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Changes of Cholesterol Levels in Different Tissues of Adult *Lohita grandis*. Effect of Allatectomy, Brain-cauterization and Juvenoids Treatment

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Allatectomy, brain-cauterization and the operation of both at a time in an insect, *Lohita grandis* produce more or less the similar trend of effect regarding the cholesterol content. After the operation the cholesterol accumulated in all the tissues is more than that of control insects. The application of juvenoids into the allatectomized insects reverses the effect of allatectomy. The physiological significance of this finding is discussed in relation to the endocrine control of cholesterol metabolism.

Key Words: *Lohita grandis*, **Cholesterol, Allatectomy, Brain-cauterization, Juvenoid**

Introduction

Cholesterol is essential for normal growth, reproduction, larval moulting and metamorphosis (Gilbert 1967, Robbins et al. 1971) in insects. Without having the ability of their own to synthesize sterol, a dietary supply of the same is indispensable for their normal growth (Clayton 1964. Clark & Bloch 1959, Norris et al. 1969). The importance of cholesterol as a pre-requisite in the synthesis of different steroid hormones (Kaplanis et al. 1966, Thompson et al. 1975, Robbins et al. 1971) and its conversion into different steroid hormones occurring in different tissues of insects, under the stimulation of different endocrine glands (Gilbert & King 1973, Katz et al. 1971, Goodfellow et al. 1971) and the existence of a close functional relationship between the brain's neurosecretory cells, corpora-allata and the prothoracic gland, are well known.

The present work is undertaken to investigate the role of corpora-allata, brain and synthetic juvenoids on the cholesterol metabolism in both the sexes of adult *Lohita grandis*, a pyrrhocorid bug.

Material and Methods

The insects were maintained in the laboratory by following the methods described earlier (Mandal & Choudhuri 1982). Both sexes of adult *Lohita* (just 3 hr after adult ecdysis) were used in this

experiment. The allatectomy and brain-cauterization were performed by following the method described by Stay and Tobe (1977) and Girardie (1966), respectively. The juvenoids used were the analogue FMC-23509 (CRD-9499) obtained from FMC Corporation, New York, by Dr David S Pincus, and a derivative of N-geranylaniline (N-2, 5-dichlorophenyl-3, 7-dimethyl-2, 6-octadienylamine) obtained from Universidade Federal do Rio de Janeiro e Institute de Pesquisas da Marinha, Brazil, by Dr A M De Oliveira Filho. These were dissolved in double distilled acetone and applied by injection into the abdomen using a microsyringe (Hamilton) with a dose of 20 μg/insect 10 μl acetone. The control insects received only the equivalent volume of acetone in the same manner as the hormone-treated ones. The haemolymph was obtained by cutting the femur of the hind leg. It was pooled from a large number of insects of the required sex in the small ice-cooled centrifuge tubes which were previously coated with phenylthiourea to inhibit tyrosinase activity. The haemolymph was then centrifuged to obtain a supernatant free from haemocytes. The cholesterol content was directly estimated by following the colorimetric method of Zaltikis et al. (1953). The protein content of haemolymph and other tissues were determined by the methods of Lowry et al. (1951). The results were analysed statistically by following the Duncan's Multiple Range Test.

Results

The results obtained here show the differential cholesterol concentration in different tissues of both sexes. Allatectomy in both sexes produces more or less similar effect i.e., the concentration of cholesterol increases in different tissues after the operation of corpora-allata (table 1). The quantity of cholesterol augmentation was parallel in all the tissues from both sexes after operation. Treatment with a large dose of juvenoid into the allatectomized insects reverses the effect of allatectomy (table 1). The application of two different juvenoids into the allatectomized insects separately, produces more or less similar effect but the most effective compound regarding its action on cholesterol was the N-geranylaniline derivative. The responsiveness of different tissues from both sexes to the juvenoid action was also different. Brain-cauterization produces also the same effect as in the case of allatectomy but the amount of increase in cholesterol content after brain-cauterization is much less than that from the allatectomized ones (table 2). The increase of cholesterol level in different tissues is at its highest after the removal of both corpora-allata and brain. Here almost all of the tissues exhibit more or less two-fold increase in cholesterol level than that of the control. Time of exposure after different treatment also produces significant effect.

Discussion

Though the actual pathways of sterol metabolism and its regulation in insects were not clearly explicit but its importance regarding different steroid hormones (like ecdysone, testosterone, etc.), their biosynthesis and other structural roles were well established (Sovoboda et al. 1975). The synthesis of different steroid hormones from cholesterol occurred mainly in fat bodies, gonads and partly in other tissues of adult insects (Gilbert & King 1973). Extra accumulation of cholesterol in all the tissues of both sexes after allatectomy, brain-cauterization

and operation of both a time compared to control was probably due to the inhibition of cholesterol-utilization for different synthetic activity. According to Lea (1972) and Gilbert and King (1973) the neurosecretory hormone and corpus allatum hormone stimulated the production of ecdysone. So, the extra accumulation of cholesterol after allatectomy and brain-cauterization as found here in all the tissues was probably due to the inhibition of ecdysone-synthesis. It might also be possible that this extra accumulation of cholesterol in all the tissues after allatectomy or brain-cauterzation was due to the rapid release of cholesterol from different sources where cholesterol remained in bound-form with other chemical substances (e.g. cell membranes) to maintain the structural integrity (Choudhuri & Mandal 1982); because the absence of corpora allata or brain (major neuro-endocrine centers) might cause the cellular disintegration (Gilbert & King 1973). The treatment of juvenoids into the allatectomized insects caused cholesterol to drop rapidly compared to that of only allatectomized and also of control insects, which demonstrated a positive role of juvenoids (JHa), juvenile harmone (JH) and corpora allata in the cholesterol metabolism of insects. This observation as regards the role of JHa on cholesterol content in insects

