

Effects of myoinhibitory peptides on food intake in the German cockroach

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Abstract

Insect myoinhibitory peptides were discovered through their inhibitory activity on visceral muscle contraction. We tested the antimyotropic gut properties of three galanin-related myoinhibitory peptides (Mas-MIP II: GWQDLNSAW-NH₂, Grb-AST-B1: GWQDLNNGW-NH₂ and Grb-AST-B3: AWRDLSGGW-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 were studied on previously starved female cockroaches. 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 50 µg dose), whereas Mas-MIP II is inactive even at the 50 µg dose. The differences between the three peptides could 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.

Short title: Antifeeding effect of myoinhibitory peptides

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

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Introduction

Insect peptides belonging to the myoinhibitory peptide family are usually nonapeptides, characterised 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 to 5: Lorenz *et al.*, 1995; 1999), the Dictyoptera *Periplaneta americana* (Pea-MIP: Predel *et al.*, 2001) and the Phasmida *Carausius morosus* (Cam-AST-B1 to 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 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 (JH) 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).

Anatomical localization of myoinhibitory peptides using immunocytochemical techniques has given further physiological support to 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 neurons 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 on 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, which means that the adult female requires a proteinaceous meal before starting vitellogenesis and oogenesis (Osorio *et al.*, 1998). Thus, the female has a feeding cycle that almost parallels that of vitellogenesis. In the *B. germanica* colony studied here, 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). As 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 have been shown to 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, suggest that the myoinhibitory peptides might be involved in the regulation of food intake in insects. It is to test this hypothesis that 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* have been studied.

Material 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 $30 \pm 1^\circ\text{C}$ and 60-70% relative humidity.

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 μl standard organ bath, as described elsewhere (Maestro *et al.*, 2001; Aguilar *et al.*, 2003). A FSG-01 transducer (Experimetria Ltd, Budapest, 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 reported 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 (*c.* 90%) of the peptides were assessed by amino-acid analysis, MALDI-TOF MS (matrix-assisted laser desorption ionization time-of-flight mass spectrometry) and HPLC.

Results

Before studying possible antifeeding effects of myoinhibitory peptides in *B. germanica* females, it was first assessed where these peptides could elicit antimyotropic effects in gut tissues. Therefore, we studied 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 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).

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 5-50 µg per specimen were used. Grb-AST-B3 was the most active peptide, resulting in *c.* 60-80% inhibition of food intake at doses between 15 and 50 µg (Fig. 3). Grb-AST-B1 resulted in *c.* 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 was postulated that myoinhibitory peptides could affect food intake in insects as a consequence of their effects on gut motility. Correspondingly, three galanin-related myoinhibitory peptides were 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 antimyotropic activity on both foregut and hindgut, and it was suggested that this activity was responsible for its antifeedancy (Aguilar *et al.*, 2004). Therefore, in the case of the myoinhibitory peptides studied here, both gut antimyotropic 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 antimyotropic 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 and 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 and Fig. 1). In contrast to the strong myoinhibitory effect of the three myoinhibitory peptides tested here in *B. germanica*, the myotropic peptide Pea-MIP cause only a moderate inhibition in foregut and hindgut muscle assays in the cockroach *P. americana*, with activity threshold concentrations as high as 5 x 10⁻⁹ M and 1 x 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. On the contrary, the peptides Grb-AST-B1 and B3 inhibit food intake in *B. germanica*, with Grb-AST-B3 the most active at *c.* 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) show the highest activity, resulting in *c.* 60% inhibition of food intake at doses between 5 and 50 µg, whereas BLAST-1 (LYDFGL-NH₂) is the least active, inducing only *c.* 50% inhibition at a dose of 50 µg (Aguilar *et al.*, 2003). LMS (pQDVDHVFLRF- 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 inhibited gut motility in *B. germanica in vitro* to a similar extent, only Grb-AST-B3 and, to a lesser extent, Grb-AST-B1 inhibited 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 this study of the myoinhibitory peptides it is clear that myomodulatory gut activity *in vitro* and antifeeding effects do not always correlate.

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Table 1. Comparison of the sequences of some myoinhibitory peptides with the N-terminal sequences of two typical vertebrate galanins. The coincident residues between the two groups of peptides have been highlighted in bold.

Galanins

Human galanin 1	GWTLNS AGYLLGPHAVGNHRSFSDKNGLTS (1)
Porcine galanin	GWTLNS AGYLLGPHAIDNHRSFHDKYGLA-NH ₂ (2)

Myoinhibitory peptides

Mas-MIP I	AWQDLNS AW-NH ₂ (3)
Mas-MIP II	GWQDLNS AW-NH ₂ (3)
Mas-MIP IV	GWNDMSS AW-NH ₂ (4)
Mas-MIP V	GWQDMSS AW-NH ₂ (4)
Grb-AST-B1	GWQDLN GGW-NH ₂ (5)
Grb-AST-B2	GWRDLN GGW-NH ₂ (5)
Grb-AST-B3	AWRDL SGGW-NH ₂ (5)
Cam-AST-B3	GWQDLQ SGW-NH ₂ (6)
Cam-AST-B6	AWQDLG SAW-NH ₂ (6)
Pea-MIP	GWQDLQ GGW-NH ₂ (7)

(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.

FIGURE LEGENDS

Fig. 1. Inhibitory effect of the myoinhibitory peptides Mas-MIP II (GWQDLNSAW-NH₂), Grb-AST-B1 (GWQDLNNGGW-NH₂) and Grb-AST-B3 (AWRDLSGGW-NH₂) on foregut (top) and hindgut (bottom) motility in *B. germanica* females. Results (mean \pm SEM; n=5-7) are expressed as the difference of the mean of the force produced by the tissue during one minute 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.

Fig. 3. Effect of the myoinhibitory peptides Mas-MIP II (GWQDLNSAW-NH₂), Grb-AST-B1 (GWQDLNNGGW-NH₂) and Grb-AST-B3 (AWRDLSGGW-NH₂) on food intake in *B. 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 (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).





