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LEUCOKININ II A NEUROPEPTIDE HORMONE AFFECTING BIOLOGICAL AND BIOCHEMICAL ACTIVITIES OF THE EGYPTIAN COTTON LEAFWORM *Spodoptera littoralis* (BOISD.)

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ABSTRACT

The insect neuropeptide, leucokinin II was injected into larvae of the cotton leafworm *Spodoptera littoralis*(Boisd.) to investigate the influence on development, digestive enzyme release and heamolymph main component. One injected dose of ($2x10^{-6}$ to $4x10^{-6}$M) of leucokinin II into the 5^{th} instar larvae induced prolongation in pupal period in all experimental groups, while it had no effect on the timing of larval ecdysis and length of feeding period. Also leucokinin II caused a great reduction in pupal weight, adult longevity, oviposition period, fecundity and fertility. A significant increase in larval mortality 66.66 to 86.66% was observed. Leucokinin II inhibited the release of digestive, protease, amylase, trehalase and invertase. A significant reduction was obtained in total protein level, whereas total carbohydrate and total lipid levels were significantly increased in dose dependent manner.

INTRODUCTION

Insect neuropeptides are chemical transmitters of short chains of amino acids that produced in a nerve cell and that can be released at synaptic junction to excite or inhibit post functional cells; they function as hormones and as neuromodulators (Callec, 1974).

The large number of neuropeptides structurally identified from different insect groups highlights the complexity of neurosecratory system in regulating various physiological process (Predel and Eckert, 2000).

The search for new forms of chemical and biological control of the major agricultural pests has provided the efforts towards the development of insect neuropeptide mimics that are resistant to the process insects normally use to degrade neuropeptids because of their potential value to the insecticide industry.

Insect kinin is a class of insect neuropeptides regulates water and mineral balance as well as digestive process (Nachman *et al* ., 1997). The probable role of these neuropeptides in biological and biochemical activities needs to investigate. This study clarify the effects of leucokinin II, a member of a family of eight peptides isolated from *Leucophaea maderae* on some biological aspects, *in vivo* release of digestive enzymes and heamolymph main component in the lepidopteran insect, *S. littoralis.*

MATERIALS AND METHODS

Rearing technique:

Larvae of *S. littoralis* 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±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).

One day 5 th instar larvae were starved for 8 hrs., arrested by cooling in refrigerator for about 5-10 min. Leucokinin II was injected into the larvae with a 20 µl microsyringe through the segmental membrane between the fifth and sixth abdominal segments (Tanaka *et al.*, 2002) and immediately provide with castor bean leaves.

Biochemical assays:

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

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.

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

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

Biological studies

Special attention was paid to pupal weight and duration, emergence percentage, also for moths fecundity and egg fertility in relation to the leucokinin II doses.

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 Cochran, 1972).

RESULTS AND DISCUSSION

Insect leucokinins are as group of myotropic peptides, that stimulate lipid mobilization and inhibit protein synthesis in *L.moderae* (Goldsubrthy *et al.*, 1992). They function in the control of water and ion balance in numerous insects (Schoofs *et al.*, 1993). In *Drosophilla melanogaster*, leucokinins are reported to increase the intracellular calcium in the stellate cells (O'Donnell *et al* ., 1998).

The insect midgut has been previously described as one of the larges endocrine organs in insect (Lang, 2001). Neuropeptides, including those previously mentioned, have been implicated in altering midgut activity by eliciting a short circuit ion current, as in *Manduca Sexta* (Lee *et al.*, 1998). Regarding the structural activity relationships of the leucokinins (Nachman and Holman 1991) indicated that the leucokinin structure is recognized by the midgut receptors.

In this study leucokinin II has been bioassayed for evaluating its effect on digestive enzymes and heamolymph main component in the larvae of *S. littoralis* then try to conjugate these results with observed biological events.

Data in Table (1) showed that larval mortality is directly proportion with dose increasment. Statistically, high differences between all doses and untreated group were obtained. On the other hand pupation percentages decrease by increasment of dose, while it shows the same significant changes. Leucokinin II enhances a significant reduction in pupal weight. Such finding are in agreement with the results previously stated on *Helicoverpa zea* F. larvae by Nachman *et al.*, 2003. The prolongation in pupal duration takes place and that results was mentioned before on the silk worm, *Bombyx mori* L. (Tanaka *et al* ., 2002). The treatment with different doses of Leucokinin II statistically reduced adult emergence, also no emergence was obtained in high doses.

