

Effects of myoinhibitory peptides on food intake in the German cockroach

RUTH AGUILAR*, JOSÉ L. MAESTRO and XAVIER BELLÉS

Department of Physiology and Molecular Biodiversity, Institut de Biologia Molecular de Barcelona (CSIC), Jordi Girona, Barcelona, Spain.

Abstract. Insect myoinhibitory peptides were discovered through their inhibitory activity on visceral muscle contraction. The present study tests the antimitropic gut properties of three galanin-related myoinhibitory peptides (Mas-MIP II: GWQDLNSAW-NH₂; Grb-AST-B1: GWQDLNGGW-NH₂; and Grb-AST-B3: AWRDLSSGW-NH₂) in adult females of the cockroach *Blattella germanica* (L.) (Dictyoptera, Blattellidae). The three peptides elicit a strong inhibitory effect on both foregut and hindgut contractions, with ID₅₀ values in all the cases within the nanomolar range. In addition, the modulatory effects of these three peptides on food intake are studied on previously starved female cockroaches. The results show that Grb-AST-B3 is the most active peptide, inhibiting food intake by 60–80% at doses between 15 and 50 µg, followed by Grb-AST-B1 (45% inhibition of food intake at the 50 µg dose), whereas Mas-MIP II is inactive even at the 50 µg dose. The differences between the three peptides may be due to a differential effect of their structure on activity or to a differential degradation. These results show that myomodulatory gut activity *in vitro* and antifeeding effects do not always correlate.

Key words. *Blattella germanica*, food intake regulation, German cockroach, myoinhibitory activity, myoinhibitory peptides.

Introduction

Insect peptides belonging to the myoinhibitory peptide family are usually nonapeptides, characterized by a residue Trp at positions 2 and 9 and by an amidated C-terminus. The first member of this family was identified from extracts of brain-corpora cardiaca-corpora allata-suboesophageal ganglion from *Locusta migratoria*, and was called Lom-MIP (from MyoInhibitory Peptide), in accordance with its inhibitory activity on hindgut and oviduct contractions (Schoofs *et al.*, 1991). Later, other peptides belonging to this family were identified in the Lepidoptera *Manduca*

sexta (Mas-MIP-I to VI: Blackburn *et al.*, 1995, 2001); *Bombyx mori* (Bom-PTSP = Mas-MIP-I: Hua *et al.*, 1999); *Lacanobia oleracea* (Mas-MIP-VI: Audsley & Weaver, 2003); the Orthoptera *Gryllus bimaculatus* (Grb-AST-B1-5: Lorenz *et al.*, 1995, 1999); the Dictyoptera *Periplaneta americana* (Pea-MIP: Predel *et al.*, 2001); and the Phasmida *Carausius morosus* (Cam-AST-B1-6: Lorenz *et al.*, 2000). In addition, the cDNA of the preproprotein containing the five myoinhibitory peptides of the dipteran *Drosophila melanogaster* (Williamson *et al.*, 2001) and a partial cDNA containing three previously described peptides and a novel peptide of *G. bimaculatus* (Wang *et al.*, 2004) have also been reported. Biological activities described for these peptides include the inhibition of visceral muscle contraction in locusts, moths and cockroaches (Schoofs *et al.*, 1991; Blackburn *et al.*, 1995, 2001; Predel *et al.*, 2001); the inhibition of juvenile hormone production in crickets (Lorenz *et al.*, 1995, 1999, 2000); the inhibition of ecdysteroidogenesis in moth prothoracic gland (Hua *et al.*, 1999) and in cricket ovary (Lorenz *et al.*, 1997); and the inhibition of adipokinetic hormone release in locusts (Vullings *et al.*, 1999).

Correspondence: J. L. Maestro, Department of Physiology and Molecular Biodiversity, Institut de Biologia Molecular de Barcelona (CSIC), Jordi Girona 18-26, 08034 Barcelona, Spain. Tel.: +34 93 4006135; fax: +34 93 2045904; e-mail: jmagr@ibmb.csic.es.

*Present address: Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, U.S.A.

