Precursor related peptides functionality within the central pattern generator in the cardiac neuromuscular system in the American lobster, *Homarus americanus*

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The cardiac neuromuscular system of the lobster is a neurogenic heart. Unlike the human heart, which is myogenic and can contract independent of neural input, the lobster's heart requires neural impulses to contract. These neural impulses come from a cluster of neurons on the heart wall known as the cardiac ganglion. This is a network of nine neurons with four posterior pacemaker interneurons and five anterior motor neurons. The neural communication between these neurons create bursts of action potentials that are responsible for the muscle contractions of the heart.

The cardiac ganglion is one of the lobster's central pattern generators (CPGs). CPGs are networks of neurons that generate rhythmic patterns of output to drive behaviors such as chewing, walking, and breathing. These CPGs are relatively fixed networks of neurons that produce consistent, stereotypical patterns in the absence of other inputs. However, neuromodulators like peptides acting on the network enable flexibility in the motor outputs. This flexibility allows organisms to adjust to changes in the environment and sensory input. The presence of neuropeptides in the nervous system permits flexibility in the CPGs.

Neuropeptides are synthesized as long chains of peptides (preprohormones) composed of both neuropeptides and intervening amino acid chains, known as precursor related peptides (PRPs). Once synthesized, the preprohormones go through post translational processing, where enzymes cleave the peptide at specific sites, making smaller chains of amino acids, i.e., smaller peptides. Many of these peptides then undergo post-translational modifications, which are known to alter the peptide structure, making the peptide biologically active and stabilizing it for cellular interactions and neural output. These structural changes can include phosphorylation, disulfide bridges, and C- terminal amidation.
Once the post-translational modifications are complete, many neuropeptides exert their effects. One of these effects is the modulation of CPGs. However, very little research has been done on PRPs, so their function still remains unknown. Based on the research done thus far, PRPs are thought to be biologically inactive, with their major functions being for the integrity of the peptide and for the folding of larger neuropeptides. Because PRPs are assumed to be broken down quickly due to their biological inactivity, it is expected that they wouldn't be subject to post-translational modifications.

However, by using mass spectrometry, Professor Stemmler's lab at Bowdoin College confirmed that there were PRPs present in the lobster's nervous system, some of the PRPs having post-translational modifications. The presence of PRPs counteracts the prior notion that the PRPs break down rapidly. These new data, coupled with research that found PRPs to be highly conserved, raise the question of PRP functionality - are PRPs just structural elements or are they in fact biologically active?

The PRPs I analyzed this summer were CCAP PRP2 (DIGDLLEGKD) and three AST-B preprohormones, GDDLADAELQAAED, GWTLWa, and GEELQAAED. These PRPs are known to be present within the lobster, suggesting they may have modulatory effects. The PRPs were tested by being perfused through the heart of the lobster, while I recorded the amplitude and frequency of the muscle contractions. Changes in amplitude and frequency from baseline contractions suggested modulatory effects.

It was found that there was a significant increase from baseline contractions in frequency and amplitude for AST-B GDDLADAELQAAED and AST-B GEELQAAED when perfused at a concentration of 10^{-6}M. It was also found that there was a significant increase in baseline amplitude for CCAP PRP2 when perfused at a concentration of 10^{-6}M. These results show that PRPs can have modulatory effects on the lobster's nervous system. However, it was found that CCAP PRP2 has a very variable response from prep to prep, suggesting that there is some feature driving the CCAP PRP2 modulatory behaviors. In the future, the Dickinson lab hopes to understand why the response to CCAP PRP2 is so variable.

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