Table (1): Effect of graded doses of leucokinin II on the larval and development of *S. littoralis*.

Dose (µl)	Larval mortality %	Pupation %	Pupal weight (gm)		Pupal duration (days)		Emergence %
			♂	♀	♂	♀	
40	86.66 a	13.33 b	- ns	-	- ns	- ns	0.0 de
30	73.33 a	26.66 b	-	-	-	-	0.0 d
25	66.66 a	33.33 b	0.2552±0.00	0.3412±0.011 c	9±0.002	7.5±0.210	60 b
20	66.66 a	33.33 b	0.2998±0.005	0.3539±0.010 b	8.5±0.290	7.0±0.001	80 c
Untreated control	6.75 b	93.33 a	0.3270±0.006	0.3876±0.007 a	8.25±0.211	7.25±0.121	94.66 a

Each value represents mean ± S.E
The significance was analyzed by F-test
ns = non significant difference
- = no data obtained cause no emergence observed

Data in Table (2) indicated that pre-oviposition period, oviposition and post-oviposition period were (2.5, 2.5 and 2.0 days), (5.0, 5.0 and 7.25 days) and (1.25,0.5 and 0.5) at 25, 20 and untreated, respectively. Statically, there are no significant differences between all values at all treatments and untreated for the three biological aspects. Also male longevity was 9.25, 9.0 and 10.25 days at 25, 20µl and the untreated, the general longevity for *S. littoralis* adult were 9.0, 8.5 and 10.25 days at 25, 20 and untreated, respectively. In the same table, fecundity showed great differences from the untreated it were 772, 750 and 1330 egg/female at 25, 20 µl and the untreated. Statistically, no difference between doses were obtained while the

values are significantly different with the untreated. This dramatically decrease in fecundity is consequent upon the role of insects that accumulate the vast majority of their egg destined amino acids during larval development and in which vitellogenin synthesis and egg production takes place after eclosion. The initiation of vitellogen in synthesis in diverse Lepidoptera is known to fall anytime from the last larval instar (Davis *et al* ., 1990; Lamison *et al* ., 1991).

Table (2): Effect of graded doses of leucokinin II on the adult development of *S. littoralis*.

Dose (µl)	Adult longevity (days)					Fecundity (no. eggs/ female)
	Female longevity				Male longevity	
	Pre-oviposition	Oviposition	Post-oviposition	Total		
25	2.5±0.25 ns	5.0±0.35 ns	1.25±0.21 ns	8.75±0.31 ns	9.25±0.35 ns	772.0±5.91 b
20	2.5±0.25	5.0±0.35	0.5±0.21	8.0±0.21	9.0±0.35	750.0±3.37 b
Untreated control	2.0±0.21	7.25±0.56	0.5±0.21	9.75±0.33	10.25±0.323	1330.25±13.6a

Each value represents mean ± S.E
High doses were negligible in statistical analysis because no data were obtained in adult emergence
ns = non significant differences.

Table (3) showed high significant reduction in egg fertility, while incubation period seems equal in treated and untreated.

Table (3): Effect of leucokinin II injection on the fertility and the incubation period of *S. littoralis*.

Dose (µl)	Fertility %	Incubation period
25	81.0 b	3.06 ± 0.06 ns
20	72.0 c	3.1 0 ± 0.09
Untreated control	96.0 a	3.23 ± 0.04

All values are mean ±S.E
ns = non significant difference

Development events mirror the physiological differentiation along *S. littoralis* biochemical activities. In Table (4), leucokinin II showed a great effect in digestive enzymes activity. Leucokinin II caused a signficant increase in inhibitory effect of releasing protease and amylase. Such finding are in agreements with that obtained by Harshini *et al*., 2002, they observed significant reduction in amylase and protease releasing from midgut in *Opisina arenosella* larvae. Data revealed high reduction in trehalase activity, this result is supported by such previously studies (Lopata and Gade, 1994; Becker *et al* ., 1996). On the other hand, leucokinin II exerted a stimulatory effect by increasing invertase level.

