General and Comparative Endocrinology 280 (2019) 185–191

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Function of myosuppressin in regulating digestive function in the two-spotted cricket, *Gryllus bimaculatus*

Yi Jun Zhou[a,b], Hitomi Seike[a], Shinji Nagata[a,*]

[a] Department of Integrated Biosciences, Graduate School of Frontier Sciences, The University of Tokyo, 5-1-5 Kashiwa no ha, Kashiwa City, Chiba #277-8562, Japan
[b] Research Fellow of Japan Society for the Promotion of Science (JSPS), Kojimachi, Chiyoda-ku, Tokyo 102-0083, Japan

ARTICLE INFO

Keywords:
Gryllus bimaculatus
Feeding behavior
Peptide
Myosuppressin

ABSTRACT

Myosuppressin is one of essential peptides controlling biological processes including feeding behavior. Here we identified and characterized the cDNAs that encode myosuppressin precursor and its receptor in the two-spotted cricket *Gryllus bimaculatus*. The presence of the mature peptide (Grybi-MS) was confirmed by direct measurement of adult brain. RT-PCR revealed the tissue distribution of these transcripts; myosuppressin is expressed predominantly in the brain and central nervous system, whereas its receptor is ubiquitously expressed in the cricket body. To address the function of Grybi-MS, we performed several bioassays to test concerning feeding behavior and digestive function upon exposure to Grybi-MS. Administration of synthetic Grybi-MS resulted in increased feeding motivation, accompanied by an increase in food intake. Meanwhile, the hemolymph lipid and carbohydrate titers were both elevated after Grybi-MS injection. As the intestinal contraction is significantly inhibited by the exposure to Grybi-MS, the upregulating feeding index might be complicated in the cricket body. The current data indicate that Grybi-MS modulates feeding behavior to control the physiological processes in the cricket.

1. Introduction

Neuropeptides are evolutionarily ancient mediators of neuronal signaling in both the central nervous systems and peripheral systems and have crucial roles in regulating multiple physiological and biological processes and behaviors. Feeding is one of the most basic behaviors for survival of an individual animal and for preservation of the species. Animals must ingest food for energy production and for the maintenance of biochemical resources for the continuity of living processes, such as growth, and development (Browne, 1975). Feeding behavior is composed of several sequential stepwise behaviors, including foraging for the favorite diet that provides the appropriate nutrients, ingesting the diet, digesting the diet to absorbable forms, and finally excreting the excess and indigestible nutrients and metabolites. These feeding and digestion-related processes are thought to be modulated by a series of neuropeptides (Pool and Scott, 2014). For example, foraging, the food-seeking behavior, is modified significantly by the short neuropeptide F (sNPF) (Wu et al., 2003). Interestingly, sNPF also controls feeding motivation in the silkworm *Bombyx mori* (Nagata et al., 2012), and the digestive enzymatic regulation in the American cockroach (Mikani et al., 2012). In addition, excretion process is modulated by the diuretic hormones such as CRF/DH and CT/DH which have been reported in many insects (Maddrell, 1964; Mills, 1967). In general, neurohormones and bioactive peptides are intricately involved in each of these processes, and their orchestration determines the execution of feeding behaviors.

Among the regulatory neuropeptides and bioactive peptides, some peptides with a common C-terminal RFamide motif have been identified as RFamide peptides and RFamide-related peptides (Tsutsui and Ubuka, 2015). RFamide peptide was originally discovered in ganglia of the clam *Macrocallista nimbosa* as a factor exerting cardioexcitatory effect (Price and Greenberg, 1977). Genes encoding RFamide peptides appear to have emerged early during the neuropeptide evolution and have remained relatively well conserved (Bechtold and Luckman, 2007). To date, RFamide is considered as a transmitter associated with the feeding behavior in a wide range of species, including coelenterates and the blow fly as well as mammals (Downer et al., 2007; Mackie et al., 2003). Among RFamide and its related peptides, myosuppressin (MS) is one of the most conserved neuropeptides in insects. It has been shown to exhibit inhibitory activities against various visceral muscles isolated from the Madeira cockroach *Leucophaea maderae*, and is known for its ability to reduce the frequency of hindgut contractions (Holman et al., 1986).

The structure of MS peptides is represented by the consensus amino

* Corresponding author.
E-mail address: shinjin@edu.k.u-tokyo.ac.jp (S. Nagata).

https://doi.org/10.1016/j.ygcen.2019.05.001
Received 1 October 2018; Received in revised form 22 April 2019; Accepted 1 May 2019
Available online 02 May 2019
0016-6480/ © 2019 Elsevier Inc. All rights reserved.

acid sequence of XDXXHXFLRFamide, but MS in *Rhodnius prolixus* has a unique amino acid sequence with methionine[8] instead of leucine (Lee et al., 2012). Similar to the conserved structure of MS, the receptor for MS (MSR) has a conserved amino acid sequence, forming a G-protein coupled receptor. Even though MSs are often grouped together with insect FMRFamides, MSRs are not activated by *Anopheles* FMRFamide and other FMRFamide peptides (Egerod et al., 2003). Owing to the different structural characteristics in the N-terminal portion of MS in addition to the FLRFamide motif between MS and FMRFamides, these domains are important for the activation of MSR (Egerod et al., 2003). Indeed, MSRs are not evolutionarily related to the insect FMRFamide receptors (Vilaplana et al., 2004). In addition to its original function in inhibition of hindgut contraction, MS also shows inhibitory activities against the actions of the visceral muscles of the heart, oviduct, Malpighian tubules, and salivary gland in insects (Bendena et al., 1997). Like other members of RFamide peptide family, MS also inhibits feeding activity in the cockroach *Blattella germanica* and in the tobacco cutworm *Spodoptera littoralis* (Audsley and Weaver, 2009). In addition to its contractive functions, MS inhibits the release of adipokinetic hormones (AKHs) in the migratory locust *Locusta migratoria* (Vullings et al., 1998), indicating that MS functions as a pleiotropic factor modulating feeding-related function and then controlling digestive function.

