ELSEVIER

Regulatory Peptides 77 (1998) 163–168

REGULATORY PEPTIDES

The muscular contractions of the midgut of the cockroach, *Diploptera punctata*: effects of the insect neuropeptides proctolin and leucomyosuppressin

Megumi Fusé*, Ian Orchard

Department of Zoology, University of Toronto, 25 Harbord Street, Toronto, Ontario, Canada M5S 3G5

Received 10 June 1998; received in revised form 1 July 1998; accepted 1 July 1998

Abstract

We have previously shown differential expression of leucomyosuppressin (LMS) mRNA in apparent endocrine cells in the anterior region of midguts of the cockroach *Diploptera punctata*, using in situ hybridization. In contrast, other FMRFamide-related peptides, as revealed by immunohistochemistry, have been found most abundantly in the posterior region in both apparent endocrine cells and nerve tracts [1]. Here, we partially purified extracts of anterior and posterior cockroach midguts, using HPLC coupled with radioimmunoassay, and found, among multiple FMRFamide-like immunoreactive fractions, one fraction co-eluting with LMS in both regions. The presence of a co-eluting fraction in the posterior region, in the absence of LMS mRNA positive endocrine cells suggests that LMS might therefore be present in nerve tracts running along the length of the midgut. Using a circular muscle contraction assay from different portions of midgut, we determined the effects of LMS, proctolin and a variety of other midgut peptides on contractions of the midgut of *Diploptera*. Proctolin caused a sustained tonic contraction in the 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 spontaneous phasic contractions, which were elicited by proctolin application in only a few preparations. Other postulated midgut peptides did not induce or inhibit contractions, nor augment the proctolin-induced contractions. The *C*-terminal truncated sequences of LMS, HVFLRFamide and VFLRFamide, were sufficient to reduce the amplitude of the proctolin-induced contractions. This work illustrates a possible physiological role for LMS in *Diploptera* midguts, in the passage of food along the alimentary canal.

Keywords: FMRFamide; SchistoFLRFamide; Myosuppressin; HPLC; Endocrine cells

1. Introduction

The cardioacceleratory neuropeptide FMRFamide (Phe–Met–Arg–Phe–NH_2), first isolated from mollusks [2], is the smallest member of a broad family of structurally-related peptides. This family of FMRFamide-related peptides (FaRPs) all share the common RFamide (Arg–Phe–NH_2) *C*-terminal moiety, and include the basic tetrapeptide FMRFamide, its close structural variant FLRFamide, as well as a host of *N*-terminally extended RFamides. The FaRPs are found throughout the animal kingdom in both nervous and non-nervous tissues [3], and can act on a wide variety of tissues, apparently as neurotransmitters, neuromodulators or neurohormones.

The insect myosuppressins form a subfamily of the FaRPs, and were originally named for their ability to inhibit visceral muscle contractions. They are decapeptides which share the common amino acid sequence XDVXHX-

Abbreviations: FMRFamide, Phe–Met–Arg–Phe–NH_2; HPLC, high performance liquid chromatography; LMS, leucomyosuppressin; FaRPs, FMIRFamide-related peptides; RIA, radioimmunoassay

*Corresponding author. Tel.: +1-416-978-4602; fax: +1-416-978-3522; e-mail: fuse@zoo.utoronto.ca

0167-0115/98/$ – see front matter
PII: S0167-0115(98)00110-4

FLRFamide, and appear to be encoded on a gene separate from the other FaRPs [4,5]. Leucomyosuppressin (LMS), the first member of the myosuppressins to be sequenced, was identified by its ability to inhibit spontaneously contracting hindgut muscle in the cockroach, *Leucophaea maderae* [6]. Its gene sequence has since been deduced in the cockroach, *Diploptera punctata* [5]. Leucomyosuppressin has been shown to act on other visceral tissues, such as the locust oviduct [7] and midgut [8], where it can inhibit spontaneous and proctolin-induced contractions. A preliminary report has indicated that LMS may act on *Diploptera* midguts as well, to inhibit proctolin-induced contractions [9]. The myosuppressins in general have now been shown to have a variety of actions on tissues, including the stimulation of skeletal muscle contraction [10] and the stimulation of enzyme secretion from digestive tissue [11].

