REGULATION 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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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 insects (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 regulating the synthesis and secretion of digestive enzymes.

The present study demonstrates *in vivo* digestive enzymes activity and main metabolites formed in response to leucokinin II application in the larvae of two lepidopteran moths, *Agrotis ipsilon* and *Helicoverpa armigera*.

MATERIAL AND METHODS

Rearing technique

Larvae of *A. ipsilon* and *H. armigera* were kept in groups of 20 in 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 leaves *Ricinus commnuis* and kept in a thermostatically regulated room at $27 \pm 2°C$ 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 5th larval instar, which was starved for 8hrs. The larvae of the two insect species, *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 by puncturing the prolegs of larvae after 5 hrs (Maestro *et al.*, 2001), and stored at -20°C 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 lipids 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 the different used 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 is 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/ ml at 40, 30, 25, 20 μl and control 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 the lowest level by 134.67 unit/ min. / ml 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 of 170.33, 164.0, 126.0, 113.0 and 96.0 unit/ min. / ml at 40, 30, 25, 20 μl and the control, respectively.

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

TABLE (I)

Effect of different doses of leucokinin II on secretionactivity 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 ± 0.72e	170.33 ± 1.29 a	185.0 ± 0.47 d
30	177.67 ± 0.98d	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
Control	249.67 ± 0.72 a	263.0 ± 0.94 a	96.0 ± 0.94 e	208.0 ± 0.47 a
L.S.D $_{1\%}$	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. /ml at 40, 30, 25, 20 μl and the control respectively.

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 an inhibition of amylase activity in treated larvae, however its activity extremely decreased to reach zero; no amylase activity was found after treatment with all doses. On the other hand, amylase activity in normal state averaged a value of 55.0 unit/ min. / ml.

Data in Table (2) indicated that leucokinin II caused significant increases in invertase activity of *H.armigera* larvae, while it was a reverse relationship occurred between trehalase activity and the increase of leucokinin II dose.

TABLE (II)

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 ± 0.00 b	253.0 ± 1.24 a	105.0 ± 0.47 e
30	209.67 ± 0.72 d	0.0 ± 0.00 b	235.67 ± 0.54 b	153.33 ± 0.72 d
25	294.33 ± 0.54 c	0.0 ± 0.00 b	210.33 ± 0.72 c	160.67 ± 0.54 c
20	346.33 ± 0.54 b	0.0 ± 0.00 b	184.33 ± 0.72 d	171.0 ± 0.47 b
Control	361.33 ± 0.72 a	55.0±0.94 a	172.33 ± 1.29 e	193.33 ± 0.27a
L.S.D $_{1\%}$	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.

Main metabolites concentration

1. *A. ipsilon*

Data in 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 control, respectively.

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

TABLE (III)

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
Control	3572.67 ± 1.44 a	10930.33 ± 0.72 e	1373.33 ± 0.98 e
L.S.D $_{1\%}$	5.57	4.47	6.09

*concentration = μg/ml.

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 (Table 4). However, these values were 1342.67, 1334.33, 1155.33, 1118.33 and 937.33 μg/ml at 40, 30, 25, 20 μl and the control, respectively.

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

TABLE (IV)

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
Control	6236.33 ± 0.54 a	937.33 ± 1.29 e	5356.0±0.72 e
L.S.D $_{1\%}$	5.36	3.73	6.44

*concentration = μg/ml.

The previous study of biochemical activity of leucokinin II demonstrated that, leucokinin II inhibited the release of protease and 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 production of main metabolites.

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 neurosecretory 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 is supported by the fact that the protein metabolism is linked to digestive enzymes production (Englemenn, 1969). While the stimulation activity in invertase release could be explained that tested larvae were at 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 directed 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 are 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 by glycogen phosphorylase or lipase activation by Leucokinin II in fat body (Gade, 2004). An additional explanation to that stimulatory effect in larval instars, that are 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.

SUMMARY

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. Meanwhile, 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.

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