Anatomical localization of myoinhibitory peptides using immunocytochemical techniques provides further physiological support for most of the known biological activities. For example, myoinhibitory peptide-like immunoreactivity has been localized in several areas of the brain and in the *corpora cardiaca-corpora allata* complex of the cockroach, *P. americana* (Predel *et al.*, 2001), the locust, *L. migratoria* (Schoofs *et al.*, 1996), and the cricket, *G. bimaculatus* (Witek *et al.*, 1999). These locations fit well with the described activities of myoinhibitory peptides on adipokinetic hormone release in locusts, and on juvenile hormone production in crickets. In addition, immunoreactivity has been observed in neurones from the ventral nerve cord innervating the heart, oviduct and hindgut of locust (Schoofs *et al.*, 1996), and the foregut and hindgut of the American cockroach (Predel *et al.*, 2001), which is compatible with myotropic functions in these organs. The occurrence of immunoreactivity in different neurohemal organs suggests that the myoinhibitory peptides act as true hormones (Predel *et al.*, 2001; Davis *et al.*, 2003). Furthermore, *in situ* hybridization experiments in *D. melanogaster* larvae have shown myoinhibitory peptide expression in the central nervous system and in midgut endocrine cells (Williamson *et al.*, 2001), which suggests that these peptides have a role with respect to feeding and digestive processes.

The amino acid sequence of some myoinhibitory peptides shows a certain degree of similarity with the N-terminal sequence of vertebrate galanins (Table 1). Galanins are 29/30-residue neuropeptides found in the gastrointestinal tract and nervous system of vertebrates, predominantly in the hypothalamus. They are linked to a number of functions, including the stimulation of food intake (Crawley, 1999; Halford & Blundell, 2000). In addition, galanins show myotropic action on the smooth musculature of mammal gastrointestinal tract, stimulating or inhibiting motility depending on the particular region or tissue (Ekblad *et al.*, 1985; Rattan & Tamura, 1998).

Blattella germanica (L.) is an anautogenous cockroach, where the adult female requires a proteinaceous meal before starting vitellogenesis and ovogenesis (Osorio *et al.*, 1998). Thus, the female has a feeding cycle that almost parallels that of vitellogenesis. In the *B. germanica* colony investigated in the present study, food consumption in the adult female starts 24 h after the imaginal moult, peaks on day 4 and decreases steadily thereafter until day 7, when oviposition occurs (Osorio *et al.*, 1998). Because this pattern suggests that food intake is finely regulated, the regulatory mechanisms have been investigated, and the peptide perisulfakinin identified as a putative satiety factor (Maestro *et al.*, 2001). In addition, a number of YXFGL-NH₂ allatostatins and leucomyosuppressin inhibit food intake, an activity possibly based on the antimyotropic gut effects of these peptides (Aguilar *et al.*, 2003, 2004).

The antimyotropic activity of myoinhibitory peptides on insect gut motility (Schoofs *et al.*, 1991; Blackburn *et al.*, 1995, 2001; Predel *et al.*, 2001), their gene expression in midgut endocrine cells of *D. melanogaster* (Williamson *et al.*, 2001) and their sequence similarity with the N-terminal region of vertebrate galanins, all suggest that the myoinhibitory peptides might be involved in the regulation of food intake in insects. To test this hypothesis, the present study investigates the activity of three galanin-related insect myoinhibitory peptides on foregut and hindgut motility, and on food intake in adult females of the German cockroach, *B. germanica*.

Materials and methods

Insect rearing

Adult females of the German cockroach, *B. germanica* (L.) (Dictyoptera, Blattellidae), were obtained from a colony fed on dog chow and water and reared in the dark at 30 ± 1 °C and 60–70% relative humidity.

Table 1. Comparison of the sequences of some myoinhibitory peptides with the N-terminal sequences of two typical vertebrate galanins.

