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Juvenile hormone synthesis by corpora allata of tomato moth, *Lacanobia oleracea* (Lepidoptera: Noctuidae), and the effects of allatostatins and allatotropin in vitro*

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Abstract. The nature and rate of juvenile hormone (JH) biosynthesis and effects of allatostatins and allatotropin have been investigated in isolated corpora allata (CA) of adults and larvae of the noctuid tomato moth, *Lacanobia oleracea*. In adult female CA, mean rates of synthesis were relatively constant (10–16 pmol/pr/h) at all times. However, the range of JH synthesis by individual CA of similarly aged insects was quite large (2–30 pmol/pr/h). High performance liquid chromatography (HPLC) separation shows that adult female moth CA synthesise predominantly JH I and JH II. Rates of JH synthesis in vitro are dependent on methionine concentration. Synthetic *Manduca sexta* allatostatin (Mas-AS) caused a dose-dependent inhibition of JH synthesis by adult female CA but only to a max. of 54%, whilst 10 μM synthetic *M. sexta* allatotropin caused a 37% stimulation of CA activity. At 1 mM the cockroach allatostatin, Dip-allatostatin-2, had no significant effect on JH synthesis. In larval *L. oleracea*, rates of JH biosynthesis were very low (< 10 fmol/pr/h) and could only be measured by incubating several pairs of CA with a methionine radiolabel of high specific activity (2.96 TBq/mmol). Using LC and enzyme linked immunosorbent assay (ELISA) to Mas-AS, two peaks of Mas-AS-like immunoreactivity were detected in larval brain homogenates of *Spodoptera littoralis*, but none in *Tenebrio molitor*.

INTRODUCTION

Juvenile hormones (JHs) in insects are known to influence a number of physiological processes, including reproduction, development, diapause, polymorphism and pheromone production (Tobe & Stay, 1985; Riddiford, 1994; Wyatt & Davey, 1996). Although a number of JHs have been identified, it appears that most insects produce predominantly JH III. In lepidopterans, however, JHs other than JH III have been identified (Schooley et al., 1984).

Juvenile hormones are synthesised by the corpora allata (CA) which are in turn regulated by stimulatory allatotropins and inhibitory allatostatins, both produced in the brain. To date, only one allatotropin (Mas-AT: G-F-K-N-V-E-M-M-T-A-R-G-F-NH_2) has been isolated and characterised (Kataoka et al., 1989), this from the moth *Manduca sexta* (Lepidoptera: Sphingidae). In contrast, a large extended family of up to 14 allatostatins per species has been characterised from three species of cockroaches: *Diploptera punctata* (see Bendena et al., 1997), *Periplaneta americana* (Weaver et al., 1994) and *Blattella germanica* (Bellés et al., 1994). These peptides have a common C-terminus, Y/F-X-F-G-L/I-NH_2 (where X is A, G, S, or N). Cockroach-like allatostatins have also been identified in a number of other insects (flies, crickets, locust, moths, honey bee; Duve et al., 1993, 1994, 1995, 1997; Lorenz et al., 1995; Veelaert et al., 1996; Veenstra et al., 1997; Davis et al., 1997), but have not been shown to be functional as allatostatins in the native insect (Duve et al., 1994, 1995; Vanden Broeck et al., 1996; Veelaert et al., 1996). Kramer et al. (1991) characterised a distinct allatostatin from *M. sexta*, (pE-V-R-F-R-Q-C-Y-F-N-P-I-S-C-F-OH). This *M. sexta* allatostatin (Mas-AS) was shown to inhibit JH biosynthesis in the CA of *M. sexta* fifth instar larvae and newly emerged adult females, and also in CA of adult female *Heliothis virescens* (Lepidoptera: Noctuidae). In the true army worm, *Pseudaletia unipuncta* (Lepidoptera: Noctuidae), a brain cDNA which encodes a 125 amino acid polypeptide containing the Mas-AS sequence has been characterised (Jansons et al., 1996), but Mas-AS only produces a 60% inhibition in five day old adult females. In addition, Kramer et al. (1991) have shown that Mas-AS had no effect on the CA of the beetle *Tenebrio molitor* (Coleoptera: Tenebrionidae), the grasshopper *Melanoplus sanguinipes* (Orthoptera: Acrididae) or the cockroach *Periplaneta americana* (Dictyoptera: Blattidae). Although little is known of the regulation of JH biosynthesis in Lepidoptera generally, it is perhaps possible that Mas-AS allatostatic functionality is specific to this group.

