ECDYSTEROID MEDIATED DIFFERENTIAL UPTAKE OF LARVAL HAEMOLYMPH PROTEINS DURING POST-EMBRYONIC DEVELOPMENT IN RICE MOTH, *CORCYRA CEPHALONICA* (LEPIDOPTERA)

APARNA DUTTA-GUPTA* AND S.M.ISMAIL

School of Life Sciences, University of Hyderabad

Hyderabad, 500 134 (A.P.) INDIA

ABSTRACT

Three larval haemolymph proteins (LHPs), LHP 1, LHP 2 and LHP 3 are syntheised in stage and tissue specific manner by the fat body cells of *Corcyra cephalonica* and secreted into the haemolymph during larval development. The focus of this study is towards the receptor mediated differential uptake of LHPs during the larval, prepupal and pupal development of *Corcyra*. The fat body shows a low uptake of all the three LHPs during the last larval instar. During the prepupal stage of development, there is a significant increase in LHP uptake by the fat body cells and LHP 1 is incorporated more than LHP 2 and LHP 3. However, during the early pupal development the LHPs uptake remains high but LHP 2 and LHP 3 are sequestered more than LHP 1. Addition of 20-hydroxyecdysone significantly stimulates the uptake of LHPs by the larval, prepupal and pupal fat body *in vitro*. We suggest that 20-hydroxyecdysone activates the cryptic receptors present in the membrane of fat body cells. Juvenile hormone, low temperature and metabolic inhibitors inhibit the LHPs uptake.

Key words: *Corcyra*, larval haemolymph proteins, uptake, 20-hydroxyecdysone, juvenile hormone.

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*Author for correspondence

INTRODUCTION

In holometabolous insects, the fat body synthesises large number of proteins including larval haemolymph proteins (LHPs) and releases them into the haemolymph during active feeding phase of last larval stadium (Riddiford and Law, 1983; Levenbook, 1985). These proteins are variously designated as storage proteins (Munn and Greville, 1969) or larval serum proteins (Roberts *et al.*, 1977). The protein synthesising function of the fat body greatly diminishes after cessation of feeding (Martin *et al.*, 1971; Price, 1973; Pau *et al.*, 1979; Tojo *et al.*, 1981; Powell *et al.*, 1984; Caglayan and Gilbert, 1987; Ray *et al.*, 1987) and it starts sequestering LHPs (Levenbook, 1985; Marinotti and de Bianchi, 1986) which are deposited in the form of protein granules (Tojo *et al.*, 1978; Ueno and Natori, 1982). The process of LHP uptake is mediated by receptors present in the plasma membrane of the fat body cells (Ueno *et al.*, 1983; Ueno and Natori, 1984; Matzner and Scheller, 1989). Several studies in dipterans (Marinotti and de Bianchi, 1986; Ueno and Natori, 1982, 1984; Ueno *et al.*, 1983; Matzner and Scheller, 1989) and lepidopterans (Tojo *et al.*, 1981; Levenbook, 1985; Webb and Riddiford, 1988) showed that ecdysteroids stimulate LHPs uptake by the fat body.

In *Corcyra cephalonica*, larval fat body synthesises three LHPs which are designated as LHP 1, LHP 2 and LHP 3 (Ismail and Dutta-Gupta, 1990). Juvenile hormone I (JH I) inhibits the synthesis of LHP 1 and LHP 2 (Ismail and Dutta-Gupta, 1988) while 20-hydroxyecdysone (20-HE) stimulates LHP synthesis in *Corcyra* (unpublished observation).

In this paper we present evidence for a differential uptake of LHPs by fat body cells during the last larval, prepupal and pupal development, and also report on the effect of certain metabolic inhibitors, temperature and hormones on the *in vitro* LHPs uptake by the fat body cells of *Corcyra*.

MATERIALS AND METHODS

Insects: *Corcyra cephalonica* larvae were mass reared in the laboratory at 26 ± 1 °C temperature, 70 ± 5 % relative humidity, 14 : 10 light-dark period on coarsely crushed *Sorghum* seeds. For the present study, mid-last instar larvae, late-last instar larvae, prepupae and early pupae were used.

