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NEUROENDOCRINE INFLUENCE ON HAEMOLYMPH PROTEIN CONCENTRATION AND MIDGUT PROTEASE ACTIVITY IN THE FINAL INSTAR LARVA OF *TRAMEA VIRGINIA* (RAMBUR) (ANISOPTERA: LIBELLULIDAE)

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The haemolymph protein concentration increased throughout most of the intermoult period of the final instar larvae, but decreased sharply a few days before emergence. Treatment with Farnesyl methyl ether caused no significant change in the haemolymph protein concentration. However, cauterization of the medial neurosecretory cells (MNC) of the brain caused depletion, while administration of an aqueous extract of corpora cardiaca (CC) elevated the level of haemolymph protein. Cauterization of the MNC and removal of the CC caused significant reduction in the midgut protease activity. These results collectively suggest the profound influence of the MNC hormone on protein metabolism, mostly mediated through the midgut protease activity.

INTRODUCTION

Earlier studies on the hormonal control of protein metabolism in insects reveal that the neurohormones of the medial neurosecretory cells (MNC) of the brain influence the haemolymph protein concentration (LEVENBOOK, 1985; RIDDIFORD, 1985) and the juvenile hormone (JH) is also reported to be involved, especially during vitellogenesis (RAABE, 1982; KUNKEL & NORDIN, 1985).

Similarly, midgut protease activity is found to be stimulated by the quantity of food ingested (ENGELMANN, 1970) and also by the hormones secreted by the MNC of the brain (RAABE, 1982; PRABHU, 1988).

TEMBHARE & THAKARE (1976) noticed enhancement of haemolymph protein level during vitellogenesis in the dragonfly *Orthetrum chrysis* after treatment with corpora cardiaca extract and Farnesyl methyl ether (FME). THAKARE et al. (1980) analysed the composition of haemolymph, including

protein, in the final instar larva of the same species. In Odonata little information is available on protein metabolism in relation to metamorphosis.

The present work has, therefore been undertaken to determine haemolymph protein concentration during the intermoult period of the final instar larva of *Tramea virginia* and to examine the neuroendocrine influence on the haemolymph protein level and midgut protease activity.

MATERIAL AND METHODS

Penultimate instar larvae of *Tramea virginia* (Rambur) were collected from local freshwater ponds and acclimatized in the laboratory in dechlorinated water (renewed twice a day) at a constant photoperiod of 10 hours light and at 20 ± 2° C. The larvae were fed daily on mosquito larvae ad libitum. As soon as the penultime instar larvae had moulted into the last instar, they were separated and maintained under similar, constant laboratory conditions.

The last instar larvae were sampled at four day intervals following ecdysis until their transformation into adults in order to find any changes in the concentration of protein in the haemolymph.

12-day old last instar larvae were subjected to various treatments. Some were injected with a quantity of extract equivalent to a single corpus cardiacum, others with 1 μg FME (a JH analogue) and those treated with an equivalent quantity of saline and olive oil served as their respective controls. In other larvae, the pars intercerebralis region of the brain containing the MNC was cauterized. In another set of experiments, the CC were removed to evince effect of their absence on protease activity.

The collection of haemolymph, treatment with FME and with aqueous extract of CC, cauterization of the MNC and removal of the CC were performed as described earlier (TEMBHARE & THAKARE, 1976; TEMBHARE, 1984). Protein concentration and protease activity were determined with the methods of LAYNE (1957) and ISHAAYA et al. (1971), respectively.

RESULTS

The concentration of haemolymph protein in the last instar larvae of *T. virginia* was determined from ecdysis until emergence i.e. throughout the entire intermoult period (Fig. 1).