Recently, Audsley et al. (1998) reported the presence of Mas-AS immunoreactivity in the nervous system and haemolymph of the tomato moth (or bright-line brown-eye), *Lacanobia oleracea* (Lepidoptera: Noctuidae), but virtually nothing is known of JH biosynthesis or its control in this moth. This study investigates the nature of JH biosynthesis in larval and adult female *L. oleracea* and the regulatory effects of Mas-AS. For comparative purposes, data concerning both the effects of an allatotropin,

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Mas-AT, and of a cockroach allatostatin, Dip-allatostatin 2 (Dip-AS2: A-Y-S-Y-V-S-E-Y-K-R-L-P-V-Y-N-F-G-L-NH_2), on the CA of *L. oleracea*, and of Mas-AS on the CA of *M. sexta*, are also included. Further, the brain content of Mas-AS-like immunoreactive peptide during the development of *L. oleracea* is discussed in relation to the known titres of JH in this insect. Additional data are also presented on the enzyme linked immunosorbent assay (ELISA) detection of a Mas-AS-like immunoreactive peptide in the Egyptian cotton leafworm, *Spodoptera littoralis* (Lepidoptera: Noctuidae), and its absence from the beetle, *T. molitor*.

MATERIAL AND METHODS

Animals

L. oleracea and *S. littoralis* were reared at 20°C and 65% r.h. under a 16L : 8D photoperiod. Larvae were fed on a maize-flour based noctuid artificial diet (Korano Ltd, Montalieu, France), and adults on a 10% honey, 2 g/l (w/v) methyl-4-hydroxybenzoate and 2% (v/v) mixed vitamin solution. *M. sexta* were reared from eggs supplied by Prof. S.E. Reynolds, University of Bath, UK. *T. molitor* were bred at 27°C, on a mixture of wheatfeed, rolled oats and yeast (5 : 5 : 1 w/w/w).

JH biosynthesis assay

Corpora allata were dissected from larval and adult *L. oleracea* and the rate of JH biosynthesis determined by the incorporation of a radiolabel using procedures based on those previously described by Pratt & Tobe (1974), Feyereisen & Tobe (1981), and Cusson et al. (1990). In routine assays, pairs of CA were incubated in 100 µl tissue culture medium (TC-199; Gibco) which contained Hanks salts and 25 mM HEPES buffer, pH 7.2, and to which was added 2% Ficoll, 0.1% BSA, 0.3% bacitracin, 5 mM $CaCl_2$, 53 mM sodium propionate, 30 mM sodium acetate, and 0.37–3.7 MBq/ml of ^{14}C or ^{3}H methionine. Radiochemicals were as follows: L-[methyl-^{14}C] methionine, 2.04–2.10 GBq/mmol; L-[methyl-^{3}H] methionine, 7.4 GBq/mmol (both purchased from NEN Life Sciences); L-[methyl-^{3}H] methionine, 2960 GBq/mmol (purchased from Nycomed Amersham plc). Tissues were incubated at 30°C for 3 h, after which CA were removed and 20 µg synthetic JH III (Fluka) was added as cold carrier. Juvenile hormones secreted by the CA were extracted in 400 µl iso-octane. Samples were vortexed briefly and centrifuged at 1,100 g for 10 min at ambient temperature. Two hundred microlitres of the organic phase were added to 3 ml Ecoscint A (National Diagnostics) for counting on a Beckman LS 6000TA liquid scintillation spectrometer. For analysis by high performance liquid chromatography (HPLC) a mixture of synthetic JHs (JH I, II, III) and methyl farnesoate (MF) was added and samples extracted in 3 × 500 µl hexane. Supernatants were pooled after centrifugation, dried under a stream of nitrogen, and resuspended in 100 µl hexane for injection onto HPLC column. Hexane extracts of samples incubated with high specific activity radiolabel (^{3}H methionine, 2.96 TBq/mmol) were routinely back-extracted in 2 volumes of water to reduce amount of unincorporated label.