Radiolabelling of larval haemolymph proteins: Mid-last instar larvae were injected with 20 μCi [^{35}S] methionine (specific activity 800 Ci/ mmol, Amersham, U.K.) and incubated for 16 h. Haemolymph was collected in an Eppendorf tube prerinsed with 0.1 % phenyl thiourea and diluted with equal volume of PMSF (0.1 %). It was then centrifuged at 10,000 x g for 2 min at 4 °C to remove the haemocytes. The supernatant was used for SDS-PAGE analysis which was carried out according to the procedure of Laemmli (1970) using 7.5 % gel. LHPs were identified and excised out seperately from the gels and electroeluted using 10 mM phosphate buffer, pH 7.4 (Maniatis *et al.*, 1982). The electroeluted LHPs were dialysed against 50 mM tris-glycine buffer, pH 8.3 and concentrated by lyophilization. The specific activity of each LHP was determined by radiolabel counting. The homogenity of electroeluted LHPs was checked

by SDS-PAGE. Protein was estimated by Folin-Ciocalteau method (Lowry *et al.*, 1951).

In vivo LHP uptake studies: [^{35}S] methionine labelled LHP preparations were injected into the haemolymph of late-last instar, prepupa and early pupa of *Corcyra*. The insects were sacrificed 12 h after injection. The fat body was dissected out, rinsed thoroughly in insect Ringer and processed to determine the recovered radioactivity, using Beckmann liquid scintillation counter.

In vitro fat body culture and low temperature treatment: For *in vitro* studies, the fat body was dissected out from the prepupae and incubated in 100 μl Grace's medium at 25 ^{o}C in sterile conditions. Known amounts of [^{35}S] methionine labelled LHPs were added to the incubation medium. For low temperature treatment, the fat body cultures were incubated at 4 ^{o}C for 8 h.

Inhibitors and hormone treatment: Metabolic inhibitors such as sodium azide and sodium fluoride were selected for the present study. The fat body was cultured either with 0.5 μg sodium azide (in 5 μl distilled water) or 5 μg of sodium fluoride (in 5 μl distilled water) in 100 μl of Grace's medium. The fat body was collected after 8 h treatment and recovered radioactivity was determined as stated above. For hormone studies, either 0.25 μg of 20-HE (in 10 μl of 10 % ethanol) or 0.25 μg of JH I (in 5 μl acetone) or carrier was added to the fat body samples contained in the culture medium. The samples were collected 8 h after hormone treatment and processed for radiolabel counting.

RESULTS

Changes in LHP uptake by the fat body during post-embryonic development: Table 1 shows the uptake of [^{35}S] methionine labelled LHPs by the fat body during late larval, prepupal and early pupal development. There was no significant incorporation of radiolabelled LHPs in the fat body during late larval development. However, the fat body of prepupae and early pupae revealed a generally increased incorporation of radiolabelled LHPs. The amount of LHP 1 sequestered by prepupal fat body was three to four fold higher as compared to LHP 2 and LHP 3. On the other hand the fat body of early pupae incorporated less of LHP 1 than the prepupal fat body and showed a preferential uptake of LHP 2 and LHP 3.

Effect of inhibitors on the *in vitro* LHPs uptake: Table 2 shows the effect of metabolic inhibitors, sodium azide and sodium fluoride on the *in vitro* uptake of LHP 1, LHP 2 and LHP 3 by prepupal fat body. The data shows that 0.5 μg sodium azide treatment for 8 h resulted in lowering the uptake of LHPs. Similar results were also obtained with sodium fluoride treatment. Unlike sodium azide, the inhibition of uptake of LHP 2 and LHP 3 was more drastic than LHP 1 in sodium fluoride treated fat body.

Effect of temperature on *in vitro* LHP uptake: In order to determine the effect of temperature on LHPs sequestration, the fat body from prepupae were incubated in Grace's medium at 4^{o}C for 8 h. The results

presented in table 2 show that exposure to low temperature reduced the uptake of all the three categories of LHPs by the fat body cells.

Effect of hormones on the *in vitro* LHPs uptake: The fat body from prepupae was incubated with [^{35}S] methionine labelled LHPs in the presence of either 0.25 μg 20-HE or 0.25 μg JH I and the results are set forth in table 3. JH I treatment for 8 h partially blocked the incorporation of all the three LHPs by the prepupal fat body. It is seen that incorporation of LHPs by the fat body was reduced by JH I treatment. On the other hand, 0.25 μg 20-HE treatment for 8 h significantly stimulated the uptake of all the three LHPs by the fat body. However, the stimulatory effect was more pronounced in the case of LHP 1.

