

Biochemical Changes in Testis and Ovary of *Chrysocoris stoll* Wolf. after the Application of Juvenoid and Ecdysterone

L. M. Saha, S. Mandal * and D. K. Choudhuri

Entomology Laboratory, Zoology Department
The University of Burdwan
Burdwan - 713 104, W. Bengal (India)

(Received on October 16, 1984)

L. M. SAHA, S. MANDAL and D. K. CHOUDHURI. *Biochemical Changes in Testis and Ovary of Chrysocoris stoll* Wolf. after the Application of Juvenoid and Ecdysterone. *Rev. esp. Fisiol.*, 41, 249-258. 1985.

The changes in the metabolic status of both testis and ovary of *Chrysocoris stoll* following the treatment with juvenile hormone analogue (JHa) and ecdysterone were studied. After the exogenous application of JHa in selective dose, total carbohydrate, glycogen, trehalose, cholesterol, ascorbic acid and inorganic phosphorus increased significantly whereas free fatty acid (FFA), phospholipid, total protein, RNA and DNA decreased significantly in comparison to control of both testis and ovary. Total lipid significantly decreased in testis and significantly increased in ovary after JHa injection. The activities of cellular enzymes like alkaline phosphatase, 5'nucleotidase, catalase and peroxidase significantly decreased while acid phosphatase and GPT significantly increased after the JHa application in comparison to control both in testis and ovary. Activities of GOT and general esterase significantly decreased in testis and increased in ovary after JHa application. The exogenous application of ecdysterone also brought about the similar kind of responses as was noticed in case of JHa treatment but these two treatments differed in some cases such as ecdysteroid that produced some results which were just the reverse of what was produced by JHa treatment. The results obtained here were explained in terms of mode of action of these two hormones.

Key words: *C. stoll*, Ecdysterone, Juvenoid, Ovary, Testis.

The role of different hormones viz. juvenile hormone, ecdysone, neurosecretory hormones on the adult insect life have been extensively studied (14, 27-28, 33, 44, 51). The literature so far published

mainly deals with the role of juvenile hormone, ecdysone and neurosecretory hormone in controlling the sexual maturity, oogenesis and spermatogenesis in many insect species (31, 34, 44). Though there are ample evidences that juvenile hormones are necessary for gonadal maturation (16), whether 20-hydroxyecdysone or ecdysterone is required for normal gonadal sexual maturation in insects

* Present address: Zoology Department, A.M. College, Jhalda, Purulia, W. Bengal (India).

is still unsolved (22, 24). Recently it has been shown that ecdysteroid is synthesized not only in larva but also in the adult insects (4-6). On the other hand there are suggestions that ecdysteroid has a biological function not only in females but also in adult male insects (25). Most of the workers have tried to establish the mechanism of action of juvenile hormone, and ecdysterone at the biochemical level (29). According to ENGELMANN (14) the juvenile hormone induces the synthesis of female sex specific protein. BASSI and FEIR (1, 2) have shown that JH increases the synthesis of acid phosphatase. GILLOTT and FRIEDEL (17) have demonstrated that allatectomy prevents the normal increase in soluble protein in testis. On the other hand VAN PELT VERQUIL (48) has shown that ecdysteroid induces the activity of acid phosphatase in the adult insects.

It will be of academic interest to know whether both JHa and ecdysterone produce the similar quality of responses in the insect gonads (testis and ovary) in respect of different biochemical parameters and for that matter the present study is aimed to explore the effects of exogenous application of juvenoid and ecdysterone on some biochemical components of gonads of *C. stoll*i, a pentatomid bug.

Materials and Methods

Adults of *Chrysocoris stoll*i were collected from the plant, *Croton bonplandium* (Baill) and cultured in the laboratory by providing them with the same host plant at the temperature of $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$, and 80 % RH and 12:12 D:L photoperiod maintained in BOD incubator. Four days after the emergence the adult males and females were taken from the culture for experimental purpose. Juvenile hormone analogue (JHa) used was a derivative of N-geranyl-

aniline with its chemical structure: N-(2,5-dichlorophenyl)-3,7-dimethyl-36-octadimethylamine and it was injected at a dose of $1.5 \mu\text{g/insect}$ dissolved in $30 \mu\text{l}$ olive oil. Similarly $5 \mu\text{g}$ ecdysterone/insect (ecdysterone, $\text{C}_{27}\text{H}_{44}\text{O}_7$, Rhoto Pharmaceutical Co.) dissolved in $10 \mu\text{l}$ 0.9 N NaCl was also injected separately. The control insects received the olive oil and 0.9 N NaCl respectively maintaining the same dose as in case of experimental ones. Both the treated and control insects were sacrificed 24, 48 and 72 h after each treatment. Before the dissection the insects were mildly anesthetised with ether vapour.

