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BIOSYNTHETIC ACTIVITY OF CORPORA ALLATA, GROWTH OF SEX ACCESSORY GLANDS AND MATING IN THE MALE MOTH *AGROTIS IPSILON* (HUFNAGEL)

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Summary

The involvement of both juvenile hormone acid (JHA) and the sex accessory glands (SAGs) in the reproduction of the male moth *Agrotis ipsilon* was studied as a function of age and mating status. Total protein content analysis followed by gel electrophoresis of the SAGs, radiochemical assay for JHA biosynthesis and surgical and behavioural experiments were performed. Both the protein content of the SAGs and the biosynthetic activity of the corpora allata (CA) increased with age. Allatectomy and JHA/JH treatments showed that the protein content of the SAGs is linked with the activity of the CA. The protein content of the glands, but not the rate of JHA biosynthesis, decreased just after mating, and both increased sharply 24 h later. Injection of fluvastatin, an inhibitor of JH biosynthesis, in males immediately after mating prevented the increase in JHA synthesis and lowered the total protein content of the SAGs. Moreover, fluvastatin disrupted normal spermatophore transfer during the next mating of the injected males. Our results show that JHA controls the reproduction of *A. ipsilon* males by its separate actions on the sex accessory glands and on sexual behaviour.

Key words: Noctuidae, *Agrotis ipsilon*, reproduction, juvenile hormone acid, sex accessory gland, corpora allata, mating, protein, fluvastatin, cholesterol inhibitor.

Introduction

In male moths, sexual attraction and mating occur only when the female sexual pheromone is detected by the antennae and the information is subsequently integrated in the central nervous system (for a review, see Hansson, 1995). During mating, a spermatophore originating in the sex accessory glands (SAGs) of the male is transferred into the female. The SAGs of insects grow and produce proteinaceous material that is associated with sperm production and transfer (see Happ, 1992) and has various modulatory functions on female behaviour (Leopold, 1976). In most insect species, growth and protein synthesis in the SAGs seem to be regulated by both ecdysteroids and juvenile hormone (JH), with development and differentiation being under the control of ecdysteroids and protein secretion being regulated by JH (see Gillott and Gaines, 1992).

This general scheme is well documented in many insect orders including the butterflies *Danaus plexippus*, *Vanessa cardui* and *Nymphalis antiopa* among the Lepidoptera (Herman, 1973; Herman and Bennett, 1975; Herman and Dallmann, 1981). In moths, nothing is known about the role of JH in SAG maturation. The corpora allata of all male moths studied so far are known to produce JH acids (JHAs) instead of JHs (Peter *et al.* 1981; Bhaskaran *et al.* 1988; Cusson *et al.* 1993; Ho *et al.* 1995). In a few cases, such as in *Hyalophora cecropia* and *Manduca sexta*, SAGs have been shown to contain JHA methyltransferase (JHAMT) activity which enables the conversion of JHAs into JH (Bhaskaran *et al.* 1988). However, little is known of the role of either JHA or JH in male reproduction in moths.

In the noctuid moth *Agrotis ipsilon*, mating does not occur until a few days after eclosion (Swier *et al.* 1976, 1977; Gadenne, 1993). Moreover, the presence of the CA is required for the male to respond to the sex pheromone (Gadenne *et al.* 1993). Allatectomy suppressed male responsiveness, and both JHA and JH were able to restore the sexual behaviour of the operated males (Duportets *et al* .1996). The CA of male *A. ipsilon* produce JHAs (Duportets *et al.* 1996), and no JHAMT has been detected at any site, including the SAGs (L. Duportets, unpublished observation).

To clarify the role of JHAs in males of the moth *A. ipsilon*, we performed electrophoretic studies of SAG contents as a function of age and JHA biosynthesis. In this paper, we studied SAG maturation, the role of JHAs in this maturation and the possible links between SAG maturation and male sexual responsiveness.

Materials and methods

Insects

The colony of *Agrotis ipsilon* (Hufnagel) originated from moths caught in southern France. Adults from field catches are introduced into the colony each spring. Larvae were reared on an artificial diet and maintained in individual plastic cups until pupation under a 16 h:8 h light:dark cycle at 21±1 °C (modified from Poitout and Buès, 1974). Pupae were observed each day to identify day 0 (newly emerged) adults. Adults were held in plastic boxes and had access to 20 % sucrose solution. Males and females were kept in different chambers.

