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VISCERAL MUSCLES**

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**KEY WORDS :*Locusta migratoria*, Foregut, Hindgut, Spontaneous Activity
(Spt.Act), K⁺-responses, L-glutamate, Proctolin, Leucomyosuppressin (LMS).**

ABSTRACT: Locust foregut and hindgut muscles were seen to have significantly different characteristics in both their natural baseline and their responses to added drugs. Although, locust hindgut preparations were spontaneously active and consisted of mainly uniform amplitude, the foregut muscles were more active. K⁺-induced tonic contractions in both hindgut and foregut above a concentration of 20mM K⁺, but the response in hindgut was smaller than in foregut preparations. Maximum responses occurred in both muscles around 80mM K⁺. L-glutamate responses were very inconsistent, but in the foregut concentrations below 10⁻⁵M caused small reductions in baseline tension. Above 10⁻⁵M L-glutamate induced a contraction. The hindgut exhibited a threshold of 10⁻⁷M, above which bursts of phasic contractions were induced. Proctolin induced a tonic contraction in foregut with a maximum response at around 5x10⁻⁷M and phasic contractions in the hindgut with a maximum response around 5x10⁻⁸M. Above these concentrations both foregut and hindgut showed considerable tachyphalaxis. LMS reduced both spontaneous activity and baseline tension of foregut and hindgut muscles. It also increased 80mM K⁺-responses of foregut muscle at 10⁻⁹M, and of hindgut muscles at 10⁻⁶M LMS. However, 10⁻⁷M LMS inhibited 80mM K⁺-responses of foregut and hindgut muscle. This could be due to the LMS acting as to block the cyclic

Ca²⁺-movements in and out of the cell, responsible for Spt.Act. L-glutamate (10⁻⁴M) inhibited foregut muscles, but it increase hindgut Spt.Act. Although, LMS inhibited both gut divisions Spt.Act, yet these natural activities were persisted in presence of L-glutamate. A linear reduction of responses induced by L-glutamate in relation to LMS concentration were observed in foregut and hindgut. LMS reduced the responses induced by proctolin up to a threshold concentration of 10⁻⁸M in foregut and hindgut. Above this concentration, it acted to increase the responses. This may occurs by the LMS competitive action with Ca²⁺-channels. The results of this study show that each *Locusta* visceral muscle type has its own unique response profile to LMS and support the idea that peptides may be multifunctional regulators.

INTRODUCTION

The locust foregut has a reasonably homogeneous longitudinal fiber population, as well developed as the circular fibres (Mutwally, 1990). However, in the hindgut the longitudinal fibres are exceptionally well developed compared with the circular fibres, and are collected together into strong external superior and inferior straps of powerful cyclical shortening of the rectum (Dunbar, 1980). The visceral muscles of the foregut and hindgut posses an intrinsic myogenic rhythm (Oldfield & Huddart, 1982; Mutwally & Jamel Al-Layl, 1992 & 1993). It appears that these intrinsic rhythmus are modulated by the extrinsic innervation from the stomatogastric and caudal regions of the autonomic nervous system (Dunbar, 1980; Mutwally, 1990).

Both L-glutamate and proctolin have been proposed, and relatively conclusively proven, to be neurotransmitters in the *Locusta* gut (Dunbar, 1980; Dunbar & Huddart, 1982; Banner *et. al.*, 1986). Although, there are still gaps to

be filled before the full picture can be seen. The recently fashionable field of neuropeptide transmission in the vertebrates has spread to insect physiology with the demonstration of neurotransmission or neuromodulation by a host of same neuropeptides and neurohormones synthesized both centrally and locally (Huddart, 1975 & 1985). Holman and Cook (1970) isolated four biologically active substances from *Leucophaea* and *Periplaneta* hindguts and two of these were identified as L-glutamate and L-aspartic acid. A number of reports now suggest that L-glutamate may be the excitatory neuromuscular transmitter in a number of insect visceral muscles (Holman & Cook, 1970; Cook & Holman, 1975; 1979a; Dunbar, 1980) and also acted as an inhibitory agent in *Locusta* foregut muscles (Mutwally, 1990; 1993 & 1994).

Cook and Holman (1979b) reported that low L-glutamate concentrations $1.5 \times 10^{-6} \text{M}$ potentiate's nearly evoked events, while high concentrations $8.5 \times 10^{-5} \text{M}$ caused suppression of response in locust hindgut. Moreover, L-glutamate elicited responses, while at $9.5 \times 10^{-5} \text{M}$ precipitated dramatic phasic and occasionally tonic response (Dunbar, 1980). Cook and Holman (1978 & 1979b) concluded that proctolin stimulated cockroach foregut and hindgut spontaneous activity (Spt.Act), the hindgut was more sensitive to this drug than foregut.

Dunbar (1980) and Dunbar and Huddart (1982) reported that proctolin affected Ca^{2+} -mobilization in locust visceral muscles. They suggested that proctolin activation of contractility be due to an inwardly directed Ca^{2+} -pules, which release Ca^{2+} from intracellular sites. They added that proctolin may have dual mode of action: one *via* the conventional postsynaptic membrane and a second *via* action on extrajunctional receptors (Cook & Holman, 1980; Dunbar & Huddart, 1982).