Peptide	Sequence	Reference
Galanins		
Human galanin 1	GW TLNSAGYLLGPHAVGNHRSFSDKNGLTS	(1)
Porcine galanin	GW TLNSAGYLLGPHADNHRSFHDKYGLA-NH ₂	(2)
Myoinhibitory peptides		
Mas-MIP I	AW QDLNSAW-NH ₂	(3)
Mas-MIP II	GW QDLNSAW-NH ₂	(3)
Mas-MIP IV	GW NDMSSAW-NH ₂	(4)
Mas-MIP V	GW QDMSSAW-NH ₂	(4)
Grb-AST-B1	GW QDLNGGW-NH ₂	(5)
Grb-AST-B2	GW RDLNGGW-NH ₂	(5)
Grb-AST-B3	AW RDLSGGW-NH ₂	(5)
Cam-AST-B3	GW QDLQSGW-NH ₂	(6)
Cam-AST-B6	AW QDLGSAW-NH ₂	(6)
Pea-MIP	GW QDLQGGW-NH ₂	(7)

Coincident residues between the two groups of peptides are highlighted in **bold**. (1) Bersani *et al.* (1991); (2) McDonald *et al.* (1992); (3) Blackburn *et al.* (1995); (4) Blackburn *et al.* (2001); (5) Lorenz *et al.* (1995); (6) Lorenz *et al.* (2000); (7) Predel *et al.* (2001).

Myotropic bioassay

Peptides belonging to the myoinhibitory peptide family were tested on the foregut and hindgut from 3-day-old adult females of *B. germanica* maintained in a 500- μ L standard organ bath, as described previously (Maestro *et al.*, 2001; Aguilar *et al.*, 2003). A FSG-01 transducer (Experimetria Ltd, Hungary) was used for isometric recording. The activity was calculated as the difference of the mean of the force produced by the tissue 1 min after and 1 min before treatment.

Feeding bioassay

The feeding bioassay was carried out as described previously (Maestro *et al.*, 2001). Freshly ecdysed adult females were starved for 48 h, injected with saline or with the corresponding myoinhibitory peptide, and provided with carrot *ad libitum*. The females had then access to food for 5 h (in which time carotenoids do not appear in the faeces), after which the whole gut was dissected out and extracted with methanol. Carotenoid concentration in the methanolic extracts was estimated by spectrophotometric measurement of the absorbance at 450 nm. The weight of ingested carrot was estimated by interpolation on a standard curve obtained with the carotenoid values of methanolic extracts containing increasing weights of lyophilized carrot (Maestro *et al.*, 2001).

Synthetic myoinhibitory peptides

Peptides Mas-MIP II from *M. sexta* (Blackburn *et al.*, 1995) and Grb-AST-B1 and Grb-AST-B3 from *G. bimaculatus* (Lorenz *et al.*, 1995) were synthesized using standard Fmoc chemistry. The identity and purity (approximately 90%) of the peptides were assessed by amino-acid analysis, matrix-assisted laser desorption ionization time-of-flight mass spectrometry, and high-performance liquid chromatography.

Results

Before studying the possible antifeeding effects of myoinhibitory peptides in *B. germanica* females, we assessed whether these peptides could elicit antimyotropic effects in gut tissues. Therefore, the dose-response effect of three peptides belonging to the myoinhibitory peptide family (Mas-MIP II, Grb-AST-B1 and Grb-AST-B3) on *B. germanica* foregut and hindgut motility was studied using a myotropic bioassay. Each of the three myoinhibitory peptides showed a strong myoinhibitory effect in terms of frequency and force on both tissues (Fig. 1). The three peptides gave an ID₅₀ value within the nanomolar range, and the maximum activity was between 40 and 60 mg of decreasing force in the foregut, and between 6 and 10 mg of decreasing force in the hindgut. The effect was very rapid, although the rate of contraction recovered partially within a few minutes of the treatment (Fig. 2).