Table 1 *Showing the levels of cholesterol in different tissues of adult* Lohita grandis *after allatectomy and treatment of juvenoids into the allatectomized insects. Contents represents as µg/mg tissue protein*

Tissues	Sex	Allatectomized		Allatectomized + Juvenoids treatment			
		24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
				JHa-1		JHa-2	
Haemolymph	M	14.0 (0.1)	14.8 (0.6)	8.2* (0.1)	7.1** (0.2)	10.0* (0.1)	9.9** (0.3)
	F	30.7 (0.1)	32.0 (0.2)	9.0** (0.1)	8.0** (0.2)	16.2** (1.0)	16.0** (0.8)
Fat-body	M	50.3 (0.4)	54.3 (0.2)	24.4** (0.8)	22.1** (1.1)	32.5** (0.2)	31.0** (0.5)
	F	82.9 (0.4)	92.2 (1.1)	30.1** (1.1)	25.9** (0.9)	44.1** (0.2)	40.7** (0.3)
Intestine	M	3.9 (0.1)	4.8 (0.2)	3.0 (0.1)	2.8* (0.2)	3.8 (0.1)	3.1 (0.1)
	F	4.5 (0.2)	8.0 (0.2)	2.9* (0.5)	2.9* (1.2)	4.0 (0.1)	4.0 (0.1)
Testis		18.2 (1.2)	18.5 (1.5)	16.2* (0.3)	16.0* (0.9)	18.0 (0.2)	16.2* (1.6)
Ovary		28.0 (0.7)	36.2 (0.7)	11.5** (0.2)	10.3** (0.3)	17.5** (0.1)	16.1** (0.8)

JHa-1 : N-geranylaniline derivative; *JHa-2*; FMC-23509 (CRD-9499)

Number within the parentheses indicates the ± S.E. ($n=18$)

* = Significant at $p < 0.05$; ** = Significant at $p < 0.01$; in comparison with the allatectomized one

F, Female; *M*, Male

further strengthened the view that juvenile hormone was necessary for the proper utilization of cholesterol in insects (Choudhuri & Mandal 1982). But it is still unknown why one type of juvenoid is more active than other type in one insect, at least regarding its effect on cholesterol metabolism and utilization. The present results showed that N-geranylaniline derivatives (JHa-1, see table 1) was more active than the compound FMC-23509 (CRD-9499) (JHa-2, see table 1) in *L. grandis* and this result also supported the contention of Filho et al. (1981), according to whom N-geranylaniline derivatives were the most active juvenoids (JHa) in bugs regarding their effects on morphogenesis. Another inference which could be made from the results obtained here was that the potentiality of different tissues from both sexes were not equally sensitive to the different froms of juvenoid action. It was apparent that unlike males, the females were more responsive to juvenoid action on cholesterol metabolism, and of the tissues from both sexes. Fat-body and gonads responded more than the other tissues.

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Table 2 *Showing the fluctuation of cholesterol level in the different tissues of adult* Lohita grandis *after brain-cauterized and allatectomized + brain-cauterized. Contents represents as μg/mg tissue protein ± S.E. (n=18)*

Tissues	Sex	Control	Brain-cauterized		Allatectomized + Brain-cauterized	
			24 hr	48 hr	24 hr	48 hr
Haemolymph	M	10.4 (0.1)	11.5* (0,1)	13.9** (0.1)	16.8** (0.9)	18.6** (0.1)
	F	18.9 (0.3)	22.0* (0.3)	25.3** (0.2)	32.0** (1.2)	34.3** (0.8)
Fat-body	M	36.0 (0.4)	38.9 (0.3)	45.3** (0.9)	58.2** (2.1)	60.2** (0.1)
	F	52.0 (0.2)	62.0* (0.3)	66.4** (0.5)	98.0** (1.2)	98.5** (2.5)
Intestine	M	3.9 (0.1)	5.9* (0.1)	5.9* (0.1)	6.1* (0.8)	6.5* (0.4)
	F	4.0 (0.5)	6.0 (0.1)	6.9* (0.4)	6.4* (0.2)	7.5* (0.1)
Testis		18.0 (0.6)	20.9 (0.3)	25.0* (0.3)	25.3* (0.4)	26.0** (0.3)
Ovary		22.2 (0.7)	28.2** (0.2)	33.0** (1.2)	30.0** (0.4)	39.5** (0.6)

Number within the parentheses indicates the ± S.E.

* = Significant at $p < 0.05$; ** = Significant at $p < 0.01$; in comparison with the control (as there were no significant differences found in between the control of each treatment, only the mean values are given)

F, Femal; *M*, Male

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