In this study, we identified contigs encoding the precursor of *Gryllus bimaculatus* MS (Grybi-MS) and MSR (Grybi-MSR) from RNA sequencing data, which were derived from the brain and other ganglia of the two-spotted cricket. The presence of Grybi-MS was biochemically confirmed in the cricket brain by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). Moreover, to assess the physiological roles of Grybi-MS *in vivo* in terms of feeding behavior, we measured food intake, and excretion, and the mobilization of carbohydrate and lipid *in vivo*. In addition, we analyzed its regulatory function of foregut contraction in response to the administration of Grybi-MS mature peptide.

2. Materials and methods

2.1. Insects

Synchronously growing male of the two-spotted cricket *G. bimaculatus* were used, from a colony maintained in our laboratory colony breeding for 15 years. The crickets were reared in plastic containers (55 cm × 39 cm × 31 cm) at 27 ± 1 °C, and under a 16 h light-8 h dark photoperiod as previously described (Zhou et al., 2018).

2.2. RNA-sequencing

RNA-sequencing data from total RNA from more than fifty adult male brains, subesophageal ganglia, and corpora cardiaca was used from our previous study (Tsukamoto and Nagata, 2016). Contigs were *de novo* assembled newly using the CLC workbench (https://www.qiagenbioinformatics.com/products/clc-main-workbench/) and comprehensive annotation was performed using tblastx.

2.3. Chemically synthesis of Grybi-MS

The mature peptide of Grybi-MS was synthesized manually based on the Fmoc method using Rink-amide resin (Merck Millipore, Darmstadt, Germany) (Zhou et al., 2018). The crude synthesized peptide was then subjected to reverse-phased HPLC (Jasco SC-802, PU-880, UV-875; Jasco INt., Tokyo, Japan) on a Senshu Pak PEGASIL-300 ODS column (4.6 mm i.d. × 250 nm; Senshu Kagaku, Tokyo, Japan). The adsorbed peptide was eluted with a linear gradient of 10%–60% acetonitrile containing 0.1% TFA over 30 min, at a flow rate of 1 ml/min, and was monitored by measuring the absorbance at 225 nm. The isolated synthetic peptide was confirmed by measurement on a MALDI-TOF MS system.

2.4. MALDI-TOF MS and MS/MS analyses

MALDI-TOF MS was carried out using a TOF/TOF 5800 System (AB SCIEX, Framingham, MA, USA). Peptide solutions were mixed with a half-saturated solution of α-cyano-4-hydroxycinnamic acid (CHCA; 1:1 v/v) in 60% acetonitrile containing 0.1% TFA and dried on a stage for measurement. MS spectra were acquired with a total accumulation of 250–1000 laser shots until the generation of visible ion peaks.

2.5. Expression analysis

RNA was extracted from the adult tissues of the adult crickets using TRI reagent® (Molecular Research Center, Inc., Cincinnati, OH, USA) according to the manufacturer's protocol. Using the extracted RNAs, cDNAs were synthesized with an oligo dT_{18} primer by ReverTra Ace® (Toyobo Co. Ltd., Osaka, Japan). The cDNAs were used as DNA templates for RT-PCR with Gotaq Green Master Mix (Promega Corporation, Madison, WI, USA). PCR was performed under the following conditions: 120 s at 96 °C, followed by 30 cycles of 20 s at 94 °C, 20 s at 55 °C, and 45 s at 72 °C and 240 s of 72 °C in the end. The primers used for amplification of Grybi-MS are 5′-AGAGGCAAGACGTCGATCAC-3′ as primer and 5′-ACATGCTTTGTACAGGGCCG-3′ as reverse primer; the primers used for amplification of Grybi-MSR are 5′-CCACCAACTCCATCCTCACC-3′ as forward primer and 5′-CGTTGTGGTCGCTCTTGTTG-3′ as reverse primer. PCR products were electrophoresed on a 1.0% agarose gel and visualized by staining with ethidium bromide. Amplified cDNAs in the gel were extracted and purified using Wizard SV Gel and PCR Clean-up system (Promega Corporation, Madison, WI, USA) and subsequently subcloned into the pGEM-T Vector System (Promega Corporation, Madison, WI, USA) according to the manufacturer's protocol. The inserted cDNAs encoding the precursor of Grybi-MS and Grybi-MSR were confirmed using a 3500 Genetic Analyzer (Applied Biosystems, Waltham, MA, USA).

2.6. Lipid and carbohydrate extraction from hemolymph

Lipid and carbohydrate were extracted following the procedure described in a previous report (Zöllner and Kirsch, 1962).

2.7. Measurement of lipid

Extracted lipid fraction of hemolymph, obtained by the processes described above was subjected to the sulfo-phospho-vanillin method, following the previous report (Gore and Schal, 2005).

2.8. Measurement of trehalose

For quantification of hemolymph carbohydrate, trehalose, the extract of carbohydrate fraction was subjected following an enzymatic digestion in our previous report (Zhou et al., 2018).

