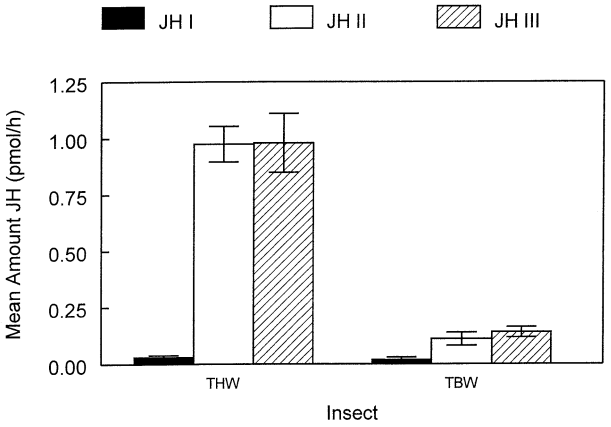

Fig. 1. Mean amounts of JH I, JH II and JH III recovered from incubations of RCs of day 1 females of THW and TBW moths. Incubations of tissue from THW females (n = 6) lasted 4 h while those for THW (N = 10) lasted 24 h. Error bars represent SEM.

3. Results

3.1. In vitro production of JH

Analyses of extracts obtained from in vitro incubations of RCs from both species, in medium containing no supplemental propionate, indicated the presence of JH I, JH II and JH III (Fig. 1). No other homologs of JH were found despite analysis of pooled extracts containing as much as 1 ng of JH III and the fact that the lower limit of sensitivity for full spectra (60–350 amu) for the JH homologs is ca. 3.0 pg (see [22]). JH II and JH III were present in approximately equal amounts. Amounts of JH I detected were always significantly lower than those of either JH II or III (Fig. 1). Extracts obtained from incubations of tissue from TBW females of different ages showed that total JH production was low during the first 24 h post eclosion, peaked between days 3–4 and declined to low levels by day 8 (Fig. 2a). The ratio of components remained relatively constant for all ages with JH II and III being the predominant homologs (Fig. 2b).