Mating experiments

The following symbols were used to identify males according to their age following emergence and their mating status: A+*x* denoted *x*-day-old adults and M+*y* denoted the number of days after mating. For example, A+6, M+2 would mean 6-day-old adults, 2 days after mating. M_1 represents the first mating and M_2 the second mating. Pairs were formed between day 4 males (A+4 males) and females before the onset of scotophase. Mating always occurs at mid-scotophase (Gadenne, 1993). Males were removed from the boxes just after the termination of mating (A+4, M_1+0) and 24 h (A+5, M_1+1) or 48 h (A+6, M_1+2) later for extirpation of the sex accessory glands (SAGs) and corpora allata (CA). At the end of scotophase, successful mating was always checked by tactile location of the spermatophore by gently pressing the abdomen of the live female between two fingers. At the end of the experiments, the females were dissected and checked again for the presence of the spermatophore.

To analyse the effect of a second mating (M_2) of males on both JH biosynthetic activity and protein synthesis by the sex glands, mated females were removed and replaced by new virgin day 4 females that were allowed to mate with males that had mated the preceding night. Mating was observed, and the SAGs and CA were removed either immediately after the second mating of the males (A+5, M_2+0) or the next day (A+6, M_2+1).

Surgical treatments

Removal of the CA was performed according to Gadenne (1993). Newly emerged day 0 males (A+0 males) were allowed free access to drink and were allatectomized less than 3 h after emergence. Care was taken to ensure that only the CA and not the corpora cardiaca were removed. Sham operations were performed by opening the head and touching, but not removing, the CA.

Chemicals

[^{14}C]Sodium acetate was purchased from Amersham International (Buckinghamshire, UK). JH-III and Ficoll were purchased from Sigma (Taufkirschen, Germany). JH-II was purchased from SciTech (Prague, Czech Republic). Fluvastatin (Sandoz compound XU 62-320) was a gift from Dr F. Kathawala (Sandoz Research Institute, Hanover, NJ, USA). JHA was obtained by an enzymatic procedure, using pig liver carboxyesterase (Sigma) (Duportets *et al.* 1996), in which three enzyme units (1 unit will hydrolyse 1.0 μmol of ethylbutyrate to butyric acid and ethanol per minute at pH 8.0 at 25 °C, Sigma) converted 1 μmol of JH into JHA in 1 h at 40 °C in a phosphate buffer (50 mmol l^{-1}, pH 7.4). The JHA formed was subsequently purified by reverse-phase liquid chromatography (RPLC).

Juvenile hormone injections

Injections were performed using a 10 μl Hamilton syringe. Allatectomized 0-day-old (A+0) males of *A. ipsilon* were injected on day 1 with either 2 μl of olive oil or 2 μl of olive oil containing 5 μg of synthetic JH-III (Sigma) or JH-III acid.

Fluvastatin injections

In our rearing conditions, scotophase starts at 09:00 h and ends at 17:00 h, and mating always occurs between 12:00 h and 15:00 h. Fluvastatin, a hydroxymethylglutaryl-CoA reductase inhibitor, inhibits juvenile hormone biosynthesis in insects (Debernard *et al.* 1994) including *A. ipsilon* (Duportets *et al.* 1996). Fluvastatin (30 μg in 2 μl of water) was injected into the mated males just after lights on (i.e. 17:00 h). Twenty-four hours later, both SAGs and the CA from mated/injected males were removed, and the SAGs were analysed for protein content and the CA for JH biosynthesis. Water injections were used as control experiments.

A second experiment was conducted with fluvastatin using the same injection procedures as described above. The following day, however, mated/injected males were placed with virgin females to monitor their ability to re-mate. After observing re-mating of these males, the mated females were checked for the presence of a spermatophore. Fertility was measured by counting the number of females laying viable eggs.

Dissection and protein analysis

The SAGs of *A. ipsilon* males are long paired glands of a pink colour originating from the ductus ejaculatorius duplex (Gemeno *et al.* 1998). The glands were dissected under methanol (100 %) and stored in Eppendorf tubes at −20 °C under methanol. Proteins were precipitated, sonicated and centrifuged at 24 000 ***g*** in pure methanol at 4 °C for 15 min. Pellets were homogenized in ammonium formate buffer, pH 4.8. After protein extraction and centrifugation, supernatants were collected and stored at −80 °C. Total protein contents were measured following the method of Bradford (1976).