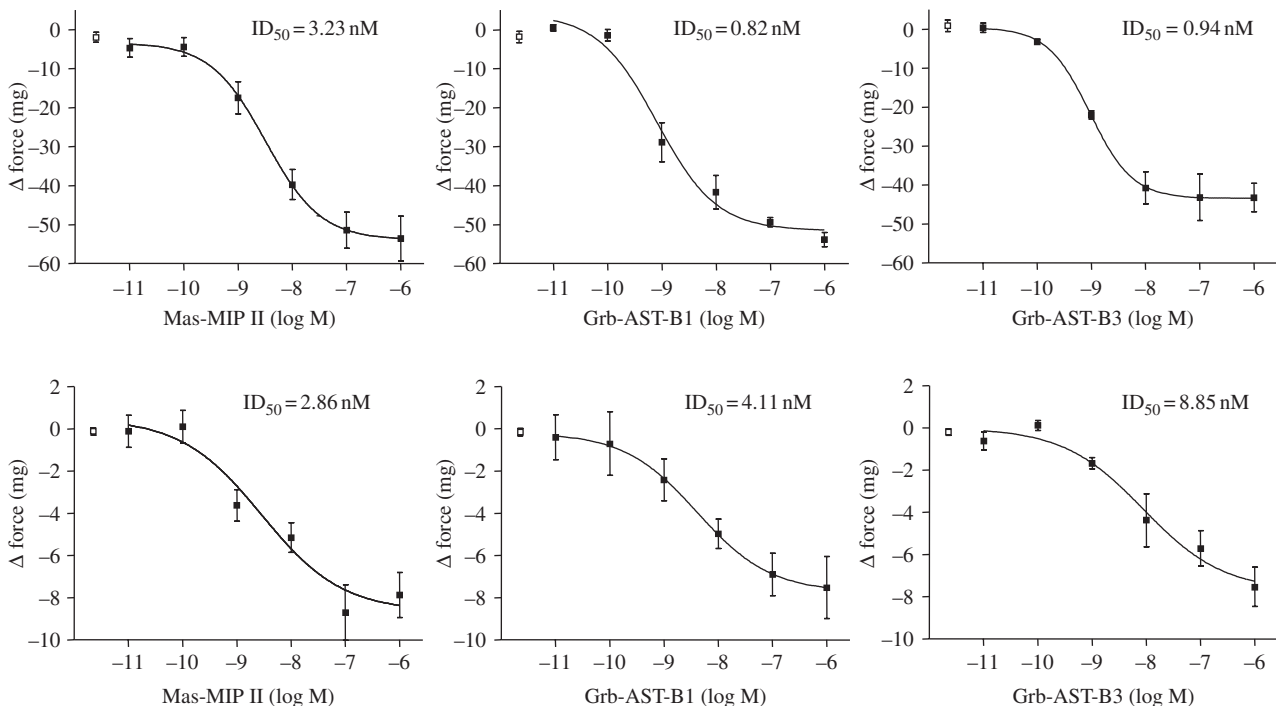


Fig. 1. Inhibitory effect of the myoinhibitory peptides Mas-MIP II (GWQDLNSAW-NH₂), Grb-AST-B1 (GWQDLNGGW-NH₂) and Grb-AST-B3 (AWRDLSSGGW-NH₂) on foregut (top) and hindgut (bottom) motility in *Blattella germanica* females. Results (mean \pm SEM; $n = 5-7$) are expressed as the difference of the mean of the force produced by the tissue 1 min after and before the treatment. The ID₅₀ for each tissue is also shown. Empty squares indicate values for water control experiments.

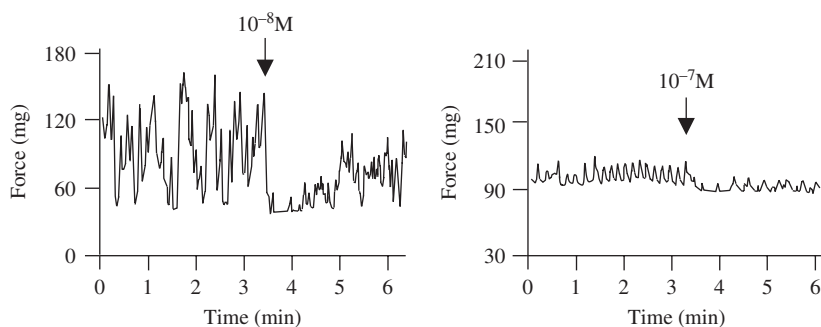


Fig. 2. Representative response of foregut (left) and hindgut (right) organ bath preparations to the myoinhibitory peptide Mas-MIP-II. Arrows indicate application of the peptide and the concentration used.