2.9. Food intake assay

On the fifth day after adult emergence, crickets were injected with 100 pmol synthetic Grybi-MS. Each cricket was kept isolated in a plastic container. Weight difference on an artificial diet (33% casein, 33% dextrin, and 33% soybean oil) and counting the number of fecal pellets after 24 h of injection represented the amount of food intake, as an index of feeding activity as previously defined (Gore and Schal, 2005).

2.10. Direct MALDI-TOF MS and MSMS analysis of brain

After washing the tissue in insect saline for three times, fresh brain was directly applied on a stage, then overlaid with a half-saturated solution of CHCA (1:1 v/v) in 60% acetonitrile containing 0.1% TFA and dried on the stage for measurement.

2.11. Gut contraction assay

After removal of head, crickets on the fifth days after adult emergence are spread out along the abdomen by showing foregut clearly with no harm on peripheral neurons in the 0.9% NaCl. Their foreguts were exposed to 100 pmol synthetic Grybi-MS for the foregut was conducted observation of gut contraction for 40 s. Experimental control was treated with PBS in the same methods. The contractile movement was observed and recorded using a digital microscope USB2.0 DigiScope II v2™ (CHRONOS, Taiwan). The contractile movements were analyzed using the ImageJ software (https://imagej.net/Fiji) by Kymograph analysis plug. Incubation and observation duration were used shorted than 120 s due to the weakened gut by *ex vivo* incubation.

2.12. Statistical analyses

Two groups comparison was statistically analyzed by Student's *t* test or Mann Whitney *U* test. Crop contraction comparison was statistically analyzed by one-way ANOVA, post hoc Tukey's test. *P*-value less than 0.05 was considered to be statistically significant. Reproducibility in this study was confirmed by at least two independent experiments.

2.13. Bioinformatic analyses

N-glycosylation sites of MSR sequence were predicted by NetGlyc 1.0 Server (http://www.cbs.dtu.dk/services/NetNGlyc). Transmembrane domains were predicted by TMHMM (http://www.cbs.dtu.dk/services/TMHMM). TBLASTN searches were performed at http://blast.ncbi.nlm.nih.gov/blast.cgi. Sequences were aligned using CLUSTALW (http://clustalw.ddbj.nig.ac.jp) and visualized by using BoxShade Server (https://embnet.vital-it.ch/software/BOX_form.html). Sequences were aligned by WEBLOGO (http://weblogo.berkeley.edu/logo.cgi). Sequences used are shown as the following GenBank™ accession numbers: *Drosophila melanogaster*-MS (NP_001287503.1), *Tribolium castaneum*-MS (EFA12055.1), *Mamestra brassicae*-MS (BAV57610.1), *Rhodnius prolixus*-MS (AEX08672.1), *Apis mellifera*-MS (ACI90289.1), *Cimex lectularius*-MS (XP_024084761.1), and MS in *G. bimaculatus*.

3. Results

3.1. Identification of cDNAs encoding Grybi-MS and Grybi-MSR

Contigs encoding candidate myosuppressin (MS) and MS receptor (MSR) homolog in sequences of other insect species were identified in the in-house RNA-sequencing database derived from the brain and other ganglia of the two-spotted cricket, which has been established in our previous report (Tsukamoto and Nagata, 2016). Using Blastx, a contig of 72 bp encoding 23 amino acids was identified as a partial sequence of MS precursor homologous gene (Fig. 1A). Similarly, a contig of 863 bp encoding 287 amino acids was identified as partial sequence of MSR homologous gene (Fig. 1B). Hereafter, we thus designated them as partial sequences of Grybi-MS and Grybi-MSR, respectively. Within the deduced amino acid sequence of the Grybi-MS precursor, there was a glycine residue (which is required for C-terminal amidation) immediately before the di-basic amino acid sequence for proteolytic cleavage to produce the mature peptide. A high level of homology was confirmed from the mature peptide alignment of Grybi-MS with MSs of other insects (Fig. 1C). Within the deduced amino acid sequence of Grybi-MSR, there were seven transmembrane domains and it exhibited approximately 70% identity with the MSRs of other insects.

3.2. Confirmation of presence of Grybi-MS in the crickets

To confirm the presence of Grybi-MS peptide predicted by the contigs (Fig. 1A), direct measurement by MALDI-TOF MS of the isolated cricket brain was performed. Predicted ion peaks corresponding to the predicted mature form of Grybi-MS were observed in the brain of the adult cricket (Fig. 2A), which was consistent with that of the chemically synthesized standard Grybi-MS.

3.3. Tissue distribution analysis of Grybi-MS and Grybi-MSR

RT-PCR confirmed the tissue distribution of Grybi-MS and Grybi-MSR expression in adult male crickets, three days after adult emergence. Results revealed that Grybi-MS is expressed in the CNS, ventral neural system (VNS) and foregut. In contrast, Grybi-MSR is expressed in CNS and peripheral organs, such as fatbody, foregut, midgut (Fig. 2B), consistent with the expression pattern of MSs and MSRs in other insect species (Orchard and Lange, 2006).

3.4. Effects of Grybi-MS on food consumption and excretion

To examine the effects of Grybi-MS on feeding behavior, we next measured the food consumption and excretion amount after mature peptide application (Fig. 3AB). Data indicated that application of 100 pmol of Grybi-MS resulted in a significant increase of both food consumption and excretion amount, when compared with the control group of crickets injected with PBS (Fig. 3AB).