Dunbar (1980) concluded that the relative actions of two transmitter candidates' proctolin and L-glutamate modulated electromechanical activity of locust hindgut in a dose-dependent manner, and he suggested a synaptic site of action. Furthermore, proctolin often exhibited long-term effects on contractility suggesting that it may have an additional effect on cell metabolism.

Ca²⁺-channel voltage-depending on membrane depolarization with K⁺, the more depolarized the muscle the more Ca²⁺-channel opens (Stanfield, 1986). Reuter (1983) suggested that Ca²⁺-channel had a voltage sensor made up of a protein with dipole properties, a change in membrane potential (MP) causing a reorientation of the charged sensor, which open the gating mechanism.

During initial isolation of several myotropic peptides from cockroach head extracts (Holman *et. al.*, 1984) a material, later was called leucomyosuppressin (LMS), which suppressed the Spt.Act of the isolated cockroach hindgut muscles in dose-dependent and reversible manner. Holman *et. al.* (1986) suggested that LMS may be present in the tissues of the hindgut in a distribution paralleling that of the brain gut peptides in vertebrates. It is reasonable to suppose LMS may be present in the gut of *Locusta*. During this study, LMS was seen to have a definite effect on the foregut and hindgut Spt.Act and responses to KCl, L-glutamate and proctolin. Although, locustamyoinhibiting peptide (LOM-MIP) differed from LMS structurally, however, LOM-MIP suppresses the Spt.Act of the hindgut and oviduct of *Locusta migratoria* and the hindgut of *Leucophaea maderae* (Schoofs *et. al.*, 1991). Moreover, LMS did not show a uniform inhibition profile for all visceral muscle types in the cockroach, the heart and the oviduct were less sensitive to LMS than either the foregut and the hindgut (Cook & Wagner, 1991).

An antiserum raised against LMS, the first insect neuropeptide shown to inhibit contraction of both visceral and skeletal muscles of insects, revealed the presence of LMS-like material in neurons of the adult stable fly, *Stomoxys calcitrans* (L.) (Meola *et. al.*, 1991). The appearance of LMS m-RNA in the central nervous system, stomatogastric nervous system, and midgut suggests that LMS may play a central role in *Diptera* and may be associated with feeding and digestion (Fuse *et. al.*, 1998).

There were many studies with L-glutamate on *Locusta* foregut and hindgut (Dunbar, 1980; Oldfield & Huddart, 1982; Mutwally, 1990), and proctolin on the foregut (Banner *et. al.*, 1986) and on hindgut (Dunbar, 1980). However, LMS was mainly used with cockroach hindgut (Holman *et. al.*, 1986), and work was done on *Locusta* foregut (Mutwally, 1990). Therefore, the aim of this present study is to extend the investigation of LMS action on the foregut and hindgut of *Locusta*. In addition, this study is intended to study LMS effect on baseline activity, K⁺-responses and contractions induced by L-glutamate and proctolin, in a variety of concentrations.

MATERIALS AND METHODS

In this study, the locust normal saline was composed of (g / l): NaCl (150mM) 8.8g, KCl (10mM) 0.75g, Tris-HCl (10mM) 1.58g and CaCl₂ (2mM) 0.29g. Drugs used in this investigation were as follows: leucomyosuppressin (LMS), proctolin, L-glutamate and KCl. All drugs were obtained from Sigma Co., except LMS, which is kind gift from Dr. H. Huddart, Institute of Biological and Environmental Sciences, Lancaster University, Lancaster, UK. Drugs were freshly made up to a concentrated stock solution, so only small aliquots needed to be added to the organ baths to give the required concentration.

RESULTS

K⁺-responses

The locust foregut and hindgut muscles were seen to have significantly different characteristics in both their natural baseline tension and their responses to added drugs.

i) Foregut: On adding KCl to isolated foregut preparations a direct depolarization occurred, which resulted in a contraction of the longitudinal muscles. Beyond the threshold of about 20mM K⁺ progressive increases in [K⁺]_o of the bathing medium induced tonic contractions. Some typical K⁺-induced contractions are shown in Figure 1.

ii) Hindgut: Hindgut preparations were found to be rather less responsive to sequential K⁺-application than foregut preparations. As in foregut, the hindgut showed a threshold at around 20mM K⁺. Subsequently higher concentrations induced tonic contractions, which were much lower in amplitude compared to the foregut. No phasic contractions were seen throughout the range of K⁺-concentrations (Figure 2). As the maximum observed response to K⁺-concentration was around 80mM in both the foregut and hindgut, this concentration was chosen as the control for testing L-glutamate, proctolin and LMS action on the K⁺-induced contraction.

