Published in final edited form as:
Gen Comp Endocrinol. 2008 April ; 156(2): 395–409.

Mass spectral characterization of peptide transmitters/hormones in the nervous system and neuroendocrine organs of the American lobster *Homarus americanus*

Mingming Ma[1], **Ruibing Chen**[2], **Gregory L. Sousa**[3], **Eleanor K. Bors**[3], **Molly Kwiatkowski**[3], **Christopher C. Goiney**[4], **Michael F. Goy**[5], **Andrew E. Christie**[3,4], and **Lingjun Li**[1,2*]

1*School of Pharmacy, University of Wisconsin, 777 Highland Avenue, Madison, Wisconsin 53705-2222 USA*

2*Department of Chemistry, University of Wisconsin, 1101 University Avenue, Madison, Wisconsin 53706-1396 USA*

3*Mount Desert Island Biological Laboratory, P.O. Box 35, Old Bar Harbor Road, Salisbury Cove, Maine 04672 USA*

4*Department of Biology, University of Washington, Box 351800, Seattle, Washington 98195-1800 USA*

5*Department of Cell and Molecular Physiology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599 USA*

Abstract

The American lobster *Homarus americanus* is a decapod crustacean with both high economic and scientific importance. To facilitate physiological investigations of peptide transmitter/hormone function in this species, we have used matrix-assisted laser desorption/ionization Fourier transform mass spectrometry (MALDI-FTMS), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS) and nanoscale liquid chromatography coupled to electrospray ionization quadrupole time-of-flight tandem mass spectrometry (nanoLC-ESI-Q-TOF-MS/MS) to elucidate the peptidome present in its nervous system and neuroendocrine organs. In total, 84 peptides were identified, including 27 previously known *H. americanus* peptides (*e.g.* VYRKPPFNGSIFamide [Val^1-SIFamide]), 23 peptides characterized previously from other decapods, but new to the American lobster (*e.g.* pQTFQYSRGWTNamide [Arg^7-corazonin]), and 34 new peptides *de novo* sequenced/detected for the first time in this study. Of particular note are a novel B-type allatostatin (TNWNKFQGSWamide) and several novel FMRFamide-related peptides, including an unsulfated analog of sulfakinin (GGGEYDDYGHLRFamide), two myosuppressins (QDLDHVFLRFamide and pQDLDHVFLRFamide), and a collection of short neuropeptide F isoforms (*e.g.* DTSTPALRLRFamide, and FEPSLRLRFamide). Our data also include the first detection of multiple tachykinin-related peptides in a non-brachyuran decapod, as well as the identification of potential individual-specific variants of orcokinin and orcomyotropin-related peptide. Taken collectively, our results not only expand greatly the number of known *H. americanus* neuropeptides, but also provide a framework for future studies on the physiological roles played by these molecules in this commercially and scientifically important species.

*Correspondence to: Dr. Lingjun Li, School of Pharmacy, University of Wisconsin, 777 Highland Avenue, Madison, Wisconsin 53705-2222 USA; Phone: 608-265-8491; Fax: 608-262-5345; Email: lli@pharmacy.wisc.edu.

Keywords

Homarus americanus; matrix-assisted laser desorption/ionization-Fourier transform mass spectrometry (MALDI-FTMS); electrospray ionization quadrupole time-of-flight tandem mass spectrometry (ESI-Q-TOF MS/MS); matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS); neuropeptide; neurohormone; peptide sequencing; supraoesophageal ganglion; suboesophageal ganglion; thoracic ganglia; abdominal ganglia; eyestalk ganglia (ESG); stomatogastric ganglion (STG); sinus gland (SG); pericardial organ (PO); ventral nerve cord (VNC)

1. Introduction

The American lobster *Homarus americanus* provides a useful model organism in many areas of physiology, including the study of neuroendocrine control/modulation of behavior. For example, this species has long served as a model for studies of hormonal control of aggression (Kravitz, 1988; Huber et al., 1997; Kravitz 2000). Likewise the neural circuits contained within the *H. americanus* cardiac ganglion and stomatogastric nervous system (STNS) have served as important models for the study of modulatory control of rhythmic behavior, specifically cardiac output and the swallowing, chewing and filtering of food items, respectively (Cooke, 2002; Clarac and Pearlstein, 2007; Marder and Bucher, 2007).

One surprising limitation, given the commercial and biological importance of *H. americanus*, is the lack of a complete catalog of the signaling molecules that lobsters use to affect their behavior, particularly their complement of neuropeptides. In fact, only about two-dozen neuropeptides have been fully characterized from this species (*e.g.* Schwarz et al., 1984; Trimmer et al., 1987; Chang et al., 1990; Tensen et al., 1991a; Tensen et al., 1991b; Tensen et al., 1991c; Li et al., 2002; Skiebe et al., 2003; Stemmler et al., 2005; Fu et al., 2005a; Christie et al., 2006; Stemmler et al., 2006; Dickinson et al., 2007a; Dickinson et al., 2007b; Dickinson et al., 2007c; Stemmler et al., 2007a). Since complete knowledge of the neuromodulators/hormones present in an organism is necessary for an accurate assessment of its behavioral control mechanisms, we have undertaken a mass spectral characterization of the peptide transmitters/hormones present in the *H. americanus* nervous system using a combination of matrix-assisted laser desorption/ionization Fourier transform mass spectrometry (MALDI-FTMS), matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and nanoscale liquid chromatography coupled to electrospray ionization quadrupole time-of-flight tandem mass spectrometry (nanoLC-ESI-Q-TOF-MS/MS). This combined approach identified 84 neuropeptides, including 27 known *H. americanus* peptides, 23 peptides that were known previously from other arthropods, but new to the American lobster, and 34 peptides sequenced/detected here for the first time. Some of these data have appeared previously in abstract form (Li et al., 2007).

2. Materials and methods

2.1. Animals

American lobsters, *H. americanus*, were purchased from Downeast Lobster Pound (Trenton, ME) or Commercial Lobster and Seafood Company (Boston, MA) and were maintained in flow-through natural seawater aquaria at ambient seawater temperature (approximately 8–12 °C) or recirculating artificial seawater aquaria at 10–14°C. In total, approximately 150 individuals, including both males and females, were used in this study.

2.2. Tissue collection

All animals were anesthetized by packing them in ice for 30–60 minutes, after which the dorsal carapace was removed from each individual and its CNS (eyestalk ganglia [ESG], supraoesophageal ganglia [brain] and ventral nerve cord [VNC; consisting of the suboesophageal, thoracic and abdominal ganglia]), neuroendocrine tissues (sinus gland [SG] and pericardial organ [PO]), and/or stomatogastric ganglion (STG) dissected free from surrounding muscle and connective tissues in chilled (approximately 10 °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, 5.00 HEPES [pH 7.4]). Following dissection, tissue samples were either immediately assayed or placed in acidified methanol (90% methanol [Fisher Scientific, Pittsburgh, PA]: 9% glacial acetic acid [Fisher]: 1% deionized water) and stored at −80°C until utilized for peptide extraction or direct tissue mass spectral analysis.

2.3. Tissue extraction and HPLC fractionation

For some experiments, tissues were pooled, homogenized, and extracted with acidified methanol (see 2.2). Extracts were dried in a Savant SC 110 SpeedVac concentrator (Thermo Electron Corporation, West Palm Beach, FL) and re-suspended in approximately 100 µl of 0.1% formic acid. The re-suspended extracts were then vortexed and briefly centrifuged. The resulting supernates were subsequently fractionated via high performance liquid chromatography (HPLC).

HPLC separations were performed using a Rainin Dynamax HPLC system (Rainin Instrument Inc., Woburn, MA) equipped with a Dynamax UV-D II absorbance detector. The mobile phases were: deionized water containing 0.1% formic acid (Solution A), and acetonitrile (HPLC grade, Fisher Scientific) containing 0.1% formic acid (Solution B). For each separation, 20 µl of extract was injected onto a Macrosphere C18 column (2.1 mm i.d. × 250 mm length, 5 µm particle size; Alltech Assoc. Inc., Deerfield, IL). The separation consisted of a 120 minute gradient of 5%–95% Solution B with fractions automatically collected every two minutes using a Rainin Dynamax FC-4 fraction collector.

2.4. Formaldehyde derivatization

For some experiments, peptides in HPLC fractions were derivatized with formaldehyde prior to mass spectral analysis. Specifically, 0.3 µl of a given fraction was spotted on the MALDI plate, followed by the addition and mixing of 0.3 µl of 26 mM sodium cyanoborohydride (Sigma-Aldrich, St. Louis, MO), and subsequent addition of 0.3 µl of formaldehyde (20% in H_2O vol/vol, Sigma-Aldrich). The droplet was left at room temperature for 5 minutes, after which 0.3 µl of 50 mM ammonium bicarbonate solution was added to the reaction mixture. Finally, 0.3 µl of a saturated 2,5-dihydroxybenzoic acid (DHB; ICN Biomedical Corp., Costa Mesa, CA) matrix (150 mg/ml in a 50:50 v/v mixture of deionized water and purge and trap grade methanol [Fisher Scientific]) was added to the droplet and crystallized at room temperature.

2.5. MALDI-FTMS and sustained off resonance irradiation-collision induced dissociation

MALDI-FTMS experiments were performed on an IonSpec ProMALDI Fourier transform mass spectrometer (Lake Forest, CA) equipped with a 7.0 Tesla actively-shielded superconducting magnet. This FTMS instrument contains a high pressure MALDI source where ions from multiple laser shots can be accumulated in the external hexapole storage trap before the ions are transferred to the ICR cell via a quadrupole ion guide. A 337 nm nitrogen laser (Laser Science, Inc., Franklin, MA) was used for ionization/desorption. The ions were excited prior to detection with an rf sweep beginning at 7050 ms with a width of 4 ms and amplitude of 150 V base to peak. The filament and quadrupole trapping plates were initialized

to 15 V, and both were ramped to 1V from 6500 to 7000 ms to reduce baseline distortion of peaks. Detection was performed in broadband mode from *m/z* 108.00 to 4500.00.

Peptide fragmentation was accomplished by sustained off resonance irradiation-collision induced dissociation (SORI-CID). An arbitrary waveform from 2000 ms to 2131 ms with a ±10 Da isolation window was introduced to isolate the ion of interest. Ions were excited with SORI Burst excitation (2.648V, 2500–3000 ms). A pulse of nitrogen gas was introduced through a pulse valve from 2500 to 2750 ms to introduce collision activation.

For direct tissue analysis, tissue fragments were desalted by briefly rinsing in a solution of DHB prepared in deionized water (10 mg/ml). The tissue was then placed onto the MALDI sample plate along with 0.3 μl of saturated DHB matrix (prepared as described in 2.4) before allowing the DHB spot to crystallize at room temperature.

Off-line analysis of HPLC fractions (see 2.3) was performed by spotting 0.3 μl of saturated DHB on the MALDI sample plate and adding 0.3 μl of the HPLC fraction of interest. The resulting mixture was allowed to crystallize at room temperature. The MALDI-FTMS analysis was then performed as described above.

2.6. MALDI-TOF MS

MALDI-TOF mass spectra were obtained using a Voyager DE STR (Applied Biosystems, Framingham, MA) time-of-flight mass spectrometer equipped with delayed ion extraction. A pulsed nitrogen laser (337 nm) was used as the desorption/ionization source, and positive-ion mass spectra were acquired using both linear and reflectron modes. Each representative mass spectrum shown is the smoothed average of 128–256 laser pulses. Mass calibration was performed externally using a mixture of synthetic peptide standards (angiotensin II and bovine insulin, Sigma-Aldrich, St. Louis, MO). Mass accuracy was better than 0.01%.

