EFFECT OF BRAIN HORMONE AND CORPUS ALLATUM HORMONE ON THE PROTEIN CONCENTRAION IN CYBISTER CONFUSUS (DYTISCIDAE: COLEOPTERA)

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ABSTRACT

Quantitative assays of protein of the haemolymph, fat body, Testis and ovary of Cybister confuses have been done. A gradual rise in total protein concentration after the treatment with both the hormone was noticed in fat body testes and ovary, whereas early increase and later decline in haemolymph. Protein concentration is suggestive of greater demand of Protein in Oogenesis and Spermatogenesis under the influence of both the hormone.

Keywords : Brain hormone, Corpus allatum, Protein, Haemolymph, Cybister Confusus.

INTRODUCTION

Protein constitutes one of the fundamental structural component of all living system owing to its profound importance in different metabolic aspects. Proteins seems to be indispensable for cellular differentiation during morphogenesis, metamorphosis as well as during maturation.

Many of Morphogenetic changes are under the direct control of hormones which act directly on the chromosomes to affect gene action and thereby regulate protein synthesis and control the one going changes. [Lewenhook (1966), Chippendale and Killry (1961), Pant and Morrib (1972), Ring (1973), Sharma & Enteshamuddin (1988)].

This analysis of the pattern of variation of nitrogenous constituents during the development and maturation periods serve as the index of dynamics of developmental trend. These experiments clearly indicate that protein metabolism is partly under the control of Brain hormone or Corpus allatum hormone secreted by the corpora allata or by both.

MATERIAL AND METHODS

The mature and immature insects were collected from local ponds and were brought to laboratory and put in glass aquaria. Some water hyacinth and Hydrilla and small fish were kept in the aquaria and the insects were acclimatized at least for five days prior to performing experiments.

The rearing insects were divided into three groups. Some insects were injected with 0.025ml. acetone diluted with distilled water (1:4). This served as the control of the latter two groups (each group consisting 78 to 100 insects), one group was injected with corpus allatum homone (0.025 ml. in diluted acetone) with the help of an insulin syringe.

Isolation of Corpus allatum hormone

Corpora allata were dissected out from 50 insects and they were kept in 1ml. of acetone. They were homogenized in a glass homogenizer. Then 4ml. of acetone was added to the homogenate and centrifused at 3000 rpm for 10 minutes. The clear liquid was poured in a clean glass tube and stored at 4^0 to5^0C in a refrigerator. This served as a Corpus allatum hormone.

Isolation of Brain hormone

Brains (Pars intercerebralis) were dissected out from 50 insects and were kept in 1 ml. of acetone. They were homogenised in glass homogenizer, 4 ml. of acetone was added to homogenate and centrifuged at 3000 rpm. for 10 minutes. The clear liquid was poured in a clean tube and stored at 4^0 to 5^0C in refrigerator. This served as brain hormone.

Haemolymph was drawn by puncturing the tip of the head with a fine needle and then centrifuging the insects in a centrifuge tube at 3000 rpm. for 10 minutes 5ml of 10% trichloro acetic acid was added to 1 ml. haemolymph in order to precipitate the protein and then centrifuged at 3000 rpm. The supernetant obtained was used the Quantitative estimation for carbohydrates. The residues of the precipitated protein was dissolved in 5 ml. of 0.1N, NaOH, for the Quantitative estimation of haemolymph protein. The fat body, Testis and ovary were dissected out from the adult insect weighed and homogenized. Protein was precipated and removed as mentioned above. Quantitative estimation of Protein was done by employing Lowry's method.

The absorption was read in an Erma (Japan) Calorimeter at 620 μm.

	Sex	Stage	Control	Treated	
				Brain hormone	Corpus allatum
MALE	Haemolymph	24h	13.5 ± 2	16 ± 2.5 N.S.	22 ± 2.5 xxx
		48h	14 ± 2	28 ± 3 xxx	11 ± 2 x
		72h	20 ± 2	25 ± 2.5 xx	13 ± 2.5 xx
		7 days	21 ± 2.5	15 ± xx	10 ± 1.5
	Fat body	24h	115 ± 4	125 ± 5 xx	152 ± 8 xxx
		48h	120 ± 5	165 ± 8 xxx	105 ± 5 xx
		72h	120 ± 6	120 ± 5 xx	130 ± 4 x
		7 days	120 ± 5	120 ± 5 N.S.	120 ± N.S.
	Testes	24h	110 ± 5	110 ± 5	160 ± 9 xxx
		48h	110 ± 6	110 ± 6	155 ± 8 xxx
		72h	110 ± 5	110 ± 5	150 ± 6 xxx
		7 days	108 ± 4	108 ± 4	140 ± 5 xxx
FEMALE	Haemolymp	24h	13.5 ± 2	30 ± 5 xxx	18 ± 2 xx
		48h	14 ± 2	45 ± 5 xxx	11 ± 2 xxx
		72h	20 ± 2	40 ± 4 xxx	18 ± 3 xxx
		7 days	21 ± 2.5	40 ± 5 xxx	20 ± 4 xxx
	Fat body	24h	115 ± 4	150 ± 8 xx	50 ± 2 xxx
		48h	120 ± 5	160 ± 6 xxx	100 ± 3 N.S.
		72h	120 ± 6	130 ± 5 xx	125 ± 5 xxx
		7 days	120 ± 5	120 ± 5 N.S.	135 ± 5 xxx
	Ovary	24h	105 ± 3	150 ± 8 xx	175 ± 5 xxx
		48h	110 ± 4	185 ± 9 xxx	120 ± 4 xx
		72h	110 ± 4.5	130 ± 6 xx	160 ± 3 xxx
		7 days	115 ± 4	120 ± 5 N.S.	180 ± 5 xxx