High performance liquid chromatography

Normal-phase HPLC was performed using a Beckman System Gold chromatographic system, utilising a single pump programmable solvent module 126. Samples were loaded via a Rheodyne loop injector, and elution was monitored at 230 nm using a Beckman 166 UV detector. The hexane extract (containing cold and labelled JH) was injected onto a Zorbax 5 mm, 100 Å normal phase column (250 mm × 4.6 mm) fitted with a guard column (30 mm × 4.6 mm) of similar packing material. The column was eluted with 12% methyl-tert-butyl ether in 50% water saturated hexane over 15 min at a flow rate of 1 ml/min, and 0.5 ml fractions for liquid scintillation counting were collected into 3 ml Ecoscint A using a RediFRAC fraction collector. After each elution of the samples deriving from incubations with higher specific activity radiolabelled methionine (L-[methyl-^{3}H]-methionine, 2.96 TBq/mmol), the HPLC column was eluted with 100% methanol for 30 min (to remove contaminating levels of radio-isotope), and then equilibrated with ether/hexane for 30 min before the next run.

Synthetic peptides

Manduca sexta allatostatin and Dip-allatostatin2 were custom synthesised using solid phase methodology (Fmoc procedure) on an Applied Biosystems model 431A automatic peptide synthesiser at the Advanced Biotechnology Centre, Charing Cross and Westminster Hospital Medical School, London. *Manduca sexta* allatotropin was purchased from Sigma.

ELISA of *M. sexta* immunoreactive peptide in brain extracts

Relative amounts of Mas-AS-like immunoreactivity in LC fractions of brain extracts from last larval instar *S. littoralis*, and of adult female beetle, *T. molitor*, were measured using the ELISA procedure developed by Audsley et al. (1998). Details of the treatment of brain homogenates, and of the LC separation of brain extracts, were as previously described (Audsley et al., 1998).

RESULTS

JH biosynthesis by adult female *L. oleracea*

Using L-[methyl-^{14}C] methionine as label, the mean total amounts of JH produced by the CA in vitro of differently aged adult female *L. oleracea* (day 1 – day 7, day 10, 14) are represented in Fig. 1. Mean rates of synthesis between different days were relatively constant at all times (10–16 pmol/pr/h). However, if we examine the range of JH biosynthesis by isolated CA of day 2–4 adult female *L. oleracea* (Fig. 2), there is a vast variation (2–29 pmol/pr/h) between CA activities of individual, similarly-aged insects. Despite the wide range of JH production,

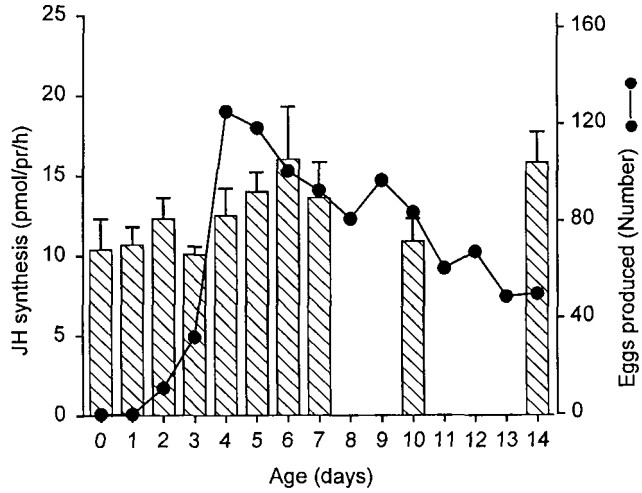

Fig. 1. Bar graph shows rates of juvenile hormone synthesis in vitro by corpora allata from adult female *Lacanobia oleracea* at different ages (means ± S.E., n = 4–14; no bar = no data collected). Also shown (line graph) are mean numbers of eggs laid by similarly aged females over the same period (egg production data adapted from Corbitt et al., 1996).