The glutamate induced response

i) Foregut: At concentrations below 5x10⁻⁷M glutamate, no response was noted. At this threshold concentration, and up to around 10⁻⁵M glutamate, small decreases in the amplitude of the foregut Spt.Act were seen. In addition, at concentrations above 10⁻⁵M small increases in Spt.Act were seen, which were slow in onset and displayed mechanical summation. (Figure 3).

ii) Hindgut: No responses were seen to glutamate concentrations below 10^{-7}M . Subsequently higher concentration above this threshold induced short bursts of contractions, which increased in amplitude and duration as the concentration increased (Figure 4). The time lapse between addition of glutamate and a subsequent response is much longer in hindgut than in foregut. Once initiated, however, the responses of the hindgut were larger and more consistent. With higher concentrations than 10^{-7}M the responses increased until $5 \times 10^{-6}\text{M}$, after which no apparent increase in contracture were noted. When 10^{-5}M glutamate was added to a quiescent preparation phasic contractions were induced and Spt.Act restored.

The proctolin induced response

i) Foregut: On adding proctolin to the bathing medium a tonic contraction was induced in isolated foregut preparations. The initial tonic contraction is maintained for less time, but the overall size of the response increases in relation to increases in proctolin concentration. Maximum contraction occurred around $5 \times 10^{-7}\text{M}$ proctolin, with considerable tachyphalaxis occurring above this concentration (Figure 5).

ii) Hindgut: Adding external proctolin to the bathing medium of the isolated hindgut, phasic contractions were induced after a latent period. The duration and maximum amplitude of the contractions increased with subsequent higher amplitude of the contractions increased with subsequent higher concentrations. There was considerable mechanical summation present, an example of which can be seen in (Figure 6). After the period of phasic contractions the hindgut maintained a small tonus, which was eliminated by washing the preparation in locust normal saline. These results indicate that the hindgut is far more sensitive

to proctolin than the foregut. Since the foregut and hindgut have maximum responses at markedly different proctolin concentrations, it was considered wise to choose two separate control concentrations to be used in phase two. In both the foregut and hindgut the concentration producing maximum response were chosen as the controls. Hence 5×10^{-7} M proctolin was used for the foregut, and 10^{-7} M for the hindgut.

The action of LMS on baseline activity

i) Foregut: On adding LMS to a Spt.Act preparation a lowering of the baseline and a decrease in the contracture amplitude, were noted (Figure 7). When washed with locust normal saline, the baseline tension and Spt.Act were restored. Subsequently higher concentrations of LMS induced a larger suppression of Spt.Act, until at 5×10^{-5} M completely eliminated it. In addition to this subsequently higher LMS concentrations induced larger hyperpolarization and hence relaxation of the baseline tension.

ii) Hindgut: Both Spt.Act and baseline tension were reduced by adding LMS to isolated hindgut preparations (Figure 8). The hindgut appeared to be more sensitive than the foregut in its Spt.Act and baseline responses to the range of LMS concentrations. At 10^{-5} M LMS, the hindgut stopped being naturally active, compared to 5×10^{-5} M in the foregut, and showed much large relaxation of the baseline tension. These responses were easily reversible by washing the preparation in locust normal saline.

The action of LMS on the K^+ -induced response

i) Foregut: Isolated foregut preparations were subjected to a range of LMS concentrations and after a period of time (2-3 minutes), to each respectively dosed

preparation 80mM K⁺ was added. When the LMS was added a considerable lowering of baseline tension and Spt.Act was observed. On adding 80mM K⁺, a rapid smooth tonic contracture was observed which reached a maximum in about 1 minute. This maximum contracture was short-lived and then rapidly fell to a tonus approximately half of the maximum response (Figure 9).

ii) Hindgut: At 10⁻⁹M, LMS had very little effect on the hindgut response to 80mM K⁺. However, at higher concentrations up to 10⁻⁷M, LMS caused a decrease in the hindgut response, with a maximum effect at 5x10⁻⁸M. At concentrations above 10⁻⁷M, LMS slightly began to increase the response of the hindgut until at 10⁻⁶M the response was greater than that of the control (Figure 10). On adding to the preparation, a drop in baseline tension and Spt.Act was observed. Rapidly after adding the KCl to the bathing medium, the hindgut exhibited a tonic contraction. In addition to the tonic contraction, slow phasic contractions were induced. The general tonus then gradually reduced until washed with locust normal saline, when it returned to normal baseline tension (Figure 11).

The action of LMS on the glutamate response

i) Foregut: A concentration of 10⁻⁴M L-glutamate was used as the foregut control when testing the action of LMS. Additional of LMS in a range of concentrations, an apparent linear relationship was found between LMS concentration and depression of the Spt.Act amplitude. When the L-glutamate was added it initiated the return of phasic contractions, but their amplitude was decreased by the presence of the LMS. The latent period before a response was seen to increase also (Figure 11).

ii) Hindgut: As in foregut, addition of LMS to hindgut preparations caused a decrease in the observed responses to the control L-glutamate concentration (10⁻⁴

⁴M). The hindgut showed a linear relationship between LMS concentration and reduction of the control L-glutamate response. However, it proved to be affected less than the foregut, with smaller reductions in L-glutamate responses at corresponded LMS concentrations. The presence of LMS caused lowering amplitude in the induced phasic contractions, and a lengthening of the latent period between L-glutamine introduction and contraction (Figure 12).