3.5. Effects of Grybi-MS on lipid and carbohydrate mobilization

Because the significant effects of Grybi-MS on feeding activities were observed (Fig. 3AB), we next measured the hemolymph lipid and carbohydrate titers in response to injection of Grybi-MS by 120 min. The application of 100 pmol of Grybi-MS resulted in significant elevation on both lipid and carbohydrate level in hemolymph compared with those of crickets injected with PBS (Fig. 3CD).

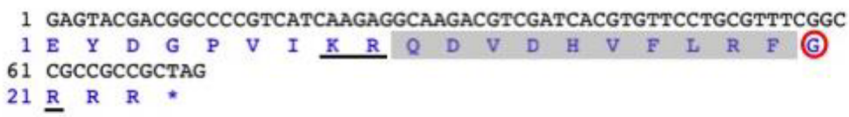
3.6. Effects of Grybi-MS on foregut contraction

Corresponding to the food intake and excretion alteration observed in the crickets after Grybi-MS application, we further examined the contractile activity of the foregut of crickets after Grybi-MS application *ex vivo* by directly exposing on the foregut of abdomen-opened crickets. Significant inhibition on the contraction was observed after 100 pmol of Grybi-MS application; contraction per minutes was decreased from 19.2 to near 0, whereas no obvious difference was observed after PBS application (Fig. 4AB). Moreover, the amplitude of contraction was nearly impaired compared with the control (Fig. 4A).

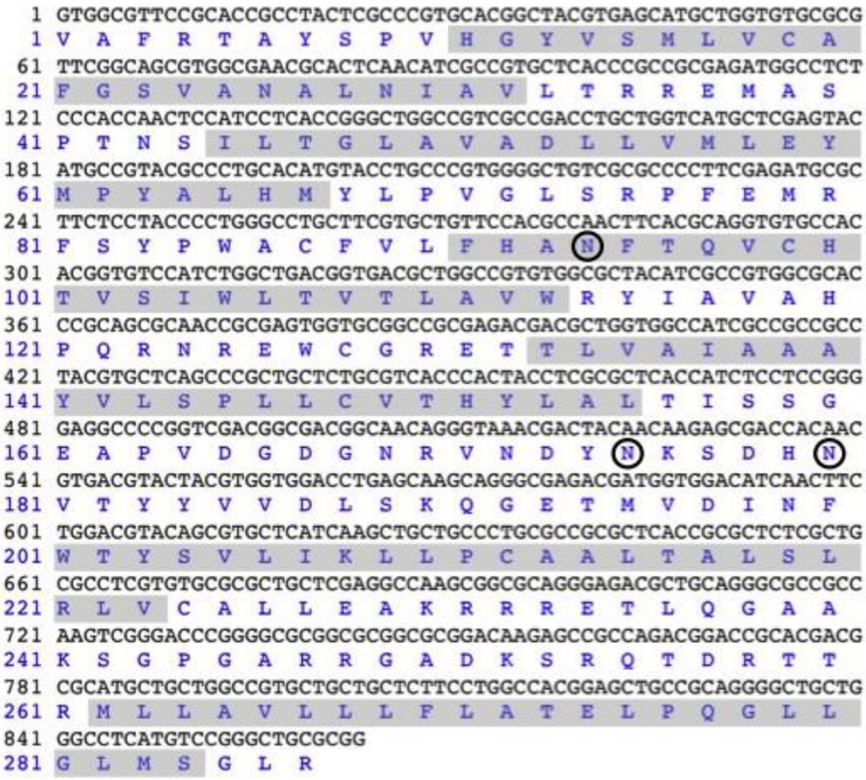
4. Discussion

The Myosuppressin was firstly isolated from the cockroach for its ability to inhibit hindgut contractions (Holman et al., 1986). In this study, we identified MS and its receptor in the cricket, *G. bimaculatus*. Within the *de novo* assembled unigenes from RNA-seq data of cricket, only one contig encoding a G-protein coupled receptor (GPCR) has been identified as the homolog of MSR, which was somewhat different from the fact of the presence of two MSR isoforms in *D. melanogaster* (Egerod et al., 2003). However, transcriptomic analyses revealed the number of MSR isoform is mostly singular over the insect species (Scholler et al., 2005; Yamanaka et al., 2005; Yamanaka et al., 2008). In this study, only partial sequences of Grybi-MS and Grybi-MSR were identified due to the limitation of RNA-seq contigs, which contaminate miss contigs or gaps even if coming across high homology sequences with MS and MSR. Fortunately, because we could identify the mature portion of Grybi-MS in the partial sequence, we confirmed the existence of MS mature peptide by MALDI-TOF MS, clearly showing the presence of Grybi-MS in the brain of cricket (Fig. 2A). This result is consistent to the data of RT-PCR (Fig. 2B). According to the previous study in many species, immunohistochemical studies (Mccormick and Nichols, 1993; Meola et al., 1991; Swales and Evans, 1995) and in situ studies (Donly et al.,