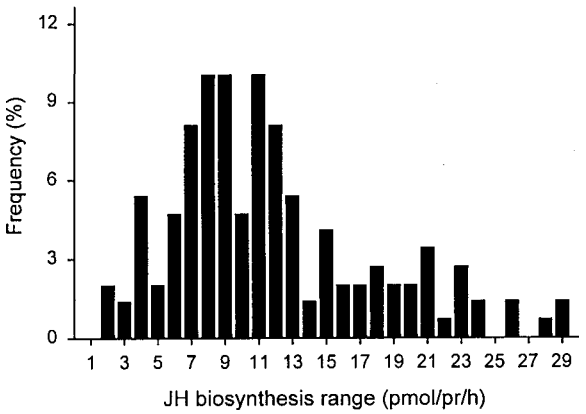

Fig. 2. JH biosynthesis of adult female *L. oleracea* CA in vitro as frequency of occurrence. Each bar represents a span of biosynthesis rates, 1.00–1.99, 2.00–2.99 etc. (pmol/pr/h; n = 145).

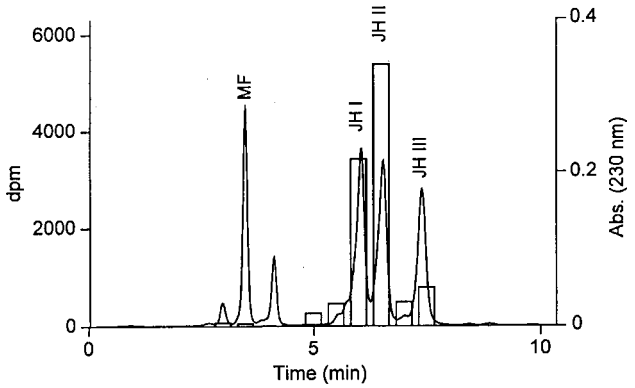

Fig. 3. HPLC separation of JH homologues secreted by adult female *L. oleracea* CA incubated in vitro. HPLC profile shows JH standards, open bars represent amount of radiolabelled JH in each fraction. MF = methyl farnesoate, JH I, II and III = juvenile hormone homologues. Note that the major portion of incorporated radioactivity elutes with authentic markers JH I and II.

52% of measured CA activities fall within the range 7.00–12.99 pmol/pr/h. The mean response for all values is 12.48 ± 0.55 pmol/pr/h (n = 145). Also shown in Fig. 1 are the values for mean egg production by adult females on successive days throughout the same period (data adapted from Corbitt et al., 1996).

Fig. 3 shows the HPLC separation of JH analogues (JH I, II, III & MF) and in each LC fraction the relative amounts of radiolabel (dpm) incorporated into JHs synthesised by the CA of day 3 adult female *L. oleracea*, and shows that adult females produce predominantly JH I and JH II. Table 1 shows the relative amounts of the various JH homologues detected by a series of in vitro incubations of 2–3 day adult female CA, compared to the proportions detected in vivo previously in 1–5 day old mated females (Edwards et al., 1995). Both studies show JH I and JH II predominating in broadly equivalent amounts. The present study shows rather more JH II than JH I, whereas the GC-MS data generally has this the other way round. Edwards et al. (1995) detected only relatively small amounts of JH III (< 0.06 ng/g wet weight; < 1%) and even lower amounts of JH 0 (0.15% of the total). Our present assignment of an average value of 8.38% to JH III from the in vitro incubations must therefore be somewhat tentative.

TABLE 1. Relative amounts of various JH homologues detected using the in vitro assay of JH synthesis by isolated *L. oleracea* CA compared to the proportions measured in vivo[a].

JH homologue	JH synthesised by adult female CA in vitro (%)	Total body JH measured by GC-MS/SIM in vivo[b] (%)
JH 0	n.d.	0.15
JH I	39.37	57.96
JH II	52.25	40.93
JH III	8.38	0.96

[a] In vivo data derived from Day 1 to Day 5 mated females (Edwards et al., 1995), average values.
[b] JH titre determination carried out using the method of Bergot et al. (1981).