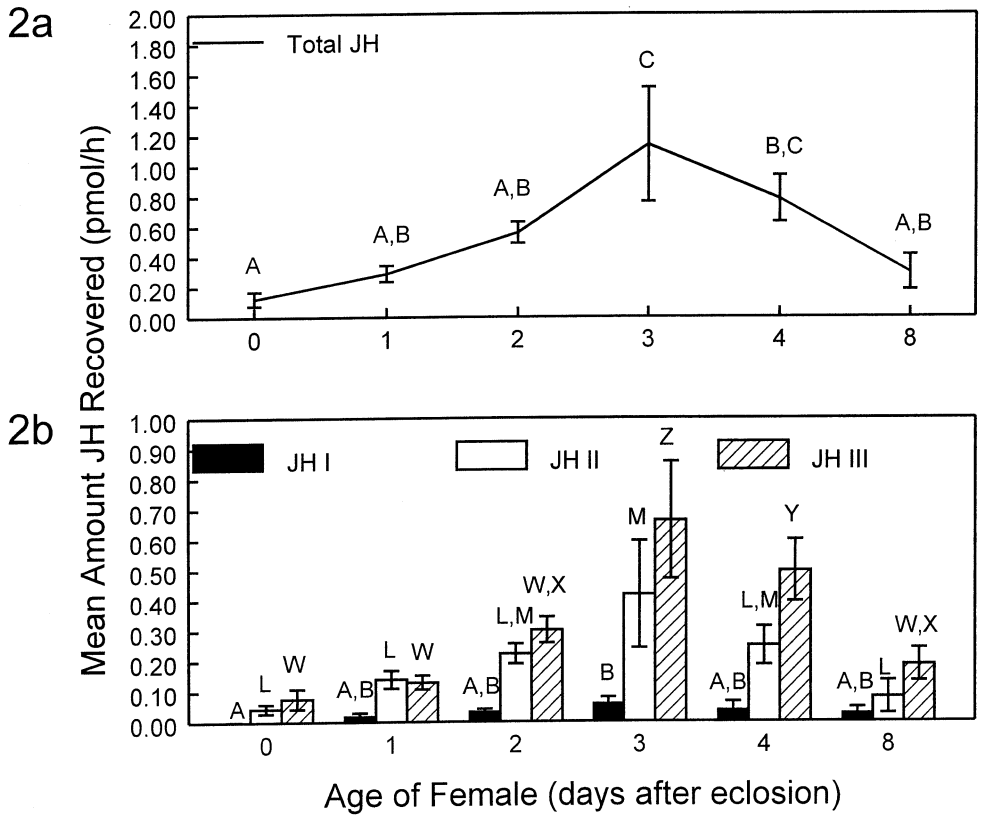

Fig. 2. Effect of age on in vitro biosynthesis of JH homologs by RCs of female TBW (n = 10/age). 2a: Mean total amounts of all three homologs (±SEM). 2b: Mean amounts (±SEM) of JH I, JH II and JH III. Means of each individual homolog superscribed by the same letter are not different in a Fishers Least Significant Difference Test (P = 0.05) performed after an analysis of variance indicted differences among the means.

3.2. Effect of in vitro incubation with Manse-AT and/or precursors of JH

Addition of NaOPr, in equal molar concentration to that of NaOAc present in the medium (0.6 mM) induced significant increases in the amounts of JH I and JH II produced by both species. Amounts of JH III were elevated but not to the extent that the higher homologs increased (Figs. 3, 4). Similarly, incubation of RCs from day 0 females of both TBW and THW with Manse-AT induced significantly more production of all three homologs than was produced by control tissue incubated without the neuropeptide (Figs. 3, 4). However, relative increases in amounts of JH I and JH II were significantly greater than that for JH III for both THW and TBW. The relative increases in all three JH homologs were similar to increases obtained when RCs were incubated in media containing equimolar amounts of sodium acetate and sodium propionate (Figs. 3, 4). Indeed, when tissue from THW was incubated in media containing equimolar amounts of sodium acetate and sodium propionate plus 10 nmol Manse-AT the amounts of JH produced (JH III = 1.08 ± 0.14 [t = 0.94, 8 df]; JH II = 1.57 ± 1.0 [t = 1.36, 8 df]; JH I = 0.031 ± 0.015; [t = 0.18, 8 df] n = 5) were no different from amounts produced when equimolar amounts of sodium acetate and sodium propionate were used (JH III = 0.88 ± 0.17; JH II = 1.21 ± 0.05; JH I = 0.029 ± 0.027; n = 5).

3.3. Effect of in vitro incubation with Manse-AST with or without farnesol, or JH III acid

In vitro incubation of RCs from 3-day-old TBW females in media containing Manse-AST resulted in a significant decrease in amounts of JH recovered (Fig. 5). However, incubation of tissue from day-0 TBW females in the presence of Manse-AST did not reduce production of JH when compared with tissue incubated without the peptide. By comparison, co-incubation of tissue from either 0-day old (Fig. 5) or 3-day-old females (data not shown) with both Manse-AT and Manse-AST had a significant affect on JH production. In fact, the amounts of each JH homolog recovered from these samples were no different from those recovered from either control incubations or incubations in which only Manse-AST was added. No evidence for the presence of farnesol, farnesal or farnesoic acid was found in any of the samples incubated with Manse-AST or Manse-AST plus Manse-AT.

To determine if Manse-AST inhibited JH biosynthesis, either before or after formation of the sesquiterpene alcohol precursors, RCs were incubated with either Manse-AST alone or Manse-AST plus farnesol. Extracts from incubations with Manse-AST alone contained essentially no JH. However, inclusion of farnesol in the incubation medium stimulated significant production of JH III (Fig. 6). Simi-

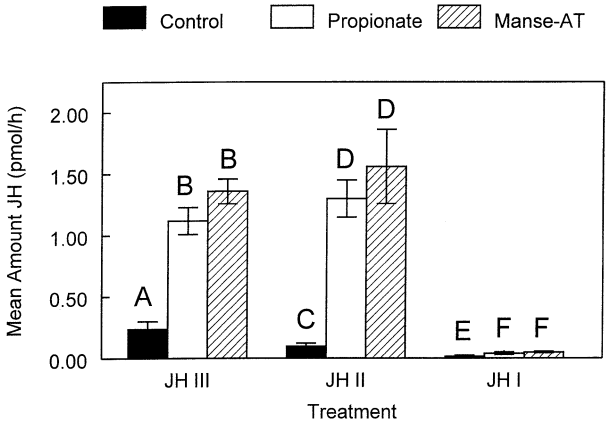

Fig. 3. Effect of addition of either 0.6 mM NaOPr or 10nM Manse-AT to culture medium on production of JH homologs by RCs of newly eclosed THW females. Tissue was incubated for 4 h in the control media (without NaOPr or Manse-AT) (n = 12) and then 6 were transferred to media containing NaOPr and another 6 were transferred to media containing Manse-AT and incubated for an additional 4 h. Mean amounts (±SEM) of the individual homologs for each treatment superscribed by the same letter are not different in a Fishers Least Significant Difference Test (P = 0.05) performed after an analysis of variance indicted differences among the means.