2.7. Capillary LC-ESI-Q-TOF MS/MS

Nanoscale LC-ESI-Q-TOF MS/MS was performed using a Waters capillary LC system coupled to a Q-TOF Micro mass spectrometer (Waters Corp., Milford, MA). Chromatographic separations were performed on a C18 reverse phase capillary column (75 μm internal diameter ×150 mm length, 3 μm particle size; Micro-Tech Scientific Inc., Vista, CA). The mobile phases used were: deionized water with 5% acetonitrile and 0.1% formic acid (A); acetonitrile with 5% deionized water and 0.1% formic acid (B); deionized water with 0.1% formic acid (C). An aliquot of 6.0 μl of an HPLC fraction (see 2.3) was injected and loaded onto the trap column (PepMap™ C18; 300 μm column internal diameter × 1 mm, 5 μm particle size; LC Packings, Sunnyvale, CA, USA) using mobile phase C at a flow rate of 30 μl/min for 3 minutes. Following this, the stream select module was switched to position the trap column in line with the analytical capillary column, and a linear gradient of mobile phases A and B was initiated. A splitter was added between the mobile phase mixer and the stream select module to reduce the flow rate from 15 μl/min to 200 nl/min.

The nanoflow ESI source conditions were set as follows: capillary voltage 3200 V, sample cone voltage 35 V, extraction cone voltage 1 V, source temperature 120°C, cone gas (N_2) 10 l/hr. Data dependent acquisition was employed for the MS survey scan and the selection of precursor ions and subsequent MS/MS of the selected parent ions. The MS scan range was from *m/z* 300–2000 and the MS/MS scan was from *m/z* 50–1800. The MS/MS *de novo* sequencing was performed with a combination of manual sequencing and automatic sequencing by PepSeq software (Waters Corp.).

2.8. Figure production

MALDI-FTMS figures were produced by converting the spectra obtained using IonSpec version 7.0 software (IonSpec Corp.) to a bitmap image using Boston University Data Analysis (BUDA) software (version 1.4; Boston University, Boston, MA). The BUDA files were then pasted into Fireworks MX 2004 (Macromedia, Inc., San Francisco, CA) and resampled to improve the resolution. All MS/MS figures were produced using a combination of Fireworks MX 2004 and Microsoft Windows paint tool (Microsoft Corporation, Redmond, WA).

3. Results

3.1. Strategy for the mass spectral analysis of peptides in the nervous system of *H. americanus*

The characterization of a neuropeptidome is inherently difficult due to its extreme chemical complexity and the often-minute amounts of the expressed peptides. Here, we have used a strategy combining MALDI-based high resolution mass profiling (direct tissue and off-line HPLC fraction analysis; Fig. 1) and nanoscale biochemical separation/derivatization coupled to tandem mass spectrometric sequencing (Fig. 2–Fig. 4) to characterize the peptide complement of the *H. americanus* nervous system. Our analyses include all of the major components of the ventral nerve cord, as well as the stomatogastric ganglion and two prominent neurosecretory organs (the sinus gland and the pericardial organ). For ease of later discussion, we have grouped the identified peptides into families of related isoforms, whenever possible, and these are presented below in alphabetical order based on family name. Novel peptides for which there are no known families are presented at the end of the Results section. The tissues that express each identified peptide are given in Table 1. It is important to note that the methodologies used in this study do not differentiate between isobaric amino acids, e.g. isoleucine and leucine, and thus for some peptide sequences, e.g. most of the novel FMRF-related peptides, the assigned amino acid(s) were predicted and assigned based on homology to related family members. Thus, it is possible that some leucine residues have been mis-assigned, and care should be taken by readers in interpreting these sequences until they are confirmed biochemically and/or genetically.

3.2. A-type allatostatins

Ten peptides possessing -Y*X*FGLamide C-termini (where *X* is a variable amino acid) were sequenced via ESI-Q-TOF MS/MS from the brain of *H. americanus* (Table 1). This C-terminus classifies these peptides as members of the A-type allatostatin (A-type AST) family (Stay and Tobe, 2007). Among these peptides, EPYAFGLamide, SPYAFGLamide, SGPYAFGLamide, ASPYAFGLamide, AGPYAFGLamide, TPSYAFGLamide, SQYTFGLamide, and AGGAYSFGLamide are identical in structure to previously identified A-type ASTs from the crabs *Carcinus maenas* and *Cancer productus* or the shrimp *Penaeus monodon* (Duve et al., 1997;Duve et al., 2002;Fu et al., 2005b), but are new to the American lobster. The remaining two isoforms, SGPYSFGLamide and VGPYAFGLamide, are described here for the first time.

Outside of the brain, AGPYAFGLamide was also found in the VNC and SGPYAFGLamide, ASPYAFGLamide and TPSYAFGLamide were detected in the STG (Table 1).

3.3. B-type allatostatins

A novel peptide possessing the C-terminal motif -W(X)$_6$ Wamide (*X* indicating variable amino acids), which is the hallmark of the B-type allatostatin (B-type AST) family (Stay and Tobe, 2007), was sequenced via ESI-Q-TOF MS/MS from the brain of *H. americanus* (Fig. 2 and Table 1). The newly characterized B-type AST is TNWNKFQGSWamide, whose C-terminus -WNKFQGSWamide is identical to that of two previously identified *Cancer productus* B-type

allatostatins, *i.e.* GNWNKFQGSWamide and NWNKFQGSWamide (Fu et al., 2005b). This peptide was also detected via direct tissue/off line HPLC fraction MALDI-FTMS analysis of brain tissue/extract (Fig. 1), and was detected in both the PO and STG using direct tissue MALDI-TOF MS (Table 1).

It should be noted that the initial MS/MS *de novo* sequencing of TNWNKFQGSWamide revealed ambiguity at the N-terminus, which could have been either TN- or SK-. This sequence discrepancy was resolved using a combination of chemical derivatization and MALDI-FTMS internal calibration. Specifically, upon formaldehyde labeling, a 56 Da mass shift was observed, which indicated that there are two primary amine groups in the peptide, thus suggesting a TN-rather than SK- N-terminus. An N-terminus of TN- rather than SK- was further substantiated by the internal calibration of MALDI-FTMS spectra, which showed mass measurement accuracies (MMAs) of 4.3 versus 24.4 ppm, respectively, for the two possible termini. Thus, TNWNKFQGSWamide, rather than SKWNKFQGSWamide, was derived as the correct sequence.

3.4. Corazonins

The peptide pQTFQYSRGWTNamide, commonly referred to as Arg^7-corazonin (Veenstra, 1989), was identified via direct tissue MALDI-FTMS analysis of both brain and eyestalk ganglia tissue fragments (Table 1). Internal calibration of the spectra containing this peptide showed MMA of approximately 1.1 ppm, which strongly supported this attribution. While Arg^7-corazonin has been identified previously from the PO of the crab *Cancer borealis* (Li et al., 2003), here we report the first detection of this peptide in *H. americanus*.

3.5. Crustacean cardioactive peptide

The peptide PFCNAFTGCamide, commonly referred to as crustacean cardioactive peptide or CCAP (Stangier et al., 1987), was sequenced from the brain of *H. americanus* via ESI-Q-TOF MS/MS (Table 1). This peptide was also detected in the brain via direct tissue/off-line HPLC fraction MALDI-FTMS analysis and in the PO via direct tissue MALDI-TOF MS (Table 1). While authentic CCAP has been detected/predicted in the nervous systems of a number of decapod species (Stangier et al., 1987; Chung et al., 2006), and has been shown to be capable of modulating neural output in the STNS of *H. americanus* (Richards and Marder, 2000), this study reports the first direct demonstration that this peptide is present in authentic form in the American lobster.

3.6. CCAP precursor-related peptides

In addition to CCAP itself, another peptide, DIGDLLEGKD, was *de novo* sequenced from the *H. americanus* brain and VNC via ESI-Q-TOF MS/MS (Table 1). This peptide is identical to one predicted from the European lobster *Homarus gammarus* prepro-hormone encoding CCAP (Chung et al., 2006), but is shown to exist for the first time here.

3.7. Crustacean hyperglycemic hormone (CHH) superfamily members

In decapods, the crustacean hyperglycemic hormone (CHH) superfamily consists of a large group of structurally-related 70+ amino acid peptides that possess six conserved cysteine residues which form three characteristic disulfide bridges (Fanjul-Moles, 2006). In addition to the CHHs proper, this superfamily also encompasses moult-inhibiting hormones (MIHs), gonad-inhibiting hormones (GIHs), vitellogenesis-inhibiting hormones (VIHs) and mandibular organ-inhibiting hormones (MOIHs). In *H. americanus*, multiple CHH superfamily members have been identified/predicted previously, including several isoforms of CHH (Tensen et al., 1991a; Tensen et al., 1991b; de Kleijn et al., 1995), an isoform of MIH (Chang et al., 1990) and an isoform of GIH (de Kleijn et al., 1994). Here, via direct tissue

MALDI-FTMS peptides with mass similar to that of *H. americanus* CHH A and CHH B (Tensen et al., 1991a) were detected in the SG with average mass errors of 0.01% and 0.02% (Table 1). CHH A isoform was also detected in the PO and STG using MALDI-TOF. Likewise, a peptide with a mass corresponding to that of *H. americanus* moult-inhibiting hormone MIH (Chang et al., 1990) was detected in the STG via direct tissue MALDI-TOF with an average mass error of 0.01% (Table 1).

3.8. CHH precursor-related peptides (CPRPs)

In addition to encoding an isoform of CHH, all CHH precursor proteins also contain an isoform of a second peptide, crustacean hyperglycemic hormone precursor-related peptide or CPRP (Fanjul-Moles, 2006). To date, numerous CPRPs have been identified from decapod species, including several isoforms from *H. americanus* (Tensen et al., 1991a; Tensen et al., 1991c; Fu et al., 2005a). Here, two of the previously identified *H. americanus* CPRPs, RSVEGASRMEKLLSSSNSPSSTPLGFLSQDHSVN and RSVEGVSRMEKLLSSISPSSTPLGFLSQDHSVN (Tensen et al., 1991a; Tensen et al., 1991c; Fu et al., 2005a), were sequenced via ESI-Q-TOF MS/MS and direct tissue MALDI-FTMS analysis from both the SG and eyestalk ganglia (Table 1). The former peptide was also detected in the PO via MALDI-TOF MS (Table 1). In addition to these full-length CPRPs, ten truncations were also sequenced from the SG via ESI-Q-TOF MS/MS (Table 1): PLGFLSQDHSV, PLGFLSQDHSVN, RSVEGVSRME, RSVEGASRMEKL, RSVEGASRMEKLL, RSVEGASRMEKLLS, RSVEGVSRMEKLLS, RSVEGASRMEKLLSS, RSVEGASRMEKLLT and RSVEGVSRMEKLLT, the former eight peptides being previously identified from the *H. americanus* SG (Fu et al., 2005a), with the latter two being identified here for the first time.