With mRNA probes from the cloned gene encoding LMS in *Diploptera*, Fusé et al. [1] were able to localize the production of LMS mRNA, by in situ hybridization, to specific cells of the nervous system and alimentary canal. Of particular interest was the differential distribution of apparent endocrine cells containing LMS mRNA in the midgut, located to a discreet band in the anterior midgut, near, but not within the gastric caecae. In contrast, the majority of RFamide-like immunoreactive material in *Diploptera* midguts was found towards the posterior region of the gut, near the Malpighian tubules, in apparent endocrine cells and nerve tracts, suggesting that FaRPs other than LMS were localized to the posterior midgut.

With these results in mind, we have partially purified extracts of anterior and posterior midguts, using HPLC coupled to a radioimmunoassay (RIA) for RFamide-like peptides. We have further studied in greater detail, the effects of LMS and other neuropeptides on muscle contractions in the anterior or posterior regions of the midgut. We found the anterior midgut to provide a robust preparation for bioassay, whereas the posterior midgut was less robust and responded weakly to proctolin.

2. Materials and methods

2.1. Animals, tissue extraction and purification

The colony of *Diploptera punctata* was maintained on lab chow and water at 27°C on a 12:12 h light:dark cycle as described previously [12]. Three hundred insect midguts were dissected as two pieces under physiological saline (composition in mM: 150 NaCl; 12 KCl; 10 $CaCl_2$ 3 $MgCl_2$ 4 HEPES; 40 glucose). For the contraction assay, the midgut was defined as the portion of gut between the gastric caecae and the origin of the Malpighian tubules at the hindgut junction. The anterior piece of gut was cut immediately posterior to the gastric caecae, and consisted of 1/3 of the entire midgut. The posterior piece consisted of the rest, and excluded the Malpighian tubules and ampullae (see Fig. 1). Tissues were processed for reversed-phase HPLC separation coupled to RIA. Homogenates were passed through a C_{18} and a phenyl column with an increasing acetonitrile gradient, as previously described [5]. FMRFamide-like peptides were detected using a commercially available polyclonal anti-FMRFamide antiserum (Incstar, Stillwater, MN), which detects the presence of many extended RFamide peptides [13].

2.2. Midgut contraction assay

The midgut contraction assay, also referred to as the ring preparation, was based on the protocol of Lange and Orchard [8]. This preparation monitors the movement of the circular muscles using a force transducer. The midgut preparation (either anterior or posterior midgut, excluding the gastric caecae) was isolated and threaded with two strands of silk thread; one strand was loosely tied and pinned to a Sylgard-coated dish, the other strand was

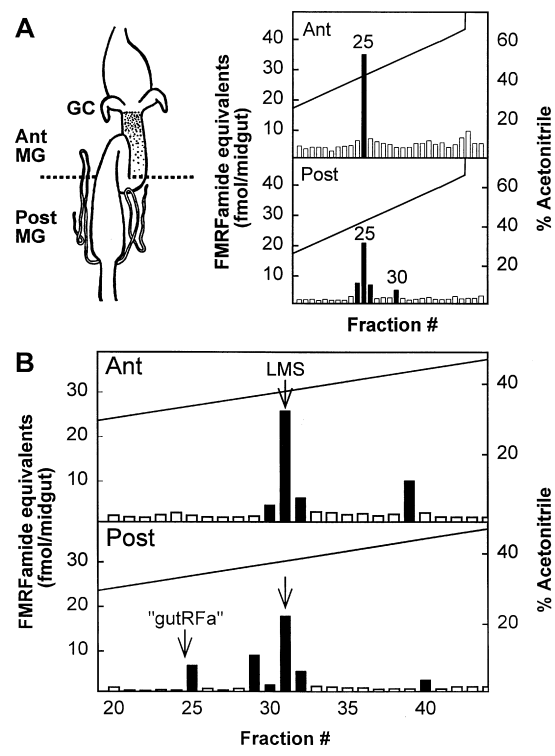

Fig. 1. HPLC purification of *Diploptera* midguts through C_{18} and phenyl columns. (A) Diagram of the cockroach midgut defining regions dissected for HPLC purification. The anterior midgut (ant MG) has apparent endocrine cells staining positively for LMS mRNA. The posterior midgut (post MG) is defined as the region which appears to lack these cells, and is delineated by the dashed line. The gastric caecae (GC) were not used in the purification. Homogenates of 300 anterior or posterior midguts were passed through a C_{18} column and shaded fractions were pooled and collected and run on a phenyl column. (B) Phenyl column; profiles of RFamide-like immunoreactive fractions from (A) are represented as shaded bars. The retention times for LMS and ANRSPSLRLRFamide ('gutRFa') are marked with arrows.