A



B



C

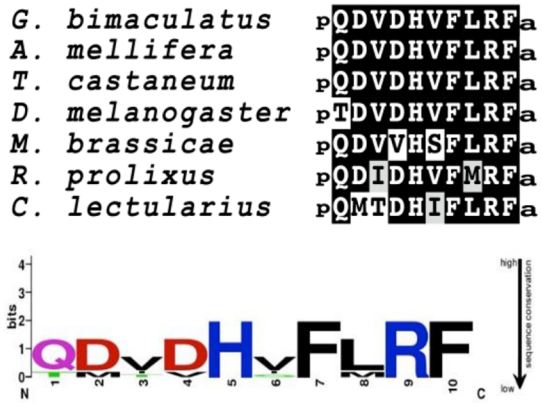


Fig. 1. Partial cDNA and deduced amino acid sequences encoding Grybi-MS precursor and Grybi-MS receptor (MSR). (A) cDNA sequence encoding a partial precursor Grybi-MS. The sequence with grey background indicates the mature peptide of Grybi-MS; a red circle indicates the amidation site and the underlined sequence indicates a cleavage site. (B) cDNA and deduced amino acid sequences encoding partial Grybi-MSR. The sequences with grey background indicate seven trans-membrane domains of Grybi-MSR; black circles are potential *N*-glycosylation sites. (C) Alignment of cDNA and amino acids sequences of Grybi-MS mature peptide and SequenceLogo over the aligned insect MSs.

1996; Fuse et al., 1998) have demonstrated that MS is mainly expressed in the central nervous system (CNS) and stomatogastric nervous systems in many insects as a endocrine neurohormone. Since MS was proved to be multifunction as hindgut contraction (Holman et al., 1986) and trehalose release (Orchard et al., 2001), its reasonable for Grybi-MSR express in many peripheral organs, such as fat body and gut.

Like other typical brain-gut peptides, MS has been also proved as an antifeeding neurohormone in both phytophagous and omnivorous insects, *Blattella germanica* (Aguilar et al., 2004) and *Spodoptera littoralis* (Vilaplana et al., 2008) and elicited latency in feeding initiation (Nagata et al., 2011). Similar to inhibitory effect of MS in other insects, Grybi-MS elicited inhibition on foregut contraction (Fig. 4).

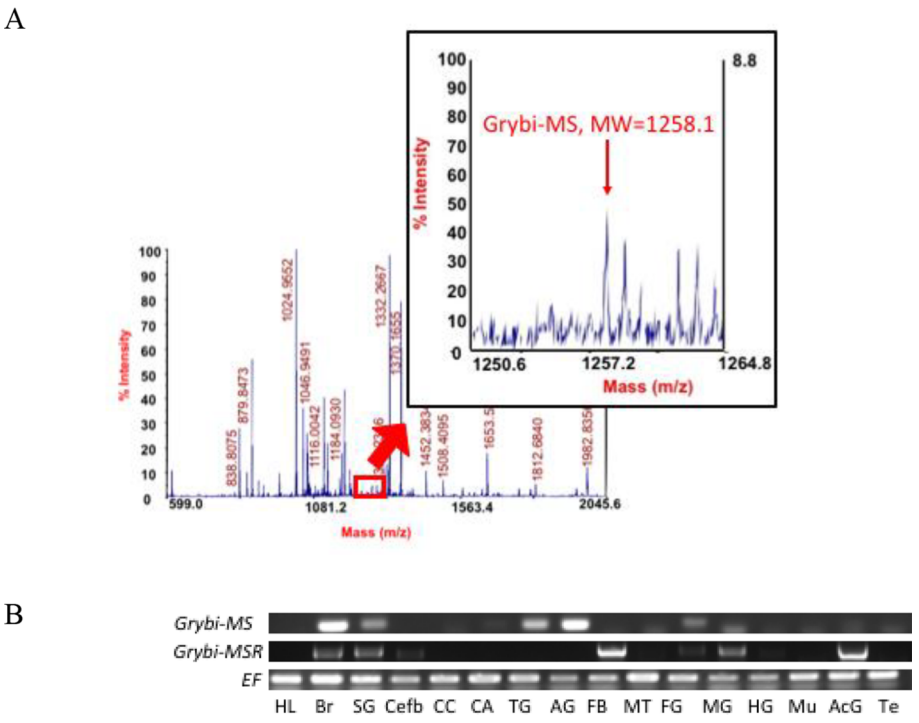


Fig. 2. Confirmation of the presence of Grybi-MS. (A) Direct measurement of the isolated cricket brain by MALDI-TOF MS. Inset represents the zoomed spectrum around the ion peak corresponding to the calculated molecular mass of Grybi-MS. (B) RT-PCR analysis of Grybi-MS and Grybi-MSR in the adult cricket. EF: elongation factor; HL: hemolymph; Br: brain; SG: subesophageal ganglion; Cefb: cerebral fat body; CC: corpora cardiaca; CA: corpora allata; TG: thoracic ganglia; AG: abdominal ganglia; FB: fat body; MT: Malpighian tubules; FG: foregut; MG: midgut; HG: hindgut; Mu: muscle; AcG: accessory gland; Te: Testis.