3.9. FMRFamide-related peptides

The FMRFamide family is a large and diverse grouping of peptides found in both invertebrates and vertebrates (Zajac and Mollereau, 2006). In arthropods many subfamilies have been identified, including the sulfakinins, the myosuppressins and the neuropeptide Fs (Brown et al., 1999; Nichols, 2003; Garczynski et al., 2006). In our study, 19 FMRFamide-related peptides were sequenced/detected from *H. americanus* neural tissues using a combination of ESI-Q-TOF MS/MS sequencing, direct tissue/off line HPLC fraction MALDI-FTMS analysis and/or direct tissue MALDI-TOF MS (Table 1). One peptide, GGGEYDDYGHLRFamide, possessed the C-terminal motif -YGHM/LRFamide, which clearly places it within the sulfakinin subfamily. This peptide was previously predicted from an *H. americanus* prepro-sulfakinin cDNA (Dickinson et al., 2007c), but is shown to exist for the first time here (Table 1). Two *de novo* sequenced peptides, QDLDHVFLRFamide (Fig. 1 and Fig. 3) and pQDLDHVFLRFamide, possess the C-terminal motif -HVFLRFamide, which places them into the myosuppressin subfamily (Table 1). The presence of the N-terminal pyroglutamine residue in the latter isoform was confirmed by formaldehyde labeling, where no mass shift was observed for the peptide after derivatization. Six peptides, SMPSLRLRFamide, SM(O) PSLRLRFamide (where M(O) represents an oxidized methionine residue), GPPSLRLRFamide, PSLRLRFamide, FEPSLRLRFamide and DTSTPALRLRFamide (Fig. 3), exhibit -R*X*RFamide C-termini (where *X* represents a variable residue), which places them into the short neuropeptide F (NPF) subfamily. This subfamily has been proposed to be the invertebrate homolog of the vertebrate neuropeptide Ys (McVeigh et al., 2005). All of the *H. americanus* NPFs are novel to the species, with all but SMPSLRLRFamide, which was identified previously in both the shrimp *Penaeus monodon* and the crab *Cancer borealis* (Sithigorngul et al., 2002; Huybrechts et al., 2003), being sequenced here for the first time. Of the remaining 10 peptides, seven possess C-terminal sequence -NFLRFamide, with the remaining three exhibiting -YLRFamide C-termini. Four of the -NFLRFamide isoforms, GGRNFLRFamide, GNRNFLRFamide, GDRNFLRFamide and APQRNFLRFamide, were

identified previously from the PO of the crab *Cancer productus* (Fu et al., 2005b), but are described for the first time here from *H. americanus*. Three other NFLRFamide-containing peptides, SGRNFLRFamide (Fig. 1 and Fig. 3), DQNRNFLRFamide and NFLRFamide were *de novo* sequenced for the first time from any crustacean species. Among the identified -YLRFamide, SDRNYLRFamide and GYSDRNYLRFamide were novel peptides sequenced in this study, while GAHKNYLRFamide has been previously described from several species of *Cancer* crabs (Cruz-Bermudez et al., 2006), but is new to *H. americanus*.

3.10. Orcokinins

Four full-length orcokinins, NFDEIDRSSFGFN, NFDEIDRSGFGFV (Fig. 1), NFDEIDRSGFGFN (Fig. 1) and NFDEIDRSGFGFH (Fig. 1), and four putative orcokinin truncations, NFDEIDRSGF, NFDEIDRSGFG (Fig. 1), NFDEIDRSGFGF (Fig. 1), and NFDEIDRSGFA (Fig. 1), were characterized from *H. americanus* neural tissues via ESI-Q-TOF MS/MS, direct tissue/off line HPLC fraction MALDI-FTMS analysis and/or direct tissue MALDI-TOF MS (Table 1). Each of these peptides has been described previously from crustacean neural tissues (Yasuda-Kamatani et al., 2000; Fu et al., 2005), with all but NFDEIDRSSFGFN and NFDEIDRSGFA identified previously from *H. americanus* (Li et al., 2002;Skiebe et al., 2003;Stemmler et al., 2005;Dickinson et al., 2007a). In addition, a novel amidated truncation, NFDEIDRSGFamide, was *de novo* sequenced via ESI-Q-TOF MS/MS from the brain, VNC and SG (Table 1). This peptide was also detected via MALDI-TOF MS in the STG (Table 1).

3.11. Orcokinin-precursor-related peptides

Simultaneous with our study, several cDNAs encoding *H. americanus* prepro-orcokinins were identified and characterized (Dickinson et al., 2007a). In addition to the full-length orcokinin isoforms NFDEIDRSGFGFV, NFDEIDRSGFGFN and NFDEIDRTGFGFH, several other peptides were predicted from the encoded prepro-hormones, including the orcokinin-like peptide SSEDMDRLGFGFN, and the orcomyotropin-related peptide FDAFTTGFGHN, as well as the five additional peptides which bear no sequence homology to any known peptide family: APARSSPQQDAAGYTDGAPV (encoded within prepro-orcokinin I), GPIKVRFLSAIFIPIAAPARSSPQQDAAAGYTDGAPV (encoded within prepro-orcokinin II), VYGPRDIANLY, GDYDVYPE, SAE (Dickinson et al., 2007a). Direct tissue MALDI-FTMS analysis showed that all of these peptides, save APARSSPQQDAAGYTDGAPV, GPIKVRFLSAIFIPIAAPARSSPQQDAAAGYTDGAPV and SAE, are detectable in *H. americanus* neural tissues (Dickinson et al., 2007a).

In our study, ESI-Q-TOF MS/MS sequencing, direct tissue/off line HPLC fraction MALDI-FTMS analysis and/or MALDI-TOF MS identified SSEDMDRLGFGFN in the brain (Fig. 1), VNC, eyestalk ganglia, sinus gland and STG (Table 1). Moreover, a novel methionine oxidized form of this peptide, SSEDM(O)DRLGFGFN was *de novo* sequenced via ESI-Q-TOF MS/MS from both the brain and VNC (Table 1). In addition, two related, putative truncations, SSEDMDRLGFG and SSEDMDRLGFA, were also identified by ESI-Q-TOF MS/MS sequencing, direct tissue/off line HPLC fraction MALDI-FTMS analysis and/or MALDI-TOF MS from the brain, VNC and SG; the former peptide was described previously from *H. americanus* (Fu et al., 2005a), however the latter peptide was *de novo* sequenced here as a new peptide. SSEDMDRLGFG was also detected in the STG via direct tissue MALDI-TOF MS (Table 1).

The peptide FDAFTTGFamide, described previously from crayfish *Orconectes limosus* (Dircksen et al., 2000) and named orcomyotropin, was also sequenced from the brain of *H. americanus* via ESI-Q-TOF MS/MS (Table 1), which was the first identification of this peptide from the American lobster. In addition, FDAFTTGFGHN, was identified in the brain, VNC,

SG and STG via ESI-Q-TOF MS/MS sequencing, direct tissue/off line HPLC fraction MALDI-FTMS analysis (Fig. 1) and/or MALDI-TOF MS (Table 1). Interestingly, a second isoform of orcomyotropin-related peptide, FDAFTTGFGHS, was also detected in our study, specifically in the brain and eyestalk ganglia via direct tissue/off line HPLC fraction MALDI-FTMS analysis (Fig. 1 and Table 1). This peptide is identical in sequence to an isoform described previously from numerous brachyuran species, one thalassinidean, and the crayfish *Cherax quadricarinatus* and *Pacifastacus leniusculus* (Skiebe et al., 2003), but is described here from *H. americanus* for the first time.

The orcokinin precursor-related peptide VYGPRDIANLY was identified in the brain, VNC, SG and STG via ESI-Q-TOF MS/MS sequencing (Fig. 4), direct tissue/off line HPLC fraction MALDI-FTMS analysis and/or MALDI-TOF MS (Table 1). This peptide is described for the first time simultaneously in our study and by Dickinson et al. (2007a). It should be noted that the identities of isoleucine and leucine, as reported here, were determined based on knowledge of orcokinin prepro-hormone sequence described in Dickinson et al. (2007a).

3.12. Pigment dispersing hormones

The peptide NSELINSILGLPKVMNDAamide, commonly known as β-pigment dispersing hormone (β-PDH; Rao et al., 1985), was sequenced via ESI QTOF MS/MS from both the SG and brain (Table 1). NSELINSILGLPKVMNDAamide is a previously known *H. americanus* peptide (Fu et al., 2005a).

3.13. Proctolin

The pentapeptide RYLPT, commonly referred to as proctolin (Brown, 1975; Starratt and Brown, 1975), was dectected in the PO of *H. americanus* via direct tissue MALDI-TOF MS (Table 1). The presence of RYLPT in the American lobster nervous system has been described previously (Schwarz et al., 1984).

3.14. Pyrokinins

Recently, peptides possessing the C-terminal motif -FXPRLamide (where *X* is a variable amino acid) were identified from the penaeid shimp *Penaeus vannamei* and the crab *Cancer borealis* (Torfs et al., 2001; Saideman et al., 2007). This sequence places them in the pyrokinin/(PBAN) family of peptides (Torfs et al., 2001). Here, we have *de novo* sequenced the pyrokinin FSPRLamide using ESI-Q-TOF MS/MS from both the brain and VNC of *H. americanus* (Table 1).

3.15. Red pigment concentrating hormone

The peptide pQLNFSPGWamide, commonly known as red pigment concentrating hormone or RPCH (Fernlund, 1974), was sequenced via ESI-Q-TOF MS/MS from the SG of *H. americanus* (Table 1). The presence of RPCH in the *H. americanus* nervous system has been described previously (Jaffe et al., 1984; Fu et al., 2005a; Stemmler et al., 2006).

3.16. SIFamides

The peptide VYRKPPFNGSIFamide (Val^1-SIFamide; Christie et al., 2006) was identified via ESI-Q-TOF MS/MS and/or direct tissue/offline HPLC fraction MALDI-FTMS analysis (Fig. 1) from the brain, VNC and eyestalk ganglia of *H. americanus* (Table 1). This peptide was also detected via MALDI-TOF MS in the STG of this species (Table 1). This isoform of SIFamide is a known *H. americanus* variant (Christie et al., 2006). In addition to this full-length isoform, the putative truncation RKPPFNGSIFamide was *de novo* sequenced via ESI-Q-TOF MS/MS from the brain (Table 1).

3.17. Tachykinin-related peptides

Two full-length tachykinin-related peptide (TRP) isoforms, APSGFLGMRamide and TPSGFLGMRamide, their methionine oxidized forms APSGFLGM(O)Ramide and TPSGFLGM(O)Ramide, and two putative truncated forms, PSGFLGMRamide and SGFLGMRamide, were sequenced via ESI-Q-TOF MS/MS from the brain and VNC of *H. americanus* (Table 1). Likewise, the putative precursor of APSGFLGMRamide, APSGFLGMRG, was sequenced from both tissues via ESI-Q-TOF MS/MS (Table 1). APSGFLGMRamide was also sequenced from the SG via ESI-Q-TOF MS/MS and was detected via direct tissue MALDI-TOF MS in the STG (Table 1). APSGFLGMRamide, TPSGFLGMRamide, APSGFLGMRG, and SGFLGMRamide are previously described crustacean peptides (Christie et al., 1997;Stemmler et al., 2007b), though all except APSGFLGMRamide (Stemmler et al., 2007a) are described here from the lobster for the first time. PSGFLGMRamide and the methionine oxidized isoforms APSGFLGM(O)Ramide and TPSGFLGM(O)Ramide were *de novo* sequenced.

