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THE RELATION BETWEEN CORPORA ALLATA SECRETION AND PROTEIN SYNTHESIS IN MALE SEXUAL ACCESSORY GLANDS OF *Spodoptera littoralis* (Boisd.)

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ABSTRACT

The relation between juvenile hormone (JH) released from the corpora allata of the *Spodoptera littoralis* male moths and protein synthesis in the sexual accessory glands (SAGs) of the reproductive system had been studied in the present work. The amount of total protein in SAGs of the newly emerged male moth was the lowest. It increased as the age of the unmated male progressed to reach the maximum in males 3 day old. Then, the amounts gradually decreased towards the end of the male life span, The highest amount was relatively similar to that found in the SAG of 3 day old male previously sham-operated and allatectomized soon after emergence. Injection of 5 µl of JH dissolved in 2 µl of olive oil per individual *S. littoralis* virgin male previously allatectomized as newly emerged moths moderately increased the amounts of total protein in SAGs of 3 day old individuals. Soon after mating and separation, the male lost an average of 51.13 µg of protein in the single SAG. One day after the first mating, sex protein increased by 25.12 µg in the SAG, however, the increase in this case did not reach that in SAG of the unmated 3 day old male. After the second mating, the amount of sex protein in the same males again decreased and the compensation in protein content in SAG was very slight one day after the second mating. This indicates that the efficiency of males decreased when successive mating occurred.

Keywords: Protein synthesis, Corpora allata, Sexual accessory glands, Male moth, *Spodoptera littoralis*, Mating

INTRODUCTION

The male antennae of moths detect the sexual pheromone of the female of the same insect species. Thus, sexual attraction and mating occur. The information is subsequently integrated by the central nervous system of the males (Hansson, 1995). During mating, spermatophore(s) of the male transferred into the female reproductive system. Such spermatophore(s) originate in the male accessory glands (AGs), which grow and produce proteinaceous material that is associated with sperm production and transfer (Happ, 1992) and has various functions on female behaviour (Leopold, 1976). In most insect species. Protein synthesis and secretion in the accessory glands seem to be regulated by juvenile hormone (Gillott and Gaines, 1992). The corpora allata (CA) of male moths are known to produce juvenile hormone acids (JHAs) instead of juvenile hormones (JHs) (Peter *et al*., 1981; Bhaskaran *et al*., 1988; Cusson *et al*.,1993; Ho *et al*., 1995). However, little is known about the role of JHAs or JHs in male reproduction of moths.

In Lepidopteran adult males, the major release products of CA are JH acid (JHA) I and II for *Hyalophora cecropia* (Peter *et al*., 1981), and JHA I, JHA II, and homo-farnesoic acid (FA) for *Pseudaletia unipuncta*. The release of these JHA homologues increases with age under different rearing

conditions (Cusson *et al.* 1993). *Mythimna (Leucania) loreyi* adult moths possess isolated cell type CA, and that the male hypertrophic CA is more than 20 times larger than that of the female (Kou *et al*. 1995). The major release products of CA in *M. loreyi* are identified as JH II and JH III for females, and JHA I, JHA II, Iso-JHA II, and JHA III for males (Ho *et al.* 1995).

In noctuid moths, the presence of CA is required for male to respond to sex pheromone (Gadenne *et al*.,1993). Allatectomy suppressed male responsiveness, and both JHAs and JHs were able to restore the sexual behaviour of the operated males (Duportets *et al.*, 1996).

The aim of the present work is to clarify the role of JHs in *S. littoralis* male moths in synthesize the protein in the sexual accessory glands and the relation between the CA secretion and the protein synthesis in these glands as being affected by the age of male moths and mating process

MATERIALS AND METHODS

The cotton leafworm*, Spodoptera littoralis* (Boisd.) was obtained as egg masses from different cotton fields located in Kaluobia Governorate. In the laboratory, egg masses were placed in small glass jars covered with clean muslin, held with a rubber band. The rearing technique adopted by Gomaa (2001) was adopted. Towards the end of the last (sixth) instar larvae, moist saw dust was placed at the base of the rearing jars to provide a pupation site. Newly formed pupae were carefully collected daily, sexed and placed in clean jars until adult emergence to be used in the present experiments.