The action of LMS on the proctolin induced response

i) Foregut: At concentration up to about 5×10^{-8} M LMS proved to inhibit the response induced by the control (5×10^{-7} M) proctolin. At this threshold concentration LMS suddenly becomes an agonist and increases the proctolin response to around double that of the control. The agonist character peaks at 10^{-7} M LMS, after which higher concentrations cause less of an increase in the proctolin response. The character of the LMS / proctolin-response is very similar to that of the LMS / K^+ -response. Addition of LMS reduces Spt.Act and baseline tension. Subsequent addition of proctolin then induces a rapid tonic contraction, which has a short-lived maximum (Figure 13).

ii) Hindgut: Both Spt.Act and baseline tension were reduced on adding LMS to isolated hindgut preparations. After a period of 2-3 minutes, proctolin was added to produce control concentration of 5×10^{-8} M. The hindgut showed a decrease in the induced proctolin response, when compared to the control, at LMS concentrations below 10^{-8} M. Above this threshold, LMS acted as to increase the proctolin response, peaking at 10^{-7} M. The relative increases in sensitivity were much smaller in the hindgut than the foregut. The tonic contractions induced by adding the proctolin were accompanied by a revival of Spt.Act immediately after the increase in tonus (Figure 14).

DISCUSSION

The result of this study do not comply with the findings of Dunbar (1980) who noted that phasic contractions at a threshold of 30mM K⁺, and large tonic responses at higher concentration. The fact that the present results confirmed the finding of Oldfield and Huddart (1982) and Mutwally (1993 & 1994) whom noted that the hindgut was highly irregular in its response to sequential K⁺-application, and hence a meaningful correlation of tension with K⁺-concentration, was impossible.

This raised intracellular free Ca²⁺ can either become 'activator' Ca²⁺ directly or, as is often the case, cause Ca²⁺-induced Ca²⁺-release from intracellular pools. Activator Ca²⁺ then binds to a receptor protein such as calmodulin, in smooth muscle, which in turn binds to inactive enzymes activating them in doing so. One such enzyme modified by calmodulin is 'myosin light chain kinase' (MLCK), whose active complex causes phosphorylation and subsequent crossbridging between actin / myosin filaments, leading to a contraction. Relaxation then occurs by closing of the Ca²⁺-channels and the extrusion, *via* the Na⁺ / Ca²⁺-exchange pump, or, ATP-dependant Ca²⁺-extrusion pump, of free Ca²⁺ back to the resting level of 10⁻⁷M (Huddart, 1975 & 1985; Campbell, 1985; Stanfield, 1986).

The foregut and hindgut of *Locusta migratoria* show clear difference in both structure and intrinsic Spt.Act (Dunbar, 1980; Oldfield & Huddart, 1982; Mutwally, 1990; Mutwally & Jamel Al-Layl, 1992). The foregut shows continuous small amplitude irratic rhythms, where as the hindgut shows cycles of strong contractions of regular amplitude, often with large periods of quiescence in-between. The differences between the rhythms of locust both tissues were

almost certainly a reflection of the differences in the functions of the two gut divisions. This result confirmed previous studies of Oldfield and Huddart (1982) and Mutwally and Jamel Al-Layl (1992).

The *Locusta* gut responds to K⁺-depolarization in a way more like that of vertebrate smooth visceral muscle than insect or vertebrate skeletal muscle (Huddart, 1975; Huddart & Hunt, 1975). The extent of tension-induced, seems to be directly related to the degree of depolarization caused by KCl (Huddart, 1985; Mutwally & Jamel Al-Layl, 1992 & 1993). In addition, Dunbar (1980) showed that tension development is dependent on trigger influxes of [Ca²⁺]_o, which are voltage-dependent (*i.e.*, *via* voltage operated channels; VOC).

In recent years many advances in electrophysiological techniques have been made providing greater understanding into the workings of the voltage-operated channel (VOC) (Stanfield, 1986). Another Ca²⁺-channel is the receptor-operated channel (ROC), which are opened by the interaction of an agonist, such as ACh to a postsynaptic receptor. Bolton (1979) suggested that the ROC's are closely associated with receptors but it is still not known what biochemical event links them. The relative inability of ROC's to open on depolarization and close on contact with Ca²⁺-antagonists suggests they have different gating properties to VOC's.

Hindgut also proved to be more responsive to L-glutamate, at concentration above 10⁻⁷M the hindgut display short-lived bursts of phasic contractions. In the foregut L-glutamate proved to inhibit Spt.Act up to a concentration of 10⁻⁵M above which small increases in the Spt.Act were seen. The foregut responses to glutamate were very inconsistent, which complies with the

findings of Oldfield and Huddart (1982), but a correlation of the induced response with L-glutamate concentration will be attempted.

In this study, hindgut muscle proved to be significantly more sensitive to the effects of applied proctolin than the foregut, which required higher dose of proctolin to cause maximum contracture. However, L-glutamate was inhibitory in foregut up to 10^{-5} M, but showed small increases in the Spt.Act of the hindgut at concentration above 10^{-7} M. The responses to proctolin were far more intense than its responses to L-glutamate, suggesting that proctolin are not a neuromodulator of L-glutamate, but in fact, the major neurotransmitter. The inhibitory nature of L-glutamate on the foregut could act as a modulator of the proctolin response, in that it could suppress its action. It was found a great difference in L-glutamate threshold between the foregut and hindgut of *Locusta* reached a similar conclusion (Dunbar, 1980; Oldfield & Huddart, 1982; Dunbar & Piek, 1983; Mutwally, 1990 & 1994). They suggested that L-glutamate was likely to only have a transmitter role in the foregut and hindgut.