3.18. Other peptides

In addition to the above mentioned peptides, six peptides that do not fit into any known peptide family were *de novo* sequenced via ESI-Q-TOF MS/MS from the brain of *H. americanus*: DLPKVDTALK, KPKTEKK, AVLLPKKTEKK, EVEEPEAPAPPAK, LRVAPEEHPVLL and GPSGGFNGALAR (Table 1). DLPKVDTALK was also sequenced from the VNC via this technique (Table 1).

4. Discussion

4.1. Peptide discovery in *H. americanus* using high-resolution mass profiling and tandem mass spectrometric sequencing

The American lobster *H. americanus* is arguably one of the most important decapod species, given its combined economic and scientific significance. As investigations of peptidergic control of behavior are among the major uses of this species scientifically, and are of critical importance for understanding the physiological control of this species in terms of its fishery, it is significant to note that little work has focused on identifying the lobster neuropeptidome prior to this and several contemporaneous molecular studies (Dickinson et al., 2007a; Dickinson et al., 2007b; Dickinson 2007c). Here, we have used a combination of mass spectral techniques to identify the peptide complement present in the nervous system and neuroendocrine organs of *H. americanus*, complementing and augmenting the ongoing molecular analyses.

In our study, neuropeptides were identified using a strategy combining MALDI-based high resolution mass profiling (direct tissue and off-line HPLC analysis) and nanoscale biochemical separation/derivatization coupled to tandem mass spectrometric sequencing. Specifically, the highly sensitive and accurate mass measurements provided by MALDI-FTMS and MALDI-TOF-MS (both performed with internal calibration) were used to identify known peptides based on predicted *m/z*, while the sequencing power of nanoLC-ESI-Q-TOF MS/MS was exploited to confirm the identity of the known peptides and to *de novo* sequence novel ones. This combined approach resulted in the identification of 84 peptides from 17 peptide families in the lobster nervous system, including 27 previously known *H. americanus* peptides (*e.g.* β-PDH [Fu et al., 2005a], proctolin [Schwarz et al., 1984] and Val^1-SIFamide [Christie et al., 2006]), 23 peptides identified in other species, but new to the American lobster (*e.g.* Arg^7-corazonin [Li et al., 2003] and crustacean cardioactive peptide [Stangier et al., 1987; Chung et al., 2006]), and 34 new peptides that were *de novo* sequenced/detected for the first time (*e.g.* the pyrokinin isoform FSPRLamide). The truncated forms and the methionine oxidized forms were also included. The origin of these peptides is unknown. It could be either enzymatic processing

product or due to sample preparation artifact. Collectively, these data have nearly tripled the number of fully characterized *H. americanus* neuropeptides, and thus provide a stronger framework for future investigations of the physiological roles these molecules play in this species. Moreover, in combination with several recently constructed *H. americanus* cDNA libraries with ESTs (*i.e.* Stepanyan et al., 2006; Towle and Smith, 2006), these data will provide a strong foundation for future peptide discovery, as well as studies directed at the expression and regulation of peptide modulators/hormones in the American lobster.

4.2. Most, but not all, known *H. americanus* peptides were detected using combined mass spectral analyses

As stated earlier, approximately two dozen neuropeptides had been characterized from *H. americanus* prior to our study, and the majority of those were re-identified here. Interestingly, however, several well-known lobster peptides were not detected in our study, including the FMRFamide-related peptides TNRNFLRFamide and SDRNFLRFamide (Trimmer et al., 1987). The lack of detection of these two peptides is of particular note as members of the FMRFamide family were by far the largest single group of peptides identified in our study (19 in total), and the tissues assayed included those used for the original biochemical isolation and subsequent sequence analysis of both peptides.

The reason for the lack of detection of TNRNFLRFamide and SDRNFLRFamide in our study remains unknown. It is possible that the different approaches to peptide discovery used here versus those employed by Trimmer et al (1987) may be at play; however both peptides possess structures that should make them readily ionizable and detectable via the MS methods we used, and both freshly dissected tissues and tissue extracts were assayed. Moreover, FMRFamide-related peptides with very similar structures to both TNRNFLRFamide and SDRNFLRFamide were identified, *e.g.* GNRNFLRFamide, GDRNFLRFamide and SDRNYLRFamide. It is possible that the three N-terminal residues in each peptide were in some way labile under the conditions used here and that the two peptides were truncated to NFLRFamide, which we did sequence via ESI-Q-TOF MS/MS. Again, this seems unlikely given the sequencing of SDRNYLRFamide, which possesses the same three N-terminal residues seen in SDRNFLRFamide. It is also possible that TNRNFLRFamide and SDRNFLRFamide are regional-specific variants that were not present in the population of animals used in our study; however, this possibility seems remote as some of the animals used in our study were from the same general geographic area as those used by Trimmer et al (1987). Clearly additional experiments will be needed to determine why these two well-known *H. americanus* peptides were absent in our study; however, their lack of detection does impart a cautionary note that, while extensive, the catalog of *H. americanus* peptides described here undoubtedly represents only subset of the total peptidome of *H. americanus* as a species, missing peptides that possess structures that are not readily ionizable, one in very minute abundance, as well as those that may be population- and/or individual-specific variants.

4.3. Correspondence of mass spectrally-identified peptides with previous immunohistochemical data

While sequence data on *H. americanus* neuropeptides was limited prior to our study, many immunohistochemical studies had been conducted on neuronal tissues from this species, and thus a wealth of data exists on the putative localization of many of the peptides identified here. For the most part, there is good correspondence between the tissues from which we identified peptides and the extant anatomical data concerning their putative tissue distributions (*e.g.* Siwicki and Bishop, 1986; Kobierski et al., 1987; Goldberg et al., 1988; Mortin and Marder, 1991; Bungart et al., 1994; Skiebe, 1999; Li et al., 2002; Christie et al., 2005), however, discrepancies were also noted (*e.g.* Marder et al., 1986; Bungart et al., 1994; Skiebe, 1999; Li et al., 2002; Pulver and Marder, 2002). For example, in a recent study, A-type AST-, CCAP-,

FMRFamide-related peptide-, orcokinin-, proctolin- and TRP-like immunoreactivities were seen in the *H. americanus* PO (Pulver and Marder, 2002). While we detected CCAP, proctolin and multiple isoforms of FMRFamide-related peptide in this tissue, no A-type ASTs, orcokinins or TRPs were identified. It is possible that our failure to identify members of these peptide families via mass spectrometry resulted from their low abundance in the PO and a lack of sufficient sensitivity to detect them via our MS instrumentation. It is also possible that the endogenous isoforms of these families possess sequences that do not ionize efficiently and hence were not detected on our instruments. Alternatively, it is possible that no authentic members of the A-type AST, orcokinin and TRP families exist in the *H. americanus* PO, and that the antibodies used for their detection in the PO were simply cross-reacting with structurally unrelated peptides, though this scenario seems unlikely for at least the A-type ASTs as the PO has been shown to be a rich source of them in other decapods (*e.g.* Fu et al., 2005b). Clearly additional studies will be required to resolve these and other discrepancies that exist between our mass spectral and previous anatomical studies.

4.4. The presence of B-type allatostatins appears broadly conserved in decapod species

The B-type allatostatins are a family of peptides possessing the C-terminal motif - WX_6Wamide (where X_6 is six variable amino acids; Stay and Tobe, 2007). While these peptides bear no sequence identity to the A-type allatostatins (characterized by -Y/F*X*FGLamide C-termini [where *X* is also a variable amino acid]), they do inhibit the biosynthesis of juvenile hormone in crickets, where they were first identified, and hence have been named accordingly due to this function (Stay and Tobe, 2007). Since their original description, B-type allatostatins have been identified in a number of other insect groups, though they do not necessarily appear to inhibit juvenile hormone synthesis in many of these species (Stay and Tobe, 2007).

Recently, we demonstrated the existence of peptides exhibiting the -WX_6Wamide C-terminal motif in decapod crustaceans (Fu et al., 2005b). Specifically, we characterized several B-type allatostatins from the pericardial organs of the crabs *Cancer productus* and *Cancer borealis* (Fu et al., 2005b; Fu et al., 2007). In the latter species, the peptide VPNDWAHFRGSWamide was identified and shown to exhibit inhibitory action on the pyloric motor pattern, which drives the rhythmic filtering of food between the foregut and midgut (Fu et al., 2007). Interestingly this inhibitory action was described as essentially identical to that of A-type allatostatin, though, again, the two families share no sequence homology (Fu et al., 2007). The presence of bioactive B-type allatostatins in Brachyurans raised the question as to the prevalence of these peptides in members of other decapod infraorders. In our study, we identified the peptide TNWNKFQGSWamide from *H. americanus*, showing that B-type allatostatin isoforms are present in at least some Astacidean decapods. Moreover, we also identified an EST encoding a putative B-type AST precursor from the shrimp *Marsupenaeus japonicus* during a recent *in silico* search for unannotated neuropeptide encoding transcripts (Christie et al., 2008). Thus, with the identification of B-type ASTs in members of three decapod infraorders, which include both a basal and two derived taxa, it would appear that this family of peptides is broadly conserved within the decapods.

4.5. The presence of multiple tachykinin-related peptides is not limited to Brachyuran species

The invertebrate homologs of the vertebrate tachykinins are characterized by the C-terminal motif –FX_1GX_2Ramide, where the *X*s are variable amino acids (Nässel, 1999). In insects, large families of species-specific TRP isoforms are common (Nässel, 1999), while in decapod crustaceans only two full-length isoforms are known: the ubiquitously conserved APSGFLGMRamide and TPSGFLGMRamide, thus far identified only from *Cancer* crabs, and not detected by molecular cloning or mass spectrometry in either the crayfish *Procambarus clarkii* or the spiny lobster *Panulirus interruptus* (Christie et al., 1997; Yasuda-Kamatani and

Yasuda, 2004; Stemmler et al., 2007a; Stemmler et al., 2007b). While the phylogeny of the decapods is controversial, it is generally agreed that the Brachyurans are more derived than either the Palinurans (spiny lobsters) or the Astacideans (clawed lobsters and freshwater crayfish). Thus, it was proposed that APSGFLGMRamide represented the common ancestral decapod TRP with TPSGFLGMRamide being an evolutionary advancement in the derived Brachyurans (Stemmler et al., 2007b).

Here, we have identified both APSGFLGMRamide and TPSGFLGMRamide in the nervous system of *H. americanus*. This finding is in sharp contrast to the hypothesis proposed above, as this species is a member of the same infraorder as *P. clarkii* (Astacidea), where no TPSGFLGMRamide is present (Yasuda-Kamatani and Yasuda, 2004). Moreover, it is generally agreed that the freshwater crayfish are derived from marine ancestors (Crandall et al., 2000), and hence the derived Astacidean lacks the peptide whereas the more basal species is the one possessing it. Clearly additional mass spectral and molecular studies will be needed to determine the evolutionary origin and complexity of the tachykinin-related peptides in decapods.