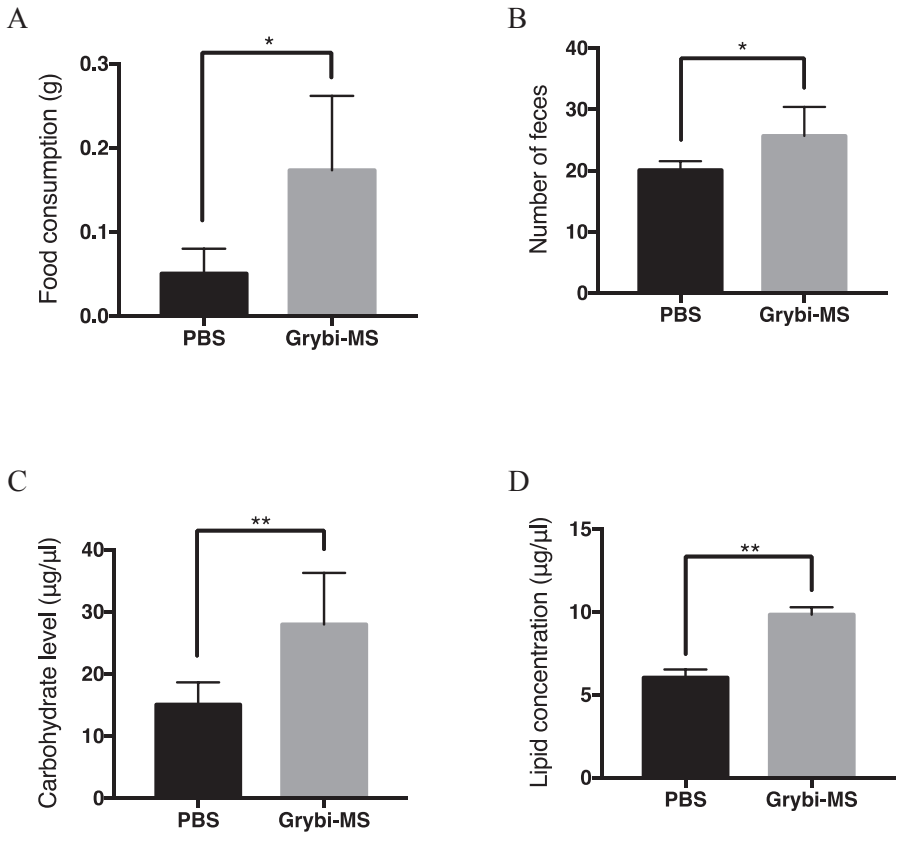


Fig. 3. Effects of Grybi-MS on feeding activities. (A) Amount of food intake after Grybi-MS injection (100 pmol). (B) Number of fecal pellets after Grybi-MS injection (100 pmol). The examined crickets were treated on the third day after adult emergence. Food intake and fecal pellets were measured after 24 h of peptide injection. (N = 8 for (A) and (B), Mean + S.D. $*p < 0.05$; $**p < 0.01$, Student's t test). (C) Lipid titer in the hemolymph of crickets after application of Grybi-MS. (D) Carbohydrate titer in the hemolymph of crickets after application of Grybi-MS. (N = 12 for (C) and (D), Mean + S.D. $*p < 0.05$, $**p < 0.01$, Student's t test). Lipid and carbohydrate fraction were extracted from 5 μl hemolymph collected at 2 h after peptide injection.

Surprisingly, we observed increases of both food consumption and excretion after 24 h at a dose of 100 pmol (Fig. 3AB). Those data indicate that in *G. bimaculatus*, Grybi-MS application affected much stronger on food intake than on excretion. In the abdomen of Grybi-MS-treated cricket, no food accumulation in the foregut and no depletion of food in the hindgut were observed at two hours after Grybi-MS application. Therefore, the functions of MS on the feeding in the cricket may be different from the fact previously reported (Aguilar et al., 2004). One considerable reason for this difference is that Grybi-MS may cause different alteration in larvae or in the adult. Also, the inhibitory effects of Grybi-MS may oppositely cause a complementariness after action time. In this study, we also measured the carbohydrate and lipid titers

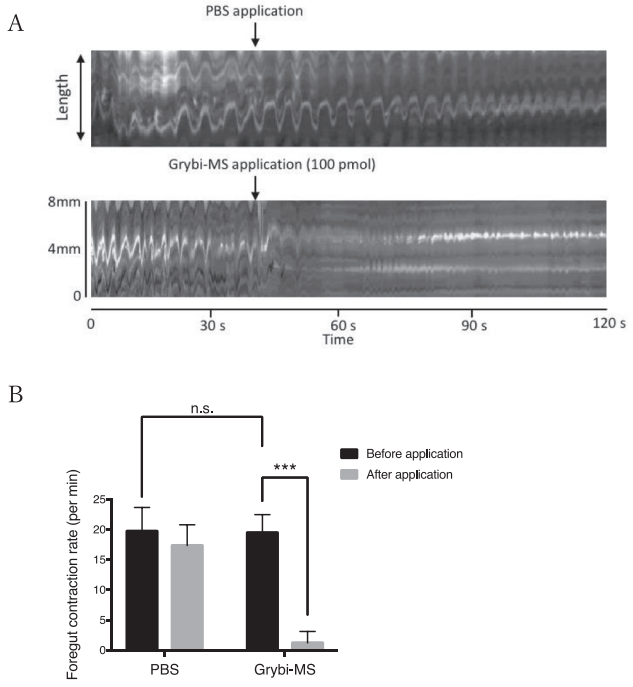


Fig. 4. Effects of Grybi-MS on foregut contraction. (A) Representative kymographs of foregut contraction after exposure to Grybi-MS (100 pmol) and PBS (control). Samples were perfused at the timing which arrows indicate. (B) Frequency of foregut contraction from kymographs. Exposure of the contracting foregut to Grybi-MS (100 pmol). (N = 6, Mean + S.D. $*p < 0.05$, one-way ANOVA, post hoc Tukey's test).