loosely tied and attached to a miniature force-displacement transducer (Model FT03C; Grass Instruments, Quincy, MA). The midgut thereby formed a ring structure, and circular muscle contractions were monitored on a linear chart recorder when the two loops were pulled apart. The muscles were bathed in 300–500 μl of saline and washed for at least 45 min prior to application of each test solution.

2.3. Chemicals

LMS (pQDVDHVFLRFamide), ANRSPSLRLRFamide and locustatachykinin 3 (LomTK III; APQAGFYGVR-amide) were custom synthesized by Research Genetics (Huntsville, AL). LomTK II (APLSGFYGVRamide), SchistoFLRFamide (PDVDHVFLRFamide) and proctolin (RYLPT) were purchased from Peninsula Laboratories (Belmont, CA). Truncated versions of LMS (HVFLRFamide and VFLRFamide) were synthesized by the Queen's University Core Facility for Protein and Peptide Chemistry (Kingston, Ontario). Other chemicals were purchased from Sigma Chemicals (St. Louis, MO).

3. Results

In this study, multiple FaRPs were detected in an extract of 300 anterior or posterior pieces of midgut, using HPLC separation couple to RIA. After separation through a C_{18} column, the anterior region was found to include one single RFamide-like immunoreactive peak co-migrating with LMS at 25 min, while the posterior homogenate contained a broader peak of immunoreactivity, eluting around the same time as the anterior region, along with a smaller fraction at 30 min (Fig. 1). When the major peaks were subsequently run on the phenyl column, 80% of the RFamide-like immunoreactivity of the anterior region co-eluted with LMS. In the posterior region, 50% co-eluted, with the remaining fractions scattered throughout the HPLC run (Fig. 1).

The anterior midgut contracted in the presence of proctolin, in a dose-dependent manner, with a threshold at about 2×10^{-9}M and a maximal response at 5×10^{-6}M (Fig. 2). A sustained, tonic contraction was seen as a positive inflection on the chart recorder, and usually no spontaneous phasic contractions were noted (Fig. 2). However, on occasion, proctolin induced longer lasting phasic contractions of the midgut, and these contractions increased in amplitude with subsequent applications of proctolin (Fig. 3). The midguts continued to produce these phasic contractions even after prolonged washing. These phasic contractions were inhibited by LMS, and by a related myosuppressin, SchistoFLRFamide, in a dose-dependent manner (Fig. 3). Basal tonus was not affected. The phasic contractions did not reappear after washing away the myosuppressin, and only returned upon renewed application of proctolin.

