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Effect of Juvenoid and Allatectomy on the Biochemical Components of Gonads in *Gryllotalpa gryllotalpa* (Gryllotalpidae: Orthoptera: Insecta)

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Effects of allatectomy, juvenoid, on allatectomized and sham-operated insects on the levels of protein, lipid, glycogen, cholesterol and acid phosphatase in testis and ovary were investigated in *Gryllotalpa gryllotalpa*. After allatectomy, the total lipid, cholesterol and glycogen levels increases while the total protein and acid phosphatase decrease significantly both in testis and ovary. Treatment of allatectomized insects with juvenile hormone analogue reverses the effect of allatectomy whereas treatment of sham-operated insects with JHA causes a further significant decrease of lipid, cholesterol and glycogen levels significantly and increase in total protein, and acid phosphatase levels in comparison to the control and JHa treated allatectomized insects.

Key Words: **Juvenoid, Allatectomy, Biochemical components,** *Gryllotalpa gryllotalpa*, **Gonads**

Introduction

Much of the literature has been directed towards the role of juvenile hormone (JH) in controlling the sexual maturity, oogenesis and spermatogenesis in many insect species (Englemann 1970, 1971, Englemann & Friedel 1974, Englemann & Penny 1966, Chen et al. 1976, Blaine & Dixon 1973, Gillott & Friedel 1976, Friedel & Gillott 1976 and Maria et al. 1979). In most insect species it has been found that reproduction is possible only if sufficient juvenile hormone is available (Schooneveld et al. 1979) and corpora allatum (CA) activity is correlated with the reproductive maturation in variety of insect species (Tobe & Pratt 1975, Tobe & Stay 1977 and Weaver et al. 1975). Recently it is shown that the rate of JH synthesis as well as breakdown in the haemolymph depend on the age and on the photoperiod (Kramer & De Kort 1976 and Kramer 1978). Englemann and Penny (1966), Englemann (1971), and Englemann and Friedel (1974) have shown that JH induces female stage specific proteins, and Bassi and Feir (1971 a,b,c, 1972, 1977) have

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demonstrated that JH increases the synthesis of acid phosphatase in an insect. Similarly allatectomy prevents the normal increase in soluble protein in testis as well as accessary reproductive glands (Gillott & Friedel 1976 and Friedel & Gillott 1976).

So, it would be of some academic interest to learn whether the levels of various biochemical components in insect gonads are initiated or inhibited by the action of JH. The present study is an attempt to explore the effects of exogenous application of juvenoid and allatectomy on the biochemical components of reproductive system of the insect *Gryllotalpa gryllotalpa*.

Material and Methods

Material

The insect material used was the emerging (after 6 hr) adult males and females of *Gryllotalpa gryllotalpa* reared in the laboratory at 25°C, 12 hr:12 hr photoperiod.

Allatectomy

Allatectomy was performed on newly emerged males and females within 6hr of adult emergence after the method of Stay and Tobe (1977). The ringer solution used here was always mixed with phenyl thiourea.

Hormone Treatment

The juvenoid used was the analogue FMC-23509 (CRD-9499) obtained from Dr David S Pincus of the FMC Corporation, New York. It was dissolved in peanut oil and kept in a refrigerator at a temperature of $4°\pm0.5°C$. Hormone was injected into the abdomen by means of a micrometer syringe. A dose of 25 μg in 19 μl peanut oil per insect (both allatectomized and in non-allatectomized) was employed. Control insect received equivalent quantities of peanut oil alone in the same manner as the hormone-treated ones. Mercury was used to separate the syringe medium (peanut oil) from the hormone.

Weight Measurement

After the operation the testis and ovary were removed and dried to constant weight (14-29 hr at 60-70° C) and weighed to the nearest 0.001 mg in (an electrically operated pan-balance).

Estimation of Cholesterol, Glycogen, Protein, Lipid and Phosphatase

These were assayed 24 and 48 hr after each treatment. The animals were dissected under ringer solution (mixed with phenyl thiourea) for obtaining the reproductive organs. The various biochemical components mentioned above were measured by following the methods of:

(a) Total protein by Folin-phenol method, using BSA (Bovine serum albumin) as standard (Lowry et al. 1955),

(b) Glycogen by van Handel's (1965) method,

(c) Cholesterol by Zaltkis et al. (1953) method,

(d) Total lipid by vanilline reactive method (Holwerda et al. 1977),

(e) Acid phosphatase assay was the sigma colorimetric determination (Technical bulletin 104) at 35°C for 30 min. Here the unit of enzyme is defined as the amount that catalyzes the cleavage of 1 μ mol of substrate (or bond) per min.