Effect of methionine concentration on JH biosynthesis by adult female *L. oleracea*

The amount of JH biosynthesis by the CA of day 2–3 adult female moths is dependent on methionine concentration in the incubation medium, as demonstrated using differently radiolabelled methionine (Table 2). At a methionine concentration of 175 µM (using ^{14}C methionine: specific activity = 2.1 GBq/mmol) the mean rate of JH biosynthesis is 12.6 pmol/pr/h. This rate of biosynthesis drops by 45 and 88% when using ^{3}H methionine concentrations of 50 µM (7.4 GBq/mmol) and 1.25 µM (2.960 TBq/mmol), respectively. However, by increasing the methionine concentration of the latter radiolabel from 1.25 to 100 µM (reducing specific activity from 2.96 TBq/mmol to 35 GBq/mmol) the rate of JH biosynthesis can be increased from approximately 1 to 15 pmol/pr/h, demonstrating that in adult female *L. oleracea* JH biosynthesis is methionine dependent.

TABLE 2. Effect of radiolabel and methionine concentration on rate of total JH synthesis by the CA of 3 day adult female and Vth (CHC) larval instar *L. oleracea* (means ± S.E., n = 4–16).

Methionine radiolabel[a]	[Methionine] (µM)	Adult CA JH (pmol/pr/h)	Larval CA JH (fmol/pr/h)
^{14}C [2.1]	175	12.6 ± 1.13	0
^{3}H [7.4]	50	6.71 ± 0.93	0
^{3}H [2960]	1.25	0.97 ± 0.47	0.25 ± 0.09
^{3}H [35[b]]	100	15.1 ± 1.21	7.35 ± 1.61

[a] Final specific activity [GBq/mmol] shown in brackets.
[b] Diluted from 2960 GBq/mmol using cell culture tested L-methionine (Sigma, M 2893).

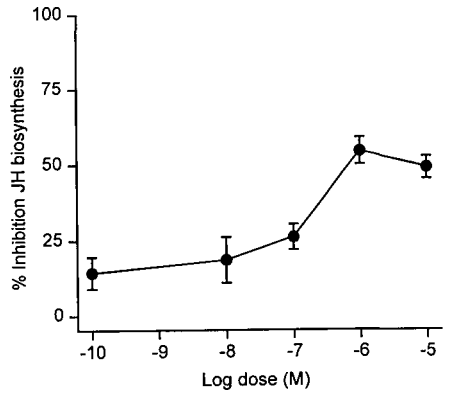

Fig. 4. The effect of Mas-AS on total JH secreted by adult female *L. oleracea* CA in vitro represented as a percentage inhibition compared to control values (means ± S.E., n = 7–9).

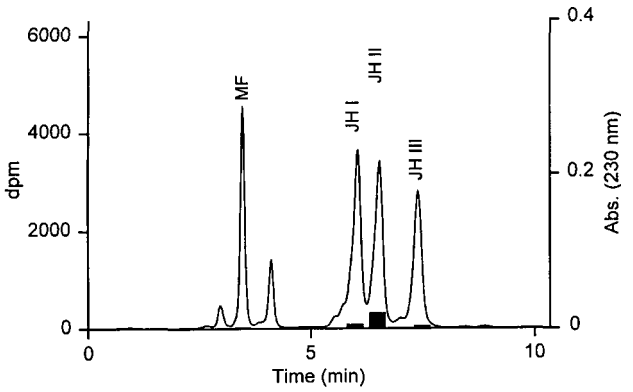

Fig. 5. HPLC separation of JH homologues secreted by fifth instar clear head capsule *L. oleracea* CA. HPLC profile shows JH standards, solid bars represent amount of radiolabelled JH in each fraction. MF = methyl farnesoate, JH I, II and III = juvenile hormone homologues. (Scale of the y-axis represents typical values obtained for radiolabel incorporation in adult CA).

Effect of Mas-AS on CA of adult female *L. oleracea*

Fig. 4 shows the dose-response effect of synthetic Mas-AS (0.1 nM–10 μM) on the inhibition of JH biosynthesis by isolated CA of day 2–3 adult female *L. oleracea*. Mas-AS did not completely inhibit JH biosynthesis, producing at best an average reduction of 54% compared to control values. As observed for individual rates of JH biosynthesis, levels of inhibition by Mas-AS varied widely from 0–92%. Concurrent assays of the same peptide preparation, using CA from *M. sexta*, revealed a median inhibition of 92% ($P < 0.0001$) for day 0, V^{th} (= last larval instar) larval insects, but only ca. 40% ($P < 0.02$) inhibition for CA from day 3 adult females (Table 3).