4.6. Identification of orcokinin and orcomyotropin-related peptide isoforms not encoded on the known *H. americanus* prepro-hormone

As stated in Sections 3.10 and 3.11, cDNAs encoding *H. americanus* prepro-orcokinin were recently identified and characterized (Dickinson et al., 2007a). Present within the predicted prepro-hormones are the orcokinin isoforms NFDEIDRSGFGFH, NFDEIDRSGFGFN and NFDEIDRSGFGFV, the orcokinin-like peptide SSEDMDRLGFGFN, and the orcomyotropin-related peptide FDAFTTGFGHN, as well as the five additional peptides which bare no sequence homology to any known peptide family: APARSSPQQDAAGYTDGAPV or GPIKVRFLSAIFIPIAAPARSSPQQDAAAGYTDGAPV, VYGPRDIANLY, GDYDVYPE, SAE (Dickinson et al., 2007a). Direct tissue MALDI-FTMS analysis of *H. americanus* neural tissues confirmed the production of all peptides from the prepro-hormones except APARSSPQQDAAGYTDGAPV and GPIKVRFLSAIFIPIAAPARSSPQQDAAAGYTDGAPV (Dickinson et al., 2007a).

In our study, the same complement of peptides identified by Dickinson et al. (2007a), save GDYDVYPE and SAE, was identified from the lobster CNS. In addition, we also identified the full-length orcokinin NFDEIDRSSFGFN, the orcokinin truncation NFDEIDRSGFA, the C-terminally truncated orcokinin-like peptide SSEDMDRLGFA and the orcomyotropin-related peptide FDAFTTGFGHS within the *H. americanus* nervous system. NFDEIDRSSFGFN, NFDEIDRSGFA and FDAFTTGFGHS have been identified previously from other decapod species (Bungart et al., 1995; Yasuda-Kamatani and Yasuda, 2000), whereas SSEDMDRLGFA is novel. The identification of these four peptides in *H. americanus* is curious as none are encoded in any of the fully characterized prepro-orcokinins (Dickinson et al., 2007a), and none have been identified in previous mass spectral studies focusing on this species (Li et al., 2002; Skiebe et al., 2003; Fu et al., 2005a; Stemmler et al., 2005; Dickinson et al., 2007a). One possible origin for the discrepancy between previous molecular and mass spectral analysis, and the data we present here, is that additional alleles of the orcokinin prepro-hormone exist. If one or more alleles encoding NFDEIDRSSFGFN, NFDEIDRSGFA, FDAFTTGFGHS and/or SSEDMDRLGFA are present at low frequency in the population, it is possible that individuals possessing them might have been missed during the previous studies, which used far fewer animals than were included in the starting material used for our study. Direct tissue MALDI-FTMS of individual sinus glands of the crab *C. productus* has shown that this situation exists for isoforms of CPRP in this species, with individual crabs exhibiting one of three distinct patterns of CPRP isoforms, one pattern being present over 60% of the population while another was found in only 10% of the individuals

assayed (Stemmler et al., 2007c). It is also possible that variant isoforms are derived from individual-specific mutations in the gene(s) encoding prepro-orcokinin and that single variant animals were included in our pooled tissue. Mass spectrometric detection of an individual-specific peptide has recently been described for a variant of Gly^1-SIFamide, *i.e.* Gly*1*-PIFamide, in the hermit crab *Pagurus pollicarus* (Cashman et al., 2007).

Clearly additional studies will be needed to determine the origin of the orcokinin and orcokinin-precursor related peptides detected here, but not reported in the earlier studies (Li et al., 2002; Skiebe et al., 2003; Fu et al., 2005a; Stemmler et al., 2005; Dickinson et al., 2007a). Regardless, our detection of them again raises a cautionary note with regard to interpreting the presence of large families of peptides in a species as being ubiquitously present in all individuals of that species rather than a result of the inclusion of animals potentially possessing distinct peptidomes in the starting material used for a peptide survey (also see 4.2).

Acknowledgements

We thank the University of Wisconsin School of Pharmacy Analytical Instrumentation Center for access to the MALDI-FTMS instrument. We wish to thank Dr. Michael Nusbaum (University of Pennsylvania School of Medicine) for his generous donation of the Rainin Dynamax HPLC system to the Li laboratory. Dr. Peter O'Connor from Boston University is thanked for the use of BUDA software to make FTMS figures. Christopher Cazzolla from the Goy laboratory is acknowledged for technical assistance with lobster tissue collection. L.L. acknowledges financial support provided by the University of Wisconsin School of Pharmacy, Wisconsin Alumni Research Foundation, National Science Foundation CAREER Award CHE-0449991, National Institutes of Health through grant 1R01DK071801 and a research fellowship from the Alfred P. Sloan Foundation. M.F.G. acknowledges support by a National Science Foundation grant (IBN 0236320), and A.E.C. thanks grants from the National Center for Research Resources' Maine INBRE Program (NIH P20 RR-016463 to Mount Desert Island Biological Laboratory [MDIBL]) and the National Science Foundation's Research Experience for Undergraduates Program (NSF DBI-0453391; to the MDIBL REU site), as well as the MDIBL High School Fellowship Research Program and an MDIBL New Investigator Award (from the Salisbury Cove Research Fund provided through the Thomas H. Maren Foundation).

References

Brown BE. Proctolin: a peptide transmitter candidate in insects. Life Sci 1975;17:1241–1252. [PubMed: 575]

Brown MR, Crim JW, Arata RC, Cai HN, Chun C, Shen P. Identification of a *Drosophila* brain-gut peptide related to the neuropeptide Y family. Peptides 1999;20:1035–1042. [PubMed: 10499420]

Bungart D, Dircksen H, Keller R. Quantitative determination and distribution of the myotropic neuropeptide orcokinin in the nervous system of astacidean crustaceans. Peptides 1994;15:393–400. [PubMed: 7937311]

Bungart D, Hilbich C, Dircksen H, Keller R. Occurrence of analogues of the myotropic neuropeptide orcokinin in the shore crab, *Carcinus maenas*: evidence for a novel neuropeptide family. Peptides 1995;16:67–72. [PubMed: 7716076]

Cashman, CR.; Hsu, YA.; Messinger, DI.; Christie, AE.; Dickinson, PS.; de la Iglesia, HO.; Stemmler, EA. Program No. 140.4. 2007 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2007; 2007. Identification of individual-specific variations in peptide complement of crustacean neural tissues using direct tissue MALDI-FTMS. Online

Chang ES, Prestwich GD, Bruce MJ. Amino acid sequence of a peptide with both molt-inhibiting and hyperglycemic activities in the lobster, *Homarus americanus*. Biochem. Biophys. Res. Commun 1990;171:818–826. [PubMed: 2169734]

Christie AE, Cashman CR, Brennan HR, Ma M, Sousa GL, Li L, Stemmler EA, Dickinson PS. Identification of putative crustacean neuropeptides using *in silico* analyses of publicly accessible expressed sequence tags. Gen. Comp. Endocrinol. Submitted. 2008

Christie AE, Lundquist CT, Nassel DR, Nusbaum MP. Two novel tachykinin-related peptides from the nervous system of the crab *Cancer borealis*. J. Exp. Biol 1997;200:2279–2294. [PubMed: 9316266]

Christie AE, Stemmler EA, Peguero B, Messinger DI, Provencher HL, Scheerlinck P, Hsu YW, Guiney ME, de la Iglesia HO, Dickinson PS. Identification, physiological actions, and distribution of

VYRKPPFNGSIFamide (Val^1-SIFamide) in the stomatogastric nervous system of the American lobster *Homarus americanus*. J. Comp. Neurol 2006;496:406–421. [PubMed: 16566002]

Chung JS, Wilcockson DC, Zmora N, Zohar Y, Dircksen H, Webster SG. Identification and developmental expression of mRNAs encoding crustacean cardioactive peptide (CCAP) in decapod crustaceans. J. Exp. Biol 2006;209:3862–3872. [PubMed: 16985202]

Clarac F, Pearlstein E. Invertebrate preparations and their contribution to neurobiology in the second half of the 20th century. Brain Res. Rev 2007;54:113–161. [PubMed: 17500093]

Cooke IM. Reliable, responsive pacemaking and pattern generation with minimal cell numbers: the crustacean cardiac ganglion. Biol. Bull 2002;202:108–136. [PubMed: 11971808]

Crandall KA, Harris DJ, Fetzner JW. The monophyletic origin of freshwater crayfish estimated from nuclear and mitochondrial DNA sequences. Proc. R. Soc. Lond. B 2000;267:1679–1686.

Cruz-Bermudez ND, Fu Q, Kutz-Naber KK, Christie AE, Li L, Marder E. Mass spectrometric characterization and physiological actions of GAHKNYLRFamide, a novel FMRFamide-like peptide from crabs of the genus *Cancer*. J. Neurochem 2006;97:784–799. [PubMed: 16515542]

de Kleijn DP, de Leeuw EP, van den Berg MC, Martens GJ, Van Herp F. Cloning and expression of two mRNAs encoding structurally different crustacean hyperglycemic hormone precursors in the lobster *Homarus americanus*. Biochim. Biophys. Acta 1995;1260:62–66. [PubMed: 7999796]

de Kleijn DP, Sleutels FJ, Martens GJ, Van Herp F. Cloning and expression of mRNA encoding preprogonad-inhibiting hormone (GIH) in the lobster *Homarus americanus*. FEBS Lett 1994;353:255–258. [PubMed: 7957869]

Dickinson PS, Cashman CR, Rus S, Brennan HR, Stemmler EA, McClintock TS, Christie AE. Molecular and mass spectral analyses of orcokinins and orcokinin precursor-related peptides in the American Lobster *Homarus americanus* and red swamp crayfish *Procambarus clarkii*. Gen. Comp. Endocrinol. Submitted. 2007a

Dickinson PS, Stemmler EA, Cashman CR, Brennan HR, Dennison B, Huber KE, Peguero B, Rabacal W, Goiney CC, Smith CM, Towle DW, Christie AE. SIFamide peptides in clawed lobsters and freshwater crayfish (Crustacea, Decapoda, Astacidea): a combined molecular, mass spectrometric and electrophysiological investigation. Gen. Comp. Endocrinol. Submitted. 2007b

Dickinson PS, Stevens JS, Rus S, Brennan HR, Goiney CC, Smith CM, Li L, Towle DW, Christie AE. Identification and cardiotropic actions of sulfakinin peptides in the American lobster *Homarus americanus*. J. Exp. Biol 2007c;210:2278–2289. [PubMed: 17575033]

Dircksen H, Burdzik S, Sauter A, Keller R. Two orcokinins and the novel octapeptide orcomyotropin in the hindgut of the crayfish *Orconectes limosus*: identified myostimulatory neuropeptides originating together in neurones of the terminal abdominal ganglion. J. Exp. Biol 2000;203:2807–2818. [PubMed: 10952880]

Duve H, Johnsen AH, Maestro JL, Scott AG, Jaros PP, Thorpe A. Isolation and identification of multiple neuropeptides of the allatostatin superfamily in the shore crab *Carcinus maenas*. Eur. J. Biochem 1997;250:727–734. [PubMed: 9461295]

Duve H, Johnsen AH, Scott AG, Thorpe A. Allatostatins of the tiger prawn, *Penaeus monodon* (Crustacea: Penaeidea). Peptides 2002;23:1039–1051. [PubMed: 12126730]

Fanjul-Moles ML. Biochemical and functional aspects of crustacean hyperglycemic hormone in decapod crustaceans: review and update. Comp. Biochem. Physiol. C Toxicol. Pharmacol 2006;142:390–400. [PubMed: 16403679]

Fernlund P. Structure of the red-pigment-concentrating hormone of the shrimp, *Pandalus borealis*. Biochim. Biophys. Acta 1974;371:304–311. [PubMed: 4433569]

Fu Q, Goy MF, Li L. Identification of neuropeptides from the decapod crustacean sinus glands using nanoscale liquid chromatography tandem mass spectrometry. Biochem. Biophys. Res. Commun 2005a;337:765–778. [PubMed: 16214114]