The peptides Mas-MIP II, Grb-AST-B1 and Grb-AST-B3 were tested for effects on food intake by *B. germanica* using the carrot assay, and peptide doses from 5 to 50 µg per specimen were used. Grb-AST-B3 was the most active peptide, resulting in approximately 60–80% inhibition of food intake at doses between 15 and 50 µg (Fig. 3). Grb-AST-B1 resulted in approximately 45% inhibition of food intake at the highest dose of 50 µg (Fig. 3), whereas Mas-MIP II was inactive even at this dose (Fig. 3).

Discussion

It is postulated that myoinhibitory peptides affect food intake in insects as a consequence of their effects on gut motility. Correspondingly, three galanin-related myoinhibitory peptides are tested as feeding modulators in adult females of the German cockroach, *B. germanica*. A standard feeding assay on adult females of *B. germanica* (Maestro *et al.*, 2001) has shown that a number of peptides effectively inhibit food intake in this cockroach. These peptides include perisulfakinin (Maestro *et al.*, 2001), YXFGL-NH₂ allatostatins (Aguilar *et al.*, 2003) and

leucomyosuppressin (LMS) (Aguilar *et al.*, 2004). Of these, LMS has the strongest antimyotrophic activity on both foregut and hindgut, and it is suggested that this activity was responsible for its antifeedancy (Aguilar *et al.*, 2004). Therefore, in the case of the myoinhibitory peptides investigated in the present study, both gut antimyotrophic activity and antifeeding effect were assessed.

The three myoinhibitory peptides, Mas-MIP II, Grb-AST-B1 and Grb-AST-B3, induce a strong myoinhibitory action on the foregut and hindgut of *B. germanica*. When compared with other antimyotrophic gut peptides studied in this species, the three myoinhibitory peptides are more active than YXFGL-NH₂ allatostatins. Allatostatins are inactive on foregut, and show hindgut ID₅₀ values similar to those of myoinhibitory peptides (Aguilar *et al.*, 2003) (Fig. 1). Conversely, myoinhibitory peptides are less active than LMS, with both foregut and hindgut ID₅₀ values around one order of magnitude higher than those of LMS (Aguilar *et al.*, 2004) (Fig. 1). By contrast to the strong myoinhibitory effect of the three myoinhibitory peptides tested in the present study in *B. germanica*, the myotrophic peptide Pea-MIP causes only a moderate inhibition in foregut and hindgut muscle assays in the cockroach *P. americana*, with activity threshold concentrations as high as 5×10^{-9} M and 1×10^{-7} M for foregut and hindgut, respectively (Predel *et al.*, 2001).

In spite of the similarity between myoinhibitory peptides and N-terminal sequence of galanins, none of the three myoinhibitory peptides stimulate food intake. By contrast, the peptides Grb-AST-B1 and B3 inhibit food intake in *B. germanica*, with Grb-AST-B3 being the most active at approximately 65% and 80% inhibition at doses of 15 and 50 µg, respectively. However, Mas-MIP II is inactive at the maximum 50 µg dose tested. Of the YXFGL-NH₂ allatostatins tested in the carrot-feeding bioassay (Aguilar *et al.*, 2003), BLAST-2 (DRLYSFGL-NH₂) shows the highest activity, resulting in approximately 60% inhibition of food intake at doses between 5 and 50 µg, whereas BLAST-1 (LYDFGL-NH₂) is the least active, inducing only approximately 50% inhibition at a dose of 50 µg (Aguilar *et al.*, 2003). LMS (pQDVVDHVFLRF-NH₂) has a 50 and 75% inhibitory effect on food intake at doses of 15 and 50 µg, respectively (Aguilar *et al.*, 2004).