in the hemolymph after Grybi-MS application. Corresponding to its stimulation on food intake and excretion, both lipid and carbohydrate level were elevated after two hours of Grybi-MS application, which is contrary the previous report that MS shows no hyperglycemic activity in the adult *Tenebrio molitor*, but consistent to hyperglycemic activity in the larvae. (Wasielewski and Skonieczna, 2008). Also, because MS suppressed the intracellular cAMP level in a dose dependent manner in *B. mori*, its function in digestive mechanisms may be occurred by the cross-talk of the signaling Adipokinetic hormone (AKH) involved in the mobilization of lipid and carbohydrate from fat body into hemolymph through intracellular cAMP signaling (Bednářová et al., 2013). This suppression on cAMP or other energy-associated neuropeptides may lead to negative feedback signal on AKH signaling, which can explain the elevated lipid and carbohydrate titers after Grybi-MS application. In fact, there have been reported in the different functions from our present study in other insect species, such as hemolymph carbohydrate-related enzymes are activated by MS in *Diploptera punctate* (Orchard et al., 2001). Finally, we consider that the effects of MS might be different according not only to each species but also to the different growth period such as larvae and adult, which could be our next direction to address the differentiation of the regulatory role of Grybi-MS during development and growth from larvae to adult.

In summary, the present study revealed the new metabolic role of Grybi-MS in digestive function other than their antifeeding ones and showed their possible roles in insect that could become one of the vital neurohormone to corporate with other energy-associated neurohormones in adult emergence.

Acknowledgements

This study was partly supported by Grants-in-Aid for Scientific Research-Japan Society for the Promotion of Science (JSPS)-KAKENHI [grant numbers 18J22078 (ZYJ) and 16K15066 (SN)]. We thank Ms. Tao Linan, ZheJiang University, for support regarding chemical peptide synthesis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygcen.2019.05.001.

References

Aguilar, R., et al., 2004. Identification of leucomyosuppressin in the German cockroach, *Blattella germanica*, as an inhibitor of food intake. Regul. Pept. 119, 105–112. https://doi.org/10.1016/j.regpep.2004.01.005.

Audsley, N., Weaver, R., 2009. Neuropeptides associated with the regulation of feeding in insects. Gen. Comp. Endocrinol. 162, 93–104. https://doi.org/10.1016/j.ygcen.2008.08.003.

Bechtold, D.A., Luckman, S.M., 2007. The role of RFamide peptides in feeding. J. Endocrinol. 192, 3–15. https://doi.org/10.1677/JOE-06-0069.

Bednářová, A. , KodrÃk, D. , Krishnan, N. , 2013. Adipokinetic hormone exerts its anti-oxidative effects using a conserved signal-transduction mechanism involving both PKC and cAMP by mobilizing extra and intracellular Ca^{2+} stores. Comp. Biochem. Physiol. C: Toxicol. Pharmacol. 158 (2013), 142– 149 . https://doi.org/10.1016/j.cbpc.2013.07.002 .

Bendena, W.G., et al., 1997. Molecular characterization of the inhibitory myotropic peptide leucomyosuppressin1. Peptides 18, 157–163. https://doi.org/10.1016/S0196-9781(96)00237-9.

Browne, L.B., 1975. Regulatory mechanisms in insect feeding. Adv. Insect Phys. 11, 1–116. https://doi.org/10.1016/S0065-2806(08)60162-9.

Donly, B.C., et al., 1996. Characterization of the gene for leucomyosuppressin and its expression in the brain of the cockroach *Diploptera punctata*. Insect Biochem. Mol. Biol. 26, 627–637. https://doi.org/10.1016/S0965-1748(96)00015-X.

Downer, K., et al., 2007. Insect satiety: sulfakinin localization and the effect of drosulfakinin on protein and carbohydrate ingestion in the blow fly, *Phormia regina* (Diptera: Calliphoridae). J. Insect Physiol. 53, 106–112. https://doi.org/10.1016/j.jinsphys.2006.10.013.

Egerod, K., et al., 2003. Molecular cloning and functional expression of the first two specific insect myosuppressin receptors. Proc. Natl. Acad. Sci. 100, 9808–9813. https://doi.org/10.1073/pnas.1632197100.

Fuse, M., et al., 1998. In situ hybridization analysis of leucomyosuppressin mRNA expression in the cockroach, *Diploptera punctata*. J. Comp. Neurol. 395, 328–341. https://doi.org/10.1002/(SICI)1096-9861(19980808)395:3<328::AID-CNE5>3.0.CO;2-4.

Gore, J.C., Schal, C., 2005. Expression, production and excretion ofBlag1, a major human allergen, in relation to food intake in the German cockroach, *Blattella germanica*. Med. Vet. Entomol. 19, 127–134. https://doi.org/10.1111/j.0269-283X.2005.00550.x.

Holman, G., et al., 1986. Primary structure and synthesis of a blocked myotropic neuropeptide isolated from the cockroach, *Leucophaea maderae*. Comput. Biochem. Physiol. 85, 219–224. https://doi.org/10.1016/0742-8413(86)90077-0.

Lee, D., et al., 2012. An unusual myosuppressin from the blood-feeding bug Rhodnius prolixus. J. Exp. Biol. 215, 2088–2095. https://doi.org/10.1242/jeb.067447.

Mackie, G., et al., 2003. Central circuitry in the jellyfish Aglantha digitale IV. Pathways coordinating feeding behaviour. J. Exp. Biol. 206, 2487–2505. https://doi.org/10.1242/jeb.00450.

Maddrell, S., 1964. Excretion in the blood-sucking bug, Rhodnius prolixus Stål: III. The control of the release of the diuretic hormone. J. Exp. Biol. 41, 459–472. http://jeb.biologists.org/content/41/3/459.

