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STUDING OF DIGESTIVE ENZYMES ACTIVITY AND MAIN METABOLITES OF *Agrotis ipsilon* (HUFN.) AND *Helicoverpa armigera* (HBN.) LARVAE IN RESPONSE TO KININ-PEPTIDE

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ABSTRACT

Kinin-peptides are a group of structurally related neuropeptides stimulating gut motility and fluid by Malpighian tubules in insects. For studying the effect of these neuropeptides on digestive enzymes activities and concentration of main hemolymph components, leucokinin II was injected into *Agrotis ipsilon* (Hufn.) and *Helicoverpa armigera* (Hbn.) 5th instar larvae at doses of 20, 25, 30 and 40 µl. The neuropeptide leucokinin II reduced the release of digestive enzymes, protease, amylase and trehalase; also, it inhibited the formation of total protein. While it exerted a stimulatory effect by increasing the activity of invertase enzyme and the formation of total carbohydrates and total lipid in both *A. ipsilon* and *H. armigera* larvae.

INTRODUCTION

Neuropeptides are diverse chemical messengers known in the nervous system of metazoans including insects (Nassel, 1993). Neuropeptides regulate virtually all aspects of insect life and are excellent candidates for development of new methods for pest control (Holman *et al.,* 1990). A number of neuro-kinins have been identified from several insect species. Eight leucokinins, originally isolated and identified from the cockroach *Leucopheae maderae* as myotropic factors (Holman et al., 1986 a, b; 1987 a, b). These peptides were later found to stimulate fluid secretion from Malpighian tubules of *Aedes aegypti*, spontaneous muscles contractions in the midgut and inhibits proctolin induce in *Locusta migratoria* (Lang and Orchard, 1998), as well as modulate digestive enzymes activity (Nachman *et al*., 1997; Fuse *et al*., 1999 and Harshini *et al*., 2002). The insect midgut has been previously described as one of the largest endocrine organs in insect (Lang, 2001). Thus, given the fast answer to that observed in digestive enzymes activity during this study. Midgut contains numerous endocrine-like cells expressing various peptides such as kinins (Pabla and Lang, 1999). Peptides, including those mentioned, have been implicated in altering midgut activity by eliciting a short circuit ion current, as in *Manduca sexta* (Lee *et al*., 1998). These evidences can demonstrate the control mechanisms regulate the synthesis and secretion of digestive enzymes.

The present study; demonstrate *in vivo* digestive enzymes activity and main metabolites formation in response to leucokinin II application in the larvae of two lepidopteran moths, *A. ipsilon* and *H. armigera*.

MATERIALS AND METHODS

Rearing technique:

Larvae of *A. ipsilon* and *H. armigera* were kept in groups of 20 glass jars. Sawdust was placed at the bottom of the jars and the top was covered with muslin and secured with rubber bands. Larvae were fed on castor bean leaves *Ricinus commnuis* and kept in a thermostatically regulated room at 27±2ºC and 70±5 R.H.

Preparation of neuropeptide solution and treatment:

Leucokinin II with sequence; Asp-Pro-Gly-Phe-Ser-Ser-Trp-Gly-NH_2 (Leucokinin II; AMERICAN PEPTIDE, USA.) was dissolved in distilled water (1ml/1mg).

Experiments were carried out on the 5^{th} larval instar, which starved for 8hrs. The larvae of the two insects, *A. ipsilon* and *H. armigera* were anesthetized by cooling in refrigerator for 10 min. before injection. Leucokinin II was injected into the larvae with a 20 µl microsyringe with doses (20, 25, 30, 40 µl) through the segmental membrane between fifth and sixth abdominal segments (Tanaka *et al*., 2002). The castor leaves were introduced to the larvae for feeding. Three replicates contained 10 larvae/jar from each species for each treatment and also for the control experiments which carried out without any injection.