For estimation of different enzymes, both the male and female insects were dissected on the ice both at $4 \pm 1^{\circ}\text{C}$ and testis and ovary were collected separately by removing the adhering tracheae and unnecessary material, and were finally homogenized in an appropriate amount of chilled buffer solution or glass distilled water of neutral pH. For the estimation of metabolites other than enzymes both the male and female insects were dissected under the ice cold saline (mixed with few crystal of phenylthiourea for inhibiting tyrosinase activity).

Total carbohydrates, glycogen and trehalose were estimated as described by MORDUE and GOLDSWORTHY (32), VAN HANDEL (47) and ROE (37) respectively; the total lipid, by following the method described by HOLWORDA *et al.* (20), and phospholipid, by the technique of FIRSKE and SUBBA RAW (15). The total free fatty acid (FFA) and cholesterol were estimated following the methods of DUNCOMBE (13) and ZALTIKIS *et al.* (50) respectively; inorganic phosphorus, by following the technique noted by Sigma (40); total protein, by Folin phenol method (26). For the estimation of DNA and RNA the methods of CHERRY (10) for extraction purpose and that, of CERIOTTI (8) for estimation purpose were followed. Ascorbic acid was estimated

by the method of ROE and KUETHER (38). The activity of acid phosphatase (EC - 3.1.3.2) and alkaline phosphatase were measured by the methods given by Sigma (41) while the activity of amino transferase, L-alanine:2 oxoglutarate aminotransferase (GPT) and L-aspartate:2 oxoglutarate aminotransferase (GOT) were determined following the methods of REITMAN and FRANKEL (36). General esterase was estimated by the method of VAN ASPEREN (46) and 5'nucleotidase, by the method given by Sigma (39), while Catalase (EC - 1.11.1.6) and peroxidase (EC-1.11.1.7) by the methods of SNELL and SNELL (43) and KAR and MISHRA (21) respectively.

Testis and ovary were removed from the experimental insects and dried to constant weight separately (14-29 h at 70-75°C) and weighed to the nearest 0.001 mg electrically operated pan balance. All the experiments were replicated seven times to minimise the error and the mean values which were considered here were further processed statistically for obtaining the critical difference (C.D.) values by following the Duncun's Multiple Range test and Student 't' test.

Results

Concentration of some metabolites i.e. glycogen, trehalose and cholesterol were greater in ovary than in testis (table I). On the other hand the concentration of total protein, total lipid, phospholipid, free fatty acid, DNA, RNA and ascorbic acid were higher in testis than that of ovary and concentration of total carbohydrate and inorganic phosphorus were more or less same in testis and ovary (table I). The activities of some enzymes namely 5'nucleotidase and catalase were higher in ovary but the activities of alkaline phosphatase, acid phosphatase, GOT, general esterase and peroxidase were higher in the case of testis than

that of ovary and the activity of GPT was more or less the same in testis and ovary (table III).

AFTER JUVENOID (JHa) AND ECDYSTERONE APPLICATION

On carbohydrates. Concentration of total carbohydrate, glycogen and trehalose after JHa and ecdysterone treatment significantly ($P < 0.05$) increased in comparison to the control cases both in testis and ovary (table I). The only difference was that in case of ecdysterone treatment the results obtained were more pronounced quantitatively than JHa.

On lipids. After the application of JHa the content of total lipid in testis decreased significantly ($P < 0.05$) in comparison to the control while in case of ovary the result was reverse. After the treatment of ecdysterone the concentration of total lipid significantly ($P < 0.005$) increased in both the gonads. So the function of JHa and ecdysterone in case of testis for lipid metabolism was somewhat antagonistic in nature. Cholesterol concentration steadily increased ($P < 0.05$) in comparison to the control after JHa and ecdysterone injection in both testis and ovary but in case of free fatty acid the results were quantitatively reverse in both the gonads. The phospholipid content increased after the treatment of JHa and ecdysterone in testis in comparison to the control but in case of ovary the action of these two hormones was antagonistic i.e. phospholipid content decreased significantly ($P < 0.05$) in case of JHa application while after the injection of ecdysterone concentration of it increased significantly ($P < 0.05$) (table II).