Surgical technique for allatectomy and sham operation in *S. littoralis* male moths:

surgical treatments were made to the newly emerged males by dividing them to three main groups. In the first, sham operation was made and in the second , corpora allata was removed, while the third group was left without operations as control. Allatectomy and sham operation were made according to the method adopted by Gaddene (1993) and modified by Park and Ramaswamy (1998) as follows. Newly emerged adult males were anesthetized with ether for 30 seconds. Anesthetized males were placed on a modeling clay bed and clay strips were flattened on their thorax and head to immobilize the head. The neck membrane in the posterior region of the head capsule was cut open to expose the corpora cardiaca-corpora allata complex. Because the corpus cardiacum and corpus allatum are closely appressed, the complex was excised in its entirety and the integument cover pushed back in place. When corpora allata of the male moth were removed at the require age, care was taken to ensure the presence of corpora cardiaca. Sham operated males were treated similarly by cutting the nerves between each corpus cardiacum and corpus allatum, but not removing the later.

Hormonal application:

5 µl of synthetic JHIII (Sigma Company, Taufkirshen, Germany) dissolved in 2 µl of olive oil were injected in individual one day old male moth previously allatectomized 6 hrs after emergence. Injection was made using a

10 µl Hamilton syringe. Another group of one day old allatectomized males was injected with 5 µl of olive oilo only as control.

Chemical analysis of male SAGs

A one day old female and male were paired and maintained in the inverted glass lamps used for moth mating. This was replicated 10 times. It was worthmentioning that copulation and/ or insemination and uncoupling, under conditions of the present work, usually lasts nearly 10 hours. After the required time, the insects were anaesthetized and dissected in physiological saline solution (9% Na Cl). The whole male reproductive system was carefully picked out, the SAGs were carefully separated from the rest of the reproductive system, then placed in a glass vial and transferred to a freezer (-25º C) for chemical analysis. The females, soon after uncoupling, were dissected and their bursa copulatrix opened to ensure the occurrence of mating by the presence of the spermatophore(s).

1. Extraction of protein contents in *S. littoralis* male SAGs

The method adopted by Gomaa (2006) for extraction of the protein contents in *S. littoralis* male SAGs was followed. The SAGs were homogenized separately in a glass homogenizer under ice containing one ml of Tris HCl (pH 6) and 2% SDS buffer. The contents were centrifuged for 5 minutes at 5000 rev./min. then filtered. The supernatant was transferred to an Eppendorf tube and stored in the deep freezer (-25±2°C) until required for protein analysis. this procedure was carried out on SAGsobtained from males aged 0, 1, 2, 3, 4, 5 and 6 days

2. Estimation of total protein:

Estimation of total protein concentration in the prepared extracts of the male SAGs was determined according to the method described by Hammouda (2002) and applied by Gomaa (2006) using Bio-Rad protein assay. A series of concentrations (ranging from 0.1 to 1.4 mg/ ml) of each of the prepared extracts by Bovine serum albumin were incubated, Bio-Rad dye was then added and the contents mixed thoroughly. The optical density (absorbency) of the obtained colour was measured by a Bausch and Lomb Spectrophotometer at 595 nm, distilled water was considered as the blank sample A standard curve was constructed to convert the optical density values of tested extract samples into protein concentrations. From the determined protein concentrations, the amounts (µg) of proteins were gravimetrically calculated.

RESULTS AND DISCUSSION

Protein content in sexual accessory glands of *S. littoralis* virgin male moth during its longevity:

The data given in table (1) clearly show that the amount of total protein in the newly emerged male moth of *S. littoralis* was the lowest, being 11.06 µg/ individual sexual accessory gland (SAG).

Total protein content in these glands increased as the age of the male moth progressed to reach the maximum (191.36 µg/ individual SAG) in males 3 day old. Then, the amounts gradually decreased towards the end of the male life span, being 68.78 µg/ individual SAG in male 6 day old.

Relatively similar findings were given by Duportets *et al.* (1998) on *A. ipsilon* male moths, who found that in newly emerged males, SAGs had low protein content, which subsequently increased till the 4th day after emergence. They added that corpora allata regulate the development of SAGs. The same pattern of development of SAGs was found in some orders of insects including Dictyoptera, Orthoptera and Diuptera (Gillott and Gaines, 1992). According to Duportets *et al.* (1996), corpora allata of *A. ipsilon* male moth do not produce produce juvenile hormone (JH), but release juvenile hormone acid (JHA), in addition to some unknown compounds that may correspond to acid-conjungated forms of unidentified JH, which were found in *Manduca sexta* (Granger *et al*., 1995).

Table 1: Total protein content in individual sexual accessory gland (SAG) picked up from *S. littoralis* male moths of different ages (Means ± S.E.).