Biochemical assays:

Heamolymph samples (10 µl) were collected form the prolegs of larvae after 5 hrs (Maestro *et al* ., 2001), and stored at -20ºC until assayed.

Protease enzymes activity: determined by the casein digestion method described by Ishaaya *et al* ., 1971.

Carbohydrate hydrolyzing enzymes: based on the digestion of trehalose, starch and sucrose by trehalase, amylase and invertase, respectively, according to the method described by Ishaaya and Swiriski, 1976.

Total protein was determined by the method of Bradford, 1976. Total carbohydrates were estimated by the phenol sulfuric acid reaction of Dubois *et al* ., 1956. Total lipid were estimated by the method of Knight *et al* ., 1972

Statistical analysis:

All tests of all stages were replicated three times and the standard analysis of variance (ANOVA) using F-test and at least significant difference (L.S.D.) were used to compare developmental and biochemical activities) at different doses (Fisher, 1950 and Snedocor and Chochran, 1972).

RESULTS AND DISCUSSION

Enzymes Activity:

1.*A. ipsilon*

The effect of tested neuropeptides analogue leucokinin II on proteases activity of *A. ipsilon* larvae presented in Table (1), Protease activity

decreased gradually with increasing dose, protease values were 164.67, 177.67, 217.0, 233.33 and 249.67 unit/ min/ larva at 40, 30, 25, 20µl and untreated respectively.

The changes in amylase activity of *A. ipsilon* 5th instar larvae in the normal state and after leucokinin treatment were given in Table (1), amylase decreased gradually with increasing dose, however, its activity reached to the lowest level 134.67 unit/ min. / larva at 40 µl.

Data in the same table indicated that the tested neuropeptides analogue leucokinin II caused significant increases in invertase activity of *A. ipsilon* with values 170.33, 164.0, 126.0, 113.0 and 96.0 unit/ min. / larvae at 40, 30, 25, 20µl and the untreated control, respectively.

Data presented in Table (1) showed a reverse relationship between trehalase activity and the increasment of leucokinin II dose.

Table (1): Effect of different doses of leucokinin II on secretion activity of protease and carbohydrate hydrolyzing enzymes in 5th instar larvae of *A. ipsilon*.

Dose (µl)	Protease units*	Amylase units**	Invertase units**	Trehalase units**
40	164.67 ± 0.72 **e**	134.67 ± 0.72 **e**	170.33 ± 1.29 **a**	185.0 ± 0.47 **d**
30	177.67 ± 0.98 **d**	153.0 ± 1.25 **d**	164.0 ± 0.47 **b**	188.67 ± 0.72 **d**
25	217.0 ± 0.94 **c**	195.0 ± 0.47 **c**	126.0 ± 0.94 **c**	194.67 ± 0.72 **c**
20	233.33 ± 1.29 **b**	223.0 ± 1.41 **b**	113.0 ± 0.82 **d**	201.67 ± 0.72 **b**
Untreated control	249.67 ± 0.72 **a**	263.0 ± 0.94 **a**	96.0 ± 0.94 **e**	208.0 ± 0.47 **a**
L.S.D 0.01	5.86	6.35	5.73	4.06

***1 unit: amount of enzyme required to liberate 1 µg of tyrosine from casein/min.**
****1 unit: amount of enzyme required to liberate 1 µg of maltose equivalents from starch/ min.**

2.*H. armigera*

Data in Table (2) revealed that the activity of Protease decreased gradually with increasing dose, protease values were 198.0, 209.67,294.33 , 346.33 and 361.33 unit/ min. / larva at 40, 30, 25, 20µl and untreated respectively.

Table (2): Effect of different doses of leucokinin II on secretion activity of protease and carbohydrate hydrolyzing enzymes in 5th larvae of *H. armigera*.

