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 antimyotropic gut properties of three galanin-related myoinhibitory peptides (Mas-MIP II: GWQDLNSAW-NH2; Grb-AST-B1: GWQDLNGGW-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 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 cardica-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).
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 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 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).

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

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galanins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human galanin I</td>
<td>GWTNSAGYLLPGAVNHRFSFKNGLTS</td>
<td>(1)</td>
</tr>
<tr>
<td>Porcine galanin</td>
<td>GWTNSAGYLLPGHAIDNHRSHDKYGLA-NH2</td>
<td>(2)</td>
</tr>
<tr>
<td>Myoinhibitory peptides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mas-MIP I</td>
<td>AWQDLNSAW-NH$_2$</td>
<td>(3)</td>
</tr>
<tr>
<td>Mas-MIP II</td>
<td>GWQDLNSAW-NH$_2$</td>
<td>(3)</td>
</tr>
<tr>
<td>Mas-MIP IV</td>
<td>GWNMDSSAW-NH$_2$</td>
<td>(4)</td>
</tr>
<tr>
<td>Mas-MIP V</td>
<td>GWQDMSSAW-NH$_2$</td>
<td>(4)</td>
</tr>
<tr>
<td>Grb-AST-B1</td>
<td>GWQDLNGGW-NH$_2$</td>
<td>(5)</td>
</tr>
<tr>
<td>Grb-AST-B2</td>
<td>GWRDLNGGW-NH$_2$</td>
<td>(5)</td>
</tr>
<tr>
<td>Grb-AST-B3</td>
<td>AWRLSNGGW-NH$_2$</td>
<td>(5)</td>
</tr>
<tr>
<td>Cam-AST-B3</td>
<td>GWQLQSGGW-NH$_2$</td>
<td>(6)</td>
</tr>
<tr>
<td>Cam-AST-B6</td>
<td>AWQDLQGSAW-NH$_2$</td>
<td>(6)</td>
</tr>
<tr>
<td>Pea-MIP</td>
<td>GWQDLQGGW-NH$_2$</td>
<td>(7)</td>
</tr>
</tbody>
</table>

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).

**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-NH$_2$ allatostatins and leucomyosuppressin inhibit food intake, an activity possibly based on the antinmotropic gut effects of these peptides (Aguilar et al., 2003, 2004).

The antinmotropic 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.
**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 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 ID$_{50}$ 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-NH$_2$), Grb-AST-B1 (GWQDLNGGW-NH$_2$) and Grb-AST-B3 (AWRDLSGGW-NH$_2$) on foregut (top) and hindgut (bottom) motility in Blattella germanica females. Results (mean ± 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 ID$_{50}$ for each tissue is also shown. Empty squares indicate values for water control experiments.](image-url)
The peptides Mas-MIP II, Grb-AST-B1 and Grb-AST-B3 were tested for effects on food intake by *Blattella 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-NH$_2$ 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 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 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-NH$_2$ allatostatins. Allatostatins are inactive on foregut, and show hindgut ID$_{50}$ 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 ID$_{50}$ 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 myotropic 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 $5 \times 10^{-9}$ M and $1 \times 10^{-7}$ 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-NH$_2$ allatostatins tested in the carrot-feeding bioassay (Aguilar et al., 2003), BLAST-2 (DRLYSFGL-NH$_2$) shows the highest activity, resulting in approximately 60% inhibition of food intake at doses between 5 and 50 μg, whereas BLAST-1 (LYDFGL-NH$_2$) is the least active, inducing only approximately 50% inhibition at a dose of 50 μg (Aguilar et al., 2003). LMS (pQDpdfHVFRLF-NH$_2$) 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
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


Accepted 11 January 2006

First published online 8 May 2006