The two main candidates proposed are L-glutamate (Cook *et. al*, 1969; Holman & Cook, 1970; Cook & Holman, 1975) and the pentapeptide proctolin (Brown, 1965 & 1975). Subsequently, Cook and Holman (1979a) suggested that proctolin be assigned a neurotransmitter role in *Leucophaea maderae*, the real neurotransmitter possibly being L-glutamate. Dunbar and Piek (1983) demonstrated that subthreshold concentration of proctolin had no visible effect on L-glutamate induced responses of locust hindgut, hence suggesting that the peptide does not act to modulate L-glutamate activity.

When proctolin was added, the hindgut proved to be far more sensitive than foregut. Maximum contractions occurred at 5×10^{-8} M in hindgut, compared to

$5 \times 10^{-7} \text{M}$ in foregut. In this study, the maximum response dose of $5 \times 10^{-7} \text{M}$ proctolin confirms the results of Banner *et. al.* (1986 & 1987), whom found a maximum response around $2 \times 10^{-7} \text{M}$ and $5 \times 10^{-7} \text{M}$ in *Schistocera gregaria* foregut. In addition, the hindgut proved to have a maximum response at a much lower proctolin concentration ($5 \times 10^{-8} \text{M}$) than in the foregut. This complies with the findings of Banner *et. al.* (1987), who found in their work on *Schistocera gregaria*, the foregut had an ED_{50} of 160 and 30nM, and the hindgut an ED_{50} of 17.5 and 106nM.

In this study, the response of *Locusta* foregut and hindgut to LMS complies with the initial findings of Holman *et. al.* (1986). Both the frequency and amplitude of Spt.Act were reduced, and also a considerable drop in the baseline tension was observed, which returned to normal immediately upon washing the tissues with normal saline. This could be due to the LMS acting as to block the cyclic Ca^{2+} -movements in and out of the cell, responsible for Spt.Act. This may occur by the LMS competitive action with Ca^{2+} -channels. Holman *et. al.* (1986) suggested that LMS acting as to block the cyclic Ca^{2+} -movements in and out of the cell responsible for Spt.Act, and probably occurs by the LMS closing or blocking the Ca^{2+} -channels. LMS addition increased 80mM K^{+} -responses, this may be due to two types of receptors for LMS on both gut divisions. First, in foregut muscle, receptor acts to open Ca^{2+} -channels and hence increases the influx and free Ca^{2+} -ions in the cell, causing contractions in excess of those caused by K^{+} -depolarization alone. The opposite action was seen in hindgut, which close the Ca^{2+} -channels and decreased Ca^{2+} -influx. Second, it has an effect on the tonic balance and hence membrane potential of cells. At a threshold concentration, the

drug changes the MP triggers the opening of voltage-dependent and causes a massive influx of Ca^{2+} into the cell.

The LMS did not inhibit the Spt.Act of muscles of the cockroach *Leucophaea maderae* uniformly as a group but rather showed a selective suppression of activity in the foregut and hindgut. The threshold of LMS inhibition for these organs was 10^{-11}M for the foregut and $3 \times 10^{-11}\text{M}$ for the hindgut. The maximum response for each organ was generally recorded at $2.4 \times 10^{-8}\text{M}$. Both the heart and the oviduct were 100-1000 times less sensitive to LMS than either the foregut or the hindgut. Although the responses of the heart to LMS (10^{-9}M - 10^{-8}M) were somewhat inconsistent, the myocardium showed a reduction in either the amplitude or frequency of contractions in 75% of the preparations tested. The oviduct showed the lowest level of responsiveness of all the muscles tested. Even at a concentration of 10^{-7}M LMS, the amplitude and frequency of contractions showed no more than a 58% inhibition. Desensitization to LMS was observed in three of the four muscle types tested. The phenomenon occurred in 37% of the foreguts, 34% of the hindguts and 54% of the heart preparations tested. The results of this study show that each visceral muscle type has its own unique response profile to LMS and support the idea that peptides may be multifunctional regulators (Cook & Wagner, 1991).

The insect peptides LMS (pEDVDHVFLRFamide) and dromyosuppressin (TDVDHVFLRFamide) have identical chemical sequences with the exception of the N-terminal amino acid; both inhibit Spt.Act of insect visceral muscles. Neurons in the hypocerebral ganglions of horn fly, *Hematobia irritans* (L.), and stable fly, *Stomoxys calcitrans* (L.), were found to contain material immunoreactive to antiserum produced against the C-terminal of LMS, but not to

the N-terminal of dromyosuppressin. Two large lateral clusters containing 8 cells, linked dorsally and ventrally by 2 chains of 6 cells, encircled the anterior surface of the proventriculus and were immunoreactive of LMS and FMRFamide antisera. Axons from these cells were traced to the wall of the aorta and over the surface of the proventriculus. Ultrastructural analysis revealed these cells contained a singular type of elementary secretory granule that contained material of relatively low electron density, both in the cell body and at the axon terminals (Meola *et. al.*, 1996).