Statistical Analysis

The appropriate statistical analysis of the results obtained included t-test and the analysis of variance or the Dancun's Multiple range test.

Results

Total cholesterol level was greater in ovary than that of the testis. Similar results were also obtained in the case of acid phosphatase and glycogen. Total protein concentration was

higher in ovary whereas the total lipid content was very much greater in the testis (table 1).

Effect of Allatectomy

Cholesterol level increased considerably both in testis and in ovary but the effect was more pronounced in the ovary where it was almost double the amount obtained in the control. Time exposure gave the significant effect. Similarly glycogen and lipid level also increased significantly. But total protein, and acid phosphatase activity decreased gradually both in testis and ovary. Although allatectomy exerted the same effect on the male and female insects the effect was more acute and pronounced in female insects, particularly on some biochemical components (table 1).

Effect of Juvenoids on the Allatectomized Insects

Topical application of JHa reversed the effects of allatectomy described above (table 1). Within 24 hr of treatment the total protein, and acid phosphatase activity increased significantly both in testis and in ovary. However, the cholesterol, lipid and glycogen contents decreased gradually. The increase in total protein was much greater in ovary than that of the testis.

Effect of Juvenoids on the Sham-operated Insects

Table 1 showed that cholesterol total lipid and glycogen contents (both in ovary and testis) decreased significantly than that of the control and JHa treated allatectomized insects ($P<0.05$). Similarly the acid phosphatase activity increased significantly ($P<0.05$ and <0.01) both in testis and ovary than that of the control and JHa treated allatectomized insects. Total protein also showed the similar results as obtained in caseo facid phosphatases.

Discussion

Much research had already been done on the endocrine regulated levels of different macromolecules, enzymes and biochemical components (Englemann 1970, 1971, Bassi & Feir 1971a, Beel & Feir 1977, Steele 1976) in different insect species of different age groups and both in vitro and in vivo. Similarly it was also amply established that juvenile hormone played an important role in the vitellogenin synthesis in female insects (Dortland 1979) and in the synthesis of secretion by male accessory reproductive glands (Gillott & Friedel 1976, Friedel & Gillott 1976 and Tobe et al. 1979). The present experimental results clearly present an idea about the role of juvenile hormone on the synthesis and utilization of different biochemical components in both testis and ovary. In case of allatectomized insects the total protein, and acid phosphatase activity markedly decreased both in testis and in ovary. But when the allatectomized insects received the exogenous application of JH, all the components (total protein, and acid phosphatase) increased significantly (table 1) as demonstrated by Beel and Feir (1977). Elliott and Gillott (1978), Englemann and Penny (1966), and Englemann and Friedel (1974). The significant decrease of acid phosphatase activity (both in testis and ovary) after allatectomy and its increase after the JH treatment was very interesting and seemed to be contradictory to the generally accepted degradative action of acid phosphatase. Apart from the contradiction, it can easily be said that JH induced the acid phosphatase activity which was confirmed by applying JH on the allatectomized and sham-operated insects (table 1). The increased activity of acid phosphatase might be due to the JH inducing cell proliferation of the gonads (Beel & Feir 1977). The decrease of protein contents in the ovary after allatectomy and its increase after the application of

Table 1 *Changes of cholesterol, protein, lipid, glycogen and acid phosphatase in the testis and ovary of* G. gryllotalpa *after allatectomy and juvenoid treatment*