Effect of Mas-AT and Dip-AS2 on CA of adult female *L. oleracea*

At a dose of 10 μM Mas-AT caused a significant ($P < 0.02$) 37% increase in the rate of JH biosynthesis in day 3 adult female moths (Table 3). Conversely, Dip-AS2 at 1 μM caused no greater than 22% inhibition (not significant) of JH synthesis in similar preparations (Table 3).

JH biosynthesis by larval *L. oleracea*

No JH biosynthesis was detected when incubating CA of variously aged larval *L. oleracea* [V^{th} (= penultimate) instar day 4, day 5, and VI^{th} (= last) instar day 1] with ^{14}C methionine and ^{3}H methionine (specific activity: 7.4 GBq/mmol, see Table 2). However, using ^{3}H methionine of specific activity 2.96 TBq/mmol, five pairs of V^{th} instar clear head capsule slippage (CHC) larvae, and subjecting samples to HPLC it was possible to measure very small rates of JH biosynthesis in larval *L. oleracea* (Fig. 5). The total amount of JH produced was less than 1 fmol/pr/h, most of which co-eluted with JH II. Clearly the amount of JH produced by larval CA was substantially less (ca. 1,000 fold) than that produced by CA of adult female *L. oleracea*. In addition the amount of radioactivity per sample was also very low (50–300 dpm) which reduces accuracy of detection and measurement. This is also further compounded by problems associated with contamination of the HPLC column when using a high specific activity label which results in the appearance of false positives and the necessity to decontaminate the column between each sample run.

JH biosynthesis by larval CA could be increased 30-fold by raising the methionine concentration to 100 μM (Table 2). However, because this reduced the final specific activity of the radiolabel by approximately 100 fold, the amount of radioactivity per sample remained almost the same, and hence the ability to detect JH in relative terms did not increase.

Detection of Mas-AS-like immunoreactive peptide in other insects

Two peaks of Mas-AS-like immunoreactivity were detected in sub-fractions of reversed phase LC separations of Sep-pak (Waters) purified brain homogenates (75% ethanol/0.2 M HCl) of last larval instar Egyptian cotton leafworm, *S. littoralis*. Of these, the earlier eluting peak appeared to closely co-chromatograph with authentic Mas-AS. Both the relative elution positions of the peaks, and the approximate quantities detected, were closely similar to those observed previously for larval *L. oleracea* (see Audsley et al., 1998). No evidence of Mas-AS-like immunoreactivity was observed in brain extracts from adult female beetle, *T. molitor* (results not shown).

TABLE 3. Effect of Mas-AT and Dip-AS 2 on JH synthesis by CA of adult female *L. oleracea* and Mas-AS on JH synthesis by CA from larval and adult *M. sexta* (mean ± S.E., n = 4–8).

Peptide	Conc. (μM)	Insect	Stage	JH (pmol/pr/h) control	JH (pmol/pr/h) peptide
Mas-AT	10	*L. oleracea*	Adult ♀ (day 3)	22.78 ± 1.33	31.15 ± 1.83[a]
Dip-AS2	1	*L. oleracea*	Adult ♀(day 3)	20.91 ± 3.39	16.20 ± 3.66
Mas-AS	0.5	*M. sexta*	Vth larva (day 0)	4.36 ± 0.53	0.34 ± 0.07[b]
Mas-AS	0.5	*M. sexta*	Adult ♀(day 3)	3.15 ± 0.36	1.88 ± 0.25[a]

Significantly different from controls [a] $P < 0.02$, [b] $P < 0.0001$.

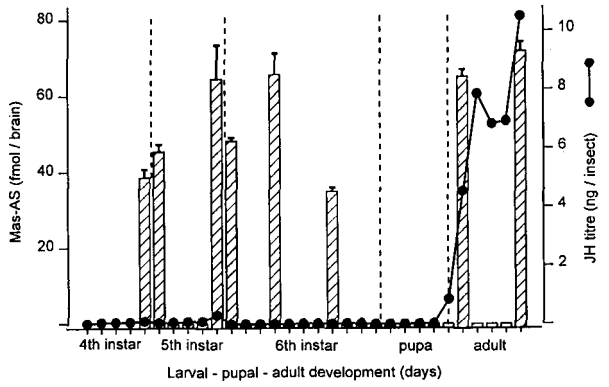

Fig. 6. Mas-AS-like immunoreactivity (hatched bars, fmol/brain) and whole body JH titre (line graph, ng/insect) of selected larval and adult *L. oleracea* (means ± S.E., n = 1–6). Mas-AS-like immunoreactivity assayed by ELISA (see Audsley et al., 1998). Whole body JH titres derived from Edwards et al. (1995).