larly, incubation of tissues with Manse-AST plus JH III acid resulted in production of significantly large amounts of JH III (Fig. 6). JH I and II were not detected in any of the samples.

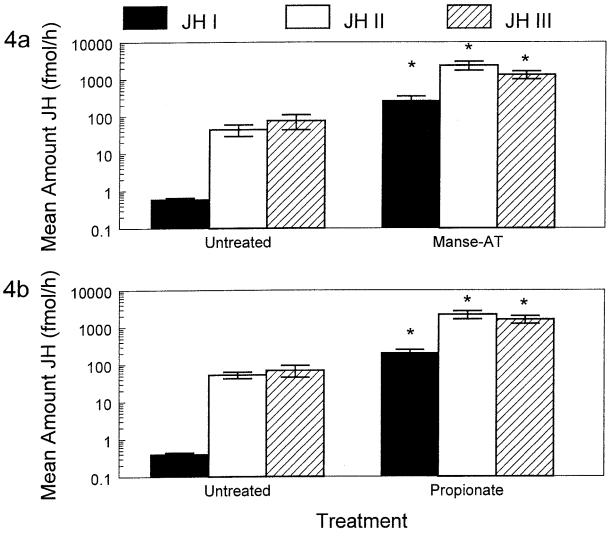

Fig. 4. Effect of addition of either 0.6 mM NaOPr or 10nM Manse-AT to culture medium on production of JH homologs by RCs of newly eclosed TBW females. RCs from day 0 females were incubated in media containing no NaOPr or Manse-AT (controls) or containing either NaOPr (4a) or Manse-AT (4b) for 24 h. Means (n = 6/treatment, ±SEM) of each homolog superscribed by * were significantly greater than those of the controls in a Students T test (P = 0.01) for each experiment. Means for both sets of controls and were not different and no differences were found between extracts of tissues incubated in NaOPr or Manse-AT (P = 0.05) although the experiments were performed on different days.

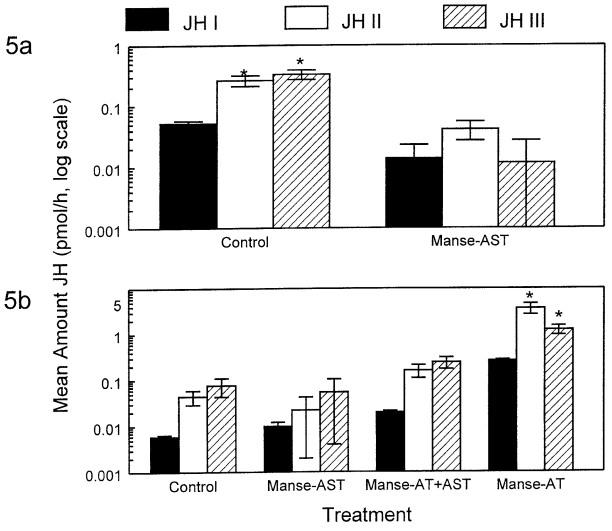

Fig. 5. Effects of Manse -AST on inhibition of JH production by RCs of TBW females. 5a: Mean amounts (± SEM) of JH I, JH II, and JH III from extracts of RCs of 3-day old females incubated in media with or without Manse-AST (n = 6/treatment). Means superscribed by * were significantly greater than those of the controls in a Students T test at P = 0.05 for JH I and P = 0.01 for JH II and JH III. 5b: Mean amounts (±SEM) of JH I, JH II, and JH III from extracts of RCs of 0-day old females incubated in media with or without Manse-AST and Manse-AT (n = 5/treatment). Means for each homolog incubated with Manse-AT were significantly greater than means for any other treatment in a Fishers Least Significant Difference Test (P = 0.01) performed after an analysis of variance indicted differences among the means. No other differences were found.