In the newly emerged last instar larva, the haemolymph protein concentration measured about 1.75 ±0.12 g/100 ml. It gradually rose and reached a peak of 8.25±0.08 g/100 ml by the 24 th day, thus

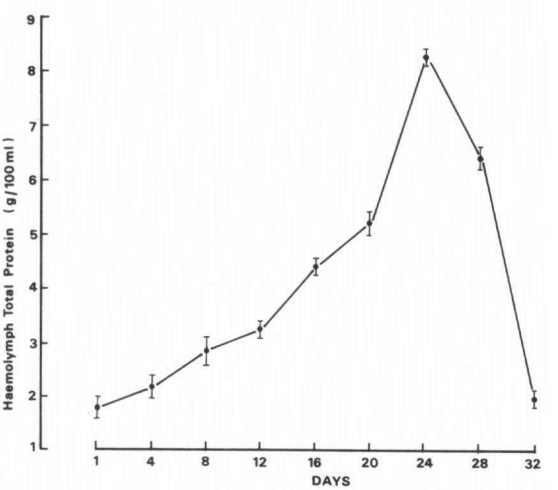

Fig. 1. Concentration of haemolymph total protein in the final instar larvae from ecdysis until the imaginal moult. — [Vertical bars represent standard error].

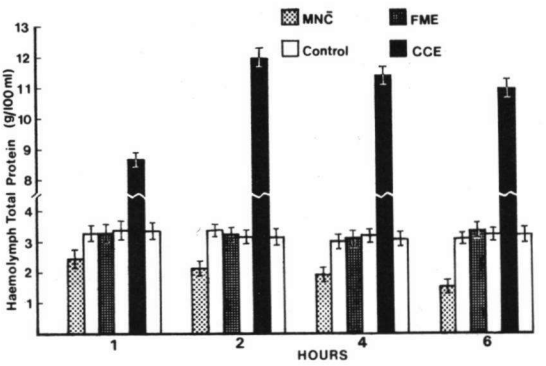

Fig. 2. Effect of cauterization of the MNC (MNC), administration of Farnesyl methyl ether (FME) and of corpora cardiaca extract (CCE) on haemolymph protein concentration.

showing an about five-fold increase. Just before the emergence of an imago (32nd day), the level had fallen to 1.85±0.16 g/100 ml.

The effect of cauterization of the MNC and of treatment with an aqueous extract of CC and with FME are presented in Figure 2.

Cauterization of the MNC caused a gradual depletion in the level of haemolymph protein. Six hours after the operation, the protein concentration was about 1.55±0.1 g/100 ml in the treated larva compared with about 3.30±0.2 g/100 ml in the control.

Treatment with an aqueous extract of CC caused a sharp rise in the level of haemolymph protein. It reached a maximum level of 11.86±0.35 g/100 ml, representing a four-fold increase over that in the controls (3.42±0.15 g/100 ml), at 2 hours. The level remained high even in the sixth hour after treatment.

FME caused no significant changes in the concentration of haemolymph protein.

The protease activity was negligible in the fore- and hindgut of the last instar larva of *T. virginia* except in the midgut region. The midgut protease activity measured about 0.78±0.05 OD at 280 nm. Cauterization of the MNC as well as removal of the CC caused a significant decline in the midgut protease activity (Tab. I).

Table I
Effect of cauterization of the MNC and removal of the CC on protease activity in the midgut of *Tramea virginia* larvae

Experiment duration	Protease activity (OD) Control	MNC	CC
0 h	0.78±0.20	0.78 ±0.20	0.78 ±0.20
1 h	0.77±0.15	0.48 ±0.05	0.35 ±0.08
2 h	0.75±0.08	0.33 ±0.06	0.25 ±0.05
4 h	0.76±0.05	0.093±0.008	0.088±0.025
6 h	0.78±0.06	0.073±0.015	0.063±0.035

MNC: Cauterization of the medial neurosecretory cells; — CC: Removal of the copora cardiaca; — OD: Optical density (280 nm)

DISCUSSION

The concentration of haemolymph protein is reported to rise rapidly in growing larvae and fall during the pupal and adult stages (CHEN, 1971; LEVENBOOK, 1985; RIDDIFORD, 1985). In *T. virginia,* a five-fold increase was recorded in the concentration of haemolymph protein prior to the final moult, and then a sharp fall immediately before emergence.

