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Review

Mass spectrometric analysis of spatio-temporal dynamics of crustacean neuropeptides ☆

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ABSTRACT

Neuropeptides represent one of the largest classes of signaling molecules used by nervous systems to regulate a wide range of physiological processes. Over the past several years, mass spectrometry (MS)-based strategies have revolutionized the discovery of neuropeptides in numerous model organisms, especially in decapod crustaceans. Here, we focus our discussion on recent advances in the use of MS-based techniques to map neuropeptides in the spatial domain and monitoring their dynamic changes in the temporal domain. These MS-enabled investigations provide valuable information about the distribution, secretion and potential function of neuropeptides with high molecular specificity and sensitivity. *In situ* MS imaging and *in vivo* microdialysis are highlighted as key technologies for probing spatio-temporal dynamics of neuropeptides in the crustacean nervous system. This review summarizes the latest advancement in MS-based methodologies for neuropeptide analysis including typical workflow and sample preparation strategies as well as major neuropeptide families discovered in decapod crustaceans. This article is part of a Special Issue entitled: Neuroproteomics: Applications in Neuroscience and Neurology.

1. Overview

Neuropeptides represent one of the most diverse classes of signaling molecules employed by the nervous system, which can mediate numerous essential physiological processes, such as feeding, pain sensing and reproduction [1]. Neuropeptides are short chains of amino acids, which are encoded within genomes as larger precursor proteins, and undergo extensive processing and modification steps to yield bioactive forms that have regulatory roles. Extensive research has been carried out to study their structures, distributions and functions. Decapod crustacean, which has a relatively simple nervous system (Fig. 1) [2] and accessible electrophysiology at the single-cell and neural circuit level, serves as an attractive model preparation for neuromodulation and neuropeptide study. Our knowledge about neuropeptides in crustacean has been significantly expanded over the past decade thanks to the development and application of MS-based techniques enabling accelerated neuropeptide discovery [3,4].

We developed a multifaceted MS-based platform for neuropeptide characterization, from sample preparation to data analysis (Fig. 2) [5]. Utilizing this platform, neuropeptide content in various tissue organs [6–11] and body fluid-hemolymph [12–14] in crustaceans has been extensively studied. Despite their relatively simple nervous system, crustacean model organisms employ diverse neuropeptides, including over two dozen neuropeptide families and many isoforms from several peptide families for each species [3]. Complementary to tandem MS-based neuropeptide discovery and identification process, the mass spectrometric imaging (MSI) technique has been utilized to map the spatial distribution of neuropeptide families and specific isoforms directly from tissue in an anatomical context [15,16]. The spatial localization of specific neuropeptides and determination of their co-localization patterns will provide critical information to help elucidate the underlying mechanism of cell–cell communication *via* signaling neuropeptide molecules.

In parallel to mapping the spatial distribution of these chemical messengers in tissue, monitoring the chemical dynamics in body fluids of live animals could be highly rewarding for functional investigation

Abbreviations: AST, allatostatin; CCAP, crustacean cardioactive peptide; CE, capillary electrophoresis; CHCA, α-cyano-4-hydroxycinnamic acid; CID, collisional induced dissociation; CoG, commissural ganglia; *dgn*, dorsal gastric nerve; DHB, 2,5-dihydroxy benzoic acid; ESI, electrospray ionization; FLPs, FMRFamide-like peptides; GABA, γ-aminobutyric acid; HPLC, high performance liquid chromatography; *ion*, inferior oesophageal nerve; *ivn*, inferior ventricular nerve; LC, liquid chromatography; LDI, laser desorption ionization; LTQ, linear trap quadrupole; IHC, Immunohistochemical; *lvn*, lateral ventricular nerve; MALDI, matrix-assisted laser desorption/ionization; MS, mass spectrometry; MSI, mass spectrometric imaging; MS/MS, tandem mass spectrometry; *mvn*, medial ventricular nerve; NIMS, nanostructure initiator mass spectrometry; OG, oesophageal ganglion; *pdn*, pyloric dilator nerve; POs, pericardial organs; PTM, post-translational modification; *pyn*, pyloric nerve; QTOF, quadrupole time of flight; SG, sinus gland; *son*, superior oesophageal nerve; STG, stomatogastric ganglion; *stn*, stomatogastric nerve; STNS, stomatogastric nervous system; TOF, time of flight; TRPs, tachykinin-related peptides; VNC, ventral nerve cord

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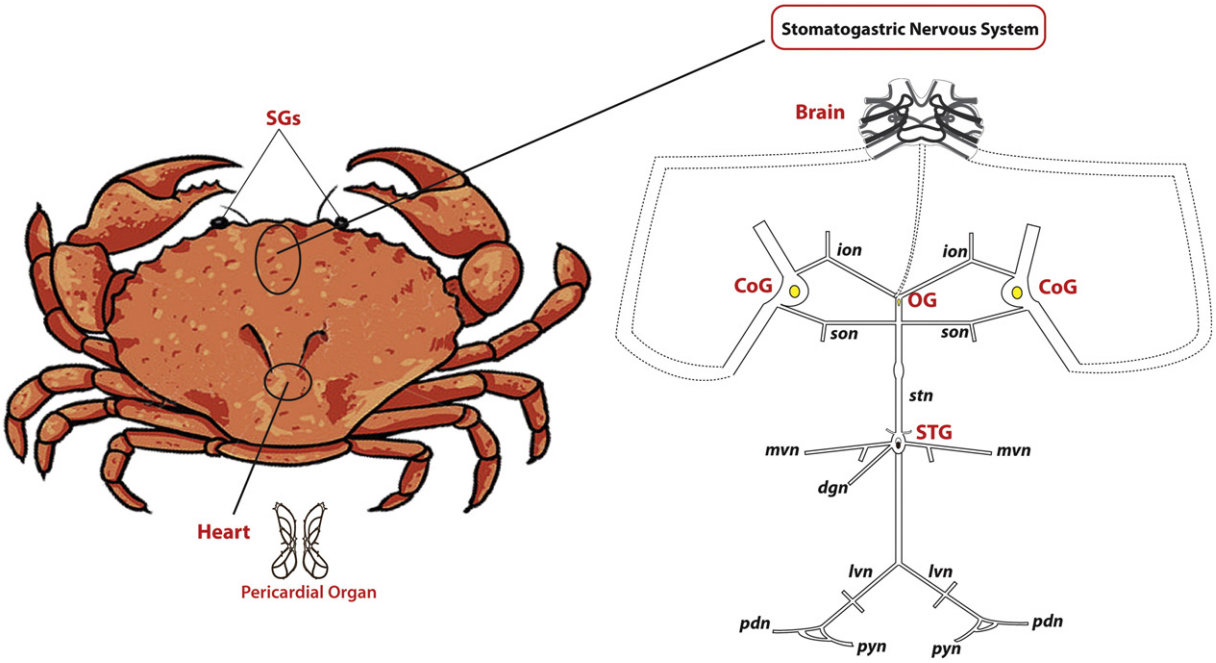