4. Discussion

In initial analyses data were obtained on the effect of age of adult females on in vitro biosynthesis of JH by isolated RCs of female TBW. As was found in earlier work with RCs of female THW [22] only three homologs, JH I, JH II and JH III, were identified from extracts from TBW females. This was expected because other in vivo and in vitro studies reported only JH I, JH II and JH III [12,20]. However, our results with TBW indicated that the ratio of the three homologs was different from that found from in vitro studies conducted by Park et al. [13]. These authors recovered significantly more JH II than JH III from HPLC analyses of extracts of CA from 3-day-old females incubated with ^{3}H-methionine. Extracts obtained from in vitro studies in medium containing NaOAC but no NaOPR indicated that the amount of JH III detected was no greater that of JH II (Figs. 1, 2). The results showed that the capacity of isolated RCs to synthesize JH was low during the first 24 h after emergence but increased with increasing age (Fig. 2). Maximal production of JH occurred between 3–4 days after emergence and by the 8th day after emergence the amount of JH biosynthesized was as low as that produced by newly eclosed females. These results were similar to those from in vitro incubations of the CA from females of other noctuid moths including the corn earworm moth, a close relative [15] and the army worm, *Pseudaletia unipuncta* [5]. How-

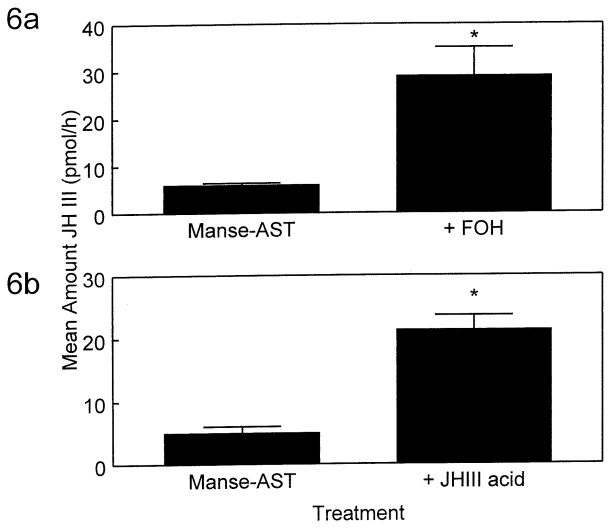

Fig. 6. Comparison of effects of incubation of either farnesol or JH III acid on JH III production by RCs of day-0 TBW females incubated in media containing Manse-AST. 6a: Mean amount of JH III (±SEM, $n = 5$/treatment) recovered from control (Manse-AST only) and farnesol (FOH) treated samples. 6b: Mean amount of JH III (±SEM, $n = 5$/treatment) recovered from control (Manse-AST only) and JH III acid treated samples. In both cases significant JH III production occurred as indicated by * (Students T test; $P = 0.01$). No differences were found between controls for each experiments and the amounts of JH III produced in the presence of farnesol or JH III acid were not different although experiments were not conducted on the same day.

ever, the results were considerably different from studies in which circulating levels of JH were determined from extracts of hemolymph of adult female moths. Indeed, amounts of JH present in extracts of hemolymph from female TBW have been reported to be the highest 24 h after adult eclosion and to drop to negligible amounts between 30–36 h after eclosion before rising again [20]. Similarly, in the pyralid moth, *Diatraea grandiosella*, circulating levels of JH are highest at eclosion and decline to low levels at 24 h after emergence [19] and in the tomato moth circulating JH is highest on the first day of adult life and then declines significantly over the next several days [7].