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) of freeze-dried sex accessory gland extracts was performed using the method of Laemmli (1970) with a Bio-Rad Mini Protean II apparatus (Richmond, CA, USA) and 7.5 % polyacrylamide slab gels. Each well was loaded with 10–15 μg of total proteins. Electrophoresis was run at 120 V for concentration and at constant voltage (150 V) for separation. Pre-stained molecular markers were obtained from Bio-Rad (high range, molecular mass 52–197 kDa). Gels were

stained with Coomassie Blue for 10–15 min. Protein levels in the gels were estimated by densitometry using a BioProfil Gel analysis system (Vilber Lourmat, France). At least three replicates of each electrophoresis experiment were performed.

Juvenile hormone biosynthesis

Rates of JH biosynthesis were determined *in vitro* using a radiochemical assay (Pratt and Tobe, 1974). Corpora allata were dissected out and incubated for 4 h at 28 °C in medium TC 199 (75 ml, Flow Laboratories, Irvine, UK) supplemented with Ficoll (20 mg ml^{-1}) and [2-^{14}C]sodium acetate (final concentration, 943 mmol l^{-1}; specific activity, 1.08 Bq pmol^{-1}). Each incubation was performed with three pairs of CA (pCA). Rates of JHA biosynthesis were calculated according to a dual-labelling procedure. Female CA incubated in medium TC 199 containing [2-^{14}C]sodium acetate and L-[methyl-^{3}H]methionine (final concentration, 101 mmol l^{-1}; specific activity, 17.27 Bq pmol^{-1}) produce dually labelled JH-III and JH-II. One methyl group from methionine was incorporated into each JH molecule and, on the basis of this, acetate incorporation was found to be 0.9 per JH-III molecule and 0.8 per JH-II molecule. We assumed an identical level of acetate incorporation into JHA in males.

Reverse-phase liquid chromatography (RPLC)

Analytical conditions were essentially those described by Halarnkar and Schooley (1990). The HPLC system (Beckman System Gold HPLC, Palo Alto, CA, USA) with ultraviolet detector (Beckmann 166) was connected to a radioactivity monitor (Berthold LB 506, Postfach, Germany). The column was a 50 mm×4.6 mm polymer column (PLRP-5, 5 mm, 100 A) protected with a 5 mm×3 mm PLRP-5 guard cartridge (Polymer Laboratories, Amherst, MA, USA). Buffer A was 5 mmol l^{-1} Hepes adjusted to pH 6.2. Buffer B was 5 mmol l^{-1} Hepes buffer (final concentration) in 80 % acetonitrile. Juvenile hormone and its catabolites were separated at a flow rate of 1 ml min^{-1} using a linear gradient from 5 % to 100 % buffer B over 20 min. Ultraviolet emission was monitored at 245 nm.

Statistical analyses

Statistical analyses of the effects of fluvastatin injection into males were performed by the comparison of proportions using the χ^2-test, $P \leqslant 0.05$.

To examine the statistical significance of differences between mean rates of JHA biosynthesis and of protein content, two-sample *t*-tests were used, $P \leqslant 0.05$. Values are presented as means ± S.E.M.

Results

Relationship between age, SAG protein content and JHA biosynthesis

In male *A. ipsilon*, a post-emergence enlargement of the SAGs occurs, and the change from thin and rather white structures to thick and reddish structures is easily detectable under the dissecting microscope. Sex gland maturation was investigated by measuring SAG protein content in males between the ages of 0 and 6 days. As shown in Fig. 1, the protein content of the SAGs increased with age to a maximum level in 4-day-old males. These showed a twenty-fold increase in protein content over 0-day-old males, and higher levels than those found in 5- or 6-day-old males.

Fig. 1 also shows that the rate of JHA biosynthesis (JHA-II and JHA-III + unknown compounds) increased gradually with age, starting at a low level (15.44±2.43 pmol pCA^{-1} h^{-1}, N=5) and reaching a level of 63.53±15.45 pmol pCA^{-1} h^{-1} (N=8) at day 4. No difference was found in the JHA-II/JHA-III ratio, and the rate of the biosynthesis of the unknown compounds increased proportionally to that of JHA-II and JHA-III.

