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Identification and cardiotropic actions of brain/gut-derived tachykinin-related peptides (TRPs) from the American lobster *Homarus americanus*

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ABSTRACT

Two tachykinin-related peptides (TRPs) are known in decapods, APSGFLGMRamide and TPSGFLGMRamide. The former peptide appears to be ubiquitously conserved in members of this taxon, while the latter has been suggested to be a genus (*Cancer*)- or infraorder (Brachyura)-specific isoform. Here, we characterized a cDNA from the American lobster *Homarus americanus* (infraorder Astacidea) that encodes both TRPs: six copies of APSGFLGMRamide and one of TPSGFLGMRamide. Mass spectral analyses of the *H. americanus* supraoesophageal ganglion (brain) and commissural ganglia confirmed the presence of both peptides in these neural tissues; both isoforms were also detected in the midgut. Physiological experiments showed that both APSGFLGMRamide and TPSGFLGMRamide are cardioactive in *H. americanus*, eliciting identical increases in both heart contraction frequency and amplitude. Collectively, our data represent the first genetic confirmation of TRPs in *H. americanus* and of TPSGFLGMRamide in any species, demonstrate that TPSGFLGMRamide is not restricted to brachyurans, and show that both this peptide and APSGFLGMRamide are brain-gut isoforms, the first peptides thus far confirmed to possess this dual tissue distribution in *H. americanus*. Our data also suggest a possible role for TRPs in modulating the output of the lobster heart.

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1. Introduction

A large family of peptides sharing the carboxy (C)-terminal motif FX_1GX_2Ramide (where the Xs represent variable amino acids) has been identified both from the nervous system and from gut epithelial endocrine cells of invertebrates (for review see Nässel [12]). Given similarities in amino acid sequence, tissue distribution, chemical/conformational requirements for receptor interaction and physiological function to members of the vertebrate tachykinins, these invertebrate peptides are hypothesized to be evolutionarily related to this vertebrate peptide family, and have collectively been termed tachykinin-related peptides (TRPs) [11,12,18,25].

In insects, the presence of multiple species-specific TRP isoforms appears to be a common trait [12]. For example, 13 members of this peptide family have been identified from the cockroaches *Periplaneta americana* and *Leucophaea maderae* [15]. In contrast, a very different picture of TRPs has emerged for decapod crustaceans. Here, a single, ubiquitously conserved isoform, APSGFLGMRamide, has been proposed [20,27].

Recently, a second decapod TRP, TPSGFLGMRamide, was identified via mass spectral methods from both neural and gut endocrine tissues of several brachyuran species, specifically the crabs *Cancer borealis*, *Cancer irroratus*, *Cancer magister* and *Cancer productus* [21]. Since TPSGFLGMRamide has not been described from any other decapod species, nor is it encoded in any known decapod TRP precursor [27], it has been proposed that this isoform may represent a genus (*Cancer*)- or infraorder (Brachyura)-specific variant [21].

Here, we present data describing the identification and characterization of a TRP-encoding cDNA from the American lobster *Homarus americanus* (infraorder Astacidea). In addition to encoding multiple copies of APSGFLGMRamide, the prepro-hormone predicted from this cDNA also contains a single copy of TPSGFLGMRamide. Via mass spectral analyses, we show that both isoforms of TRP are detectable in the supraoesophageal ganglion (brain), commissural ganglia (CoGs) and midgut of this species. Both peptides were also found to be cardioactive. Thus, our results not only demonstrate that TPSGFLGMRamide may be a broadly-conserved decapod TRP, but also show that this isoform and APSGFLGMRamide are brain-gut peptides in *H. americanus*, the first peptides thus far found to exhibit this dual tissue distribution in the lobster. Our data also suggest a possible modulatory role for the TRPs in the control of cardiac output.

2. Materials and methods

2.1. Animals

American lobsters *H. americanus* were purchased from local (Maine) retailers and were maintained in either flow-through natural seawater tables at ambient water temperature (approximately 8–14 °C) or aerated natural seawater aquaria at 10 °C.

2.2. cDNA library construction, normalization, sequencing and EST submission

The construction and normalization of the *H. americanus* cDNA library used in our study are described in detail in Towle and Smith [24], as are the methods employed for expressed sequence tag (EST) sequencing and database submission.

2.3. cDNA sequence analysis

cDNA sequence analysis and sequence trace alignments were conducted using methods modified from Dickinson et al. [7]. In brief, a sample of *Escherichia coli* possessing the insert-containing vector was cultured overnight in LB medium at 37 °C, after which the cultured bacteria were streaked on LB-agar plates containing 100 μg/ml ampicillin and grown overnight at 37 °C. Single colonies were picked from the plates and re-cultured overnight in LB medium containing 100 μg/ml ampicillin at 37 °C to create working bacterial stocks. cDNA-containing plasmid was subsequently isolated from the bacterial stocks using a Purelink Quick Plasmid Miniprep kit (Invitrogen, Carlsbad, CA, USA). The cDNA insert was then sequenced on an ABI 3100 16-capillary sequencer (Applied Biosystems, Foster City, CA, USA) using a combination of vector- and insert-specific sequencing primers (see below). Sequence trace files were read with Chromas Lite (Technelysium Pty Ltd., Tewantin, Queensland, Australia) and 4Peaks software (http://mekentosj.com/4peaks/), and the high quality regions were aligned using ClustalW2 (European Bioinformatics Institute, Hinxton, Cambridge, United Kingdom; http://www.ebi.ac.uk/Tools/clustalw2/index.html).

2.4. Nucleotide translation and structural analysis of the deduced amino acid sequence

Translation of nucleotide sequences and analyses of deduced prepro-hormone structure and post-translational modification of peptides were done using several online programs, *i.e.*, Translate tool of ExPASy (Swiss Institute of Bioinformatics, Basel, Switzerland; http://www.expasy.ch/tools/dna.html), SignalP 3.0 (Center for Biological Sequence Analysis, Technical University of Denmark, Lyngby, Denmark; http://www.cbs.dtu.dk/services/SignalP/ [1]), and Sulfinator (Swiss Institute of Bioinformatics, Geneva, Switzerland; http://www.expasy.org/tools/sulfinator/ [9]), as well as information presented in Veenstra [26], and by homology to known crustacean TRP isoforms [5,21].

2.5. Matrix assisted laser desorption/ionization Fourier transform mass spectrometry (MALDI-FTMS)

For tissue collection, animals were anesthetized by packing in ice for 30–60 min, after which the dorsal carapace was removed, and the brain, the two CoGs, and the posterior midgut caecum were dissected from each animal in chilled (approximately 4 °C) physiological saline (composition in mM: 479.12 NaCl, 12.74 KCl, 13.67 $CaCl_2$, 20.00 $MgSO_4$, 3.91 Na_2SO_4 and 5.00 HEPES; pH 7.45). The instrumentation and sample preparation techniques used for direct tissue MALDI-FTMS analyses are described in detail in Stemmler et al. [21]. For brain samples we most commonly analyzed cell cluster 6, which is located in the anterior protocerebrum (nomenclature as per Sandeman et al. [17]). Exact mass measurements were made using the internal calibration on adjacent samples (InCAS) technique [13], modified to include the accumulation