The discrepancy between the amounts of JH present in hemolymph samples of TBW females [20] and those found in in vitro studies suggested that removal of the CA from the moths may have eliminated allatotropic factors functioning in the intact moth (see [7]). To address this, in vitro studies were conducted in which tissue from both THW and TBW females were incubated in the presence of Manse-AT. The RCs from newly eclosed females were used for these experiments because they produced the least amounts of the JH homologs. Indeed, incubation with Manse-AT induced the production of significantly greater amounts of all three JH homologs for both species (Fig. 3, 4). However, relative increases in amounts of JH I and JH II were greater than the relative increase in production of JH III. The results for both species were in agreement with studies conducted by Unni et al. [23] who found that truncated analogs of Manse-AT, containing the active core of the neuropeptide, induced the CA of THW females to release significantly more of each of the three JH homologs with JH II being released at a higher rate than either JH I or JH III. The fact that JH II and JH I were produced in significantly higher relative amounts than JH III, coupled with the fact that medium 199 contains 0.6 mM NaOAC, but no propionate, suggested that Manse-AT was increasing availability of propionate. If this were so I hypothesized that it could be possible to enhance production of JH I and JH II by simply increasing supply of propionate in the medium. This was, in fact, the case because incubation of RCs from both species in medium containing equal molar amounts of NaOAC and NaOPR induced very significant increases in production of JH I and JH II. In fact, the relative increases in JH II over controls were equal for tissue incubated in either Manse-AT or medium supplemented with propionate. Interestingly, small, but significant, increases in production of JH III were also detected in extracts from both THW and TBW females. These data suggest that Manse-AT acts to increase supply of acetate and propionate required for JH biosynthesis by increasing the activity of transaminases responsible for conversion of valine to propionyl-CoA, leucine to acetyl-CoA and isoleucine to both acetyl- and propionyl-CoA [3]. Currently experiments using culture media containing no NaOAC or NaOPR but supplemented with valine, leucine and isoleucine are being conducted to more fully understand the effects of Manse-AT on increasing supply of both acetyl- and propionyl-CoA.

In initial studies on TBW females, no inhibition of JH production occurred when RCs from newly eclosed females were incubated in media containing Manse-AST. However, when RCs from 3-day-old females, the age at which JH production was at maximum (Fig. 2), were incubated with Manse-AST, production of JH I was reduced by 3.8-fold, JH II by 6.4-fold and JH III by 28.2-fold (Fig. 5). Thus, Manse-AST had a dramatic effect on JH biosynthesis. To determine if the effect could be manifest in day 0 females, RCs from one group were incubated in Manse-AT to stimulate JH production and another group were incubated in equal molar amounts of Manse-AT and Manse-AST. Results of this experiment showed conclusively that Manse-AST completely negated the effects of Manse-AT on stimulation of JH production (Fig. 5). However, only small amounts of JH were produced in all cases in which Manse-AST was added to the medium and Manse-AST had no effect on JH production by RCs obtained from day 0 females. Therefore, the CA cannot be completely inhibited from producing JH by Manse-AST.

Manse-AST inhibited production of all three naturally produced JH homologs when co-incubated with Manse-AT (Fig. 5) and no evidence for the presence of farnesol, or farnesal or any of the higher sesquiterpene homologs (e.g. homofarnesol, dihomofarnesol) of these precursors (see [17]) was found in samples incubated with Manse-AST or Manse-AST plus Manse-AT. Thus, allatotropin functioned prior to formation of the sesquiterpene alcohol precursors as

was hypothesized by Kramer et al. [11] for THW. When rescue experiments in which tissue was incubated in the presence of Manse-AST plus farnesol or JH III acid were performed and the results compared to those from tissue incubated with only Manse-AST it was obvious that addition of either farnesol or JH III acid induced significant production of JH III (Fig. 6). However, essentially no JH I or JH II was produced in either control group incubated with Manse-AST or groups incubated with Manse-AST and farnesol or JH III acid. This indicated that biosynthesis of JH III resulted solely from addition of farnesol or JH III acid to the incubation medium and that Manse-AST acts to inhibit JH biosynthesis at a point prior to production of the sesquiterpene alcohol precursors. However, the fact that JH III biosynthesis was inhibited to a greater extent by Manse-AST than production of the other two homologs, despite the fact that NaOAC was present in relatively large amounts (ca 0.6 mM) indicates that inhibition occurs after production of either acetyl- or propionyl-CoA.

In summary the results presented here have demonstrated that both Manse-AT and Manse-AST act on the corpora allata of adult TBW females. However, the effects of Manse-AST were manifest only in glands that were actively engaged in biosynthesis of significant amounts of JH. The results with both TBW and THW females shows that stimulation by allatotropin probably occurs by increasing supply of acetyl- and propionyl-CoA by inducing transaminases to convert valine, leucine and isoleucine to these CoA esters. The action of Manse-AST is also manifest early in biosynthesis because the inhibitory action of the peptide was alleviated by addition of either farnesol or JH III acid. Moreover, sesquiterpene alcohol precursors of the JH homologs were not found in extracts from incubations in media containing Manse-AST. However, Manse-AST acts after synthesis of acetyl- and propionyl-CoA, because inhibition of JH III biosynthesis was substantially greater than that of JH I and JH II despite the presence of relatively large amounts of acetate in the incubation media.

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