Effect of allatectomy and JH-III and JH-III acid injections on the protein content of SAGs

The protein content of the SAGs in allatectomized 4-day-old males was significantly different from that of untreated 4-day-old males (P<0.001), although it was not as low as that of 0-day-old males (P<0.001) (Fig. 2). The protein level of sham-operated males did not differ significantly from that of 4-day-old males.

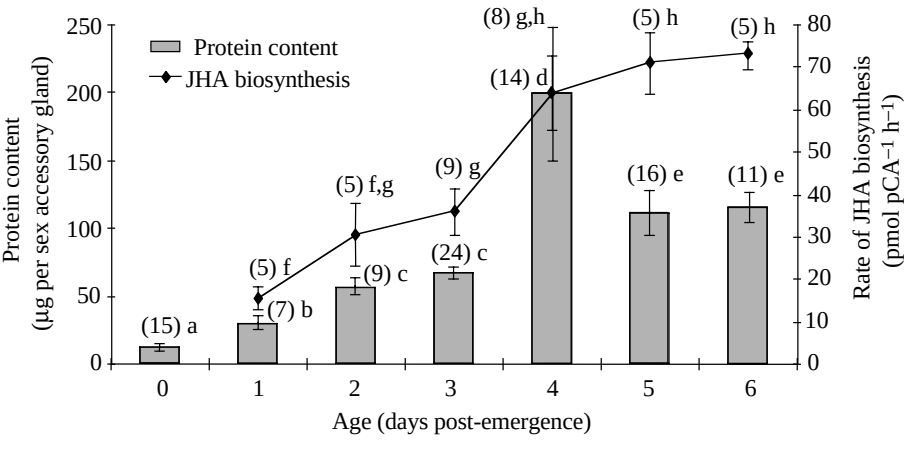

Fig. 1. Effect of age on the total rate of isoprenoid biosynthesis by corpora allata (CA) incubated *in vitro* and on the total protein content of individual sex accessory glands (SAGs) from 0-day-old to 6-day-old males of *Agrotis ipsilon* (mean values ± S.E.M.). For protein analysis, numbers in parentheses represent the number of sex glands examined. For juvenile hormone acid (JHA) biosynthesis, the number in parentheses represents the number of individual determinations each carried out using three pairs of CA (pCA). Values with the same letter are not statistically different from one another ($P \leqslant 0.05$, Student's *t*-test).

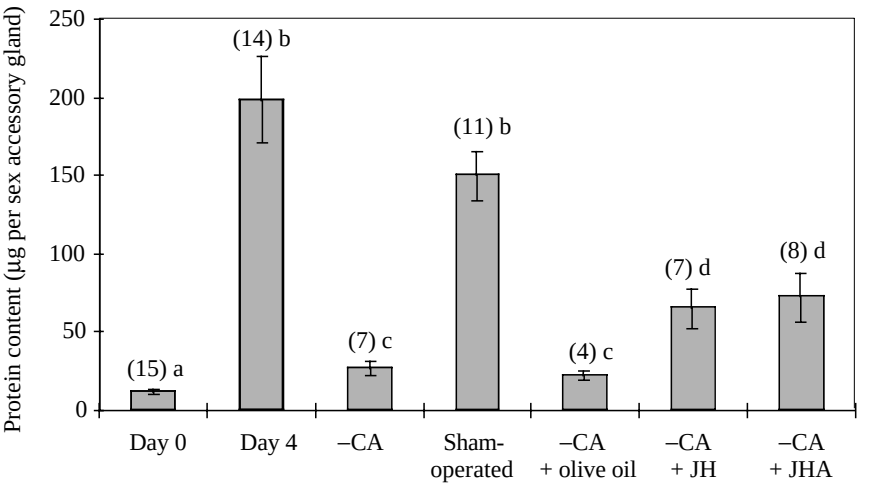

Fig. 2. Effect of allatectomy (–CA) and of juvenile hormone/juvenile hormone acid (JH/JHA) injection on the total protein content of sex accessory glands (SAGs) from 4-day-old male *Agrotis ipsilon* (mean values ± S.E.M.). Allatectomy was performed on the day of emergence (A+0), and injections were carried out on the next day (A+1). Numbers in parentheses above the columns are the numbers of individual glands used in the experiment. Values with the same letter are not statistically different from one another ($P \leqslant 0.05$, Student's *t*-test).