Dose (µl)	Protease units*	Amylase units**	Invertase units**	Trehalase units**
40	198.0 ± 0.94 **e**	0.0 ± 0.0 **b**	253.0 ± 1.24 **a**	105.0 ± 0.47 **e**
30	209.67 ± 0.72 **d**	0.0 ± 0.0 **b**	235.67 ± 0.54 **b**	153.33 ± 0.72 **d**
25	294.33 ± 0.54 **c**	0.0 ± 0.0 **b**	210.33 ± 0.72 **c**	160.67 ± 0.54 **c**
20	346.33 ± 0.54 **b**	0.0 ± 0.0 **b**	184.33 ± 0.72 **d**	171.0 ± 0.47 **b**
Untreated control	361.33 ± 0.72 **a**	55.0±0.94 **a**	172.33 ± 1.29 **e**	193.33 ± 0.27 **a**
L.S.D 0.01	4.43	-	4.99	3.43

*** 1 unit: amount of enzyme required to liberate 1 µg of tyrosine from casein/min.**
****1 unit: amount of enzyme required to liberate 1 µg of maltose equivalents from starch/ min.**

The changes in amylase activity of *H.armigera* larvae in the normal state and after leucokinin II treatment were given in Table (2), leucokinin II resulted in inhibition of amylase activity in treated larvae, however its activity extremely decreased to reach zero; no amylase activity found after treatment with all doses; on the other hand amylase activity in normal state averaged value was 55.0 unit/ min. / larvae.

Data in Table (2) indicated that leucokinin II caused significant increases in invertase activity of *H.armigera* larvae.

Data in the same table indicated a reverse relationship occurred between trehalase activity and the increasment of leucokinin II dose.

Main metabolites concentration:

1. *A. ipsilon*

Table (3) showed that Leucokinin II caused a high significant reduction in total protein levels; however, the values were 2525.33, 2824.67, 3230.0, 3422.67 and 3572.67 µg/ml at 40, 30, 25, 20 µl and the untreated control, respectively.

Table (3): Changes in main hemolymph component levels of 5th instar larvae of *A. ipsilon* after treatment with different doses of leucokinin II.

Dose (µl)	Total protein concentration*	Total carbohydrate concentration*	Total lipid concentration*
40	2525.33 ± 0.72 **e**	16209.67 ± 0.72 **a**	1861.67 ± 0.98 **a**
30	2824.67 ± 0.72 **d**	12403.33 ± 0.98 **b**	1805.0 ± 0.94 **b**
25	3230.0 ± 0.47 **c**	12290.33 ± 0.72 **c**	1606.33 ± 0.98 **c**
20	3422.67 ± 0.72 **b**	11260.0 ± 0.47 **d**	1600.0 ± 0.94 **d**
Untreated control	3572.67 ± 1.44 **a**	10930.33 ± 0.72 **e**	1373.33 ± 0.98 **e**
L.S.D 0.01	5.57	4.47	6.09

***concentration = µg/ml.**

Concerning total carbohydrates, Data presented in Table (3) appeared that gradual increase in total carbohydrates concentration in *A.ipsilon* hemolymph with increasing dose. Also, data showed the same trend in total lipid values in *A. ipsilon* treated larvae.

2. *H. armigera*

Data in Table (4) showed that leucokinin II caused a high significant reduction in total protein levels.

Gradual increase of total carbohydrates concentration in *H.armigera* larvae hemolymph was demonstrated in Table (4). However, its values were 1342.67, 1334.33, 1155.33, 1118.33 and 937.33 µg/ml at 40, 30, 25, 20 µl and the untreated control, respectively.

Data in Table (4) indicated that the concentration of total lipids increase as well as increasment in leucokinin II dose.

The previous study of biochemical activity of leucokinin II demonstrated that, leucokinin II inhibited the release of protease, amylase, the obtained data seems likely that results reported by Harshini *et al*., 2002,

and trehalase (Lopata and Gade, 1994; Becker *et al* ., 1996), while it exerted stimulatory effect by increasing invertase level. Nachman and Holman, 1991 indicated that the Leucokinin structure is recognized by the midgut receptors of *Opisina arenosella*. The Leucokinin receptor for modulation of digestive enzyme activity in the midgut may differ from the midgut muscle receptor for Leucokinin II, the interaction of muscle contraction and digestive enzyme activity in the midgut may lead to more effect on digestion, more over on the producing of main metabolites.