Table (4): Effect of different doses of leucokinin II on secretion activity of protease and carbohydrate hydrolyzing enzymes in haemolymph of 5th instar larvae of *S. littoralis*

Dose (µl)	Protease units *	Amylase units**	Invertase units **	Trehalase units**
40	156.66±0.9 e	89.66±0.2 c	211.33±1.5 a	96.0±1.6 d
30	163.66±0.5 d	91.66±0.7 b	178.66±0. b	122.0±1.3 c
25	196.33±1.3 c	97.0±1.2 b	150.66±0.9 c	146.0±0.8 b
20	203.0±1.85 b	98.33±0.9 b	137.33±1.1 d	152.3±1.4 b
Untreated control	210.66±0.9 a	161.0±0.8 a	133.33±1.4 e	174.0±2.1 a

All values are mean ± S.E of number of observations indicated between doses. The significance was analysed by F-test
ns= non significant different.
*** 1 unit: amount of enzyme required to liberate 1µg of tyrosine from casein/min.**
**** 1 unit : amount of enzyme required to liberate 1µg of maltose equivalents from starch /min.**

Data in Table (5) showed another stimulatory effect in total carbohydrate and total lipid synthesis. Recently, a similar relationship between the peptide level in heamolymph and increasing of carbohydrates and lipid mobilizations observed (Gade, 2004). Data revealed that high reduction in total protein synthesis, and this further supported by the fact the neuropeptides play a crucial role in general protein metabolism, which are linked to the digestive enzymes production (Engelmann, 1969).

In summary, physiological links between the neuropeptides effect and daily biochemical events is an important feature to reveal the relation between these peptides and regulation of insect biological aspects.

Table (5): Changes in main haemolymph component levels of *S.littoralis* after treated with different doses of leucokinin II.

Dose(µl)	Total protein concentration (mg/larvae)	Total carbohydrate concentration (mg/larvae)	Total lipid concentration (mg/larvae)
40	1992.66±0.54 e	5758.33±0.98 a	1268.33±0.9 a
30	2082.33±0.72 d	5645.33±0.27 b	1172.66±1.2 b
25	2265.33±0.72 c	4643.0±0.47 c	1167.33±1.1 b
20	3214.0±0.47 b	4009.66±1.29 d	1172.33±1.4 b
Untreated control	4490±0.94 a	3817.66±0.72 e	1169.0±2.1 b

All values are mean ± S.E of number of observation indicated between doses. The significance was analyzed by F-test.

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تأثير الببتيد العصبي الهرموني الليكوكينين٢على النشاط البيولوجي والبيوكيميائى على دودة ورق القطن المصرية

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أجريت الدراسة لمعرفة تأثير الببتيد العصبي الليكوكينين ٢ على نمو ومعدل نشاط إنزيمـات الهضم والتمثيل الغذائي في دودة ورق القطن المصرية، تم حقن الليكـوكنين ٢ بجرعـات مـن ٢×١٠$^{-٦}$ إلى ٤×١٠$^{-٢}$ في يرقات العمر الخامس لدودة ورق القطن. أظهـرت النتـائج حـدوث استطالة في فترة التعذير في جميع التجارب بينما لم يؤثر مطلقاً على توقيـت الانسـلاخ اليرقـى وطول مدة التغذية، أحدث الليكوكينين ٢ أيضا انخفاض كبير في وزن العذارى، عمـر الحشـرة الكاملة، فترة وضع البيض، عدد البيض وخصوبته .أظهرت النتائج ارتفاع في معـدلات المـوت ليرقات العمر الخامس وتراوحت من ٦٦,٦٦ إلى ٨٦,٨٦%. كما أحدث الليكوكينين انخفاض في معدلات إفراز إنزيمات البروتييز، الاميليز، التريهاليز والانفرتيز، كما حدث انخفاض حـاد فـي معدل البروتين الكلى بينما انخفض معدل الكربوهيدرات والليبيدات الكلية لزيادة الجرعة المستخدمة.