Injection of JH-III or JH-III acid (5 µg on day 1) increased the level of protein in allatectomized males, but it remained significantly lower (P=0.0035 for JH-III and P=0.0036 for JH-III acid) than those of untreated or sham-operated 4-day-old males (Fig. 2). Injection of olive oil did not induce a significant increase in protein levels in the glands of allatectomized males.

Electrophoretic studies revealed a similar number of proteins present in both control and operated males (Fig. 3). Allatectomy did not alter the pattern of proteins extracted from the SAGs, although the protein content was very much reduced. The major protein at 90 kDa, which appeared soon after emergence (see Fig. 5), continued to be present after allatectomy.

Effect of mating on SAG protein content

The protein content of SAGs from newly mated males was very low compared with that of control unmated males (P<0.001) (Fig. 4A). Sex accessory glands dissected out 24 h after mating showed an increase in the level of protein present (P<0.001), which was maintained for a further 24 h and reached levels comparable with those found in mature non-mated 6-day-old males (A+6 males; see Fig. 1). The synthesis of protein 24 h after mating was significantly inhibited (P<0.001) when fluvastatin was injected after males had mated (Fig. 4B). When males were allowed to mate for a second time during the following night, the protein content of the SAGs was very low just after the second mating, not significantly different from that of A+4, M_1+0 males, and the recovery of protein synthesis was lower 24 h after this second mating than after the first mating (Fig. 4C).

Electrophoresis studies of the protein content of SAGs revealed that the same proteins were present in mated and non-mated males (Fig. 5), even though the protein content was lower in mated males. In particular, the most abundant protein band of 90 kDa, which was hardly visible from SAGs of 0-day-old males, was present, although at low level, in SAG extracts from once-mated (A+4, M_1+0) and twice-mated (A+5, M_2+0) males.

Effect of mating on juvenile hormone acid biosynthesis

Biosynthesis of JHAs showed a sharp increase 24 h after mating (P<0.001) and levels remained high 48 h post-mating (Fig. 4A). When males were allowed to mate a second time, the level of JHA synthesis remained high after the second mating,

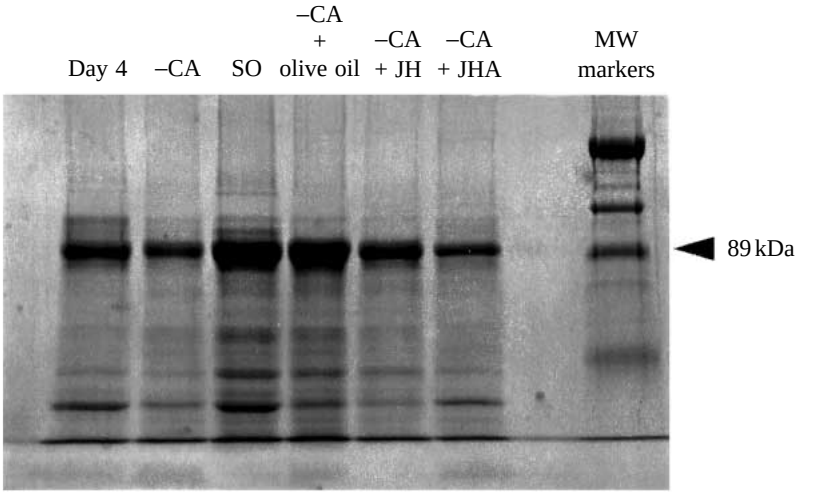

Fig. 3. Effect of allatectomy (–CA), sham-operation (SO) and juvenile hormone/juvenile hormone acid (JH/JHA) injections on the protein pattern (7.5 % SDS–PAGE) of the sex accessory glands (SAGs) of male *Agrotis ipsilon*. The arrowhead indicates the molecular mass marker. Lanes were loaded with equal concentration of protein (10–15 µg). See Materials and methods for further details.