Thus, although all three myoinhibitory peptides inhibit gut motility in *B. germanica in vitro* to a similar extent, only

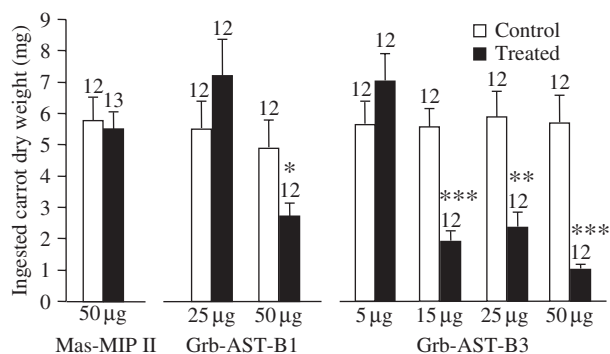


Fig. 3. Effect of the myoinhibitory peptides Mas-MIP II (GWQDLNSAW-NH₂), Grb-AST-B1 (GWQDLNGGW-NH₂) and Grb-AST-B3 (AWRDLSGGW-NH₂) on food intake in *Blattella germanica* females. Results are expressed as mean \pm SEM. The number of replicates is indicated at the top of each bar. Asterisks indicate significant differences (Student's *t*-test) with respect to the corresponding control (**P* < 0.05; ***P* < 0.01; ****P* < 0.001).

Grb-AST-B3 and, to a lesser extent Grb-AST-B1, inhibit food intake. The different effects of the three tested myoinhibitory peptides on feeding could be due to the intrinsic influence of their structure on activity or to differences of stability in the haemocoel. However, from the present study of the myoinhibitory peptides, it is clear that myomodulatory gut activity *in vitro* and antifeeding effects do not always correlate.

Acknowledgements

Financial support from the Ministry of Science and Technology, Spain (project AGL2002-01169); and the Generalitat de Catalunya (2001 SGR 003245) is gratefully acknowledged.