On protein. After the injection of JHa, the concentration of total protein in both testis and ovary decreased signif-

Table 1. Concentration of metabolites ($\mu\text{g}/\text{mg}$ dry weight) in the testis and in the ovary of *Chrysocoris stoll* before and after the injection of JHa and ecdysterone hormone.
Data are the mean values \pm S.E.M. ($n=7$). C.D.* = Critical differences.

Treatments (Number below each treatment indicates the post treatment hours)	Total carbohydrate	Glycogen	Trehalose	Ascorbic acid	Inorganic phosphorous
JHa			Testis		
24	61.82 \pm 2.51	3.86 \pm 0.11	2.73 \pm 0.14	1.64 \pm 0.13	1.66 \pm 0.16
48	68.52 \pm 3.91	9.68 \pm 0.27	4.06 \pm 0.09	2.10 \pm 0.17	1.98 \pm 0.13
72	58.08 \pm 3.54	4.93 \pm 0.25	5.13 \pm 0.48	4.69 \pm 0.51	2.54 \pm 0.17
Control**	26.00 \pm 2.02	2.70 \pm 0.51	1.39 \pm 0.12	1.57 \pm 0.31	1.05 \pm 0.11
Ecdysterone hormone					
24	72.94 \pm 3.53	6.07 \pm 0.49	2.84 \pm 0.48	2.04 \pm 0.31	1.73
48	46.32 \pm 2.89	8.39 \pm 0.74	6.32 \pm 0.76	2.48 \pm 0.27	2.05
72	27.24 \pm 2.67	11.20 \pm 1.05	3.27 \pm 0.32	2.59 \pm 0.42	2.34
C.D.* value at 5 % level	3.78	1.49	1.13	0.39	0.22
JHa			Ovary		
24	28.95 \pm 1.91	9.80 \pm 0.95	2.96 \pm 0.52	5.36 \pm 0.29	1.19 \pm 0.09
48	40.52 \pm 2.52	11.82 \pm 1.23	3.77 \pm 0.57	2.88 \pm 0.18	1.42 \pm 0.19
72	48.01 \pm 2.02	14.83 \pm 0.92	4.38 \pm 0.71	1.93 \pm 0.21	2.17 \pm 0.15
Control**	26.49 \pm 2.51	7.60 \pm 0.81	2.52 \pm 0.23	0.83 \pm 0.11	1.02 \pm 0.12
Ecdysterone hormone					
24	34.87 \pm 2.21	14.25 \pm 1.92	4.35 \pm 0.54	1.26 \pm 0.09	1.32 \pm 0.09
48	40.52 \pm 1.92	18.49 \pm 1.88	4.97 \pm 0.38	2.05 \pm 0.07	1.91 \pm 0.13
72	29.54 \pm 2.01	19.39 \pm 2.01	5.21 \pm 0.49	2.24 \pm 0.11	2.30 \pm 0.14
C.D.* Value at 5 % level	2.10	1.68	0.52	0.21	0.12

** As there were no significant differences found in between the results from the controls of different treatment and different time exposure only the mean values are given here.

Table II. Changes in the concentration of metabolites ($\mu\text{g}/\text{mg}$ dry weight) in the testis and in the ovary of *Chrysocoris stoll* before and after the injection of JHa and ecdysterone hormone.
Data are the mean values \pm S.E.M. ($n=7$). C.D.* = Critical differences.