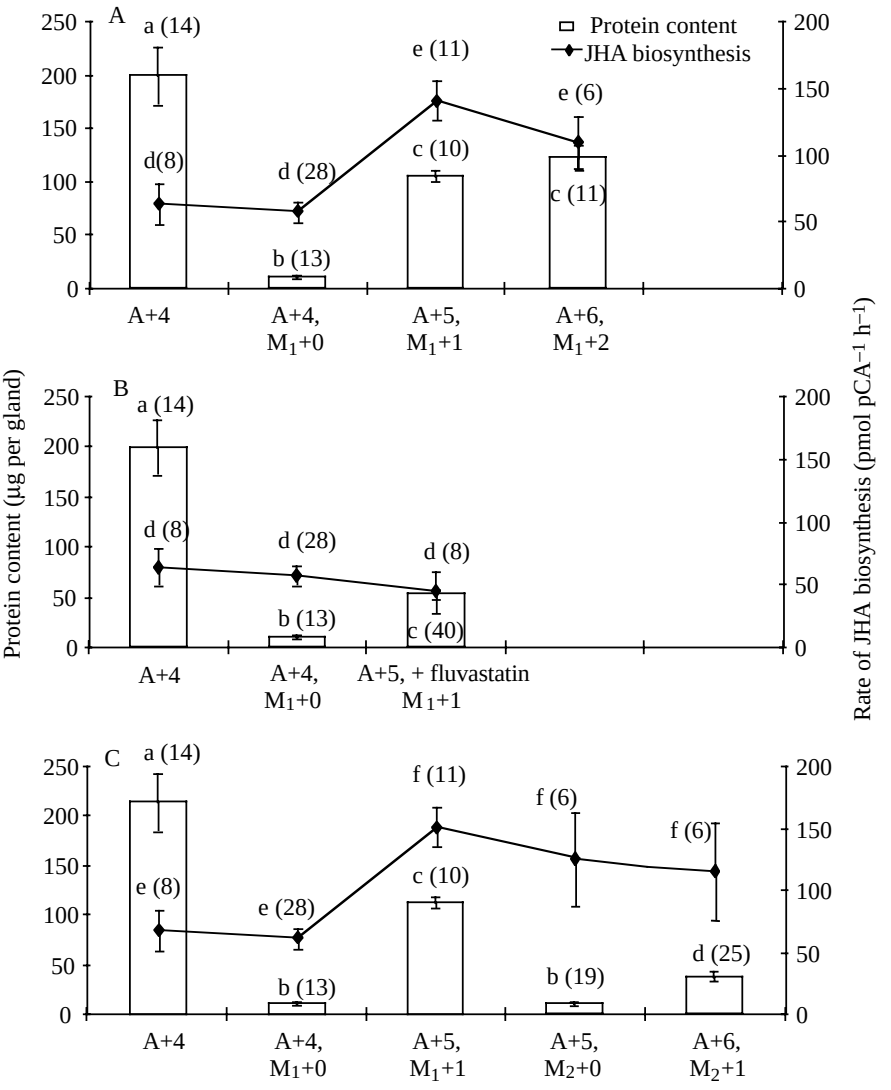

Fig. 4. Effect of mating on the rate of total isoprenoid synthesis by corpora allata (CA) incubated *in vitro* and on the total protein content of individual sex accessory glands (SAGs) from males of *Agrotis ipsilon* (mean values ± S.E.M.). For protein analysis, numbers in parentheses represent the number of SAGs used in each assay. For juvenile hormone acid (JHA) biosynthesis, the number in parentheses represents the number of individual determinations, each of which used three pairs of CA (pCA). Columns with the same letter are not statistically different from each other ($P \leqslant 0.05$, Student's *t*-test). (A) Effect of a single mating. (B) Effect of the injection of fluvastatin after the first mating. (C) Effect of a second mating. A+*x*, age (in days) after emergence; M+*y*, age (in days) after mating; M_1, first mating; M_2, second mating.

although no further significant increase was induced by this second mating (Fig. 4C). Injection of fluvastatin into males just after mating prevented an increase in JHA production by the CA on the following day (P=0.0011) (Fig. 4B).

