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EFFECT OF JH III AND β-ECDYSONE ON SEMINAL VESICLE PROTEIN SECRETION IN THE TROPICAL TASAR SILKMOTH, *ANTHERAEA MYLITTA* (DRURY) (LEPIDOPTERA: SATURNIIDAE)

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ABSTRACT
The present studies revealed an active secretory activity in the seminal vesicles soon after emergence of the male tasar silkmoth, *Antheraea mylitta*. The total concentration of DNA, RNA and proteins in the seminal vesicle tissue showed gradual reduction with advancement of age of the male moth. The SDS-PAGE analyzed about 11 protein bands ranging from 31.8 to 196.1 in molecular weight. Topical application of juvenile hormone (JH-III) on the newly emerged adult male moths showed enhancement while that of β-ecdysone (βE) caused depletion of the total seminal vesicle protein concentration.

INTRODUCTION

Various studies on hormonal regulation of male reproductive processes in the insects indicated that the testis secretes α-Ecdysone (20 - hydroxyecdysone) at adult stage which stimulates spermatogenesis (Leather and Hardie, 1995). Similarly, the role of α-Ecdysone has also been envisaged during pre-ecdysial stage in differentiation and development of the male accessory glands and glandular structures of the genital tract (Shinbo and Happ, 1992). According to large number of workers, the action of JH appears after adult ecdysis in stimulation of cellular protein synthesis mechanism and subsequently, acceleration of secretion of various proteins in order to facilitate sperm transfer (Happ, 1992; Leather and Hardie, 1995; Gillott, 2003).

In order to enrich our knowledge regarding physiology of male reproductive system in the tropical tasar silkworm, *Antheraea mylitta,* extensive studies were undertaken, to elucidate especially, the structure, development and activity of the testis, male accessory glands and seminal vesicles (SV) with prime impetus to explore hormonal regulation of various reproductive mechanisms (Pendam, 2002). The findings pertaining to the effect of juvenile hormone (JH-III) and β-Ecdysone (βE) on spermatogenesis (Tembhare and Pendam, 2005) and male accessory glands (Pendam and Tembhare, 2005) were reported earlier while the present paper deals with that of the seminal vesicle protein secretion.

MATERIALS AND METHODS

Histological methods

Soon after emergence, the male tasar silkmoths (*Antheraea mylitta*) were separated, the SV were dissected in saline and fixed in aqueous Bouin's fluid for 18- 24h. Paraffin sections were cut at 4µm and stained with Heidenhain's iron haematoxylin-eosin (Fe-HE).

Biochemical methods

The SV were removed from 0- (newly emerged), 1 and 2-day-old adults, tracheae and fat body separated and homogenized at 0°C for 5 minutes in different volumes of ice-cold distilled water, insect Ringer's solution and 0.25M sucrose solution separately. Total concentration of DNA, RNA and protein was estimated by using Burton's Diphenylamine (Searcy and MacInnis 1970a), Dische-Orcinol (Searcy and MacInnis 1970b) and biuret method (Lowry *et al*. (1951) respectively. The method of Laemmli (1970) was followed for SDS-PAGE with minor modifications. The 1mm 3% stacking gel (pH 6.8) was followed by a 10 cm 10% separating gel (pH 8.8) with 1% SDS. The SV from 0, 1 and 2-day-old adult male moths were dissected out and cut into pieces, homogenized and centrifuged as mentioned above and the supernatant was used as the sample. 50 µL of clear supernatant was mixed with 50 µL of treatment buffer (Tris-2.5mL, pH 6.8, SDS - 4mL, Glycerol -2 mL, 2-Mercaptoethanol – 1mL, distilled water - 0.5mL and a pinch of Bromophenol blue). The samples were heated for 5 minutes in a water bath. The mixture was cooled and it's 25µL, 30µL, 35µL quantity was separately applied

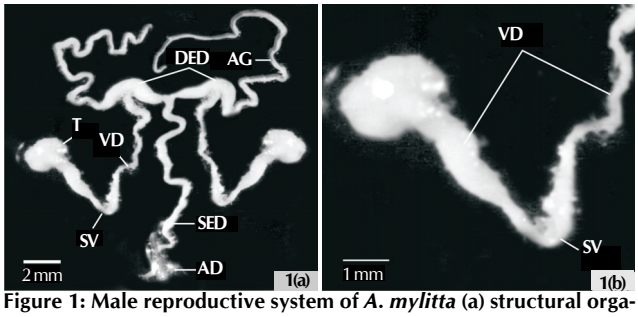
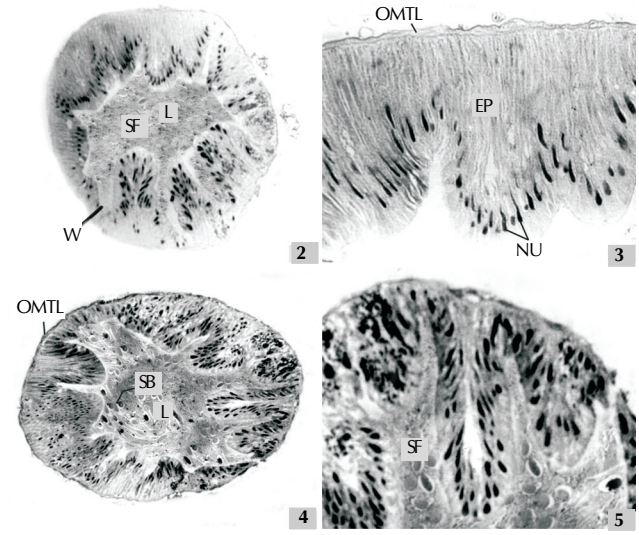