Biochemical components	Source	Control	Allatectomized		Allatectomized + JHa-injected		Sham-operated + JHa-injected		CD* value at	
			24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	5%	1%
Cholestrol (μg/100 mg	Testis	541 ± 0.05	745 ± 0.18	892 ± 0.15	502 ± 0.02	484 ± 0.08	504 ± 0.01	521 ± 0.07	5.39	—
tissue protein)	Ovary	893 ± 0.14	998 ± 0.03	1530 ± 0.13	492 ± 0.03	402 ± 0.12	500 ± 0.06	430 ± 0.07	12.80	—
Acid phosphatase (Enzyme unit/mg	Testis	14.25 ± 0.21	0.20 ± 0.05	8.15 ± 0.12	15.92 ± 0.82	17.21 ± 0.32	20.00 ± 1.19	21.93 ± 1.53	0.41	0.56
protein)	Ovary	20.05 ± 0.85	10.23 ± 0.13	11.20 ± 1.20	19.98 ± 0.07	20.12 ± 0.52	28.05 ± 0.03	30.21 ± 0.59	0.45	0.62
Glycogen (μg/100 mg	Testis	322.24 ± 2.02	525.92 ± 2.82	585.10 ± 1.85	401.05 ± 1.36	382.92 ± 0.53	290.24 ± 2.23	272.52 ± 1.25	3.36	4.56
tissue protein)	Ovary	429.00 ± 2.36	592.21 ± 1.26	623.00 ± 0.38	438.13 ± 1.63	420.98 ± 2.22	381.95 ± 1.86	350.00 ± 1.15	4.00	5.42
Total lipid (mg/100 mg	Testis	3.99 ± 0.52	4.89 ± 0.15	4.95 ± 0.23	3.52 ± 0.02	2.98 ± 0.33	3.99 ± 0.22	4.09 ± 0.26	0.097	0.13
tissue protein)	Ovary	1.26 ± 0.22	1.92 ± 0.02	1.99 ± 0.05	1.05 ± 0.07	0.95 ± 0.12	1.09 ± 0.51	1.02 ± 0.98	0.01	0.01
Total protein (μg/100 mg	Testis	670 ± 2.15	425 ± 1.25	355 ± 0.55	598 ± 0.32	692 ± 0.22	698 ± 0.83	793 ± 1.20	8.32	11.27
dry weight)	Ovary	2262 ± 3.19	895 ± 0.62	802 ± 2.26	1562 ± 1.15	1992 ± 0.39	2932 ± 1.85	3265 ± 0.88	10.24	13.87

*CD, critical difference, n = 9

JH again supported the view that in female JH acted as a gonadotropic hormone which helped in the synthesis and deposition of vitellogenin protein (Elliott & Gillott 1977, Englemann 1970, 1971, Englemann & Friedel 1974 and Englemann & Penney 1966). The effect of allatectomy and JH on protein contents of the testis showed similar results as in case of females, but what it was due to was still uncertain. According to Englemann (1970) it was probably due to the mobilization of the reserve which was mediated by the JH. As the insects were deemed to lack a sterol bio-synthetic mechanism (Robbins et al. 1971) the insects therefore required a dietary or additional source of sterol and in insects the cholesterol was mainly utilized for the synthesis of ecdysone and other steroid hormone (Schildknecht et al. 1966, 1967a,b). In allatectomized insects of both sexes, an extra accumulation of cholesterol both in testis and ovary was observed and this was probably due to the inhibition of different steroid hormone synthesis (Lagueux et al. 1977). But when the allatectomized and sham-operated insects were injected with JHa the cholesterol content decreased than that of the allatectomized and control insects. This clearly indicated a positive role of JH in the cholesterol metabolism of insects. It was generally acknowledged that JH prevented the prothoracic gland degeneration (Gilbert & King 1973) and the allatectomy in this insect might have led to the inhibition of cholesterol utilization by prothoracic and other glands.

Regarding the lipid contents, allatectomy resulted in accumulation of lipid both in testis and in ovary. Similar results were obtained by Orr (1964), Odhiambo (1966) Morohoshi and Kiguchi (1969). After the application of JH the lipid content decreased gradually both in allatectomized and sham-operated insect and there findings suggested that the corpus allatum hormone accelerated the lipid release from the tissue and conversely allatectomy led to prevent the lipid consumption. So, the present findings regarding the effect of JHa on lipid in testis and ovary followed the Morohoshi's hypothesis i.e., stronger the secretion of the corpus allatum hormone higher was the active energy metabolism and more was the lipid release from the fat-body as well as from the other tissues as a result of accelerated metabolism.

As to the effects of corpora-allata on carbohydrate metabolism, Morohoshi and Kiguchi (1969), Odhiambo (1966), Liu (1973, 1974) and Choudhuri and Mandal (1981) had already reported that allatectomy of newly emerged adult insects resulted in the accumulation of glycogen in different tissues. The effect of allatectomy on glycogen content in both testis and ovary in Gryllotalpa was also similar. The present investigation confirms the role of JH on the glycogen content, because when JHa was applied in the allatectomized insects the glycogen content decreased very significantly. So it might be possible that JH induced the glycogen release from the tissue by acting as a hyperglycemic agent or by inducing the rapid mobilization of glycogen. Another possibility might be that the JHa initiated or inhibited the brain or other endocrine portion of the insects which caused this type of effects.

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