Treatments (Number below each treatment indicates the post treatment hours)	Testis						
	Total lipid	Total cholesterol	FFA	Phospholipid	Total protein	DNA	RNA
<i>JHa</i>							
24	372.83 \pm 17.21	18.65 \pm 1.91	43.39 \pm 2.34	0.82 \pm 0.09	25.62 \pm 1.51	34.32 \pm 1.91	8.60 \pm 1.02
48	135.86 \pm 9.51	22.89 \pm 1.56	15.79 \pm 1.51	1.63 \pm 0.12	42.01 \pm 1.95	37.45 \pm 1.95	9.61 \pm 1.00
72	456.51 \pm 15.91	33.45 \pm 2.21	13.61 \pm 1.54	2.19 \pm 0.22	60.69 \pm 2.03	42.91 \pm 2.31	11.74 \pm 1.23
Control**	626.97 \pm 14.23	13.07 \pm 1.99	155.02 \pm 3.41	2.52 \pm 0.13	94.99 \pm 3.05	74.39 \pm 3.12	21.01 \pm 1.51
<i>Ecdysterone hormone</i>							
24	708.16 \pm 10.41	23.15 \pm 1.16	40.72 \pm 2.59	1.76 \pm 0.18	104.63 \pm 3.15	79.45 \pm 3.11	8.85 \pm 1.09
48	769.33 \pm 17.21	37.18 \pm 2.12	61.41 \pm 3.92	1.79 \pm 0.15	137.30 \pm 2.98	133.09 \pm 4.21	9.75 \pm 0.92
72	957.65 \pm 16.21	68.10 \pm 2.41	69.51 \pm 3.73	2.11 \pm 0.22	155.02 \pm 3.54	152.90 \pm 3.17	11.62 \pm 1.07
C.D.* value at 5 % level	12.25	3.51	2.99	0.29	2.52	4.23	2.51
<i>JHa</i>							
<i>Ovary</i>							
24	472.91 \pm 14.21	38.47 \pm 3.31	22.08 \pm 1.31	0.84 \pm 0.06	26.38 \pm 2.31	11.55 \pm 1.21	3.89 \pm 0.14
48	607.43 \pm 18.32	73.77 \pm 2.51	21.21 \pm 0.95	0.74 \pm 0.03	46.57 \pm 2.51	18.29 \pm 1.91	2.66 \pm 0.17
72	912.81 \pm 23.23	53.19 \pm 2.25	10.76 \pm 1.55	0.32 \pm 0.02	66.25 \pm 3.47	21.29 \pm 1.09	4.99 \pm 0.24
Control**	395.05 \pm 9.51	28.14 \pm 1.92	59.73 \pm 3.91	1.59 \pm 0.09	75.76 \pm 3.42	22.09 \pm 2.01	6.14 \pm 0.15
<i>Ecdysterone hormone</i>							
24	719.07 \pm 9.42	44.33 \pm 2.31	19.53 \pm 1.11	1.79 \pm 0.12	82.08 \pm 3.91	24.76 \pm 1.95	4.17 \pm 0.33
48	855.83 \pm 8.92	49.50 \pm 3.54	13.60 \pm 1.13	1.97 \pm 0.09	107.54 \pm 4.23	54.83 \pm 2.23	3.35 \pm 0.41
72	976.77 \pm 13.92	59.63 \pm 3.91	10.47 \pm 1.54	2.25 \pm 0.15	125.16 \pm 3.99	63.44 \pm 3.51	2.81 \pm 0.26
C.D.* value at 5 % level	15.73	2.91	1.91	0.24	4.42	2.05	0.85

** As there were no significant differences found in between the results from the controls of different treatment and different time exposure only the mean values are given here.

Table III. Activities of some cellular enzymes (m-unit/100 mg dry weight/min) in the testis and in the ovary of *Chrysocoris stollis* before and after the injection of JHa and ecdysterone hormone.
Data are the mean values \pm S.E.M. (n = 7). C.D. = Critical differences, * expressed as m-unit/mg dry weight/min.