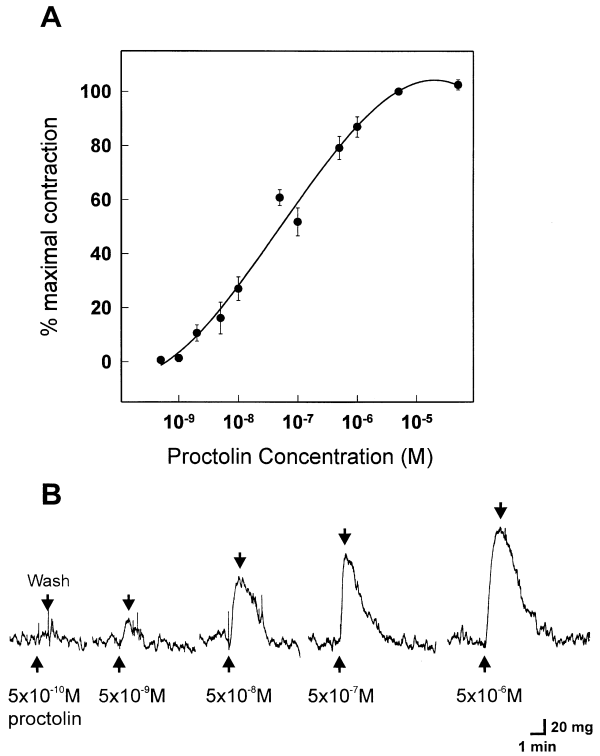

Fig. 2. Effects of proctolin on contractions of the anterior portion of cockroach midgut. (A) Dose-response curve for proctolin. Values are represented as a % of the maximal contraction elicited by proctolin (5×10^{-6}M). Points are means for 4–10 samples ± standard errors of the mean. (B) Examples of sustained tonic contractions of the midgut after application of proctolin (upward arrow) for a variety of concentrations. Washes (downward arrow) were repeated 5–6 times.

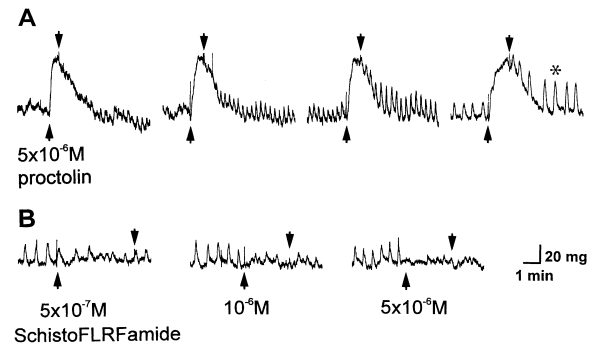

Fig. 3. Effects of proctolin or SchistoFLRFamide on spontaneous contractions of the anterior portion of cockroach midgut. (A) Application of 5×10^{-6}M proctolin (upward arrow) resulted in a sustained tonic contraction, followed by spontaneous phasic contractions (*) which persisted after washing (downward arrows). Once induced, the amplitude of these phasic contractions was increased by reapplication of proctolin. (B) Applications of different concentrations of SchistoFLRFamide (upward arrows) inhibited the spontaneous phasic contractions, and these contractions remained smaller after washing (downward arrows), unless proctolin was reapplied.

The amplitude of the proctolin-induced sustained tonic

contractions was reduced by LMS and SchistoFLRFamide, in a dose-dependent manner. The sustained tonic contractions elicited by 10^{-7}M proctolin were reduced in amplitude by 50% using a concentration of approximately 5×10^{-6}M LMS (Fig. 4), and maximal proctolin contractions elicited at 5×10^{-6}M were inhibited by 50% with 10^{-5}M LMS (data not shown). Interestingly, LMS and SchistoFLRFamide were only able to substantially reduce the amplitude of proctolin-induced contractions if they were applied immediately prior to proctolin application. When they were added along with proctolin, they were less effective. Two truncated forms of LMS, namely HVFLRFamide and VFLRFamide, were able to reduce the amplitude of proctolin-induced contractions (5×10^{-7}M), but to a lesser extent than LMS (Fig. 5).

LMS and SchistoFLRFamide did not induce any basal tonus changes when applied alone, nor did the *Periplaneta* midgut FaRP, ANRSPSLRLRFamide, or two tachykinins (LomTK II and LomTK III), up to concentrations of 10^{-5}M. ANRSPSLRLRFamide was incapable of reducing the amplitude of proctolin-induced contractions.

The posterior midgut did not respond as effectively to proctolin as did the anterior region. It contracted at most 30% of the maximal contraction produced by the anterior ring preparation, and only at the high proctolin dose of 5×10^{-6}M. It did not respond to a concentration of 10^{-5}M LomTK II either. These results may have been a reflection of a less robust tissue, or they may reflect a physiological difference in responsiveness to these peptides. With such poor and inconsistent responses, it was not possible to test the effects of LMS upon the posterior midgut.