DISCUSSION

The CA of adult female *L. oleracea* appear to synthesise JH in vitro at relative constant rates, on average, over at least the first seven days of adult life, but individual rates of biosynthesis vary considerably by more than 10 fold. Similar variations were observed in adult female *M. sexta* (Unni et al., 1991) and in the male desert locust (Avruch & Tobe, 1978). In most experiments JH biosynthesis was measured in *L. oleracea* adult females within a limited age range and stage of development (2–4 days) and CA taken at the same time each day, so this variation suggests that the CA may alternate between being switched on and off relatively frequently as determined by the presence or absence of endogenous allatostatins/allatotropins as suggested by Unni et al. (1991) for *M. sexta*. Mean rates of JH biosynthesis are 2–10 fold greater than reported for similar aged female *M. sexta* (Unni et al., 1991; Kramer & Law, 1980) and adult female *P. unipuncta* (Cusson et al., 1990, 1993). In addition to species variation, differences may also arise from methods used for in vitro incubation of CA, and extraction and measurement of JH. Regardless, relatively high levels of JH in adult female moths stimulate vitellogenesis and initiate egg production (Cusson et al., 1994), and in adult female *L. oleracea* Edwards et al. (1995) suggest that JH levels are consistent with the gonadotropic role of JH. The individual variations in JH biosynthesis observed may therefore be due to rapid increases in egg development which occur at this time.

HPLC separation of JH reveals that CA of adult female *L. oleracea* produce primarily JH I and JH II in vitro. Similarly, Edwards et al. (1995) found that total JH titres in adult female *L. oleracea* over the first 5 days were chiefly comprised of JH I and JH II, and there was only a 2–3 fold increase in JH titres from day 1 to day 5. Cusson et al. (1990, 1993) report that virgin female *P. unipuncta* also produce mainly JH I and II, and there is a general increase in the amounts produced with age. Interestingly, Unni et al. (1991) observed that newly emerged *M. sexta* adult females produce predominantly JH II, but switch to producing more JH III after 3 days. The reason for these differences is at present unknown.

In larval insects JH maintains the larval stage and hence its removal is required to progress with development to the adult. Initiation of metamorphosis could explain the low levels of JH in the final (VI^{th}) larval instar compared to IV^{th} and V^{th} instars (Edwards et al., 1995). Similar patterns also occur in the last instar larval stage (V^{th}) of *M. sexta* (Baker et al., 1987). However, JH titres (ng/g wet weight) in larval *L. oleracea* reported by Edwards et al. (1995) are at least 20 fold less than those for adult females, and biosynthesis rates in larval instars reported here are at least 1,000 fold lower than in adult females. Clearly, JH biosynthesis by CA of larval *L. oleracea* is close to the limit of detection of this assay, necessitating multiple gland pairs per assay tube to produce measurable JH. This is further compounded by the need to use a high specific activity radiolabel which contaminates HPLC columns over successive runs requiring laborious clean up of columns between samples. These low rates of JH biosynthesis observed in larval *L. oleracea* do, however, reflect the low titres reported by Edwards et al. (1995) but are very much lower than those in larval *M. sexta* (Kramer & Law, 1980) and larval *Lymantria dispar* (Jones & Yin, 1989). This would suggest that the requirement for JH by larval *L. oleracea* may be much more tightly regulated than in adults or other moths, but it is also possible that JH is rapidly degraded by esterases in CA of larval *L. oleracea*. Alternatively, larval *L. oleracea* may produce other homologues of JH which were not detected by the methods used in this study, and this is currently under investigation.