Age of virgin male moth (days)	No. of SAGs examined	Amount of protein content in individual SAG (µg)
0 (newly emerged)	8	11.06 ± 1.28 (e)
1	10	32.44 ± 2.76 (d)
2	10	65.20±11.14 (c)
3	8	191.36±15.42 (a)
4	7	119.14±12.08 (b)
5	6	77.52±11.24 (c)
6	9	68.78±12.02 (c)
" F " value		22.15 **
L.S.D. at 0.05		16.89

Juvenile hormone regulating total protein production in SAGs of virgin *S. littoralis* male moth:

As shown in table (2), the total protein found in the SAG of virgin *S. littoralis* male averaged 10.68 µg/ individual SAG. This amount increased to reach a mean of 194.42 µg/ individual SAG of virgin 3 day old male. This amount was relatively similar to that found in th SAG of 3 day old male previously sham-operated soon after emergence and those allatectomized 6 hours after emergence (186.22 µg/ individual SAG). The difference between both cases (normal and sham-operated) proved to be statistically insignificant and the L.S.D. value emphasizes the obtained results. This means that nerves connected corpora cardiaca with corpora allata did not play any role in juvenile hormone production and, in turn, in protein production in SAGs.

The present data also show that juvenile hormone required for normal protein production in SAGs released in insect haemolymph when males were less than 3 day day old, because of the remove of corpora allata from males at the beginning of the third day did not affect the amount of biosynthesized protein in SAGs. This result could be achieved by removing corpora allata from the newly emerged virgin males. In this case, the amount of sex protein in SAGs of 3 day old males reached only 24.36 µg/ individual SAG. This means that allatectomy performed in newly emerged males prevented normal development of SAGs. The total protein content remained

at a low level. The same findings were given by Herman (1975) on Monarch butterflies, who found that both JH and JHA partially restored the protein content of the SAGs of allatectomized males. In German cockroach, *Blatella germanica*, allatectomy inhibited the development of the SAGs and no particular protein could be detected, whose concentration was affected by the treatment (Vilaplana *et al.,* 1996). However, Duportets *et al.* (1998) found that allatectomy in *A. ipsilon* did not inhibit the synthesis of the main protein band of the male SAGs, which was detected by electrophoresis. The author concluded that JHA acts on the synthesis of the total protein content of the glands.

Injection of 5 µl of JH dissolved in olive oil per individual *S. littoralis* virgin male previously allatectomized as newly emerged moths moderately increased the amounts of total protein in SAGs of 3 day old individuals. Means of 71.56 µg/ individual SAG was recorded when virgin allatectomized males were injected with JH. However, this value was still 2.5 times lower than that of the normal males (194.68 µg/ individual SAG).

In the available literature, JH and/or JHA control processing of the alfactory stimulus leading to pheromone responsiveness (Gaddene *et al.*, 1993 and Duportets *et al.,*1996). Through the pheromone response, JHA is able separately to control both the production of SAG proteins and the mating process. In 1998, Duportets *et al.* stated that it is still not clear whither it is JHA and/or JH that controls the development of SAGs in *A. ipsilon* male moth. Juvenile hormone injected in vivo can readily be converted into JHA by JHA methyltransferase.

Table 2: Protein content in sexual accessory glands (SAGs) of allatectomized *S. littoralis* male moths and after injection with juvenile hormone (JH) and juvenile hormone acid (JHA).

No.	Status of male moth	No. of SAGs examined	Protein content in individual SAG (µg) (Means ± S.E.)
1	Virgin newly emerged male (NE)	10	10.68 ± 1.54 (d)
2	Virgin male three day old (3D)	6	194.42±18.76 (a)
3	Sham operation in NE & removing glands at 3D	10	186.22±16.62 (a)
4	Newly emerged virgin allatectomized male (NEA)	9	24.36±2.52 (c)
5	NEA and olive oil injection	10	21.48±2.22 (c)
6	NEA and JH injection	9	71.56±12.38 (b)
" F " value			29.08**
L.S.D. at 0.05			9.11

Effect of mating on protein production in SAGs of *S. littoralis* males:

In normal case, mated *S. littoralis* female moth has more than one spermatophore. If a male was allowed to mate with more than one female, multiple mating of male readily occurred, leading to many inseminated females. Inspection was made in the present work and found that once the males had mated, they were unable to mate a second time in the same night

but required about 18 hrs to be capable for second mating. On the other hand, a female could be mated with more than one male in the same night.