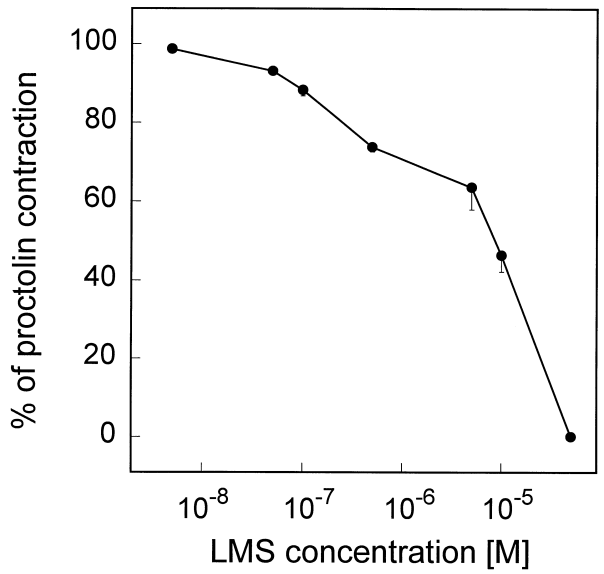

Fig. 4. Dose-response curve for the effects of LMS on the amplitude of proctolin-induced contractions of the anterior portion of cockroach midgut. Each point is the mean±standard error of the mean, for 3–5 preparations, and is expressed as a % of the contraction induced by 10^{-7}M proctolin on the same preparation.

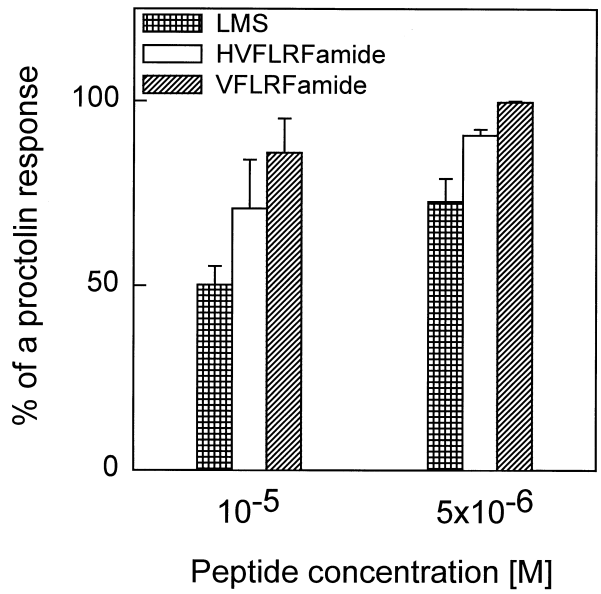

Fig. 5. The effects of 10^{-5}M or 5×10^{-6}M LMS or truncated versions HVFLRFamide and VFLRFamide, on the amplitude of proctolin-induced contractions of the anterior midgut. Each point is represented as the mean±standard error of the mean, of 3–5 preparations. Values are expressed as a % of the contraction induced by 5×10^{-7}M proctolin on the same preparation.

4. Discussion

Numerous neuropeptides have been detected in midguts of insects, using HPLC and/or immunohistochemistry, with antisera to various vertebrate and invertebrate peptides [14–16]. Among these peptides are a variety of FaRPs, including LMS. The differential expression of LMS mRNA among apparent endocrine cells [1] of the open variety, and preliminary studies measuring midgut contractions [9] have suggested that LMS might act locally to modulate midgut tissues. The myosuppressins appear to be potent inhibitors of midgut muscle contractions in other insects as well as *Diploptera*, such as in *Locusta migratoria* [17] and *Agrius convolvuli* [18], indicating a possible role for these FaRPs in regulating the movement of food along the length of the gut. Moreover, endocrine cells of the open type have been suggested to monitor nutrient contents in the gut of *P. americana* [19], possibly in a paracrine fashion. The results here describe the distribution of FMRFamide-like immunoreactive fractions of tissue homogenates after HPLC separation, in anterior and posterior portions of *Diploptera* midguts, along with the actions of LMS and other putative intestinal neuropeptides on midgut contractions. These results suggest a role for LMS and proctolin in the anterior portion of the midgut.