Treatments (Number below each treatment indicates the post treatment hours)	Ovary						
	Alkaline phosphatase	Acid phosphatase	GOT	GPT	General esterase*	5'nucleotidase	Catalase* Peroxidase
<i>JHa</i>							
24	5.61 \pm 0.18	0.63 \pm 0.09	0.72 \pm 0.04	0.94 \pm 0.11	0.71 \pm 0.05	0.75 \pm 0.12	36.84 \pm 2.91
48	4.33 \pm 0.21	0.68 \pm 0.06	0.52 \pm 0.03	1.28 \pm 0.12	0.60 \pm 0.04	0.62 \pm 0.09	38.41 \pm 3.23
72	3.73 \pm 0.24	0.72 \pm 0.06	0.46 \pm 0.05	1.62 \pm 0.12	0.43 \pm 0.04	0.61 \pm 0.06	39.87 \pm 3.91
Control**	12.19 \pm 1.04	0.25 \pm 0.03	0.93 \pm 0.08	0.35 \pm 0.04	1.48 \pm 0.11	1.84 \pm 0.11	63.23 \pm 4.27
<i>Ecdysterone hormone</i>							
24	19.45 \pm 1.82	0.40 \pm 0.02	0.16 \pm 0.02	0.66 \pm 0.05	0.82 \pm 0.07	2.60 \pm 0.21	51.66 \pm 2.91
48	33.82 \pm 2.51	0.88 \pm 0.07	0.18 \pm 0.01	0.72 \pm 0.04	0.71 \pm 0.05	2.72 \pm 0.19	55.29 \pm 3.23
72	16.25 \pm 1.67	0.77 \pm 0.05	0.21 \pm 0.02	0.78 \pm 0.06	0.66 \pm 0.06	2.94 \pm 0.14	58.79 \pm 3.41
C.D. value at 5% level	1.19	0.07	0.06	0.04	0.13	0.27	2.12
<i>JHa</i>							
24	0.53 \pm 0.05	0.63 \pm 0.02	0.37 \pm 0.01	0.40 \pm 0.02	0.72 \pm 0.05	0.15 \pm 0.01	41.02 \pm 1.25
48	0.71 \pm 0.06	0.49 \pm 0.01	0.47 \pm 0.01	0.56 \pm 0.03	0.89 \pm 0.04	0.18 \pm 0.01	23.22 \pm 0.99
72	1.74 \pm 0.13	0.33 \pm 0.10	0.58 \pm 0.05	0.69 \pm 0.04	0.99 \pm 0.04	0.19 \pm 0.01	28.30 \pm 1.31
Control**	5.87 \pm 0.51	0.24 \pm 0.02	0.25 \pm 0.03	0.35 \pm 0.01	0.25 \pm 0.02	0.38 \pm 0.06	54.53 \pm 2.51
<i>Ecdysterone hormone</i>							
24	4.84 \pm 0.27	0.12 \pm 0.01	0.32 \pm 0.02	0.26 \pm 0.02	0.12 \pm 0.01	0.19 \pm 0.03	28.28 \pm 1.92
48	5.14 \pm 0.15	0.13 \pm 0.01	0.49 \pm 0.04	0.24 \pm 0.01	0.11 \pm 0.01	0.15 \pm 0.12	46.66 \pm 2.09
72	4.17 \pm 0.19	0.15 \pm 0.01	0.68 \pm 0.07	0.27 \pm 0.03	0.14 \pm 0.02	0.12 \pm 0.10	49.79 \pm 2.19
C.D. value at 5% level	0.37	0.02	0.04	0.03	0.05	0.03	4.19

** As there were no significant differences found in between the results from the controls of different treatment and different time exposure only the mean values are given here.

icantly ($P < 0.05$) in comparison to the control (table II) and the total protein significantly ($P < 0.05$) increased after the ecdysterone treatment in both the gonads. The role of JHa and ecdysterone in protein metabolism appeared to be antagonistic in nature.

On nucleic acids. The nucleic acids i.e. DNA and RNA after the injection JHa significantly decreased ($P < 0.05$) both in testis and ovary in comparison to the control insects. But in case of ecdysterone treatment the content of DNA increased while that of RNA decreased in comparison to the control both in testis and ovary which were statistically significant (table II). The action of JHa and ecdysterone in DNA regulation appeared to be antagonistic in nature both in testis and ovary.

On ascorbic acid and inorganic phosphorus. The ascorbic acid and inorganic phosphorus in both the tissues after JHa and ecdysterone treatment increased significantly ($P < 0.05$) (table I).

On enzymes. The activities of alkaline phosphatase acid phosphatase, GPT, 5'nucleotidase and peroxidase increased significantly ($P < 0.05$) and the same of GOT, general esterase and catalase decreased significantly ($P < 0.05$) in testis after the injection of ecdysterone. Similarly in case of JHa treatment the activities of acid phosphatase and GPT significantly increased ($P < 0.05$) and the same of alkaline phosphatase, GOT, general esterase, 5'nucleotidase, catalase and periodase decreased significantly ($P < 0.05$) in testis in comparison to the control. In case of alkaline phosphatase in testis the action of ecdysterone and JHa was found to be antagonistic in nature. In case of ovary after the treatment of ecdysterone the activities of alkaline phosphatase, acid phosphatase, GPT, general esterase, 5'nucleotidase and catalase decreased significantly ($P < 0.05$)

and the activities of peroxidase and GOT increased significantly ($P < 0.05$) in comparison to the control results. Similarly the activities of acid phosphatase, GOT, GPT and general esterase increased significantly ($P < 0.05$) while the activities of alkaline phosphatase, 5'nucleotidase, catalase and peroxidase in comparison to the control cases of ovary after the injection of JHa. In case of acid phosphatase and peroxidase of ovary the action of these two hormones were antagonistic in nature (table III).