Effect of fluvastatin on the mating behaviour of males

The ability of mated males injected with fluvastatin to re-mate on the following night with virgin females was assayed. Table 1 shows that fluvastatin-injected mated males were able

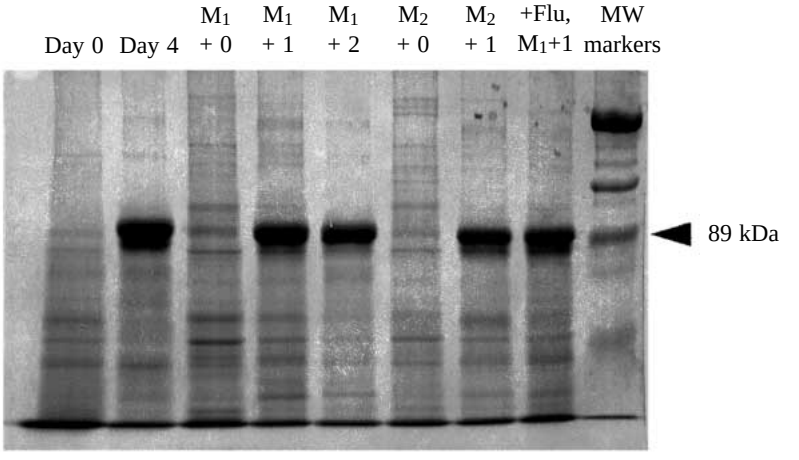

Fig. 5. Pattern of proteins from the sex accessory glands (SAGs) of male *Agrotis ipsilon* separated by SDS–PAGE (7.5% gel) showing the effects of mating (M_1+*y*, age in days after one mating), re-mating (M_2+*y*, age in days after two matings) and injection of fluvastatin (Flu). The arrowhead indicates the molecular mass marker. All lanes were loaded with the same protein concentration (10–15 µg). See Materials and methods for further details.

Table 1. *Effect of fluvastatin injection (30 μg in 2 μl) on the ability of once-mated males to re-mate on the following night*

	Number of males	Re-mated males	Females with spermatophore	Females laying fertile eggs
Control mated males	45	28 (62 %)[a]	28 (100 %)[a]	23 (82 %)[a]
Mated males				
+ Distilled water	36	24 (66 %)[a]	24 (100 %)[a]	20 (83 %)[a]
+ Fluvastatin	48	25 (53 %)[a]	14 (56 %)[b]	6 (24 %)[b]

See Materials and methods for further details.
For each column, numbers followed by the same letter are not statistically different (χ^2-test, $P \leqslant 0.05$).

to perform a second mating (53 % re-matings) as well as control males (62 % re-matings), but these matings were significantly less successful (56 % mated females with a spermatophore) than those of control males (100 % mated females with a spermatophore). While most females (82 %) that mated with control males laid fertile eggs, only 24 % of females that mated with fluvastatin-injected males laid fertile eggs (Table 1).

Discussion

In this study, we show that the CA regulate the development of the SAGs in male *A. ipsilon*. The pattern of development of SAGs in Lepidoptera is the same as that identified in several other orders of insects including Diptera, Dictyoptera and Orthoptera (for a review, see Gillott and Gaines, 1992). Just after emergence, the glands are undifferentiated and have a low protein content which subsequently increases until day 4. In *A. ipsilon*, the main protein band detected in the SAGs had a molecular mass of approximately 90 kDa. In *Blattella germanica*, four main bands have been detected ranging in mass from 15 to 65 kDa (Vilaplana *et al.* 1996). When determined *in vitro*, the rate of JHA biosynthesis showed a gradual increase with age, which is similar to observations made in another moth, *Pseudaletia unipuncta* (Cusson *et al.* 1993). As already demonstrated in male moths, the CA of *A. ipsilon* do not produce JH but release JHA (Duportets *et al.* 1996) and other unknown compounds that may correspond to acid-conjugated forms of JH that are still unidentified, as reported in *M. sexta* (Granger *et al.* 1995). The rate of total isoprenoid biosynthesis (JHA-II and its conjugated forms) in *A. ipsilon* showed a gradual age-related increase. A similar increase with age in the rates of JH and total protein synthesis by the glands has been demonstrated in *Blattella germanica* (Belles and Piulachs, 1992; Piulachs *et al.* 1992).

Allatectomy performed on day 0 prevented the normal development of the sex glands. The total protein content remained at a low level, but no single proteins seemed to be specifically affected. Both JH and JHA partially restored the protein content of the SAGs of allatectomized males, as has been shown in monarch butterflies using JH alone (Herman, 1975). This report is the first to show that JHA has a biological effect on the glands of male moths. In *B. germanica*, allatectomy inhibited the development of the sex glands, and no particular protein could be detected whose concentration was affected by the treatment, although a qualitative difference in the appearence of protein bands was seen between experiments performed at day 5 and day 10 (Vilaplana *et al.* 1996). Our experiments show that allatectomy in *A. ipsilon* did not inhibit the synthesis of the main protein band. It therefore appears that JHA acts on the synthesis of the total protein content of the glands. It is still not clear whether it is JHA and/or JH that controls the development of the SAGs. Juvenile hormone injected *in vivo* can readily be converted into JHA by JHA methyltransferase, but we have been able to show no such activity in the SAGs or in any other tissue (L. Duportets, unpublished observations).

