The Development of a Mass Spectrometric Method for the Detection of Neuropeptide Receptors in the American Lobster, *Homarus americanus*

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Neuropeptides are short chains of amino acids that are a part of a large class of signaling molecules. Neuropeptides are able to regulate animal physiology and behavior by interacting with their respective cell membrane receptors. One particular group of neuropeptides, C-type allatostatins (AST-Cs), have been found to modulate cardiac function in a number of insects and crustaceans.[1] To characterize the effect of these neuropeptides on the modulation of the lobster's rhythmic pattern generators (RPGs), or the neural circuits responsible for controlling the lobster's rhythmic behaviors, AST-C has been perfused through the lobster heart. When AST-C is perfused through the heart of the American lobster, heart contraction amplitude increases for some animals while it decreases for others.[2] Unique from the effects of other known neuropeptides, the underlying mechanism for the differences in the neuronal circuit responses to a given modulatory input is not fully understood. A working hypothesis to explain this differential response is the presence of multiple AST-C receptors for a given AST-C neuropeptide, as well as a change in the expression of these AST-C receptors. Using mass spectrometry, our goal for the summer is to develop a method to extract and detect AST-C receptors in lobster tissue to provide direct evidence for the receptors.

Mass spectrometry (MS) is an analytical technique that has played an essential role in the identification of neuropeptides in the lobster *H. americanus*. The MS spectrum provides a mass-to-charge ratio for each component in the sample. Tandem MS/MS sequencing is able to provide information about amino acid sequences by fragmenting larger peptides into smaller, charged fragment ions.[3] For this study, receptor sequences have been determined through the use of bioinformatics. These spectra are then matched against a transcriptome database that include the predicted sequences to identify the peptides present in sample. However, very few crustacean neuropeptide receptors have been identified via MS due limited information regarding receptor amino acid sequences and challenges associated with extracting and analyzing what may be low abundance proteins. Membrane proteins also contain large hydrophobic regions that are not easily extracted from tissues. To combat issues associated with the solubilization of proteins, a neuronal protein solution containing the detergent Triton X-100 was used to solubilize these proteins. Along with this, the enzyme trypsin has been used to digest the proteins, creating smaller fragment peptides that can then provide better signal when undergoing MS.

This summer, I have optimized the method for extracting and digesting cell-membrane proteins. This included introducing a high pH, reversed-phase fractionation protocol after digestion, providing further separation of peptides based on their increased hydrophobicity. This has resulted in the identification of more novel proteins. Moving forward, we will continue to optimize the method for extraction and digestion of cell-membrane proteins by testing other extraction protocols designed to isolate membrane proteins from cytosolic proteins.

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References:

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