Discussion

The present work demonstrated the functional role of juvenoid (JHa) and ecdysterone administration in *Chrysocoris stoll* in male and female insects on some biochemical parameters during maturation. The role of both juvenile hormone and ecdysterone on the ovarian maturation had already been well documented by some workers (5, 12, 14, 33), but the role of these two major hormones on testis was yet to be properly understood at least in biochemical levels (11, 14, 29). MANDAL *et al.* (29) and DEB and CHAKRAVORTY (11) for the first time demonstrated by histological and biochemical methods that juvenile hormone played important role on the structural and functional changes of testis in insects. But the role of ecdysterone on testis was still in nebular state. The present experimental results clearly demonstrated that both the exogenous JHa and ecdysterone application caused some drastic alteration in the biochemistry of both testis and ovary. The experimental data regarding the juvenoid treatment on the biochemistry of ovary fully corroborated the findings of other workers in other insect species (12, 14, 29, 30). The only exception, were the enzymes, catalase and peroxidase which showed the lesser activities after JHa treatment in ovary

thus signifying the shifting of metabolic status from oxidative state to reductive state and ultimately setting in an anabolic phenomenon. Although the ecdysteroid in reproductively competent adult female insects has been found in several insect species (4, 19); but the biological function of this ecdysterone is not yet clear and different hypothesis has been proposed in regard to its function (51). The majority of the macromolecules like total carbohydrate, glycogen, trehalose, ascorbic acid, inorganic phosphorus, total lipid, phospholipid and DNA contents in the ovary significantly increased in comparison to the control after ecdysterone treatment and such a result indicated that the ovary underwent a rapid biochemical maturation with the influence of ecdysterone as observed in case of JHa treatment in the present case and also observed earlier by others (7, 14, 27, 45, 48). But how the ecdysterone observes this function in ovary is not clear because the function of this ecdysteroid in oogenesis is still very conjectural and much variation has been observed in different species (51). The effect of JHa and ecdysterone on the biochemical components of testis showed more or less similar qualitative and quantitative responses as in case of females but frankly speaking what it was due to is still uncertain (29, 30). Both the ecdysterone and JHa produced the rapid and significant increase almost of all the major components excepting FFA, phospholipid, RNA and the enzymes catalase, GOT and general esterase in testis. According to ENGELMANN (14), the significant decline of FFA, phospholipid and protein after JHa treatment was due to the mobilization of the reserve which was mediated by JHa. The macromolecules, total lipid and protein and enzymes like peroxidase and 5'nucleotidase showed the significantly different results after JHa and ecdysterone treatment in testis

i.e. total lipid, protein, peroxidase and 5'nucleotidase increased significantly after ecdysterone treatment while the reverse results were obtained after JHa treatment. These results indicated that both ecdysterone and JHa had the similar function in gonadal maturation but the responsiveness of testis to these two hormones was somewhat different and the mechanism and mode of action of these two hormones were also different (9). The acid phosphatase was universally known as a degradative, hydrolytic and lysosomal enzyme, then, why this enzyme significantly increased after both JHa and ecdysterone treatment in both testis and ovary was not clearly understandable. However, it might be assumed that the increased activity of acid phosphatase might be due to the JHa and ecdysterone inducing cell proliferation of the gonads as already suggested by BASSI and FEIR (2).

Nowadays several hypotheses existed to explain the function of juvenile hormone and ecdysterone in both male and female insects and most of them suggested that these two hormones affected the general metabolism of insects (12, 27, 35). But SLAMA (42) demonstrated that these two hormones were gonadotropic hormones and controlled the metabolism related only to the gonadal maturation in insects. However, the comparison of the effects of these two hormones in between the different orders of insects appeared to be of no practical value and it would be unwise to compare the effects of these two hormones between different insect species belonging to same order (28). The present experimental results taken together with those of other workers (2, 48) led to suggest that these two hormones functioned only through the regulation of different enzymes. Both the treatment of ecdysterone and JHa increased the activities of most of the enzymes in testis

with the exception of catalase, GOT and general esterase. But results of this study do not permit us to say whether the increased activities of these enzymes was due to the rapid transcription/translation or to the better availability of the active metal ions which served as co-factors of these enzymes. But why the activity of catalase decreased in testis and ovary after the treatment of both these two hormones was not clearly understood. One possible reason for this sort of decline of catalase activity might be attributed to the less production of the toxic H_2O_2 from purine catabolism (3, 28), because the main role of the catalase enzyme was to reduce the oxygen cytotoxic effect of H_2O_2 (3, 49). The activity of 5'nucleotidase in both testis and ovary after application of these two hormones was very interesting because 5'nucleotidase was supposed to facilitate the adenosine transport to the membrane. The decrease in the activity of 5'nucleotidase after the JHa treatment indicated that the permeability of membrane against adenosine was impaired whereas increased activity of 5'nucleotidase after ecdysterone treatment indicated that the adenosine transport was facilitated by ecdysterone.