FaRPs appear to be differentially distributed between the anterior and posterior segments of midgut, as seen by reversed-phase HPLC purification of homogenized tissues (Fig. 1). While a single fraction co-eluting with LMS is the primary FaRP in the anterior midgut, multiple fractions, one of which co-elutes with LMS, are found in the posterior region. This is in agreement with previous in situ hybridization and immunohistochemical analyses, which noted that LMS mRNA was confined to apparent endocrine cells of the anterior region, with an abundance of FMRFamide-like immunoreactivity in posterior endocrine cells and in nerve tracts coursing the entire gut [1]. The appearance of an LMS-like fraction in the anterior midgut likely represents LMS in the apparent endocrine cells, although does not exclude the additional possibility of LMS in nerve tracts. The same fraction in the posterior midgut probably represents LMS within nerve tracts located along the gut since few if any LMS mRNA-containing endocrine cells are present. Typically, biologically active peptides have been found to occur in both gut neurons and endocrine cells in vertebrates [20] as well as invertebrates [21]. The present results suggest that LMS may play multiple roles in digestion in the cockroach midgut, via gut innervation and apparent endocrine cells.

The circular muscles of the anterior midgut contract strongly in the presence of the insect pentapeptide, proctolin (Fig. 2), which is not the case for the posterior midgut. This may suggest a differential responsiveness to proctolin in the midgut, although the possibility that the posterior tissue is too delicate for our assay cannot be ruled out. Nevertheless, these results indicate different structural properties between the anterior and posterior gut tissues. The proctolin-induced contractions are reduced in amplitude by LMS and the close relative, SchistoFLRFamide (Fig. 4), but only when they have been added immediately prior to proctolin application. Wang et al. [22] have suggested that in locust oviducts, SchistoFLRFamide prevents the influx of extracellular Ca^{+2} through Ca^{+2} channels, and have shown that once the intracellular concentration is elevated by proctolin, SchistoFLRFamide cannot inhibit the contractions. The above data support this model, since application of the myosuppressin would be necessary initially to inhibit changes in intracellular Ca^{+2} concentrations.

The proctolin-induced contractions are reduced in amplitude by the truncated forms of LMS, HVFLRFamide and VFLRFamide, but to a much lesser extent than LMS itself (Fig. 5). Nachman et al. [23] have shown similar results in *Leucophaea* hindgut preparations, in which they suggest that VFLRFamide is the biologically active core of LMS. Peeff et al. [24] on the other hand, have found that the His residue of HVFLRFamide is essential for inhibiting activity in locust oviducts. In fact, VFLRFamide may bind to another set of receptors, or activate a different G protein coupled to the same receptor [25], since it is in fact stimulatory on the oviduct preparation. Both the hindgut and oviduct contain myosuppressins, and these studies suggest that receptors in these two tissues are likely different. Our results suggest that the *Diploptera* midgut contains receptors which are pharmacologically similar to receptors on the hindgut of *Leucophaea*.

Other midgut peptides, including the *Periplaneta* midgut FaRP, ANRSPSLRLRFamide [26], and two tachykinins (LomTK II and LomTK III) do not modify midgut contractions alone or in combination with proctolin, for concentrations up to at least 10^{-5}M. No function has yet been attributed to the *Periplaneta* FaRP. The results for tachykinins differ from the work of Lange et al. [27], who showed that the locust tachykinins are capable of inducing contractions in circular muscles of the locust midgut. Related tachykinins have been isolated and sequenced from *Leucophaea* midguts [28], and we have found tachykinin-like immunoreactivity in midgut tissue homogenates of both anterior and posterior regions (unpublished data). Nevertheless, we have not yet determined the types of tachykinins present in *Diploptera* guts. While the tachykinins and proctolin did not cause contractions in the posterior midgut, it remains to be seen whether this is due to a true physiological insensitivity, or the result of a less robust bioassay using this tissue.

There are no doubt numerous other peptides present in *Diploptera* midguts (for instance, Yu et al. [21] have described differential distribution of the allatostatins in endocrine cells and nerve tracts of the midgut). Some will likely modulate the contractile responses to proctolin as does LMS. Many will possess multiple functions in midgut digestion, including the release of enzymes, as has been shown for LMS in weevil guts [11]. Studies are needed to confirm the identities of other midgut peptides, and to further characterize the responses seen by LMS and proctolin. LMS appears to be expressed in apparent endocrine cells described as the open type [29] in the anterior midgut, but may also be located in nerve tracts along the entire gut. It may therefore exert different effects within the gut, as a hormone, a paracrine or as a neurotransmitter or neuromodulator.