The data given in table (3) clearly show that 3 day old *S. littoralis* male had an average of 129.29 µg in an individual SAG just before mating. Soon after mating and separation, the male lost an average of 51.13 µg of protein in the single SAG, i.e., a mean of 141.16 µg was found in the individual SAG. One day after the first mating, sex protein increased by 25.12 µg in the SAG to reach 156.78 µg/ individual SAG. The amount of protein increase was still lower than that in SAG of the unmated 3 day old male.

After the second mating, the amount of sex protein in the same males again decreased to reach 130.64 µg / individual SAG and lost an average of 36.14 µg/ SAG. The compensation in protein content in SAG was very slight one day after the second mating, being 135.72 µg, with an increase of 5.08 µg/SAG.

Table 3: Total protein content in individual sexualaccessory glands (SAGs) picked up from *S. littoralis* male moths after single and double matings.

No.	Status of male moth	No. of SAGs examined	Protein content in individual SAG (µg) (Means ± S.E.)
1	3 day old, before mating occurrence	10	192.29±16.43 a
2	3 day old, soon after 1st mating	8	141.16±12.57 c
3	4 day old, one day after 1st mating	9	166.78±13.03 b
4	5 day old, soon after 2nd mating	7	130.64±12.22 e
5	6 day old, one day after 2nd mating	6	135.72±11.79 f
" F " value			23.47**
L.S.D. at 0.05			5.12

From the fore mentioned results, it could be concluded that the recovery of JH 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 remating experiments showing that when CA activity was not inhibited, males were able to mate successfully, even though the protein content of the SAGs had been lowered by the first mating. The mating of males induced changes in the protein content og SAGs. Just after mating, the protein content of the SAGs dropped to a low level but was restored on the following day. According to Duportets *et al.* (1998) protein restore in SAGs of *A. ipsilon* male moth was concomitant with the sharp increase in the level of JHA biosynthesis, which remained at this high level on the following day. The same authors added that the production of SAGs and the replenishment of the protein of SAGs are synchronous.

It is suggested that in *S. littoralis* a signal must be produced by the male during mating inducing the CA to produce more JH. It is probable that this mating stimulus may act on the brain of male to produce and release allatotrophic factors and/or to remove allatostatic factors. The relatively same suggestion was given by Duportets *et al.* (1998) on *A. ipsilon* male moth.

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العلاقة بين إفراز الجسم الكروى وتخليق البروتين في الغدد المساعدة الجنسية لذكور فراشات دودة ورق القطن

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قسم وقاية النبات – كلية الزراعة بجامعة عين شمس – شبرا الخيمة – القاهرة

درست العلاقة بين إفراز هرمون الشباب من الجسم الكروى لذكور فراشات دودة ورق القطن وكمية البروتينات المخلقة في الغدد المساعدة الجنسية للجهاز التناسلى . وقد أظهرت النتائج أن كمية البروتين الكلى في هذه الغدد تكون أقل ما يمكن في الذكور حديثة الخروج ، وهذه الكمية تزداد بتقدم عمر الذكور غير المتزاوجة إلى أن تصل إلى أقصاها عند عمر ثلاثة أيام . بعدها تبدأ في الانخفاض التدريجى لتصل إلى أدناها قرب نهاية الحياة .

وقد وجد أن الكمية القصوى من البروتين تعادل نسبيا تلك الكمية التي وجدت في الغدة المساعدة الجنسية لذكر عمره ثلاثة أيام سبق أن أجريت له عملية قطع العصب الذي يصل ما بين الجسمين الكروبين والجسمين القلبيين وكذلك لذكر أجريت له عملية استئصال الجسمين الكروبين، وكلتا العمليتان أجريتا بعد ست ساعات من الخروج. كما وجد أن حقن ٥ ميكرولتر من هرمون الشباب المصنع والذائبة في ٢ ميكرولتر من زيت الزيتون النقي في ذكر الفراشة والذي سبق أن أجريت له عملية استئصال الجسمين الكروبين بعد الخروج مباشرة قد زاد من كمية البروتين الكلي بدرجة معتدلة في الغدد المساعدة الجنسية للذكر عندما بلغ عمره ثلاثة أيام . بعد التلقيح وانفصال الجنسين، وجد أن الذكر قد فقد ٥١.١٣ ميكروجرام بروتين في المتوسط ، وبعد يوم واحد استعاض ٢٥.١٢ ميكروجرام فقط (أي حوالي نصف الكمية المفقودة) ، وبعد التزاوج الثاني له انخفضت كمية البروتين مرة أخرى واستعاض في اليوم الثاني كمية بسيطة . وهذا يدل على أن كفاءة الذكر تقل بتعاقب عمليات التزاوج.

قام بتحكيم البحث

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