References

- Aguilar, R., Maestro, J.L., Vilaplana, L. *et al.* (2003) Allatostatin gene expression in brain and midgut, and activity of synthetic allatostatins on feeding-related processes in the cockroach *Blattella germanica*. *Regulatory Peptides*, **115**, 171–177.
- Aguilar, R., Maestro, J.L., Vilaplana, L. *et al.* (2004) Identification of leucomyosuppressin in the German cockroach, *Blattella germanica*, as an inhibitor of food intake. *Regulatory Peptides*, **119**, 105–112.
- Audsley, N. & Weaver, R.J. (2003) Identification of neuropeptides from brains of larval *Manduca sexta* and *Lacanobia oleracea* using MALDI-TOF mass spectrometry and post-source decay. *Peptides*, **24**, 1465–1474.
- Bersani, M., Johnsen, A.H., Hojrup, P. *et al.* (1991) Human galanin: primary structure and identification of two molecular forms. *FEBS Letters*, **283**, 189–194.
- Blackburn, M.B., Wagner, R.M., Kochansky, J.P. *et al.* (1995) The identification of two myoinhibitory peptides, with sequence similarities to the galanins, isolated from the ventral nerve cord of *Manduca sexta*. *Regulatory Peptides*, **5**, 213–219.
- Blackburn, M.B., Jaffe, H., Kochansky, J. & Raina, A.K. (2001) Identification of four additional myoinhibitory peptides (MIPs) from the ventral nerve cord of *Manduca sexta*. *Archives of Insect Biochemistry and Physiology*, **48**, 121–128.
- Crawley, J.N. (1999) The role of galanin in feeding behaviour. *Neuropeptides*, **33**, 369–375.
- Davis, N.T., Blackburn, M.B., Golubeva, E.G. & Hildebrand, J.G. (2003) Localization of myoinhibitory peptide immunoreactivity in *Manduca sexta* and *Bombyx mori*, with indications that the peptide has a role in molting and ecdysis. *Journal of Experimental Biology*, **206**, 1449–1460.
- Ekblad, E., Håkanson, R., Sundler, F. & Wahlestedt, C. (1985) Galanin: neuromodulatory and direct contractile effects on smooth muscle preparations. *British Journal of Pharmacology*, **6**, 241–246.
- Halford, J.C. & Blundell, J.E. (2000) Pharmacology of appetite suppression. *Progress in Drug Research*, **54**, 25–58.
- Hua, Y.J., Tanaka, Y., Nakamura, K. *et al.* (1999) Identification of a prothoracicostatic peptide in the larval brain of the silkworm *Bombyx mori*. *Journal of Biological Chemistry*, **274**, 31169–31173.
- Lorenz, J.L., Lorenz, M.W. & Hoffmann, K.H. (1997) Factors regulating juvenile hormone and ecdysteroid biosynthesis in *Gryllus bimaculatus* (Ensifera, Gryllidae). *European Journal of Entomology*, **94**, 369–379.
- Lorenz, M.W., Kellner, R. & Hoffmann, K.H. (1995) A family of neuropeptides that inhibit juvenile hormone biosynthesis in the cricket, *Gryllus bimaculatus*. *Journal of Biological Chemistry*, **270**, 21103–21108.
- Lorenz, M.W., Kellner, R. & Hoffmann, K. (1999) Allatostatins in *Gryllus bimaculatus* (Ensifera, Gryllidae): new structures and physiological properties. *European Journal of Entomology*, **96**, 267–274.
- Lorenz, M.W., Kellner, R., Hoffmann, K.H. & Gäde, G. (2000) Identification of multiple peptides homologous to cockroach and cricket allatostatins in the stick insect *Carausius morosus*. *Insect Biochemistry and Molecular Biology*, **30**, 711–718.
- Maestro, J.L., Aguilar, R., Pascual, N. *et al.* (2001) Screening of antifeedant activity in brain extracts led to the identification of sulfakinin as a satiety promoter in the German cockroach. Are arthropod sulfakinins homologous to vertebrate gastrins-cholecystokinins? *European Journal of Biochemistry*, **268**, 5824–5830.
- McDonald, T.J., Krantis, A., Clarke, M. *et al.* (1992) Characterization of porcine gastric galanin. *Peptides*, **13**, 589–593.
- Osorio, S., Piulachs, M.D. & Bellés, X. (1998) Feeding and activation of *corpora allata* in the cockroach *Blattella germanica* (L.) (Dictyoptera, Blattellidae). *Journal of Insect Physiology*, **44**, 31–38.
- Predel, R., Rapus, J. & Eckert, M. (2001) Myoinhibitory neuropeptides in the American cockroach. *Peptides*, **22**, 199–208.
- Rattan, S. & Tamura, W. (1998) Role of galanin in the gastrointestinal sphincters. *Annals of the New York Academy of Sciences*, **863**, 143–155.
- Schoofs, L., Holman, G.M., Hayes, T.K. *et al.* (1991) Isolation, identification and synthesis of locustamyoinhibiting peptide (LOM-MIP), a novel biologically active neuropeptide from *Locusta migratoria*. *Regulatory Peptides*, **36**, 111–119.
- Schoofs, L., Veelaert, D., Vanden Broeck, J. & De Loof, A. (1996) Immunocytochemical distribution of *Locusta* myoinhibiting peptide (Lom-MIP) in the nervous system of *Locusta migratoria*. *Regulatory Peptides*, **63**, 171–179.
- Vullings, H.G.B., Diederer, J.H.B., Veelaert, D. & Van der Horst, D.J. (1999) Multifactorial control of the release of hormones from the locust retrocerebral complex. *Microscopy Research and Technique*, **45**, 142–153.
- Wang, J., Meyering-Vos, M. & Hoffmann, K.H. (2004) Cloning and tissue-specific localization of cricket-type allatostatins from *Gryllus bimaculatus*. *Molecular and Cellular Endocrinology*, **227**, 41–51.
- Williamson, M., Lenz, C., Winther, Å.M.E. *et al.* (2001) Molecular cloning, genomic organization, and expression of a B-type (cricket-type) allatostatin preprohormone from *Drosophila melanogaster*. *Biochemical and Biophysical Research Communications*, **282**, 544–550.
- Witek, G., Verhaert, P., Lorenz, M.W. & Hoffmann, K.H. (1999) Immunolocalization of two types of allatostatins in the central nervous system of the cricket *Gryllus bimaculatus* (Ensifera: Gryllidae). *European Journal of Entomology*, **96**, 279–285.

Accepted 11 January 2006

First published online 8 May 2006