The influence of the MNC on the concentration of haemolymph protein has been observed (HIGHNAM & HILL, 1969; ENGELMANN 1970; LEVENBOOK, 1985). In *T. virginia* larvae too, cauterization of the MNC caused a significant fall in the haemolymph protein level. From the results obtained after cauterization of the MNC, it becomes obvious that the elevation in haemolymph protein concentration after the administration of the CC extract is not because of the intrinsic CC hormones but is exclusively due to the MNC hormone which is stored in the CC since the CC act as neurohaemal organs in the dragonflies (TEMBHARE, 1988).

In some insects, juvenile hormone is known to be involved in regulation of protein metabolism (LEVENBOOK, 1985; PLANTEVIN et al., 1987). Moreover, in the present study, FME (JH analogue) treatment caused no significant change in the level of haemolymph protein concentration.

The MNC hormone is known to regulate protease activity (RAABE, 1982; PRABHU, 1988). In *T. virginia,* cauterization of the MNC as well as removal of the CC resulted in depletion of protease activity. The present results, thus, indicate that the MNC hormone influences the haemolymph protein level by regulating the midgut protease activity.

REFERENCES

CHEN, P.S., 1971. *Biochemical aspect of insect development.* Karger, Basel.

ENGELMANN, F., 1970. *The physiology of insect reproduction.* Pergamon Press, Oxford.

HIGHNAM, K.C. & L. HILL, 1969. *The comparative endocrinology of the invertebrates.* Edward Arnold, London.

ISHAAYA, I., I. MOORE & D. JOSEPH, 1971. Protease and amylase activity in larvae of the Egyptian Cotton Worm, Spodoptera littoralis. *J. Insect Physiol.* 17: 945-953.

KUNKEL, J.G. & J.H. NORDIN, 1985. Yolk protein. *In:* G.A. Kerkut & L.I. Gilbert, [Eds], Comprehensive insect physiology, biochemistry and pharmacology, Vol. 1, pp. 83-111. Pergamon Press, Oxford.

LAYNE, E., 1957. Spectrophotometric and turbidometric methods for measuring proteins. *In:* S.P. Colowick & N.C. Kaplan, [Eds], Methods of Enzymology, pp. 447-454. Academic Press, New York.

LEVENBOOK, L., 1985. Insect storage protein. *In:* G.A. Kerkut & L.I. Gilbert, [Eds], Comprehensive insect physiology, biochemistry and pharmacology, Vol. 10, pp. 307-346. Pergamon Press, Oxford.

PLANTEVIN, G., C. BOSQUET, B. CALVEZ & C. NORDIN, 1987. Relationships between

juvenile hormone levels and synthesis of major haemolymph proteins in Bombyx mori larvae. *Comp. Biochem. Physiol.* (B) 86: 501-508.

RAABE, M., 1982. *Insect neurohormones.* Plenum Press, New York-London.

RIDDIFORD, L.M., 1985. Hormonal action at cellular level. *In:* G.A. Kerkut & L.I. Gilbert, [Eds], Comprehensive insect physiology, biochemistry and pharmacology, Vol. 8, pp. 307-346. Pergamon Press, Oxford.

TEMBHARE, D.B., 1984. Hormonal regulation of the ionic balance in the haemolymph of the larvae of the dragonfly Orthetrum chrysis (Selys) (Anisoptera: Libellulidae) *Odonatologica* 13: 565-572.

TEMBHARE, D.B., 1988. Endocrinology of Odonata, with special reference to Orthetrum chrysis Selys from India. *Indian Odonatol.* 1: 89-125.

TEMBHARE, D.B. & V.K. THAKARE, 1976. Neuroendocrine control of vitellogenesis in the dragonfly Orthetrum chrysis (Selys) (Odonata: Libellulidae). *Z. mikrosk.-anat. Forsch.* 90: 691-704.

THAKARE, V.K., D.B. TEMBHARE, M.W. KHAN & V. PENDHARKAR, 1980. Chemical composition of haemolymph in the larvae of the dragonfly Orthetrum chrysis (Anisoptera: Libellulidae). *Odonatologica* 9: 321-324.

PRABHU, V.K.K., 1988. Endocrines and digestive enzyme secretion in insects. *Proc. natn. Symp. current State gen. comp. Endocrinol.* ISGCE, Delhi, pp. viii-ix.