Fu Q, Kutz KK, Schmidt JJ, Hsu YW, Messinger DI, Cain SD, de la Iglesia HO, Christie AE, Li L. Hormone complement of the *Cancer productus* sinus gland and pericardial organ: an anatomical and mass spectrometric investigation. J Comp Neurol 2005b;493:607–626. [PubMed: 16304631]

Fu Q, Tang LS, Marder E, Li L. Mass spectrometric characterization and physiological actions of VPNDWAHFRGSWamide, a novel B type allatostatin in the crab, *Cancer borealis*. J. Neurochem 2007;101:1099–1107. [PubMed: 17394556]

Garczynski SF, Brown MR, Crim JW. Structural studies of Drosophila short neuropeptide F: Occurrence and receptor binding activity. Peptides 2006;27:575–582. [PubMed: 16330127]

Goldberg D, Nusbaum MP, Marder E. Substance P-like immunoreactivity in the stomatogastric nervous systems of the crab *Cancer borealis* and the lobsters *Panulirus interruptus* and *Homarus americanus*. Cell Tissue Res 1988;252:515–522. [PubMed: 2456155]

Huber R, Orzeszyna M, Pokorny N, Kravitz EA. Biogenic amines and aggression: experimental approaches in crustaceans. Brain Behav. Evol 1997;50:60–68. [PubMed: 9217993]

Huybrechts J, Nusbaum MP, Bosch LV, Baggerman G, De Loof A, Schoofs L. Neuropeptidomic analysis of the brain and thoracic ganglion from the Jonah crab, *Cancer borealis*. Biochem. Biophys. Res. Commun 2003;308:535–544. [PubMed: 12914784]

Jaffe H, Loeb M, Hayes DK, Talbot N, Garvick S. Isolation of crustacean erythrophore concentrating hormone from nerve tissue of *Homarus americanus.* Comp. Biochem. Physiol. C 1984;78:397–401. [PubMed: 6149087]

Kravitz EA. Hormonal control of behavior: amines and the biasing of behavioral output in lobsters. Science 1988;241:1775–1781. [PubMed: 2902685]

Kravitz EA. Serotonin and aggression: insights gained from a lobster model system and speculations on the role of amine neurons in a complex behavior. J. Comp. Physiol. A 2000;186:221–238. [PubMed: 10757238]

Kobierski LA, Beltz BS, Trimmer BA, Kravitz EA. FMRFamide-like peptides of *Homarus americanus*: distribution, immunocytochemical mapping, and ultrastructural localization in terminal varicosities. J. Comp. Neurol 1987;266:1–15. [PubMed: 3323267]

Li L, Kelley WP, Billimoria CP, Christie AE, Pulver SR, Sweedler JV, Marder E. Mass spectrometric investigation of the neuropeptide complement and release in the pericardial organs of the crab, *Cancer borealis*. J. Neurochem 2003;87:642–645. [PubMed: 14535947]

Li, L.; Ma, M.; Chen, R.; Christie, AE. Program No. 315.17. 2007 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2007; 2007. Peptidomic analyses of the central nervous systems of the American lobster *Homarus americanus* and the European green crab *Carcinus maenas*. Online

Li L, Pulver SR, Kelley WP, Thirumalai V, Sweedler JV, Marder E. Orcokinin peptides in developing and adult crustacean stomatogastric nervous systems and pericardial organs. J. Comp. Neurol 2002;444:227–244. [PubMed: 11840477]

Marder E, Bucher D. Understanding circuit dynamics using the stomatogastric nervous system of lobsters and crabs. Annu. Rev. Physiol 2007;69:291–316. [PubMed: 17009928]

Marder E, Hooper SL, Siwicki KK. Modulatory action and distribution of the neuropeptide proctolin in the crustacean stomatogastric nervous system. J. Comp. Neurol 1986;243:454–467. [PubMed: 2869069]

McVeigh P, Kimber MJ, Novozhilova E, Day TA. Neuropeptide signalling systems in flatworms. Parasitology 2005;131:S41–S55. [PubMed: 16569292]

Mortin LI, Marder E. Differential distribution of beta-pigment-dispersing hormone (beta PDH)-like immunoreactivity in the stomatogastric nervous system of five species of decapod crustaceans. Cell Tissue Res 1991;265:19–33. [PubMed: 1913777]

Nässel DR. Tachykinin-related peptides in invertebrates: a review. Peptides 1999;20:141–158. [PubMed: 10098635]

Nichols R. Signaling pathways and physiological functions of *Drosophila melanogaster* FMRFamide-related peptides. Annu. Rev. Entomol 2003;48:485–503. [PubMed: 12414735]

Pulver SR, Marder E. Neuromodulatory complement of the pericardial organs in the embryonic lobster, *Homarus americanus*. J. Comp. Neurol 2002;451:79–90. [PubMed: 12209843]

Rao KR, Riehm JP, Zahnow CA, Kleinholz LH, Tarr GE, Johnson L, Norton S, Landau M, Semmes OJ, Sattelberg RM, Jorenby WH, Hintz MF. Characterization of a pigment-dispersing hormone in eyestalks of the fiddler crab *Uca pugilator*. Proc. Natl. Acad. Sci. USA 1985;82:5319–5322. [PubMed: 16593589]

Richards KS, Marder E. The actions of crustacean cardioactive peptide on adult and developing stomatogastric ganglion motor patterns. J. Neurobiol 2000;44:31–44. [PubMed: 10880130]

Saideman SR, Ma M, Kutz-Naber KK, Cook A, Torfs P, Schoofs L, Li L, Nusbaum MP. Modulation of rhythmic motor activity by pyrokinin peptides. J Neurophysiol 2007;97:579–595. [PubMed: 17065249]

Schwarz TL, Lee GM, Siwicki KK, Standaert DG, Kravitz EA. Proctolin in the lobster: the distribution, release, and chemical characterization of a likely neurohormone. J. Neurosci 1984;4:1300–1311. [PubMed: 6144736]

Sithigorngul P, Pupuem J, Krungkasem C, Longyant S, Chaivisuthangkura P, Sithigorngul W, Petsom A. Seven novel FMRFamide-like neuropeptide sequences from the eyestalk of the giant tiger prawn *Penaeus monodon*. Comp. Biochem. Physiol. B Biochem. Mol. Biol 2002;131:325–337. [PubMed: 11959015]

Skiebe P. Allatostatin-like immunoreactivity in the stomatogastric nervous system and the pericardial organs of the crab *Cancer pagurus*, the lobster *Homarus americanus*, and the crayfish *Cherax destructor* and *Procambarus clarkii*. J. Comp. Neurol 1999;403:85–105. [PubMed: 10075445]

Skiebe P, Dreger M, Borner J, Meseke M, Weckwerth W. Immunocytochemical and molecular data guide peptide identification by mass spectrometry: orcokinin and orcomyotropin-related peptides in the stomatogastric nervous system of several crustacean species. Cell. Mol. Biol. (Noisy-le-grand) 2003;49:851–871. [PubMed: 14528921]

Stangier J, Hilbich C, Beyreuther K, Keller R. Unusual cardioactive peptide (CCAP) from pericardial organs of the shore crab *Carcinus maenas*. Proc. Natl. Acad. Sci. USA 1987;84:575–579. [PubMed: 16593803]

Starratt AN, Brown BE. Structure of the pentapeptide proctolin, a proposed neurotransmitter in insects. Life Sci 1975;17:1253–1256. [PubMed: 576]

Stay B, Tobe SS. The role of allatostatins in juvenile hormone synthesis in insects and crustaceans. Annu. Rev. Entomol 2007;52:277–299. [PubMed: 16968202]

Stemmler EA, Cashman CR, Messinger DI, Dickinson PS, Christie AE. High mass resolution direct-tissue MALDI-FTMS reveals broad conservation of three neuropeptides (APSGFLGMRamide, GYRKPPFNGSIFamide and pQDLDHVFLRFamide) across members of seven decapod crustaean infraorders. Peptides. 2007aIn press

Stemmler EA, Gardner NP, Guiney ME, Bruns EA, Dickinson PS. The detection of red pigment-concentrating hormone (RPCH) in crustacean eyestalk tissues using matrix-assisted laser desorption/ionization-Fourier transform mass spectrometry: $[M + Na]^+$ ion formation in dried droplet tissue preparations. J. Mass Spectrom 2006;41:295–311. [PubMed: 16421875]

Stemmler EA, Hsu YW, Cashman CR, Messinger DI, de la Iglesia HO, Dickinson PS, Christie AE. Direct tissue MALDI-FTMS profiling of individual *Cancer productus* sinus glands reveals that one of three distinct combinations of crustacean hyperglycemic hormone precursor-related peptide (CPRP) isoforms are present in individual crabs. Gen Comp Endocrinol. 2007cIn press

Stemmler EA, Peguero B, Bruns EA, Dickinson PS, Christie AE. Identification, physiological actions, and distribution of TPSGFLGMRamide: a novel tachykinin-related peptide from the midgut and stomatogastric nervous system of *Cancer* crabs. J. Neurochem 2007b;101:1351–1366. [PubMed: 17437551]

Stemmler EA, Provencher HL, Guiney ME, Gardner NP, Dickinson PS. Matrix-assisted laser desorption/ionization fourier transform mass spectrometry for the identification of orcokinin neuropeptides in crustaceans using metastable decay and sustained off-resonance irradiation. Anal. Chem 2005;77:3594–3606. [PubMed: 15924394]

Stepanyan R, Day K, Urban J, Hardin DL, Shetty RS, Derby CD, Ache BW, McClintock TS. Gene expression and specificity in the mature zone of the lobster olfactory organ. Physiol. Genomics 2006;25:224–233. [PubMed: 16614458]

Siwicki KK, Bishop CA. Mapping of proctolin-like immunoreactivity in the nervous systems of lobster and crayfish. J. Comp. Neurol 1986;243:435–453. [PubMed: 3512628]

Tensen CP, de Kleijn DP, Van Herp F. Cloning and sequence analysis of cDNA encoding two crustacean hyperglycemic hormones from the lobster *Homarus americanus*. Eur. J. Biochem 1991a;200:103–106. [PubMed: 1879416]

Tensen CP, Janssen KP, Soyez D, Van Herp F. Comparative characterization of hyperglycemic neuropeptides from the lobster *Homarus americanus*. Peptides 1991b;12:241–249. [PubMed: 2067976]

Tensen CP, Verhoeven AH, Gaus G, Janssen KP, Keller R, Van Herp F. Isolation and amino acid sequence of crustacean hyperglycemic hormone precursor-related peptides. Peptides 1991c;12:673–681. [PubMed: 1788131]

Torfs P, Nieto J, Cerstiaens A, Boon D, Baggerman G, Poulos C, Waelkens E, Derua R, Calderon J, De Loof A, Schoofs L. Pyrokinin neuropeptides in a crustacean. Isolation and identification in the white shrimp *Penaeus vannamei*. Eur. J. Biochem 2001;268:149–154. [PubMed: 11121115]

Towle DW, Smith CM. Gene discovery in *Carcinus maenas* and *Homarus americanus* via expressed sequence tags. Int. Comp. Biol 2006;46:912–918.