A novel peptide termed locustamyoinhibiting peptide (LOM-MIP) was isolated from brain-corpora cardiaca-corpora allata-suboesophageal ganglion extracts of the locust, *Locusta migratoria*. The primary structure of this nonapeptide has been determined Ala-Trp-Gln-Asp-Leu-Asn-Ala-Gly-Trp-NH₂. LOM-MIP suppresses the Spt.Act of the hindgut and oviduct of *Locusta migratoria* and of the hindgut of *Leucophaea maderae*. This novel peptide is, however, structurally different from LMS, a hindgut suppressing peptide isolated from *Leucophaea maderae* heads. LOM-MIP has a Gly-TrpNH₂ carboxy-terminal in common with APGWamide, a penis retractor muscle inhibiting peptide isolated from the snail, *Lymnaea stagnalis*. In addition, it shows carboxy-terminal sequence similarities with locust AKH II, which ends in AGWamide. No sequence similarities were found with other vertebrate or invertebrate peptides. Synthetic LOM-MIP showed biological as well as chemical characteristics indistinguishable from those of native LOM-MIP (Schoofs *et. al.*, 1991).

LMS depressed contractile activity in mosquito oviducts at concentrations above 10⁻¹²M, but hindguts did not respond to concentrations below 10⁻⁶M. Hindgut Spt.Act restarted in 10⁻⁶M LMS, but only washing out LMS restored

activity in oviducts. LMS changed the amplitude of the oviduct contractions, but the dynamics of contraction remained steady. Following recovery of contractions in LMS, hindgut tissues contracted with a more regular pattern. Serotonin and octopamine had an identical action on oviduct and hindgut tissues. At concentrations greater than 10^{-8}M , serotonin eliminated the refractory period between contractions and thus increased the contraction frequency of oviducts. Proctolin failed to stimulate both oviduct and hindgut contractions at concentrations up to 10^{-6}M , but at 10^{-8}M induced contractions of cricket hindgut preparations. Aea-HP-I had no effect on either tissue at 10^{-6}M or lower concentrations. State-series analysis, based on simple manipulations of experimental data, permitted direct observation of the dynamics of oviduct and hindgut contractile activity (Messer & Brown, 1995).

In 12 experiments in which the stable fly oviduct was exposed to 10^{-7}M LMS, no change in the amplitude, frequency, or tonus of muscle contractions was detected. Octopamine caused an inhibition of muscle Spt.Act of the oviduct. Spontaneous contractions showed a graded drop in activity over the concentration range tested (10^{-8}M - 10^{-6}M) (Cook & Wagner, 1992).

In the present study, the preparations were pre-treated with LMS, and then control concentrations KCl and proctolin were added, a difference in foregut and hindgut responses was seen. Pretreating with LMS caused a marked increase in the foregut response to 80mM K^+ , correspondingly it caused a decrease in hindgut contractions up to $5 \times 10^{-7}\text{M}$. LMS also induced a smaller increase in the hindgut response to control proctolin than was seen in the foregut.

Moreover, the addition of LMS caused a decrease in Spt.Act and baseline tension in both foregut and hindgut preparations, these reductions were more

apparent in the hindgut. The effect of LMS on 80mM K⁺-response was also greatly different between foregut and hindgut. The presence of LMS in hindgut tissue reduced the K⁺-response at concentrations below 5x10⁻⁷M. Its presence in foregut, however, induced larger contractions in response to 80mM K⁺-response (Mutwally, 1990). It appears that LMS may have two types of receptors: One in the foregut, which acts as to open Ca²⁺-channels and hence increase the influx and free Ca²⁺ in the cells, causing contractions in excess of those caused by K⁺-depolarization alone. Type two in the hindgut, which acts as to close Ca²⁺-channels and hence decrease Ca²⁺-influx and resultant contraction.

In the mealworm, *Tenebrio molitor*, submicromolar concentrations of LMS reversibly attenuate evoked release of transmitter from the motor nerve terminals, LMS has no effect on the L-glutamate-induced depolarization (Yamamoto *et. al.*, 1988).

The action of LMS on the L-glutamate induced response, is reduce it in both tissues. L-glutamate increased Spt.Act, whereas, LMS acts to inhibit them. The induced increases in Spt.Act caused by L-glutamate were very small, whereas, the LMS induced decreases were large. As a result the LMS action may simply outweigh the L-glutamate action, however, as a range of LMS concentrations were used on a fixed L-glutamate concentration. It is more likely that the LMS acts as to block or close the L-glutamate opened Ca²⁺-channels, either by blocking the channel, competitive binding to the L-glutamate receptor, or by binding to a specific LMS receptor who's action is to close the channel (Mutwally, 1990).