A distinct allatostatin (Mas-AS) was characterised from pharate adult heads of *M. sexta* by Kramer et al. (1991) and was shown to completely inhibit JH biosynthesis by isolated CA of newly emerged (0–4 h) adult female and early V^{th} instar *M. sexta*. In addition, this peptide also caused a 77% inhibition of JH biosynthesis by the CA of newly emerged *Heliothis virescens* but had no significant effect on the dictyopteran *P. americana*, the orthopteran *M. sanguinipes* or the coleopteran *T. molitor*, leading to the conclusion that Mas-AS was lepidopteran specific. Audsley et al. (1998) reported a widespread distribution of a Mas-AS-like immunoreactive peptide in both larval and adult *L. oleracea*, but this did not correlate well with JH titres (Edwards et al., 1995). In all larval stages measured, JH titre was less than 0.3 ng/insect, whereas there was a rapid increase in JH titre on adult emergence to a maximum of 10.5 ng/insect at day 5 (Fig. 6). In contrast, brain levels of Mas-AS-like immunoreactivity remained within the same order of magnitude irrespective of the stage of development (Fig. 6), however, haemolymph titres of Mas-AS, which may have a greater bearing on CA activity, were not measured. Regardless, an immunoreactive peptide with a molecular mass in close agreement with that of Mas-AS was isolated providing strong evidence of the presence of this peptide in *L. oleracea* (Audsley et al., 1998). However, synthetic Mas-AS only produced a maximum of 54% inhibition on isolated CA

of adult female *L. oleracea* although the effects were variable ranging from nil effect up to apparently 92% inhibition in individual assays at the same dose (unpublished observations). In comparison, this same synthetic Mas-AS consistently reduced JH biosynthesis by greater than 90% in larval *M. sexta*. This may be due to variations in rates of JH biosynthesis by individual pairs of *L. oleracea* CA as discussed earlier. In *P. unipuncta*, Jansons et al. (1996) isolated a cDNA that encodes a polypeptide containing the Mas-AS sequence, but expression of the gene did not correlate well with JH biosynthesis in this insect. In addition Mas-AS had no effect on CA of *P. unipuncta* VIth instar larvae or newly emerged adult females, but did cause a 58% inhibition of JH biosynthesis by CA of 5 day old adult females. Although Mas-AS-like immunoreactivity is present in adult *L. oleracea* (Audsley et al., 1998) and was detected in larval brains of another noctuid, *S. littoralis*, these results are somewhat inconclusive as to whether the primary function of Mas-AS in noctuids resides in the regulation of JH biosynthesis. It is possible that other factors may be involved, or that Mas-AS has another role, such as modulation of myotropic activity as shown for cockroach-like allatostatins (Lange et al., 1993, 1995; Duve et al., 1994, 1995; Vanden Broeck et al., 1996). Duve et al. (1997) have structurally characterised a number of peptides related to cockroach-like allatostatin family from the Lepidoptera *Cydia pomonella* and *Helicoverpa armigera*, but their biological activity was not demonstrated. Weaver (unpublished observations, and results herein) found that cockroach allatostatins had no significant effect on JH biosynthesis by CA of adult female *L. oleracea*. Alternatively, because JH in adult female moths stimulates vitellogenesis and initiates egg production (Cusson et al., 1994), and in adult female *L. oleracea* egg laying is maintained over at least 25 days (Corbitt et al., 1996), there may be a continued requirement for JH and hence the CA of these moths may not be fully receptive to Mas-AS. It is therefore possible that stimulation of CA (by an allatotropin) may be more necessary to maintain JH levels, and the relatively high control rates of JH biosynthesis observed in adult female *L. oleracea* may be due to an endogenous allatotropin. This may also explain why Mas-AT has only a small, but significant effect on CA of adult female *L. oleracea*. However, in moths that migrate, such as *P. unipuncta*, JH levels fall at time of migration when females are reproductively inactive (Cusson et al., 1993), and in adult female cockroaches such as *P. americana* and *Diploptera punctata*, JH titres fluctuate according to ovarian cycles (Weaver & Pratt, 1977; Tobe & Stay, 1977; Edwards et al., 1990), suggesting that allatostatins may be more important. Finally, in larval *L. oleracea* the actions of Mas-AS on JH biosynthesis proved inconclusive due to difficulties in the detection of minute amounts of JH.

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