Table (4): Changes in main hemolymph component levels of 5th instar larvae of *H. armigera* after treatment with different doses of leucokinin II.

Dose (µl)	Total protein concentration*	Total carbohydrate concentration*	Total lipid concentration*
40	4471.33 ± 0.72 **e**	1342.67 ± 1.44 **a**	6951.33±0.72 **a**
30	4482.33 ± 0.98 **d**	1334.33 ± 0.72 **b**	6432.0±0.47 **b**
25	4489.67 ± 0.72 **c**	1155.33 ± 0.98 **c**	6417.33±0.54 **c**
20	5922.33 ± 1.29 **b**	1118.33 ± 0.72 **d**	5911.0±0.47 **d**
Untreated control	6236.33 ± 0.54 **a**	937.33 ± 1.29 **e**	5356.0±0.72 **e**
L.S.D 0.01	5.36	3.73	6.44

***concentration = µg/ml.**

Also, there is a dramatically decrease in total protein synthesis in dose dependent manner Inhibition of protein synthesis by kinin neuropeptides was reported by Gade, 2004. Hill, 1962 found that protein synthesis is controlled by hormones from neurosecratory cells of the brain, the change in neurohormone levels lead to fall of protein levels to a half or a third of the normal value. This further supported by the fact which is protein metabolism linked to digestive enzymes production (Englemenn, 1969). While the stimulation activity in invertase release could be explained in which tested larvae was in the end of their larval stage, so, the only enzyme in which adult, nectar-feeding lepidopteran need is invertase enzyme (Auclair, 1963). Thus, total protein could be direct to produce invertase enzyme only as a result of larval instar and decrease in protein synthesis.

Another stimulatory effect was observed in total carbohydrate synthesis, a similar tendency was observed by Lopata and Gade, 1994; Becker *et al*., 1996 and Gade and Auerswald, 1999.

Leucokinin II, also, showed a stimulatory effect in total lipid synthesis, it could be seen that the results recorded here in a harmony with those obtained by various authors on different insects (Gade, 1999; Oudejans *et al*.,1999; Oguri and Steele, 2003 and Gade, 2004), such stimulatory effect was caused as glycogen phosphorylase or lipase activation by Leucokinin II in fat body (Gade, 2004). An additional explain to that stimulatory effect in larval instar, in which tested larvae is largely devoted to the accumulation and storage of lipid and carbohydrates which can be used during the pupal stage for the development of the adult form.

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دراسة تأثير الببتيد الكينيني نشاط إنزيمات الهضم و نواتج الأيض في يرقات الدودة القارضة و دودة اللوز الأمريكية

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البتيدات الكينينية هي احدي مجموعات الببتيدات العصبية و التي تحفز عمل كل من القناة الهضمية و انابيب ملبيجي. لدراسة تأثير هذه المجموعة علي نشاط إنزيمات الهضم و تركيز المكونات الأساسية للتمثيل داخل هيموليمف الحشرة، تم حقن يرقات العمر الخامس لكل من الدودة القارضة و دودة اللوز الأمريكية بالجرعات التالية: ٢٠، ٢٥، ٣٠ و ٤٠ ميكرولتر.

أوضحت النتائج إنخفاض نشاط كل من إنزيم البروتييز و الأميليز و التريهاليز كما انخفض تركيز البروتينات الكلية نتيجة للمعاملة باللليكوكينين٢ داخل هيموليمف اليرقات. علي الجانب الآخر ارتفع معدل نشاط إنزيم الأنفرتيز، كما ارتفع تركيز كل من الكربوهيدرات و اللبيدات الكلية، وذلك ليرقات الحشرتين موضع الإختبار.