In most insects, the midgut is structurally and functionally differentiated along its length [30]. The differences are not always obvious structurally, but functional differences include the separation of regions for water absorption, proteinase activity and nutrient absorption. In *Diploptera*, differences such as pH along the length of the gut, and quality of pellet, are observed (unpublished observations), although no definitive studies have been conducted in this animal. The anterior midgut tissue is more robust and survives rough handling to a larger degree than the posterior segment, and the pellet in these two regions is substantially different in colour and texture. For example, regions of the anterior midgut are often observed in a contracted state, and the pellet in this area is firmer and darker than in the posterior end. Contractions are never noted in posterior segments. We postulate here that LMS

may act specifically in the anterior region to regulate contractions necessary for such pellet movement.

Acknowledgements

We are grateful to Angela Lange for her assistance in the assay techniques, and to Steve Tobe, for use of his colony of *Diploptera punctata*. This work has been funded by the Natural Sciences and Engineering Research Council of Canada.

References

[1] Fusé M, Bendena WG, Donly BC, Tobe SS, Orchard I. In situ hybridization analysis of leucomyosuppressin mRNA expression in the cockroach, *Diploptera punctata*. J Comp Neurol 1998;395(3):328–41.
[2] Price DA, Greenberg M. Structure of a molluscan cardioexcitatory neuropeptide. Science 1997;197:670–1.
[3] Greenberg M, Price DA. Relationships among the FMRFamide-like peptides. In: Joosse J, Buijs RM, Tilders FJH, editors. Progress in brain research; The peptidergic neuron. Cambridge: Elsevier, 1992:25–37.
[4] Schneider LE, Taghert PH. Isolation and characterization of a *Drosophila* gene that encodes multiple neuropeptides related to Phe–Met–Arg–Phe–NH2 (FMRFamide). Proc Natl Acad Sci USA 1988;85:1993–7.
[5] Donly BC, Fusé M, Orchard I, Lange AB, Tobe SS, Bendena WG. Characterization of the gene for leucomyosuppressin and its expression in the brain of the cockroach *Diploptera punctata*. Insect Biochem Mol Biol 1996;26(6):627–37.
[6] Holman GM, Cook BJ, Nachman RJ. Isolation, primary structure and synthesis of leucomyosuppressin, an insect neuropeptide that inhibits spontaneous contractions of the cockroach hindgut. Comp Biochem Physiol 1986;85C:329–33.
[7] Peeff NM, Orchard I, Lange AB. The effects of FMRFamide-related peptides on an insect *(Locusta migratoria)* visceral muscle. J Insect Physiol 1993;39(3):207–15.
[8] Lange AB, Orchard I. The effects of SchistoFLRFamide on contractions of locust midgut. Peptides 1998;19(3):459–67.
[9] Fusé M, Orchard I. Putative roles for the neuropeptide leucomyosuppressin in the midgut of the cockroach, *Diploptera punctata*. In: Kawashima S, Kikuyama S, editors. Advances in comparative endocrinology: Proceedings of the XIIIth Int. Cong. Comp. Endocrinol. Italy: Monduzzi Editore, 1998:619–22.
[10] Kingan TG, Teplow DB, Phillips JM, Riehm JP, Rao KR, Hildebrand JG, Homberg U, Kammer AE, Jardine I, Griffin PR, Hunt DF. A new peptide in the FMRFamide family isolated from the CNS of the hawkmoth *Manduca Sexta*. Peptides 1990;11:849–56.
[11] Nachman RI, Giard W, Favrel P, Suresh T, Sreekumar S, Holman GM. Insect myosuppressins and sulfakinins stimulate release of the digestive enzyme alpha-amylase in two invertebrates: the scallop *Pecten maximus* and insect *Rynchophorus ferrugineus*. In: Beckwith BE, Saria A, Chronwall BM, Sandman CA, Strand FL, editors. Neuropeptides in development and aging. New York: Annals N.Y. Acad. Sci. 1997;814:335–8.