DISCUSSION

In this paper we provide a clear evidence for selective uptake of LHPs by the fat body cells of *Corcyra cephalonica*, which begins after cessation of feeding during the prepupal stage and continues till early pupal development. The fat body from late-last instar larvae is able to incorporate only a small amount of radiolabelled LHPs injected into the haemolymph (Ueno *et al.*, 1983; Ueno and Natori, 1984; Marinotti and de Bianchi, 1986) and this might be due to the presence of inactive receptors for LHP on the cell membrane. In *Musca domestica*, the last instar larval fat body incorporates significant amount of storage proteins only in presence of 20-HE (Marinotti and de Bianchi, 1986) which might activate storage protein receptors in the

cell membrane (Ueno *et al.*, 1983; Ueno and Natori 1984; Marinotti and de Bianchi, 1986). By contrast, in *Galleria mellonella*, the fat body from the final instar was shown to be competent to sequester storage proteins from the haemolymph (Miller and Silhacek, 1982). The data presented here shows that the fat body of prepupae actively incorporated the injected radiolabelled LHPs from the haemolymph (Marinotti and de Bianchi, 1986). The uptake of LHPs by the prepupal fat body is a selective process in the present insect, because at this stage LHP 1 is incorporated to a greater extent than LHP 2 and LHP 3. Interestingly, there is a reversal of this trend as the development advances to early pupal stage. Undoubtedly, remarkable amounts of all the three LHPs continue to be sequestered also by early pupal fat body but with a difference that the amount of LHP 1 incorporated at this stage is much lower than in prepupal stage and at the same time, the early pupal fat body sequestered more LHP 2 and LHP 3 in comparison to LHP 1. In *Galleria mellonella*, 74 and 76 kd storage proteins are sequestered during the spinning stage but not the 81 and 82 kd proteins (Miller and Silhacek, 1982). However, both the storage proteins P1 and P2 are taken up by the prepupal fat body of *Manduca sexta* with a five fold higher uptake of P1 (Caglayan and Gilbert, 1987). The present *in vivo* uptake experiments on *Corcyra* provide a direct evidence for the sequestering ability of fat body and accumulation of LHPs during the prepupal and early pupal stage which is developmentally regulated.

In viro uptake of LHP by the fat body of *Corcyra* is partially

inhibited by sodium azide and sodium fluoride. Marinotti and de Bianchi (1986) showed that storage protein uptake by the fat body of *Musca* requires active metabolism and can be partially blocked by metabolic inhibitors. The uptake of LHPs is lowered when the fat body is incubated at low temperature. This fact suggests that the binding of LHPs to its receptors in the fat body cell is adversely affected by low temperature (Marinotti and de Bianchi, 1986).

Our present study shows that JH I inhibits the uptake of LHPs by the prepupal fat body *in vitro*. This is in accord with other studies on *Bombyx mori* where it was shown that the JH analogue, methoprene inhibits the fat body storage protein uptake (Tojo *et al.*, 1981). On the contrary it is seen here that incubation of *Corcyra* prepupal fat body with 20-HE significantly stimulates the LHPs uptake. These results suggest that 20-HE mediates LHP uptake during the post-embryonic development (Tojo *et al.*, 1981; Miller and Silhacek, 1982; Ueno and Natori, 1982; Ueno *et al.*, 1983; Marinotti and de Bianchi, 1986). In *Sarcophaga bullata* it was shown that 20-HE is essential for the activation of cryptic receptors present in the larval fat body to bind and incorporate storage proteins (Ueno *et al.*, 1983; Ueno and Natori, 1984).

Therefore, further studies are necessary to determine and characterise the LHP receptors in *Corcyra* fat body cells and clarify how these receptors are activated for LHP uptake. If each of the LHPs is, in fact, recognised by a specific receptor system, it would then be interesting to look into the intrinsic structural features of these

LHPs which confer specificity for their uptake.

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Table 1

In vivo uptake of [^{35}S] methionine labelled LHPs by the fat body of *Corcyra cephalonica* during the last larval, prepupal and pupal development.

LHPs	μg LHP injected/ insect	cpm injected/ insect	% cpm incorporated Late-last larvae	Prepupae	Early Pupae
LHP 1	7.0	48,450	3.5	20	9
LHP 2	8.0	52,625	3.2	5	18
LHP 3	6.4	43,420	3.0	7	10

Insects were injected with biosynthetically labelled LHPs and sacrificed 12 h after injection. The fat body was dissected out and processed for radiolabel counting. The specific activity of LHP 1, LHP 2 and LHP 3 was 6,921, 6,578 and 6,784 respectively. The values represent an average of two experiments. For each value, fat body from three insects was dissected out and used.