Mccormick, J., Nichols, R., 1993. Spatial and temporal expression identify dromyosuppressin as a brain-gut peptide in *Drosophila melanogaster*. J. Comput. Physiol. 338, 279–288. https://doi.org/10.1002/cne.903380210.

Meola, S.M., et al., 1991. Immunocytochemical localization of leucomyosuppressin-like peptides in the CNS of the cockroach, *Leucophaea maderae*. Neurochem. Res. 16, 543–549. https://doi.org/10.1007/BF00974872.

Mikani, A., et al., 2012. Brain-midgut short neuropeptide F mechanism that inhibits digestive activity of the American cockroach, *Periplaneta americana* upon starvation. Peptides 34, 135–144. https://doi.org/10.1016/j.peptides.2011.10.028.

Mills, R.R., 1967. Hormonal control of excretion in the american cockroach: I. Release of a diuretic hormone from the terminal abdominal ganglion. J. Exp. Biol. 46, 35–41. https://doi.org/10.1016/0010-4035(70)90012-1.

Nagata, S., et al., 2012. Effects of starvation on brain short neuropeptide F-1,-2, and-3 levels and short neuropeptide F receptor expression levels of the silkworm, *Bombyx mori*. Front. Endocrinol. 3, 3. https://doi.org/10.3389/fendo.2012.00003.

Nagata, S., et al., 2011. Effects of neuropeptides on feeding initiation in larvae of the silkworm, *Bombyx mori*. Gen. Comp. Endocrinol. 172, 90–95. https://doi.org/10.1016/j.ygcen.2011.03.004.

Orchard, I., et al., 2001. FMRFamide-related peptides: a multifunctional family of structurally related neuropeptides in insects. Adv. Insect. Phys. 28, 267–329. https://doi.org/10.1016/S0065-2806(01)28012-6.

Orchard, I., Lange, A.B., 2006. Insect myosuppressins/FMRFamides and FL/IRFamides/NPFs. Handbook Biol. Active Peptides 193–198.

Pool, A.-H., Scott, K., 2014. Feeding regulation in Drosophila. Curr. Opin. Neurobiol. 29, 57–63. https://doi.org/10.1016/j.conb.2014.05.008.

Price, D.A., Greenberg, M.J., 1977. Structure of a molluscan cardioexcitatory neuropeptide. Science 197, 670–671. https://doi.org/10.1126/science.877582.

Scholler, S., et al., 2005. Molecular identification of a myosuppressin receptor from the malaria mosquito *Anopheles gambiae*. Biochem. Biophys. Res. Commun. 327, 29–34. https://doi.org/10.1016/j.bbrc.2004.11.139.

Swales, L.S., Evans, P.D., 1995. Distribution of SchistoFLRFamide-like immunoreactivity in the adult ventral nervous system of the locust, *Schistocerca gregaria*. Cell Tissue Res. 281, 339–348. https://doi.org/10.1007/s004410050430.

Tsukamoto, Y., Nagata, S., 2016. Newly identified allatostatin Bs and their receptor in the two-spotted cricket, *Gryllus bimaculatus*. Peptides 80, 25–31. https://doi.org/10.1016/j.peptides.2016.03.015.

Tsutsui, K., Ubuka, T., 2015. RFamide peptide family. Handbook Hormones 5–15.

Vilaplana, L., et al., 2004. The cDNA for leucomyosuppressin in *Blattella germanica* and molecular evolution of insectmyosuppressins. Peptides 25, 1883–1889. https://doi.org/10.1016/j.peptides.2004.06.008.

Vilaplana, L., et al., 2008. Antifeeding properties of myosuppressin in a generalist phytophagous leafworm, *Spodoptera littoralis* (Boisduval). Regul. Pept. 148, 68–75. https://doi.org/10.1016/j.regpep.2008.02.002.

Vullings, H., et al., 1998. A possible role of Schisto FLRFamide in inhibition of adipokinetic hormone release from locust corpora cardiaca. J. Neurocytol. 27, 901–913. https://doi.org/10.1023/A:1006901123566.

Wasielewski, O., Skonieczna, M., 2008. Pleiotropic effects of the neuropeptides CCAP and myosuppressin in the beetle, *Tenebrio molitor L*. J. Comp. Physiol. B 178, 877–885. https://doi.org/10.1007/s00360-008-0276-6.

Wu, Q., et al., 2003. Developmental control of foraging and social behavior by the Drosophila neuropeptide Y-like system. Neuron 39, 147–161. https://doi.org/10.1016/S0896-6273(03)00396-9.

Yamanaka, N., et al., 2005. Identification of a novel prothoracicostatic hormone and its receptor in the silkworm, *Bombyx mori*. J. Biol. Chem. 280, 14684–14690. https://doi.org/10.1074/jbc.M500308200.

Yamanaka, N., et al., 2008. Neuropeptide receptor transcriptome reveals unidentified neuroendocrine pathways. PLoS One 3, e3048. https://doi.org/10.1371/journal.pone.0003048.

Zhou, Y.J., et al., 2018. Effects of adipokinetic hormone and its related peptide on maintaining hemolymph carbohydrate and lipid levels in the two-spotted cricket, *Gryllus bimaculatus*. Biosci. Biotechnol. Biochem. 82, 274–284. https://doi.org/10.1080/09168451.2017.1422106.

Zöllner, N., Kirsch, K., 1962. On the quantitative determination of lipids (micromethod) by means of the general sulfophosphovanillin reaction of the may natural lipids (all know plasmalipids). Z. Ges. Exp. Med. 135, 545–561. https://doi.org/10.1007/BF02045455.