[12] Szibbo CM, Tobe SS. Nervous and humoral inhibition of C_{16} juvenile hormone synthesis in last instar females of the viviparous cockroach *Diploptera punctata*. Gen Comp Endocrinol 1983;49:437–45.
[13] Tsang PW, Orchard I. Distribution of FMRFamide-related peptides in the blood-feeding bug, *Rhodnius prolixus*. J Comp Neurol 1991;311:17–32.
[14] Brown MR, Crim JW, Lea AO. FMRFamide- and pancreatic polypeptide-like immunoreactivity in midgut endocrine cells of a mosquito. Tissue Cell 1986;18:419–28.
[15] Žitnan D, Šauman I, Sehnal F. Peptidergic innervation and endocrine cells of insect midgut. Arch Insect Biochem Physiol 1993;22:113–32.
[16] Agricola HJ, Bräunig P. Comparative aspects of peptidergic signalling pathways in the nervous systems of arthropods. In: Breidbach O, Kutsch W, editors. The nervous systems of invertebrates: An evolutionary and comparative approach. Switzerland: Birkhauser, 1995:303–27.
[17] Orchard I, Lange AB. The distribution, biological activity, and pharmacology of SchistoFLRFamide and related peptides in insects. In: Coast GM, Webster SG, editors. Recent advances in arthropod endocrinology, SEB seminar series. Cambridge, Cambridge Univ. Press, 1998:278–301.
[18] Fujisawa Y, Shimoda M, Kiguchi K, Ichikawa T, Fujita N. The inhibitory effect of a neuropeptide, *Manduca*FLRFamide, on the midgut activity of the Sphingid moth *Agrius Convolvuli*. Zool Sci 1993;10:773–7.
[19] Fujita T, Yui R, Iwanaga T, Nishiitsutsuji-Uwo J, Endo Y, Yanaihara N. Evolutionary aspects of 'brain-gut peptides': an immunohistochemical study. Peptides 1981;2:123–31.
[20] Hutchinson JB, Dimaline R, Dockray GJ. Neuropeptides in the gut: quantification and characterization of cholecystokinin octapeptide-, bombesin- and vasoactive intestinal polypeptide-like immunoreactivities in the myenteric plexus of the guinea-pig small intestine. Peptides 1981;2:23–30.
[21] Yu CG, Stay B, Ding Q, Bendena WG, Tobe SS. Immunohistochemical identification and expression of allatostatins in the gut of *Diploptera punctata*. J Insect Physiol 1995;41(12):1035–43.
[22] Wang Z, Orchard I, Lange AB, Chen X. Mode of action of an inhibitory neuropeptide SchistoFLRFamide on the locust oviduct visceral muscle. Neuropeptides 1995;28:147–55.
[23] Nachman RJ, Holman GM, Hayes TK, Beier RC. Structure-activity relationships for inhibitory insect myosuppressins: contrast with the stimulatory sulfakinins. Peptides 1993;14:665–70.
[24] Peeff NM, Orchard I, Lange AB. Isolation, sequence, and bioactivity of PDVDHVFLRFamide and ADVGHVFLRFamide peptides from the locust central nervous system. Peptides 1994;15(3):387–92.
[25] Wang Z, Lange AB, Orchard I. Coupling of a single receptor to two different G proteins in the signal transduction of FMRFamide-related peptides. Biochem Biophys Res Comm 1995;212:531–8.
[26] Veenstra JA, Lambrou G. Isolation of a novel RFamide peptide from the midgut of the american cockroach, *Periplaneta americana*. Biochem Biophys Res Comm 1995;213(2):519–24.
[27] Lange AB, Pabla N, Orchard I. Distribution and function of peptides in midgut cells of *Locusta migratoria*. In: Kawashima S, Kikuyama S, editors. Advances in comparative endocrinology: Proceedings of the XIIIth Int. Cong. Comp. Endocrinol. Italy: Monduzzi Editore, 1998:1361–5.
[28] Muren JE, Nässel DR. Isolation of five tachykinin-related peptides from the midgut of the cockroach *Leucophaea maderae*: existence of *N*-terminally extended isoforms. Regul Pept 1996;65:185–96.
[29] Nishiitsutsuji-Uwo J, Endo Y. Gut endocrine cells in insects: the ultrastructure of the endocrine cells in the cockroach midgut. Biomed Res 1981;2:30–6.
[30] Billingsley PF, Lehane MJ. Structure and ultrastructure of the insect midgut. In: Lehane MJ, Billingsley PF, editors. Biology of the insect midgut. London: Chapman and Hall, 1996:3–30.