Fig. 1. Schematic drawing of the stomatogastric nervous system (STNS) of the Jonah crab, *Cancer borealis*. The SGs (sinus glands: located in the eyestalks of the crab) and the POs (pericardial organs: located in the chamber surrounding the heart) release hormones into the hemolymph. The brain is connected to the STNS *via* a tiny nerve called the inferior ventricular nerve (*ivn*), and the STNS consists of the stomatogastric ganglion (STG), oesophageal ganglion (OG), paired commissural ganglia (CoG), which are connected by motor nerves.

because it allows correlation between neurochemical content fluctuations and biological behaviors. Several techniques have been used for *in vivo* neurotransmitter measurement, among which, microdialysis sampling coupled to MS analysis is a popular choice for such measurements. This method has been well established for monitoring a wide range of analytes, from smaller molecular weight neurotransmitters, metabolites to larger molecule neuropeptides, and even proteins [17]. Fig. 2 summarizes a typical experimental workflow for crustacean neuropeptide analysis at both spatial and temporal domains.

1.1. Brief introduction to the major crustacean neuropeptide families

Here we briefly introduce the chemical features and known biological functions of the major crustacean neuropeptide families. For more in-depth discussion, readers are suggested to refer to reviews about specific crustacean and invertebrate neuropeptides [3,18,19].

1.1.1. Allatostatin

The term allatostatin (AST) was defined based on the inhibitory effect on the insect corpora allata from a peptide [20]. In crustacean, this superfamily of neuropeptides is divided into three subgroups according to their structural features. A-type AST (AST-A) peptides share a C-terminal motif of Y/FXFGLamide (X represents a variable amino acid). Their diverse inhibitory functions are tissue specific within the nervous system [21–25]. The second family of AST (B-type AST) neuropeptides are characterized with a C-terminal sequence of $W(X)_6$Wamide. The first native AST-B in crustacean (CbAST-B1) was found in the PO of *Cancer borealis* and *Cancer productus* [26]. This novel B-type AST was described to reduce the pyloric network frequency in crabs in a dose-dependent manner. Members of the AST-C subfamily share a C-terminal motif PISCF and a pyroglutamine blocked N-terminus. It is also noted that the disulfide bridge between the Cys residues at positions 7 and 14 (in a typical 15-mer AST-C) is critical for receptor binding [27]. AST-C neuropeptides exhibit inhibitory effects on the pyloric motor output and constrain the cardiac neuromuscular system to reduce the heart rate [28].

1.1.2. Crustacean cardioactive peptide

Being originally isolated *via* heart bioassays in shore crab, *Carcinus maenas*, the crustacean cardioactive peptide (CCAP) was named for its excitatory modulation on the heart [29]. This cyclic amidated neuropeptide with the sequence of PFCNAFTGCamide has a disulfide bridge between Cys^3 and Cys^9. In addition to its well-known cardioexcitatory properties [25,29,30], CCAP has also been reported to have excitatory effects on other invertebrate muscles and has been established as a neuromodulator of STG and OG [3].

1.1.3. FMRFamides

FMRFamide-like peptides (FLPs) are the largest family of crustacean neuropeptides. They share a C-terminal motif RFamide with variable preceding sequences, such as FLRFamide, RPRFamide, RLRFamide, YLRFamide and FVRFamide. The pleiotropic physiological functions of FLPs have been extensively studied among numerous species of crustacean. Their diverse neuromodulatory roles include but are not limited to muscular modulation [31], digestive system modulation [32], cardiac excitatory modulator [33] and pyloric circuit modulation [34], *etc.*

1.1.4. Orcokinins

The first orcokinin was isolated from the ventral nerve cord (VNC) of the crayfish, *Orconectes limosus* [35]. To the best of our knowledge, all full length decapod orcokinins are 13 amino acids long with an N-terminal motif of N/DFDEIDR. Orcokinins have been reported to enhance the frequency and amplitude of spontaneous hindgut contractions in crustacean, but the effect differs among different species [3]. Orcokinin is also demonstrated to be capable of modulating the motor output when applied exogenously to the STG of crabs and spiny lobster [36,37].

1.1.5. Pyrokinin

In crustacean, pyrokinin neuropeptides possess the C-terminal motif FXPRLamide (where X is a variable amino acid). In the isolated STNS of *C. borealis*, two novel pyrokinins (CabPKs) discovered by MS approach exhibited effects on the pyloric and gastric mill motor circuits when bath applied to the STG. While little effect was observed on the pyloric