From the above discussion it might be inferred that the target organ of the ecdysterone and JHa was not only fat body, the testis and ovary could well act as a target organ and the biochemical maturation of the male and female gonads are controlled by these two hormones.

Acknowledgement

The authors are grateful to Prof. A. M. De Oliveira Filho (Universidade Federal do Rio de Janeiro, Instituto de Pesquisas da Marinha, Brazil) and Rhoto Pharmaceutical Co. Ltd. (Osaka, Japan) for the kind gift of the juvenile and ecdysterone respectively. This work was supported by the Council of Scientific and Industrial Research (New Delhi).

Resumen

Se estudian los cambios en el metabolismo del testículo y del ovario del *Chrysocoris stoll* por el tratamiento con análogo hormonal juvenil (AHj) y con ecdisterona. Después de la aplicación exógena de AHj en dosis selectiva, hay incremento significativo de carbohidrato total, glucógeno, trealosa, colesterol, ácido ascórbico y fósforo inorgánico, mientras el ácido graso libre, el fosfolípido, la proteína total, el RNA y el DNA disminuyen significativamente respecto al control. El lípido total disminuye en testículo e incrementa significativamente en ovario después de la inyección de AHj. Las actividades de enzimas celulares, como la fosfatasa alcalina, 5'nucleotidasa, catalasa y peroxidasa, disminuyen significativamente mientras la fosfatasa ácida y la GPT aumentan. Las actividades de GOT y esterasa general disminuyen significativamente en testículo y aumentan en ovario. La aplicación exógena de ecdisterona produce una respuesta similar a la de la AHj, aunque difiere en algunos casos como en el del ecdististeroide en que produce resultados contrarios. Se discute el modo de acción de ambas hormonas.

References

1. BASSI, E. and FEIR, D.: *Insect Biochem.*, **1**, 428-432, 1971.
2. BASSI, E. and FEIR, D.: *J. Insect Physiol.*, **23**, 761-763, 1977.
3. BÉLPOMMÉ, M. B. and ROFF, M.: *Europ. J. Biochem.*, **121**, 349-355, 1982.
4. BRIERS, T. and DE LOEF, A.: *Int. J. Invert. Reprod.*, **2**, 263-272, 1980.
5. BRIERS, T. and DE LOOF, A.: *Int. J. Invert. Reprod.*, **3**, 145-155, 1981.
6. BRIERS, T., PERFEROERS, M. and DE LOOF, A.: *Physiol. Entomol.*, **7**, 379-386, 1982.
7. BOROVSKY, D. and VAN HANDAL, E.: *J. Insect Physiol.*, **25**, 861-865, 1979.
8. CERIOTTI, G.: *J. Biol. Chem.*, **214**, 59-70, 1955.
9. CHERBAS, P., CHERBAS, L., DEMETRI, G., MANTEUFFEL, C. M., SEVAKIS, C., YONGE, C. D. and WILLIAMS, C. M.: In «Gene Regulation by Steroid Hormones» (A. K. Roy and J. H. Clurks, eds.), Springer-Verlag, Berlin, 1980, pp. 278-308.