TABLE 2

Effect of sodium azide, sodium fluoride and low temperature on *in vitro* LHPs uptake by *Corcyra cephalonica* fat body at prepupal stage.

LHPs	μg LHP added/ insect FB	cpm added/ insect FB	% cpm incorporated Control	Sodium azide	Sodium fluoride	Low temp.
LHP 1	8.0	55,000	31.6	26.6	18.9	10.1
LHP 2	6.2	60,500	12.6	9.8	4.2	2.9
LHP 3	5.5	40,500	14.7	11.9	3.7	4.5

The fat body (FB) was dissected out from the prepupae and cultured *in vitro* in 100 μl of Grace's medium at 25 °C in sterile conditions with biosynthetically labelled LHPs. The fat body was treated with either 0.5 μg sodium azide or 5 μg sodium fluoride for 8 h. For low temperature treatment, the fat body was incubated at 4 °C for 8 h. The specific activity of LHP 1, LHP 2 and LHP 3 was 6,875, 9,758 and 7,363 respectively. These values represent an average of two experiments.

TABLE 3

Effect of JH I and 20-HE on *in vitro* uptake by the prepupal fat body of *Corcyra cephalonica*.

LHPs	µg LHP added/ insect FB	cpm added/ insect FB	% cpm incorporated			
			Control	JH I	Control	20-HE
LHP 1	8.0	55,000	29.9	12.0	32.8	49.0
LHP 2	6.2	60,500	11.1	4.1	14.0	20.5
LHP 3	5.5	40,500	13.4	4.5	15.8	25.9

The fat body (FB) was dissected out from the prepupae and cultured *in vitro* in Grace's medium at 25°C in sterile conditions with biosynthetically labelled LHPs. The fat body was either treated with 0.25 µg JH I or with 0.25 µg of 20-HE for 8 h. The specific activity of LHP 1, LHP 2 and LHP 3 was 6,875, 9,758 and 7,363 respectively. These values represent an average of two experiments.

LE TRANSIT DIFFERENTIEL DES PROTEINES DE L'HEMOLYMPHE LARVAIRE SOUS L'INFLUENCE D'ECDYSTEROIDES PENDANT LE DEVELOPPEMENT POST-EMBRYONNAIRE DE LA MITE DU RIZ, *CORCYRA CEPHALONICA* (LEPIDOPTERA)

Aparna DUTTA GUPTA et S.M. ISMAIL

School of Life Sciences
University of Hyderabad
Hyderabad, 500 134 (A.P.) India

RESUME

Trois protéines de l'hémolymphe larvaire (LHPs) d'un poids moléculaire de 49, 46 et 44 Kd sont synthétisées, dans les tissus et en plusieurs étapes qui leur sont propres, par les cellules du corps gras du *Corcyra cephalonica* et ensuite complexées dans l'hémolymphe pendant le développement larvaire. Plus tard, pendant le stade d'essaimage, ces LHPs sont stockées dans les cellules du corps gras sous forme de granules protéiques. Le point de mire de cette étude est le transit différentiel des LHPs via récepteur pendant le développement de la nymphe, le stade pré-nymphal et le stade larvaire de *Corcyra*. Le corps gras présente un transit faible des trois LHPs pendant le stade larvaire. Pendant le stade de développement pré-nymphal, il y a un accroissement net du transit des LHPs par les cellules du corps gras. Les LHP 1 sont complexées en plus grande quantité que les LHP 2 et les LHP 3. Cependant, au début du développement nymphal, le transit demeure élevé, mais les LHP 2 et les LHP 3 sont complexées en plus grand nombre que les LHP 1. L'application de 20-hydroxyecdysone stimule nettement le transit des LHPs dans le corps gras des trois stades nymphal, pré-nymphal et larvaire. Nous pensons que la 20-hydroxyecdysone active les crypto-récepteurs à LHPs présents dans les membranes des cellules du corps gras. L'hormone juvénile, une température basse et les inhibiteurs métaboliques inhibent le transit des LHPs. Les détails de l'étude ci-dessus et leur utilisation dans la gestion des déprédateurs des stocks de grains seront discutés lors de sa présentation.