Trimmer BA, Kobierski LA, Kravitz EA. Purification and characterization of FMRFamidelike immunoreactive substances from the lobster nervous system: isolation and sequence analysis of two closely related peptides. J. Comp. Neurol 1987;266:16–26. [PubMed: 3429714]

Veenstra JA. Isolation and structure of corazonin, a cardioactive peptide from the American cockroach. FEBS Lett 1989;250:231–234. [PubMed: 2753132]

Yasuda-Kamatani Y, Yasuda A. Identification of orcokinin gene-related peptides in the brain of the crayfish *Procambarus clarkii* by the combination of MALDI-TOF and on-line capillary HPLC/Q-Tof mass spectrometries and molecular cloning. Gen. Comp. Endocrinol 2000;118:161–167. [PubMed: 10753578]

Yasuda-Kamatani Y, Yasuda A. APSGFLGMRamide is a unique tachykinin-related peptide in crustaceans. Eur. J. Biochem 2004;271:1546–1556. [PubMed: 15066180]

Zajac JM, Mollereau C. RFamide peptides. Introduction. Peptides 2006;27:941–942. [PubMed: 16621148]

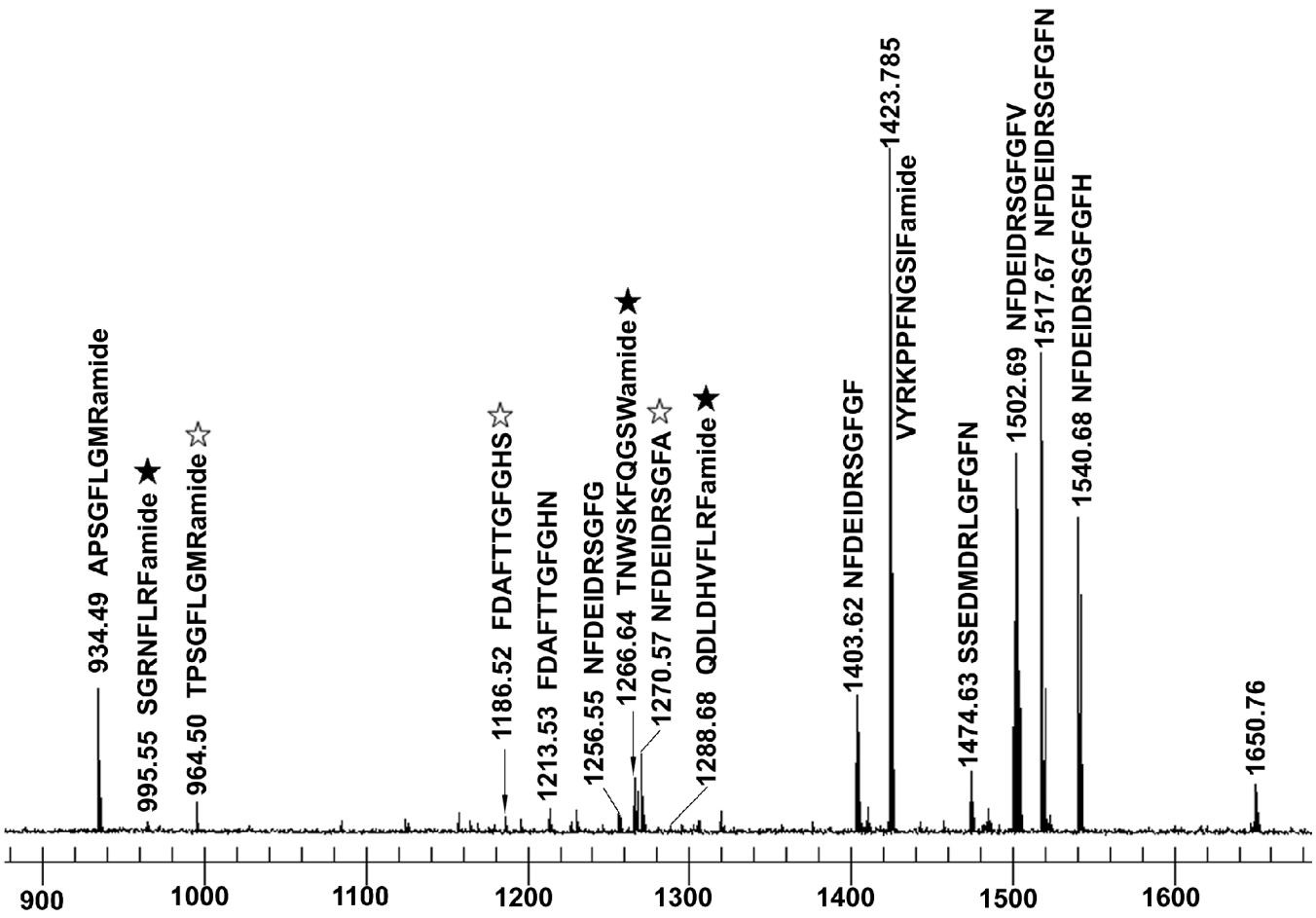

Figure 1.

Direct tissue peptide profiling of *H. americanus* brain by MALDI FTMS. Signals correspond to the protonated molecular ions, $[M+H]^+$, where M is the molecular weight of each peptide. Peptide identity was assigned based on accurate mass measurement. Novel peptides are indicated by stars with open stars indicating known peptides in other decapods but new to *H. americanus* and filled stars showing new peptides reported for the first time in this study.

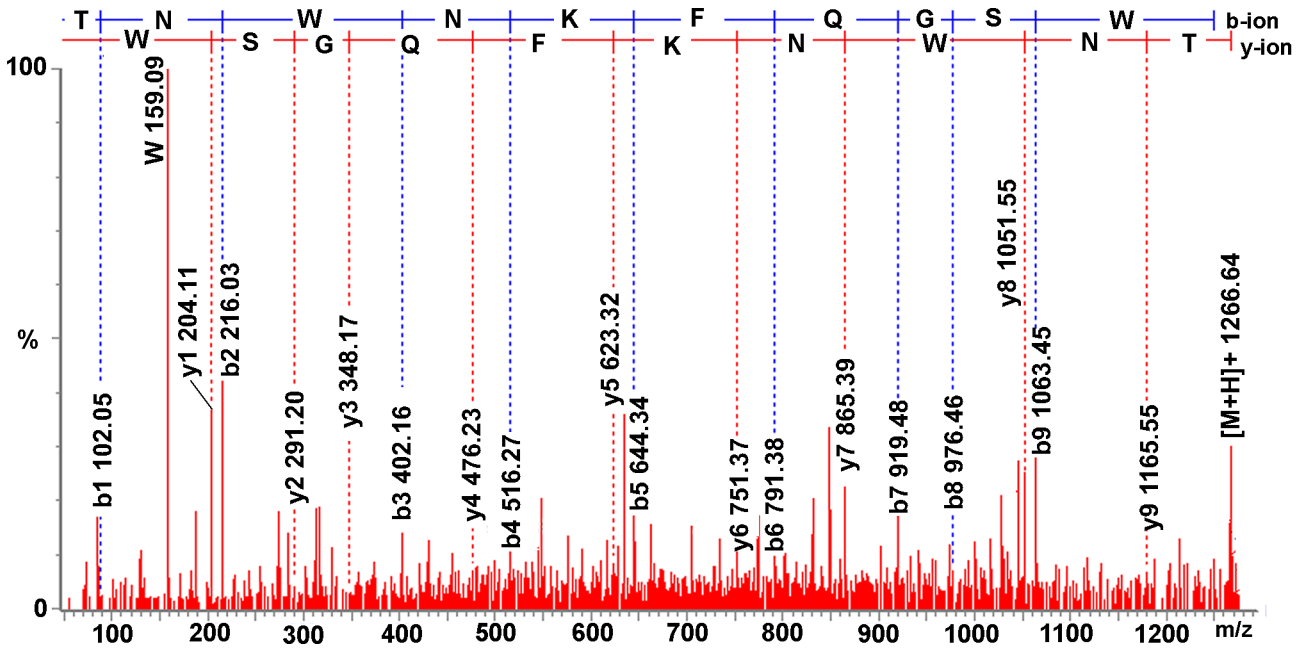

Figure 2.

Collision-induced dissociation spectrum of a *de novo* sequenced B-type allatostatin, TNWNKFQGSWamide. The sequence-specific b-type and y-type fragment ions and immonium ion characteristic of tryptophan are labeled, with derived amino acid sequence listed above the fragmentation spectrum.

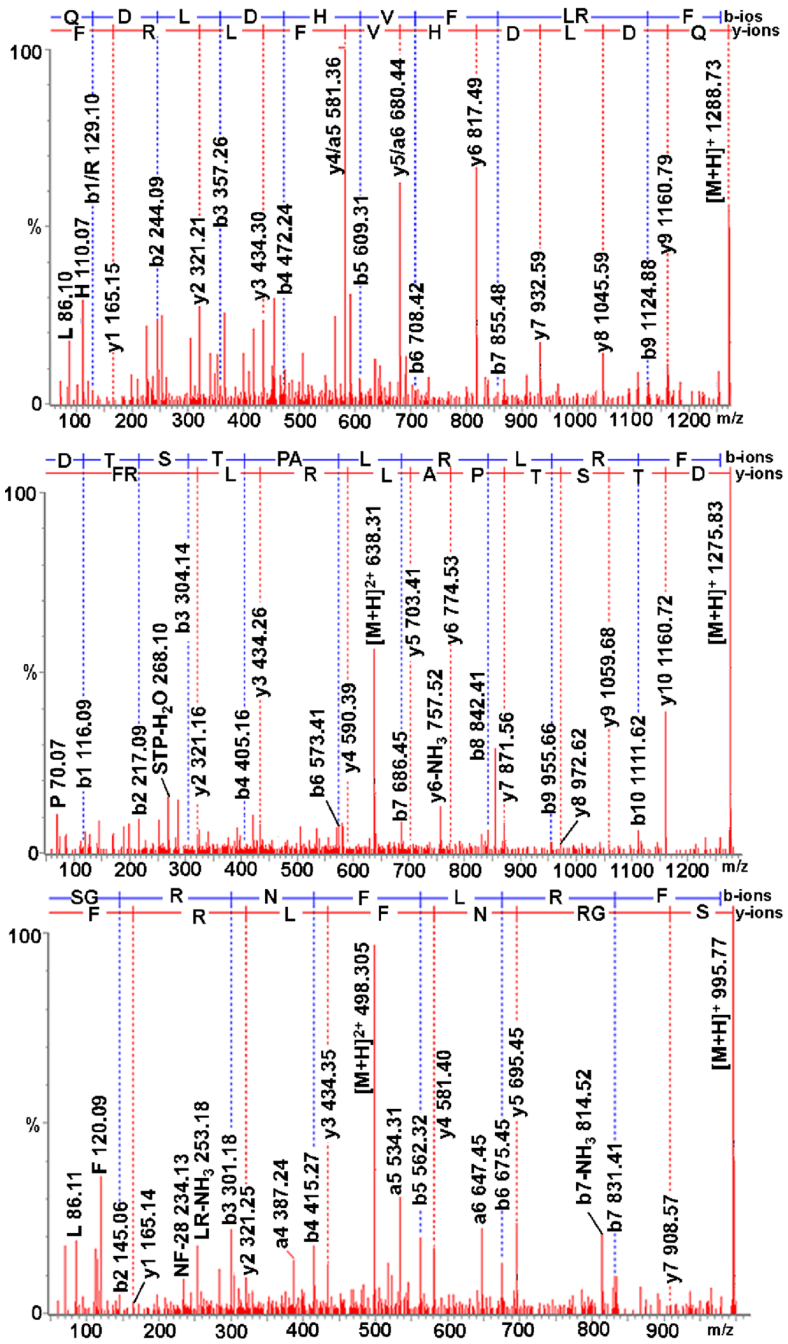

Figure 3.