Proctolin caused a sustained tonic contraction in the cockroach anterior midgut, the amplitude of which was dose-dependent. In contrast, LMS, and its

relative SchistoFLRFamide, reduced the amplitude of these contractions. LMS and SchistoFLRFamide also inhibited phasic Spt.Act, which were elicited by proctolin application in only a few preparations. This work illustrates a possible physiological role for LMS in *Diploptera* midguts, in the passage of food along the alimentary canal (Fuse & Orchard, 1998).

SchistoFLRFamide, LMS and ManducaFLRFamide were each capable of lowering basal tonus and inhibiting Spt.Act and proctolin-induced contractions of midgut muscle. It was suggested that a possible function for the FMRFamide-related peptides contained within the endocrine cells and innervation of the midgut of the locust may be in modulating midgut contraction and thereby playing a role in digestion (Lange & Orchard, 1998).

The presence of LMS increases the response to proctolin in both the foregut and hindgut muscles. Starratt and Brown (1975) found that proctolin stimulates the contraction of cockroach visceral muscles. The foregut showed a large mechanical threshold at $5 \times 10^{-8} \text{M}$ LMS, being inhibitory at lower concentrations. The presence of such a threshold suggests that may be LMS has an effect on the ionic balance and hence membrane potential of cells. At a specific concentration, this effect may cause a change in membrane potential sufficient enough to trigger the opening of VOC's, and cause a massive influx of Ca^{2+} -ions into the cell. Up to this threshold voltage, LMS may have only the effect of reducing the influx of Ca^{2+} via proctolin activated channels. At threshold concentration the induced massive influx may considerably outweigh the inhibition of the proctolin-induced fluxes and hence a larger contraction is produced. The inhibition of proctolin induced channels may be due to competitive binding between proctolin and LMS to receptors.

While this present investigation, the effect of LMS on K⁺-induced responses, a difference between male and female response to LMS was noted of two separate occasions. On both occasions the female foregut and hindgut showed an increase in baseline tension with addition of 5x10⁻⁹M LMS and higher concentrations. The degree of contracture induced in the female showed no noticeable increase as LMS concentration was increased. In addition to this the tonic contraction induced by adding KCl was greatly reduced, when measured from the LMS induced baseline, but relatively similar to the control response, when measured from the resting (pre-LMS) baseline. A possible difference between the action of LMS on male and female gut preparations was observed. With LMS being excitatory in female, and inhibitory in male, base line tension (Mutwally, Not Published).

Due to little information known about the LMS-action, or locality in the insect's body, this study postulated possible LMS-actions. The true action of LMS on gut muscles is not yet known, and only by further research such as sucrose-gap and voltage-clamped techniques, which will hopefully reveal the complex picture of LMS role in insect muscles.

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FIGURE LEGENDS

Figure 1: (A) Show two examples of spontaneous activity (Spt.Act) of locust foregut muscles (a & b). In (B) typical foregut responses to K^+ -depolarization (upper arrows): (a) 10mM, (b) 15mM, (c) 40mM and (d) 80mM. After washout with normal saline (lower arrows), the base line tension returned to normal level. Calibration: tension and time scales were applied to all traces.

Figure 2: (A) Represent two examples of locust hindgut Spt.Act (a & b). (B) Shows K^+ -induced contraction of locust hindgut muscles: (a) 15mM, (b) 20mM, (c) 35mM, (d) 45mM, (e) 60mM and (f) 80mM (upper arrows). The base line tensions were returned to normal state after washing with normal saline (lower arrows). Calibration: tension and time scales were applied to all traces.

Figure 3: Show effect of L-glutamate additions ($5 \times 10^{-6}M$, $10^{-5}M$, $10^{-4}M$ and $10^{-3}M$) on the Spt.Act of locust foregut muscles respectively. At lower doses of L-glutamate, the normal activities were enhanced. However, at $10^{-4}M$ and $10^{-3}M$ it induced muscle contracture, but the Spt.Act were reduced. Calibration: tension and time scales were applied to all traces.

Figure 4: The Spt.Act of locust hindgut muscle was increased gradually with the addition of L-glutamate ($10^{-7}M$, $10^{-6}M$ and $10^{-5}M$) (upper arrows) respectively. Moreover, at ($10^{-5}M$) the muscle induced contracture accompanied with enhancement in Spt.Act. Lower arrows indicate washout with normal saline. Calibration: tension and time scales were applied to all traces.

Figure 5: Additions of proctolin ($10^{-8}M$, $10^{-7}M$ and $5 \times 10^{-7}M$) (upper arrows) caused muscle contraction of locust foregut. Lower arrows indicate washout with normal saline. Calibration: tension and time scales were applied to all traces.

Figure 6: Additions of proctolin ($10^{-9}M$, $5 \times 10^{-9}M$, $10^{-8}M$ and $5 \times 10^{-8}M$) respectively (upper arrows), on locust hindgut muscle induced muscle contractions accompanied with high and fast twitches. Lower arrows indicate washout with normal saline. Calibration: tension and time scales were applied to all traces.

Figure 7: Locust foregut Spt.Acts were reduced and the base line tension was lowered with the addition of leucomyosuppressin (LMS) ($10^{-7}M$, $10^{-6}M$, $10^{-5}M$ and $5 \times 10^{-5}M$) respectively (upper arrows). After washout with normal saline the muscle resumed its activity faster and the base line tension returned to normal level (lower arrows). Calibration: tension and time scales were applied to all traces.