Figure 1: Male reproductive system of *A. mylitta* (a) structural organization *in situ* and (b) magnified, AD = Aedeagus; AG = Accessory Gland; DED = Duplex Ejaculatory Duct; SED = Simplex Ejaculatory Duct; SV = Seminal Vesicle; T = Testis; VD = Vasa Deferens



Figures 2-5: Histological structure of seminal vesicle (SV) stained with Heidenhain's iron haematoxylin-eosin (Fe-HE) Fig 2: Cross section of SV of newly emerged adult male X41; Fig 3: Magnified view X240 ; Fig 4: Cross section of SV of one day adult male X41; Fig 5: Magnified view X240; EP = Epithelium; L = Lumen; NU = Nucleus; OMTL = Outer Muscular Thin layer; SF = Seminal Fluid; SB = Sperm Bundles; W = Wall

onto the top of the gel. Standard wide range molecular weight marker protein was also run together. The gel was stained with Coomassie brilliant blue for 2h and destaining was done with a mixture of methanol- acetic acid- distilled water until the bands on the gel became clear. The molecular weight (mass) of the protein bands with regard to the marker proteins was estimated with the help of the Densitometer (Pendam, 2002).

Application of JH III and β-Ecdysone

The synthetic hormonal compounds (JH-III and βE) were obtained from Sigma (U.S.A.). The 200μg JH III and βE was dissolved separately in cold acetone and 20μL of quantity was applied topically on the 3-7th abdominal terga of each newly-emerged adult male separately, with the help of Hamilton's CR-700 constant syringe. Ten insects were used for treatment of each hormone. Equal numbers of adult males were treated with pure cold acetone, to serve as control insects. The SV were dissected gently from 5 JH-III– treated, βE-treated and control insects at the intervals of 24 and 48h each, homogenized separately and supernatant was used for estimation of total protein concentration and SDS-PAGE separation of proteins by the methods described above.

RESULTS

In the newly-emerged male moth, *Antheraea mylitta*, about 3.8 ± 0.37mm long middle region of each vas deferens is extensively swollen and modified into the distinct SV (Fig. 1. a, b). The SV is externally covered with a thin peritoneal sheath and the wall is composed of an outer thin muscle layer and an inner folded layer of glandular epithelium. The epithelium is composed of large columnar cells, arranged exclusively in a single tier and characterized with the large nuclei (11.2 µm in diameter) and colloidal cytoplasm. The secretory material appears first as a granular cytoplasmic inclusion in the epithelial cells soon after emergence of the male moths and thereafter, as a colloidal matter (seminal fluid, SF) in the lumen. In the mature male moths (a day after emergence), the SF contains large number of sperm bundles, migrated from the testis (Figs. 2 - 5).

In the 0- (newly emerged), 1 and 2 day old male moths, the total concentration of SV DNA, RNA and proteins, showed reduction gradually (Table. I). The SDS-PAGE separated about 11 seminal vesicle proteins (SVPs) ranging from 31.8 to 196.1 in molecular weight (Fig. 6).

After a period of 1 and 2 days, the topical application of JH III showed significant rise while that of βE caused depletion in the concentration of SVPs than that in the control insects (Fig. 7).

DISCUSSION

The SV, besides serving as the storehouse for sperm till mating, are known to be the active secretory structures producing the SF similar to the accessory glands and other regions of the male genital tract in large number of insects (Cantacuzene,1972; Riemann and Thorson, 1976; Cruz-Landim and Ferreira, 1977; Couche and Gillott, 1987; Araujo *et al.*, 2005; Viscuso *et al.*, 2005) and particularly, in the sandflies the SV alone secrete SF, as the accessory glands are totally lacking (Fausto *et al.*, 2000). Secretory nature of SV is also well evident in *Antheraea mylitta* during the present study. The chemical composition of SF in *Antheraea mylitta* is predominantly represented by the proteins similar to that in the honeybee, *Apis mellifera* (Blum *et al.*, 1962, 1967), cockroach, *Periplaneta americana* (Vijayalekshmi and Adiyodi, 1973), desert locust, *Schistocerca gragaria* (Odhiambo, 1969), stingless bee, *Scaptotrigona xanthotricha* (Araujo *et al.*, 2005) and other insects (Gillott, 2003; Poiani,