The mating of males induced changes in both the protein content of the SAGs and the rate of JHA biosynthesis by the CA. Just after mating, the protein content of the SAGs dropped to a low level but was restored on the following day concomitant with a sharp increase in the level of JHA biosynthesis, which remained at this high level on the following day. Since the production of JHA and the replenishment of the protein content of the SAGs are synchronous, we postulate that these events are linked. Further evidence to support this view was provided by injections of fluvastatin, which inhibited JHA production *in vivo* (Duportets *et al.* 1996). Complete inhibition lasted for 3 h, and JHA production was then slowly restored (Duportets *et al.* 1996). In the present study, we show that an injection of fluvastatin prevented the normal increase in the protein content of the SAGs in mated males, indicating that a reduction in JHA production could reduce the replenishment of protein in the SAGs.

Although it is possible to find mated females of *A. ipsilon* with more than one spermatophore in field-trap experiments, this happens very rarely under our rearing conditions. However, if males were allowed to mate with a different female each night, multiple mating of the males readily occurred, leading to many inseminated females. Once the males had mated, they were unable to mate a second time in the same scotophase (M. C. Dufour, unpublished observation). The protein content of the SAGs of mated males was very low, although their rate of JHA biosynthesis did not show any significant decrease. It appears that the male of *A. ipsilon* is not equipped for two matings in a single night.

In the cockroach *B. germanica*, JH biosynthesis showed a

dissimilar pattern of activity so that, although it decreased just after mating, it did not increase 24 h post-mating (Vilaplana *et al.* 1996). In *A. ipsilon*, a signal must be produced by the male during mating inducing the CA to produce more JHA. It is probable that this mating stimulus may act on the brain to produce and release allatotropin factors and/or to remove allatostatic factors. Work in progress in our laboratory shows that *M. sexta* allatotropin factors can indeed induce a marked increase in the rate of JHA production by males to levels identical with those found in mated males (L. Duportets and F. Couillaud, unpublished observation). When mated males were injected with fluvastatin, there was no increase in the rate of JHA biosynthesis and the recovery of protein synthesis was lower than that of controls (Fig. 4B). Moreover, although the fluvastatin-injected males were able to re-mate, their ability to transfer a spermatophore was greatly decreased (Table 1). JH and/or JHA have previously been shown to control the processing of the olfactory stimulus leading to pheromone responsiveness (Gadenne *et al.* 1993; Duportets *et al.* 1996). Through the pheromone response, JHA is able separately to control both the production of sex accessory gland proteins and the mating process. Fluvastatin-injected males were able to mate (to respond to the pheromone), although their SAGs were not ready for a successful mating. In a previous paper, we showed that fluvastatin temporarily inhibited the response of the males to the sex pheromone (Duportets *et al.* 1996). The strongest effect was obtained by injecting the JH biosynthesis inhibitor 3 h before the behavioural test. Here, we show that fluvastatin disturbed the re-mating behaviour and induced numerous unsuccessful spermatophore transfers (Table 1). The number of re-matings could be explained by the fact that fluvastatin was injected just after the first mating, much more than 3 h before the CA were removed for the analysis of JHA biosynthesis. The recovery of JHA production may have been sufficient to allow a pheromone response, but the restoration of the protein content of the SAGs was delayed so that protein levels were too low at the time of mating. This is confirmed by re-mating experiments showing that, when CA activity was not inhibited, males were able to re-mate successfully, even though the protein content of the SAGs had been lowered by the first mating (Fig. 4C).

To our knowledge, we have shown for the first time that JHA in a male moth acts both on the level of protein storage in the SAGs and on the responsiveness to a female-produced sex pheromone. In addition, JHA seems to act separately on these systems so that the hormone is necessary (i) for the olfactory signal to be processed by the brain, thus allowing mating behaviour (i.e. copulation), and (ii) for the sex accessory glands to develop and store proteins, thus allowing successful mating (i.e. transfer of the spermatophore).

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