Figure 8: Addition of LMS ($5 \times 10^{-9}M$ and $10^{-7}M$) lowered the base line tension and inhibited the Spt.Act of locust hindgut muscle (upper arrows). Lower arrows indicate washout with normal saline. Calibration: tension and time scales were applied to all traces.

Figure 9: In (a & b) initial 80mM K^+ -responses (control) of locust foregut muscle. Pretreatment of LMS ($10^{-9}M$ and $10^{-7}M$) (1st upper arrows), dropped base line tension and reduced 80mM K^+ -responses (2nd upper arrows). Lower arrows indicate washout with normal saline. Calibration: tension and time scales were applied to all traces.

Figure 10: In (a) initial 80mM K^+ -responses (control) of locust hindgut muscle. Addition of (10^{-7} M) LMS (1st upper arrows) reduced 80mM K^+ -responses (2nd upper arrows) (b). However, (10^{-6} M) LMS enhanced 80mM K^+ -responses slightly (c). Lower arrows indicate washout with normal saline. Calibration: tension and time scales were applied to all traces.

Figure 11: (a) Initial (10^{-4} M) L-glutamate (control) on locust foregut muscle. Addition of LMS (10^{-7} M and 10^{-5} M) (1st upper arrows) inhibited the Spt.Act, but these natural activities persisted with the addition of L-glutamate (2nd upper arrows) (b & c). Calibration: tension and time scales were applied to all traces.

Figure 12: (a) Initial (10^{-4} M) L-glutamate (control) on locust hindgut muscle. Addition of LMS (10^{-6} M and 10^{-5} M) (1st upper arrows) inhibited the Spt.Act, but these natural activities persisted with the addition of L-glutamate (2nd upper arrows) (b & c). Calibration: tension and time scales were applied to all traces.

Figure 13: (a) shows initial control of (5×10^{-7} M) proctolin-induced contracture of locust foregut muscle. (b) Addition of (10^{-8} M) LMS (1st upper arrows) reduced proctolin-responses (2nd upper arrows) and dropped base line tension. However, in (c) addition of (10^{-7} M) LMS (1st upper arrows) slightly enhanced proctolin-responses (2nd upper arrows). Lower arrows indicate washout with normal saline. Calibration: tension and time scales were applied to all traces.

Figure 14: (a) shows initial control of (10^{-7} M) proctolin-induced contracture of locust hindgut muscle. (b) Addition of (10^{-8} M) LMS (1st upper arrows) reduced proctolin-responses (2nd upper arrows) and dropped base line tension. However, at 10^{-7} M LMS (1st upper arrows) slightly enhanced proctolin-responses (2nd upper arrows). Lower arrows indicate washout with normal saline. Calibration: tension and time scales were applied to all traces.

دراسة أثر مشط الألياف العضلية البيضاء (الليوكومايوسيريسين) على العضلات الملساء للجراد الرحال

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الملخص:

أظهرت الدراسة أن لكلا معي الجراد الرحال اختلافات وظيفية واضحة في مستوى الشد العضلي الطبيعي وكذلك لاستجاباتها لأثر الأدوية المضافة على العضلات الملساء . بالرغم من ذلك ، فإن الانقباضات العضلية الطبيعية للأمعاء الأمامية كانت أكثر نشاطا من تلك التي للأمعاء الخلفية ، وكذلك فإن استجابة كلا العضلتين لاستحثاثات البوتاسيوم فأثما بدأت بوضوح عند تركيز 20 مليمول وأعلىها كان عند 80 مليمول. إضافة محلول الجلوتاميت ثبطت الأمعاء الأمامية في حين أنها زادت في نشاط الأمعاء الخلفية . أما أثر إضافة محلول البروكتالين ، فقد كان تنشيطيا على كلا معي الجرادة ولكنه كان أكثر قوة على الأمعاء الخلفية. بالنسبة لخلول الليوكومايوسيريسين ، فقد كان أثر الإضافات تثبيطيا على الانقباضات الطبيعية لكلا معي الجرادة وكان أكثر وضوحا على الأمعاء الخلفية . أما بالنسبة لأثره على الانقباضات المستحثة بالبوتاسيوم فقد كان تأثيره التثبيطي قويا على الأمعاء الأمامية . كذلك كان هذا الأثر التثبيطي لخلول الليوكومايوسيريسين إلى كلا معي الجرادة عند استحثاثها المبدئي بمحلول الجلوتاميت والبروكتالين ، ألا أنه كان قويا مع محلول البروكتالين. هذه الدراسة تقترح بأن هنالك اختلافات جوهرية بين كلا معي الجرادة ، وأن مادة الليوكومايوسيريسين قد أثرت على قنوات الكالسيوم المنتشرة على سطوح الألياف العضلية لكلا معي الجرادة ويحتمل أن أثر هذه المادة التثبيطي في هذه الدراسة قد أيد الاستنتاج المقترح بأن للبيتيدات وطانف عديدة كمادة منظمة.