Collision-induced dissociation spectra of three *de novo* sequenced FMRFamide-related peptides QDLDHVFLRFamide, DTSTPALRLRFamide, and SGRNFLRFamide. All precursor ions are doubly charged. The sequence-specific b-type and y-type fragment ions and immonium ions are labeled.

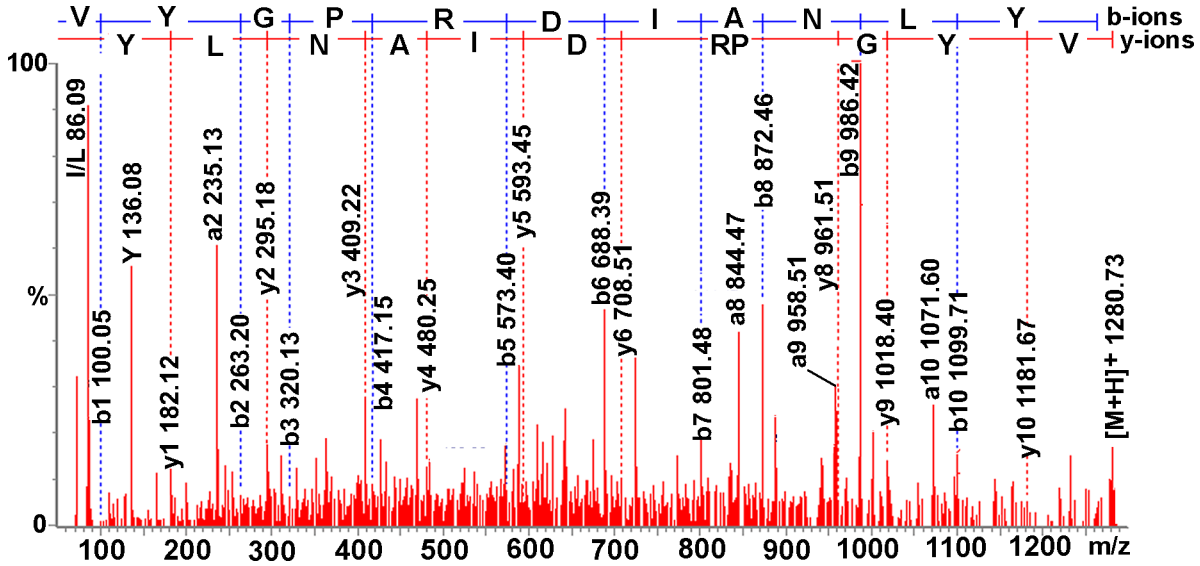

Figure 4.

Collision-induced dissociation spectrum of a *de novo* sequenced orcokinin-precursor related peptide VYGPRDIANLY. The identities of isoleucine and leucine are determined based on knowledge of orcokinin prepro-hormone sequence (Dickinson et al., 2007a).

Table 1

Neuropeptides detected in the nervous system and neuroendocrine organs of the American lobster *Homarus americanus*

Neuropeptide Family	$[M+H]^+$	Sequence	FTMS	QTOF	MALDI-TOF	Tissue location
AST-A type	795.40	EPYAFGLamide	−	+	−	Br
	753.39	SPYAFGLamide	−	+	−	Br
	810.41	SGPYAFGLamide	−	+	+	Br/STG
	826.41	SGPYSFGLamide	−	+	−	Br
	824.43	ASPYAFGLamide	−	+	+	Br/STG
	794.42	AGPYAFGLamide	−	+	−	Br/VNC
	822.45	VGPYAFGLamide	−	+	−	Br
	854.44	TPSYAFGLamide	−	+	+	Br/STG
	814.30	SQYTFGLamide	−	+	−	Br
	841.42	AGGAYSFGLamide	−	+	−	Br
AST-B type	1266.64	TNWNKFQGSWamide	+	+	+	Br/PO/STG
Corazonin	1369.65	pQTFQYSRGWTNamide	+	−	−	Br/ESG
CCAP	956.38	PFCNAFTGCamide	+	+	+	Br/PO
CCAP precursor related peptide	1074.53	DIGDLLEGKD*	−	+	−	Br/VNC
CHH	8577.89	pCHH-A[pQ^{61}–V^{132}amide]	+	−	+	SG/PO/STG
	8633.20	pCHH-B[pQ^{61}–V^{132}amide]	+	−	−	SG
MIH	8478.76	pMIH [pQ^{61}–M^{131}]	−	−	+	STG
CPRP	3604.77	RSVEGASRMEKLLSSSNSPSSTPLGFLSQDHSVN	+	+	+	SG/ESG/PO
	3544.81	RSVEGVSRMEKLLSSISPSSTPLGFLSQDHSVN	+	+	−	SG/ESG
Truncated CPRPs	1199.61	PLGFLSQDHSV	−	+	−	SG
	1313.65	PLGFLSQDHSVN	−	+	−	SG
	1149.57	RSVEGVSRME	−	+	−	SG
	1362.72	RSVEGASRMEKL	−	+	−	SG
	1503.83	RSVEGASRMEKLL	−	+	−	SG
	1562.83	RSVEGASRMEKLLS	−	+	−	SG
	1590.86	RSVEGVSRMEKLLS	−	+	−	SG
	1649.86	RSVEGASRMEKLLSS	−	+	−	SG
	1576.85	RSVEGASRMEKLLT	−	+	−	SG
	1604.88	RSVEGVSRMEKLLT	−	+	−	SG
FaRPs	695.40	NFLRFamide	−	+	−	Br/VNC
	965.54	GGRNFLRFamide	−	+	+	Br/VNC/PO/STG
	995.55	SGRNFLRFamide	+	+	+	Br/VNC/PO/STG
	1022.56	GNRNFLRFamide	−	+	+	Br/PO/STG
	1023.55	GDRNFLRFamide	−	+	+	Br/VNC/PO/STG
	1147.65	APQRNFLRFamide	−	−	+	STG
	1208.63	DQNRNFLRFamide	+	+	+	Br/VNC/PO/STG
	1271.68	pQDLDHVFLRFamide	+	+	+	Br/VNC/ESG/PO/STG
	1288.68	QDLDHVFLRFamide	+	+	+	Br/VNC/ESG/STG
	887.56	PSLRLRFamide	−	+	−	Br/VNC
	1041.63	GPPSLRLRFamide	+	+	+	Br/VNC/STG
	1105.63	SMPSLRLRFamide	+	+	+	Br/VNC/SG/PO/STG
	1121.62	SM(O)PSLRLRFamide	+	+	−	Br/VNC
	1163.67	FEPSLRLRFamide	−	+	−	VNC

Gen Comp Endocrinol. Author manuscript; available in PMC 2009 April 1.

Neuropeptide Family	[M+H]$^+$	Sequence	FTMS	QTOF	MALDI-TOF	Tissue location
	1275.72	DTSTPALRLRFamide	+	+	+	Br/VNC/STG
	1069.55	SDRNYLRFamide	−	+	−	Br
	1104.61	GAHKNYLRFamide	+	−	+	Br/PO/STG
	1289.64	GYSDRNYLRFamide	+	+	+	Br/VNC/STG
	1484.66	GGGEYDDYGHLRFamide‡	+	−	−	VNC
Orcokinin	1198.55	NFDEIDRSGFamide	−	+	+	Br/VNC/SG/STG
	1199.53	NFDEIDRSGF	−	+	−	SG
	1270.57	NFDEIDRSGFA	+	+	−	Br/VNC/SG/ESG
	1256.55	NFDEIDRSGFG	+	+	+	Br/VNC/SG/ESG/STG
	1403.62	NFDEIDRSGFGF	+	+	+	Br/VNC/SG/ESG/STG
	1502.69	NFDEIDRSGFGFV	+	−	+	Br/VNC/SG/ESG/STG
	1517.67	NFDEIDRSGFGFN	+	−	+	Br/VNC/SG/ESG/STG
	1540.68	NFDEIDRSGFGFH	+	+	+	Br/VNC/SG/ESG/STG
	1554.70	NFDEIDRSSFGFN	+	+	+	Br/VNC/STG
Orcokinin related peptides	1474.63	SSEDMDRLGFGFN	+	+	+	Br/VNC/SG/ESG/STG
	1490.62	SSEDM(O)DRLGFGFN	−	+	−	Br/VNC
	1213.50	SSEDMDRLGFG	−	+	+	Br/VNC/SG/STG
	1227.52	SSEDMDRLGFA	+	+	−	Br/VNC/SG
	904.42	FDAFTTGFamide	−	+	−	Br
	1213.53	FDAFTTGFGHN	+	+	+	Br/VNC/SG/STG
	1186.52	FDAFTTGFGHS	+	−	−	Br/ESG
	1280.66	VYGPRDIANLY#	+	+	+	Br/VNC/SG/STG
β-PDH	1927.03	NSELINSILGLPKVMNDAamide	−	+	−	Br/SG
Proctolin	649.37	RYLPT	−	−	+	PO
Pyrokinin	618.37	FSPRLamide	−	+	−	Br/VNC
RPCH	930.47	pQLNFSPGWamide	−	+	−	SG
SIFamide	1423.78	VYRKPPFNGSIFamide	−	+	+	Br/VNC/ESG/STG
	1161.65	RKPPFNGSIFamide	−	+	−	Br
Tachykinin	950.49	APSGFLGM(O)Ramide	−	+	−	Br/VNC
	980.50	TPSGFLGM(O)Ramide	−	+	−	Br/VNC
	934.49	APSGFLGMRamide	−	+	+	Br/VNC/SG/STG
	964.50	TPSGFLGMRamide	−	+	−	Br/VNC
	766.40	SGFLGMRamide	−	+	−	Br/VNC
	863.46	PSGFLGMRamide	−	+	−	Br/VNC
	992.50	APSGFLGMRG	−	+	−	Br/VNC
Other peptide	1099.64	DLPKVDTALK	−	+	−	Br/VNC
	858.54	KPKTEKK	−	+	−	Br
	1254.81	AVLLPKKTEKK	−	+	−	Br
	1363.67	EVEEPEAPAPPAK	−	+	−	Br
	1372.79	LRVAPEEHPVLL	−	+	−	Br
	1103.56	GPSGGFNGALAR	−	+	−	Br

Br, brain; VNC, ventral nerve cord; ESG, eyestalk ganglia; PO, pericardial organ; SG, sinus gland; STG, stomatogastric ganglion. AST, allatostatin; CCAP, crustacean cardioactive peptide; CHH, crustacean hyperglycemic hormone; CPRP, crustacean hyperglycemic hormone precursor related peptide; FaRP, FMRFamide-related peptide; MIH, molt inhibiting hormone; PDH, pigment dispersing hormone; RPCH, red pigment concentrating hormone

Previously known *H. americanus* peptides are shown in black; peptides previously described from other decapods, but new to *H. americanus* are shown in blue; novel peptides are shown in red.

* Predicted to exist in *Homarus gammarus* (Chung et al., 2006), but *de novo* sequenced here for the first time

≠ predicted to exist in *H. americanus* (Dickinson et al., 2007c), but detected in neural tissue for the first time here

predicted and *de novo* sequenced from *H. americanus* simultaneously and independently in Dickinson et al. (2007a)