Mean ± S.D. – Standard Deviation

N.S. = Not significant

Significant = / = • =P<0.05, •••=P < 0.001

••=P < 0.01

RESULT AND DISCUSSION

Haemolymph concentration in male at control was 13.5±2 S.D. mg / ml, 14 ± 2 S.D. mg / ml., 20 ± S.D. mg / ml., 21 ± 2.5 S.D. mg / ml. at 24 hrs., 48 hrrs, 72 hrs and 7 days respectively.

In female Haemolymph protein concentration at control was 12.5 ± 1.5 S.D. mg/ ml, 14 ± 1.5 S.D. mg / ml, 10 ± 15 S.D. mg / ml, 10 ± 1.5 S.D. mg / ml, 8 ± 1 S.D. mg/ ml. at 24 hrs, 48 hrs, 72 hrs and 7 days respectively.

After the treatment with Brain hormone protein concentration increased significantly at 24 hours, 48 hours and 72 hours but decline at 7 days. A significant increase (P<0.001) over control insect in haemolymph protein was noticed in the haemolymph Protein concentration of the female. After the treatment with Corpus allatum hormone the male haemolymph Protein concentration early increase and later decrease in haemolymph protein was noticed in male.

Fat body protein concentration at control in male was 115 ± 4 S.D. mg / gram, 110 ± 65 S.D. mg / gram, 120 ± 5 S.D. mg / gram, 120 ± 6 S.D. mg / gram, 120 ± 5 S.D. mg/ g at 24 h, 48 h, 72 h, 7 days respectively. Fat body protein concentration, at control in female was 110 ± 3.5 S.D, 120 ± 5 S.D. / mg, 110 ± 3 S.D./ mg / gram, 105 ± 4 S.D. / mg at 24 h, 48 h, 72 h and 7 days respectively.

After treatment with Brain hormone the fat body Protein shows early increase & later decline in both male & female.

After treatment with corpus allatum hormone the fat body Protein shows early increase & later decline in both male and female.

The Testes Protein concentration at control in the male was 110 ± 5 S.D. mg / gram, 120 ± 5 S.D. / mg / gram, 110 ± 5 mg / gram, 108 ± 4 S.D. mg/ gram respectively.

After treatment with brain hormone Testes protein shows significant increase over the control insects.

After treatment with Corpus allatum hormone Testes protein concentration shows significant increase over the control. Protein concentration in mature ovary in control insect was 105 ± 3 S.D. mg / gram , 110 ± 4 S.D. / mg / gram, 110 ± 4.5 S.D. mg/ gram, 115 ± 4 S.D. / mg/ gram at 24 hours, 48 h, 72 h, 7 days respectively.

After treatment with brain horne a significant increase in protein concentration shows in all stage over the control.

After treatment with Corpus allatus hormone shows a significant increase in Protein concentration in all the stages over the control. Reproduction in insects like other animals is governed by a variety of external and internal stimuli, which trigger different endocrine centres in the insect body and the hormones secreted affect the maturation process. Hormonal regulation of ovarian development in insects has been extensively studied and the role of various endocrine elements has been well established (Highnam, 1964, Gersch 1964, Novak, 1975, Engelmann, 1970, Slama et al., 1974, Laurence 1977, Zeigler 1984).

In the present investigation on C. Confusus, Brain extract treatment showed gradual increase in the protein concentration of haemolyph of male in early stage but significantly decrease after 72 hours. Male fat body also so similar pattern. In testes brain extract showed significant increase at all the stage from the picture above, it can be said that brain extract had some influence in the protein metabolism of male C. Confusus. In the testes gradual increase of protein concentration after treatment of brain extract suggest an accelerated rate of spermatogenesis.

In the female insect however brain extract had a positive effect in ovarian maturation. At all the stages of experiment protein concentration haemolymph, fat body, ovary was higher than that of control indicating the higher rate of protein synthesis under the control of brain hormone directly or through the corpora allata. The result of C.A. extract in male insect is indicative of an early activating effect and later inhibiting effect in protein synthesizing machinery. The effect of C.A. extract in female insect had in general protein raising effect in the haemolymph and ovary. Whereas an early protein lowering and then protein accelerating effect on fat body in C. confuses female.

A comparative study on the effects of Brain extract and C.A. extract on the protein concentration of both male and female C. confuses reveals the protein raising effect of two hormone sources. In male insect the possible activation of protein synthesis is far increasing rate of spermatogenesis and mating behaviour. The potency of protein synthesis activation by two hormones was understandably more in the female insect, for the greater demand of protein (vitellogenin) for yolk formation.

Brain hormone extract showed a more protein accelerating effect in female insect as compared to C.A. extract. This can be either due to the direct role of Brain hormone in ovarian maturation or due to activation of corpora allata by the additional dose of brain extract. So that C.A. hormone release of insect was more for the greater synthesis and deposition of yolk protein. This is in accordance with earlier works on other insects.

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