10. CHERRY, J. H.: *Plant Physiol.*, **37**, 670-678, 1962.
11. DEB, D. C. and CHAKRAVORTY, S.: *Ind. J. Exp. Biol.*, **20**, 742-749, 1982.
12. DE WILDE, J. WILDE, J. and DE LOOF, A.: In «The Physiology of Insecta» (M. Rockstein, ed.), Academic Press, New York, 1973, vol. 1, pp. 97-150.
13. DUNCOMBE, W. G.: *Biochem. J.*, **88**, 7-10, 1964.
14. ENGELMANN, F.: *The Physiology of Insect Reproduction*. Pergamon Press, Oxford, 1970.
15. FIRSKE, C. H. and SUBBA RAW, Y.: *J. Biol. Chem.*, **66**, 375-380, 1925.
16. GARCÍA, M. L. M., MELLO, R. P. and GARCÍA, E. S.: *J. Insect Physiol.*, **25**, 695-700, 1979.
17. GILLOTT, C. and FRIEDEL, T.: *J. Insect Physiol.*, **22**, 365-372.
18. HETRU, C., KAPPLER, C., HOFFMANN, J. A., NEARN, R., LUU, B. and HORN, D. H. S.: *Mol. Cell. Endocrinol.*, **26**, 51-80, 1982.
19. HOFFMANN, J. A., LAGUEUX, M., HETRU, C., CHARLET, M. and GOLTZENE, F.: In *Progress in Ecdysone Research* (J. A. Hoffman, ed.), 1980, pp. 431-465.
20. HOLWORDA, D. A., VAN DOORN, J. and BEENAKKERS, A. M. Th.: *Insect Biochem.*, **7**, 151-157, 1977.
21. KAR, M. and MISHRA, D.: *Plant Physiology*, **57**, 315-319, 1976.
22. KELLY, T. J. and FUCHS, M. S.: *J. exp. Zool.*, **213**, 25-32, 1980.
23. KELLY, T. J., WHISENTON, L. R. and FUCHS, M. S.: *Am. Zool.*, **20**, 863, 1980.
24. KELLY, T. J., FUCHS, M. S. and SUK-HEE KANG: *Int. J. Invert. Reprod.*, **3**, 101-112, 1981.
25. KOOLMAN, J.: In «Progress in Ecdysone Research» (J. A. Hoffman, ed.). Elsevier/North Holland Biomedical Press, Amsterdam, 1980, pp. 187-209.
26. LOWRY, O. H., ROSEBOROUGH, N. J., FARR, A. L. and RANDALL, R. J.: *J. Biol. Chem.*, **193**, 265-275, 1951.
27. MANDAL, S.: *Ph. D. Thesis*, The University of Burdwan, Burdwan, India, 1983, p. 324.
28. MANDAL, S.: *Zool. Jb. Physiol.*, **89** (in press).
29. MANDAL, S., GHOSH, B. and CHOUDHURI, D. K.: *Zool. Jb. Physiol.*, **86**, 259-265, 1982.
30. MANDAL, S. and CHOUDHURI, D. K.: *Egyptian J. Physiol.*, **10**, 1984 (in press).
31. MASLER, E. P., FUCHS, M. S., SAGE, B. and O'CONNER, J. D.: *Gen. Comp. Endocrinol.*, **41**, 250-259, 1980.
32. MORDUE, W. and GOLDSWORTHY, G. J.: *Gen. Comp. Endocrinol.*, **12**, 360-369, 1969.
33. NOVAK, V. J. A.: *Insect Hormones*, Chapman & Hall, London, 1975, pp. 118-382.
34. ODHIAMBO, T. R.: *Trans. Roy. Ent. Soc. Lond.*, **118**, 393-412, 1966.
35. ORR, C. W. M.: *J. Insect Physiol.*, **10**, 53-64, 1964.
36. REITMAN, S. and FRANKEL, S.: *Am. J. Clin. Path.*, **28**, 56-59, 1957.
37. ROE, J. H.: *J. Biol. Chem.*, **212**, 335-343, 1955.
38. ROE, J. H. and KUETHER, C. A.: *J. Biol. Chem.*, **147**, 399, 1943.
39. Sigma Chemical Co.: *Sigma Tech. Bull. No.*, 675, 1978.
40. Sigma Chemical Co.: *Sigma Tech. Bull. No.*, 670, 1982.
41. Sigma Chemical Co.: *Sigma Tech. Bull. No.*, 104, 1982.
42. SLAMA, K.: *J. Insect Physiol.*, **10**, 283-303, 1964.
43. SNELL, F. D. and SNELL, C. T.: *Colorimetric Methods of Analysis*. Van Nostrand Reinhold Pub. Comp., Amsterdam, 1971, vol. IV, pp. 7-145.
44. TOBE, S. S., MUSTERS, A. and STAY, B.: *Physiol. Entomol.*, **4**, 79-86, 1979.
45. TSUMUKI, H. and KANEHISA, K.: *Appl. Ent. Zool.*, **16**, 7-15, 1981.
46. VAN ASPEREN, K.: *J. Insect. Physiol.*, **8**, 401-416, 1962.
47. VAN HANDEL, E.: *Analyt. Biochem.*, **11**, 256-265, 1965.
48. VAN PELT VERQUIL, E.: *Ph. D. Thesis*, Dutch Efficiency Bureau, Leiden, Pijnacker, 1979.
49. VUILLAUME, M., CALVAYRAE, R., HUBERT, M., BRIAND, J. and BEST-BELPOMME, M.: *Biol. Cell.*, **43**, 189-194, 1982.
50. ZALTIKIS, A., ZAK, B. and BAYL, A. J.: *J. Lab. Clin. Med.*, **41**, 486-490, 1953.
51. ZHU, X. X., GFELLER, H. and LANZERIN, B.: *J. Insect. Physiol.*, **29**, 225-235, 1983.