Table 1: Biochemical analysis of seminal vesicle of the adult male moth, in *A. mylitta*

Age of adult male (days)	Total Concentration		
	DNA(µg/mg)	RNA(µg/mg)	Protein(µg/mg)
Newly emerged adult(0 day)	26.8 ± 0.4	7.12 ± 0.1	561.6 ± 3.2
1 day	18.08 ± 0.4	6.18 ± 0.12	474.4 ± 2.9
2 day	9.2 ± 0.3	5.06 ± 0.5	350.4 ± 2.9

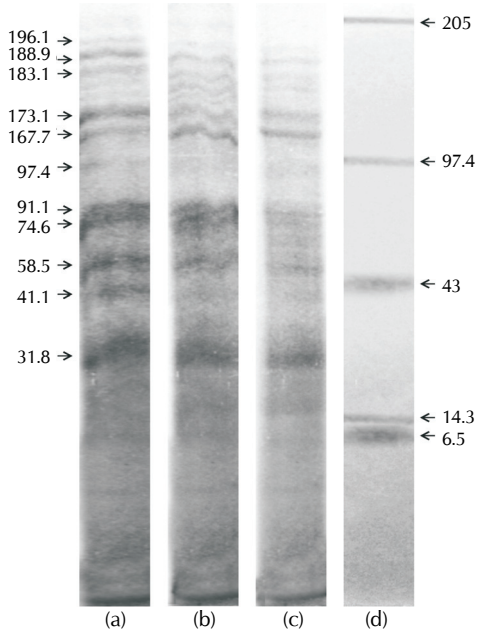


Figure 6: SDS-PAGE of SV extracts showing protein bands in *A. mylitta* a) newly emerged adult b) one day old adult c) two day old adult and d) standard molecular weight marker proteins (205-6.500 kD)

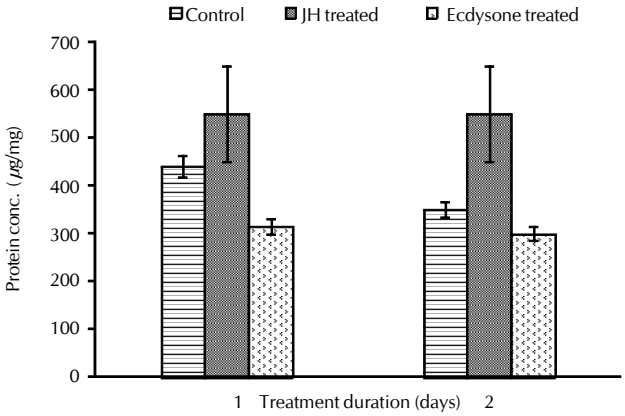


Figure 7: Effect of JH and β-E on protein concentration of seminal vesicle of the 1 & 2-day old adult male moth *A. mylitta* *p= 0.05, **p= 0.01, ± SE of mean value

2006). The present results revealed maximum concentration of total DNA, RNA and proteins in the SV of newly emerged male and thereafter, gradual reduction with the advancement of age suggesting initiation of vigorous secretory activity soon after emergence. Couche and Gillott (1988) also evinced active secretory activity in the epithelial cells of SV in the grasshopper, *Melanoplus sanguinipes*. Gradual reduction in protein concentration in the old insects seems to be related with the nutritive function of the stored sperms (Rojas-Rousse, 1972) and sperm transfer during copulation. In *Antheraea mylitta* the SDS-PAGE separated clearly about 11 distinct SVPs varying from each other in their molecular weight. The variable numbers of SVPs have been reported in other insects: 10 in the honeybee, *Apis mellifera* (Tozettto *et al.,* 2007), 7 - 8 in the grasshopper, *Zonocerus variegatus* (Muse and Balogun, 1992), 14 in the beetle, *Tribolium castaneum* (South *et al.,* 2011), 30 in crickets (Andre's *et al.,* 2006) and as maximum as 70 to 106 in Drosophila (Wolfner, 2007) while this is, perhaps, the first report in Lepidoptera and particularly, in the moths and butterflies (Walters and Harrison, 2010).

Stimulatory action of JH on development and secretory activity of accessory glands and other parts of male genitalia occurs predominantly in large number of insects (Shinbo and Happ, 1989; Happ, 1992; Leather and Hardie, 1995; Poiani, 2006) while the Ecdysteroids, on the other hand show negative regulation (Colonello and Hartfelder, 2003; Tozettto *et al.*, 2007). The present observations strongly suggest that the JH III is stimulating while the bE is inhibiting the SVPs secretion in the tasar silkworm, *Antheraea mylitta*.

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